ABSTRACT

ELLIOTT, PATSY ELIZABETH. Screening tobacco germplasm for resistance to diseases affecting transplant production. (Under the direction of Jennifer S. Levin.)

Rhizoctonia solani causes stem rot and target spot of greenhouse-produced tobacco seedlings. No fungicides are registered for control of these diseases, so sanitation is the primary disease management strategy. Seedling resistance to *R. solani* has not been characterized in current tobacco germplasm. The objective of this study was to screen seedlings of a diverse array of accessions, including several classes of tobacco cultivars and related *Nicotiana* species for resistance to a stem rot (AG-4) and a target spot isolate (AG-3) of *R. solani*. Further studies were conducted to determine if the resistance identified is heritable. Experiments were conducted in environmentally controlled growth chambers at the NCSU phytotron. Tobacco seedlings were grown in polystyrene trays floating on a nutrient solution to replicate greenhouse growth conditions. Approximately two weeks after germination, rice grains colonized by *R. solani* were placed on the surface of the growth medium to infest the medium. Symptoms, including death, stem lesions, and target spot lesions, were observed for 42 days after infesting the soil for stem rot and 56 days for target spot. Data were analyzed using a GLM procedure in SAS (SAS Institute, Cary, NC). Significant differences were observed among the accessions in level of resistance to stem rot and target spot. Disease incidence ranged from 12.5 to 100% for stem rot and 6.2 to 97.9% for target spot. This wide range of disease incidence observed among accessions for both diseases indicates that useful levels of resistance may exist to both diseases and may be useful in future breeding efforts.

Screening tobacco germplasm for resistance to diseases affecting transplant production

by

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A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of

Master of Science

Crop Science

Raleigh

2003

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DEDICATION

To my parents, for their encouragement, love and patience.

BIOGRAPHY

Patsy Elizabeth Elliott was born on October 9, 1979 in Washington, NC. She attended elementary school in her hometown of Belhaven, NC. In 1997, she graduated from Northside High School in Pinetown, NC and started her undergraduate education at Meredith College in Raleigh, NC, where she received a B. S. degree in Biology, in 2001. In the fall of 2001, she began her studies at North Carolina State University to pursue an M. S. degree in Crop Science. In late spring 2002, Patsy began her research under the direction of Dr. Jennifer Levin.

ACKNOWLEDGMENTS

I am very grateful to my advisor, Dr. Jennifer Levin, for giving me the opportunity to learn about tobacco breeding at NCSU. I am also very grateful to Dr. H. David Shew and Dr. Verne Sisson for serving on my Master of Science advisory committee. I would like to thank Dr. Janet Shurtleff for her expert guidance in the NC State Phytotron. I am grateful to Dr. Cavell Brownie for her assistance with statistical analysis.

My thesis work could not have been completed without the help of co-workers, family and friends who assisted me during "crunch times". I wish to thank Mary-Catherine Smith for her assistance over the past year and a half. I am very appreciative of Crystal Paras, who spent hours cutting trays, making mud, and transplanting. I also want to thank Carol Beth Allen, Brenda Elliott, Christine Zoul, and Daniel Zoul for their membership in the mud crew.

Finally, I am grateful to the North Carolina Tobacco Research Commission for their financial support.

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SCREENING TOBACCO GERMPLASM FOR RESISTANCE TO DISEASES AFFECTING TRANSPLANT PRODUCTION

INTRODUCTION

Since farming is an important segment of world business, crop improvement is a major aspect of current research. Tobacco, *Nicotiana tabacum* L., has been enhanced in breeding programs across the southeast USA, including North Carolina State University. A major portion of tobacco improvement involves incorporation of resistance to diseases. Research groups have been successful in development of new cultivars with resistance to multiple diseases using classical plant breeding and biotechnological techniques. In tobacco, sources of resistance to many diseases have been found in related *Nicotiana* species. In addition, some cultivars have served as sources of resistance to certain diseases. For example, the cigar wrapper cultivar Florida 301 is the source of the widely used resistance to black shank. Multiple screens for disease resistance among cultivars and tobacco types have been conducted to locate sources of resistance in present germplasm to common diseases that afflict tobacco in the greenhouse and field (Johnson, 1914; Smith-White, 1936; Clayton, 1945, Hill and Mandryk, 1962; Vinogradov et al., 1975; Chaplin and Goodling, 1968; Gwynn et al., 1986).

Rhizoctonia solani is a soil borne fungus that is commonly found around tobacco greenhouses. Two common diseases of greenhouse-produced tobacco seedlings are caused by *Rhizoctonia solani*, stem rot, characterized by brown water-soaked lesions on the seedling stem, and target spot, which is characterized by water soaked spots on leaves that turn brown and form a hole through the leaf (Shew and Lucas, 1991; Shew and Main, 1990). The high humidity and elevated temperatures found in tobacco greenhouses favor the development of stem rot and target spot.

Until recently, tobacco seedlings were grown outside in fumigated seedbeds.. Today, the majority of growers in the United States produce their seedlings using greenhouse float systems. In the float system, polystyrene trays are filled with soilless medium, direct seeded, and floated on a shallow reservoir of nutrient solution. Since this system is preferred for transplant production, disease control plays an important role in the production of healthy transplants.

Certain fungicides, such as iprodione (Rovral), do provide control of *Rhizoctonia*, but no fungicides are currently labeled for control of *Rhizoctonia* in the greenhouse. Cultural practices are currently recommended to control outbreaks of stem rot and target spot. Sanitation, such as the removal of clippings, is the primary disease management strategy. Other cultural approaches, including increased ventilation and avoidance of transplant injury, also play an important role in the management of stem rot and target spot. Since outbreaks of these diseases can cause significant losses, *R. solani* resistant tobacco lines would be highly desirable to farmers. The detection and use of genetic resistance to *R. solani* in new cultivars would aid in reducing or eliminating losses associated with stem rot and target spot.

The first step in development of *R. solani* resistant tobacco lines is the identification of sources of resistance. Several crops have been screened for *R. solani* resistance, including carrot, *Daucus carota* L., (Anderson et al., 1982), peanut, *Arachis hypogaea*, (Woodard and Jones, 1980), pepper (Muhyi and Bosland, 1995), sorghum, *Sorghum vulgare*, (Pascual et al., 2000), and sugar beet, *Beta vulgaris*, (Campbell and Altman, 1976; Scholten et al., 2001). There have been reports that some tobacco lines exhibit partial resistance to *R. solani* (Csinos and Stephenson, 1999; Dipon and Davide,

1982). These reports suggest that resistance to target spot or stem rot is present in tobacco germplasm, but seedling resistance to *R. solani* has not been well characterized.

Much of the available tobacco germplasm has been screened for a wide range of characteristics, including disease resistance (Legg and Smeeton, 1999). A reliable screening technique to identify *R. solani* resistance is vital to detect sources of resistance. The primary objective of this study is to screen a diverse array of germplasm, including several classes of tobacco cultivars and related *Nicotiana* species, for genetic resistance to *R. solani*. A secondary objective is to determine if the resistance is heritable. To quantify the data accessions were not only evaluated for death and symptom incidence, but also thennumber of lesions, lesion size, and other characteristics that might be pertinet. As demonstrated in this report, it is relatively simple to evaluate a large number of accessions to detect resistance to *R. solani*. The results may also have future implications in plant breeding for persons wishing to incorporate *R. solani* resistance into new genotypes.

MATERIALS AND METHODS

Plant Material. Ninety-nine tobacco accessions were evaluated for resistance to *R. solani*. Sixteen wild species of *Nicotiana*, 2 cigar binder types, 15 burley accessions, 1 dark type, 11 cigar-filler types, 27 flue-cured, 1 Maryland type, 16 oriental types, and 11 cigar-wrapper types were evaluated for resistance to (Table 1). The accessions chosen included popular cultivars, breeding lines, and tobacco introductions (TI) which were collected from other countries. The TIs were chosen based on low nor-nicotine content and diversity of geographic location. Eighty-five accessions were obtained from the USDA Tobacco Germplasm Collection via Verne Sisson, Oxford, NC. In a secondary experiment, crosses between resistant and susceptible accessions identified in the primary experiment were made to determine if the resistance was heritable.

Growth conditions. Growth chamber experiments were conducted in 2002 and 2003 at the North Carolina State University Southeastern Plant Environmental Laboratory (Phytotron), Raleigh, NC. The accessions were seeded and allowed to germinate in styrofoam cups containing Carolina's Choice, a specially formulated peat-lite tobacco potting mix (Carolina Soil Company, Kinston, NC). The soil was moistened thoroughly with distilled water before seeding and covered with plastic wrap after seeding.

Three-week-old seedlings were transferred to polystyrene trays (5 x 6 cells, each cell 2.3 x 6.2 cm), cut from a 288-cell float tray. The trays were uniformly filled with the moistened soilless tobacco potting mix and packed just tightly enough for proper capillary water movement (wicking). A single seedling was planted into each cell and only one type of accession was planted into each 5 x 6 cell polystyrene tray.

Transplanted trays were placed into black polyethylene pans (Hummert; 53 x 37 x 13 cm) and filled with water and fertilizer to mimic the greenhouse float-tray system used by tobacco growers. A water-soluble fertilizer (Peters 20-20-20, Scotts-Sierra Horticultural Products Company, Marysville, OH) was added to distilled water at 150 parts per million (ppm) prior to transplanting. Transplants were grown in the float tray system in a phytotron "A" chamber (8.90 x 8.90 x 2.31 m) at 28/20 ˚C day/night temperatures and a 12-hour photoperiod for 2-3 days prior to inoculation, to allow the seedlings to overcome transplanting shock. Distilled water was added to the pans as needed.

Inoculum. An AG-3 (target spot) and an AG-4 (stem rot) isolate of *R. solani* was obtained from flue-cured tobacco. The isolates were grown on potato dextrose agar (PDA) (Difco, Detroit, MI) at room temperature (22-25 °C) for five days. Colonized agar plugs were removed and transferred to 250 mL Erlenmeyer flasks containing autoclaved rice grains (Shew and Main, 1990). The fungus was allowed to thoroughly colonize the rice grains for approximately 2 weeks.

Inoculation. Tobacco seedlings were inoculated with each isolate of *R. solani* two days after transplanting. Infested rice (approximately 6 grains) was placed onto the float tray at 6 pre-planned sites (Figure 1) and *R. solani* was allowed to colonize the trays. Some cells are bordered by more than rice site, but no biases were observed on the plants in those cells. Tests for the two diseases were conducted in separate growth chambers. Plants were evaluated 1 week after the inoculation, and then every other day for disease incidence. The plants that died as a result of transplanting shock were subtracted out. Seedling death occurred in the stem rot experiment until Day 17 (days after inoculation) and in the target spot experiment until Day 15. Stem rot symptoms (Figure 2) appeared in

the stem rot experiment on Day 34. Target spot lesions (Figure 3) appeared in the target spot experiment on Day 53. Evaluations were based on seedling death and target spot or stem rot incidence.

Data analysis. The primary experiments were conducted in a randomized complete block design with four replications for the target spot analysis and three replications for the stem rot analysis. Each phytotron chamber contained a single replication and the experiment was replicated over time. Data were analyzed using the GLM procedure in SAS (SAS Institute, Inc., Cary, NC). The secondary experiments were conducted in a randomized complete block design with three replications in the same chamber. These data were analyzed similarly using the GLM procedure. Data that was ARCSIN transformed was also analyzed, but this did not provide new information, so this analysis was not used.

RESULTS

Screen for stem rot resistance.

A diverse array of 99 tobacco and wild species accessions (Table 1) were inoculated with the stem rot isolate of *R. solani*. Highly significant differences were observed among genotypes infected (Table 2). Disease incidence, which included seedling death and stem lesions, ranged from 12.5% to 100%. *Nicotiana plumbaginifolia*, a wild species from South America, and TI 1316, a cigar filler tobacco line from New Zealand, each exhibited 12.5% disease incidence, but 4.1% of the 12.5% mean disease incidence was accounted for by seedling death in TI 1316. *N. plumbaginifolia* experienced no seedling death. *Nicotiana africana*, a wild species from Namibia, was extremely susceptible to stem rot, and had 100% disease incidence, with 56.9% of those infected plants being killed. The least significant difference (LSD) of the overall disease incidence of all 99 accessions evaluated for stem rot was 34.8%.

The average of seedling death only was also calculated for the 99 accessions evaluated for stem rot resistance. Significant differences were observed among the accessions evaluated. Death ranged from 0.0 to 56.9% among the accessions evaluated, but the majority of the accessions had less than 10.0% seedling death. Five accessions, *N. plumbaginifolia*, TI 1379, TI 1241, TI 1330, and 'Pennbel 69', had no seedling death. The LSD of seedling death of accessions evaluated for stem rot resistance was 16.9%.

A frequency distribution of mean stem rot incidence of the 99 tobacco accessions following inoculation with *R. solani* was examined (Figure 4). A continuous range of disease incidence was observed and the histogram was normally distributed.

Overall disease incidence varied among tobacco types (Table 4). Significant differences were observed among the tobacco types evaluated for stem rot resistance. The oriental accessions, which had an overall type mean of 30.0%, were different from the wild *Nicotiana* species, which had an overall mean of 45.4%. The LSD of the types evaluated for stem rot resistance was 15.6%.

The distribution of phenotypes is made up of resistant to susceptible material based on their overall disease incidence. Entries were separated by tobacco type and analyzed again to determine if there were differences in susceptibility within each type. The burley accessions evaluated, including 'Burley 21' (Heggestad, 1966), 'KY 14' (Litton et al., 1969), 'L8' (Collins et al., 1971), TI 1605, 'VA 509', 'TN 90' (Miller, 1991), TI 1449, TI 819, TI 1414, 'Clay 402', DH 608, TI 1569, 'NC 5', 'NC 4' and 'R 7-11', were similar in their response to the *R. solani* AG-4 isolate (Table 5). The burley cultivars KY 14, Burley 21 and L8 had less than 20% overall disease incidence, while NC 5, NC 4 and R 7-11 had over 45% overall disease incidence. KY 14 and Burley 21 are relatively older cultivars, while NC 4 and NC 5 are fairly new cultivars. The LSD of the burley accessions was 25.4%.

Cigar filler accessions evaluated, included TI 1316, TI 835, TI 232, TI 123, TI 165, TI 672, 'Pennleaf 1', TI 1241, TI 1330, TI 1480, and 'Pennbel 69'. Significant differences were observed among these accessions (Table 6). TI 1316, a tobacco introduction from New Zealand had 12.5% disease incidence, while TI 1480, a tobacco introduction from Canada, and Pennbel 69, a cultivar from the United States were over 40%. Disease incidence among the cigar filler types ranged from 12.5 to 65.3%, and the LSD was 35.0%.

Cigar wrappers, TI 428, TI 1029, TI 1451, 'Connecticut Broadleaf', TI 79, TI 1577, FL 301, TI 1589, TI 186, TI 1518, and TI 119, were evaluated for stem rot resistance. Significant differences were also observed among these accessions (Table 7). TI 428, TI 1029, and TI 1451 were significantly different from TI 1518 and TI 119. The range of the disease incidence of the cigar wrapper type, 16.6-68.0%, was similar to the cigar filler types, and the LSD was 31.6%. Two cigar binder types from the United States, 'Havana 307' (Keller, 1958) and 'Havana 503' (Ogden, 1968), were evaluated for resistance to stem rot. No differences were observed between these two accessions, with a disease incidence of 31.1 and 39.4%, respectively.

Sixteen different oriental accessions were evaluated for stem rot resistance (Table 8). Significant differences were observed among the oriental accessions. TI 1311, a tobacco introduction from Papua, New Guinea, and 'Xanthi', a tobacco line from Greece, were significantly different from TI 1306, a tobacco introduction from Argentina. Overall disease incidence ranged from 15.3 to 47.2%, with the majority of the accessions falling between 25-35%.

Significant differences were observed among the wild *Nicotiana* species (Table 9). *Nicotiana plumbaginifolia* and *N. rustica*, two species from South America, were significantly different from *N. alata*, another South American species, and *N. africana*, a species from Namibia. The widest range of overall disease incidence was observed among the wild *Nicotiana* species. Disease incidence ranged from 12.5 to 100%.

Twenty-seven flue-cured accessions were evaluated for stem rot resistance. Significant differences were observed among the flue-cured accessions evaluated (Table 10). TI 109, a tobacco introduction from Columbia, was significantly different from 'NC

71', a popular cultivar from the United States. The majority of the overall disease incidences observed ranged from approximately 30-50%. Non-significant differences were observed among the US flue-cured cultivars evaluated for stem rot resistance. 'NC 2326' (Apple, 1964) was less infected than NC 71 and 'NC 72', two recently released hybrids.

A comparison of the 10 most resistant and the 10 most susceptible accessions evaluated for resistance to stem rot was made (Figure 5). Resistant accessions in this comparison have less than 20% mean disease incidence, while the susceptible accessions have more than 50% disease incidence. Also, the susceptible accessions had much more seedling death as a result of stem rot than the resistant accessions.

Screen for target spot resistance.

A diverse array of 99 tobacco accessions was inoculated with a target spot isolate of *R. solani* (Table 1). Highly significant differences in disease incidence were observed among genotypes (Table 3). Disease incidence, including seedling death and target spot lesions, ranged from 6.2% to 97.9%, and the LSD of the overall disease incidence of all 99 accessions evaluated for target spot was 18.4%. TI 1605, a burley accession from Japan, had 6.2% disease incidence, while TI 395, an oriental line from the USA, had 97.9% disease incidence.

Differences were observed in seedling death among the accessions evaluated. Death ranged from 0.0 to 18.5% among the accessions evaluated, but the majority of the accessions had less than 10.0% seedling death. 'SA 1214', a flue-cured accession from South Africa, had the highest seedling death at 18.5%. Seven accessions had 0.0%

seedling death. The LSD for seedling death was 8.15% for the accessions evaluated for target spot resistance.

A frequency distribution of mean target spot incidence of the 99 tobacco accessions following inoculation with *R. solani* was examined (Figure 6). Similar to the stem rot study, a continuous range of disease incidence was observed and the histogram was normally distributed, indicating that the majority of accessions have a moderate level of resistance while some entries are significantly more diseased and some significantly more resistant.

Differences were observed among the tobacco types evaluated for target spot resistance (Table 4). The burley accessions tested had relatively low incidence of target spot (20.4%), while the flue-cured and oriental cultivars had disease incidences of 45.4% and 45.9%, respectively. The LSD of the types evaluated for target spot resistance was 7.6%.

Similar to the stem rot screen, a range of phenotypes was observed among the 99 tobacco accessions evaluated for target spot resistance. The accessions were again separated by type and compared. Differences were observed among the 15 burley accessions evaluated (Table 11). TI 1605, a tobacco introduction from Japan, had the least overall disease incidence (6.2%) and was significantly different from TI 1414, a tobacco introduction from Italy that had a disease incidence of (38.5%). Disease incidence for the burley accessions evaluated ranged from 6.2 % to 38.5% and the LSD was 9.34%.

Various cigar types were evaluated for resistance to target spot. Significant differences were not observed among the two cigar binder accessions evaluated for

resistance. The overall disease incidence of Havana 307 and Havana 503 was 33.3% and 34.3%, respectively. Significant differences were observed among the 11 cigar filler types were evaluated for resistance to target spot (Table 12). TI 165, a tobacco introduction from Japan that had 21.9% disease, whereas TI 1480, a tobacco introduction from Canada, had 56.7% disease. The disease incidence ranged from 21.9% to 56.7% and the LSD was 16.4%. Significant differences were also observed among the 11 cigar wrapper accessions evaluated for target spot resistance (Table 13). TI 186, a tobacco introduction from Mexico, and TI 1029, a tobacco introduction from Venezuela, were significantly different from TI 1577, a tobacco introduction from South Africa. The disease incidence of the cigar wrapper types ranged from 26.0 to 50.0%, and the LSD was 18.6%.

Significant differences were found among the wild *Nicotiana* species evaluated for target spot resistance (Table 14). *Nicotiana africana* and *N. nudicaulis* were significantly different from *N. velutina* and *N. langsdorffii*. Disease incidence ranged from 8.3% to 42.7%, with the majority of the accessions falling between 15-25%.

Significant differences were observed among the 27 flue-cured accessions evaluated for target spot resistance (Table 15). TI 1524, TI 716, and TI 109 were significantly different from 'Speight H-20' and 'Ox 414 NF'. TI 1524, TI 716, and TI 109 had less than 30.0% overall disease incidence, while Speight H-20 and Ox 414 NF had greater than 70% overall disease incidence. The flue-cured accessions had the widest range of overall target spot disease incidence ranging from 16.6 to 83.2%.

Sixteen oriental tobacco types were evaluated for target spot resistance (Table 16). TI 1555, a tobacco introduction from Iran, had the lowest disease incidence at

28.1%, while TI 395, a tobacco introduction from the United States had a disease incidence of 97.9%. Overall disease incidence ranged from approximately 30 to 97.9%, but only five accessions, TI 88, TI 1311, 'Samsun' (nn), TI 1280, and TI 395, had a disease incidence greater than 50%.

Similar to the stem rot study, a comparison of the 12 most resistant and the 10 most susceptible accessions was made (Figure 7). Also similar to the stem rot results, the 12 most resistant accessions had less than 20% mean disease incidence, while the 10 susceptible accessions had greater than 50% disease incidence. Differences in seedling death were not noted among the resistant and susceptible accessions.

Some cultivars are susceptible to both isolates of *R. solani*, while others are resistant to one isolate and susceptible in the other. Virginia 509, Burley 21, and KY 14 had a relatively low disease incidence to both stem rot and target spot. Hicks had a relatively high disease incidence associated with both isolates. NC 2326 had a high disease incidence associated with target spot, but a relatively low disease incidence associated with stem rot. Both Havana 307 and Havana 503, the two cigar binders, were moderately susceptible to both stem rot and target spot, with disease incidences of approximately 33%. Also, MD 609, the only Maryland tobacco type evaluated, appears to be moderately susceptible to both stem rot and target spot, with disease incidences of approximately 33%.

Stem rot and target spot resistance heredity

Based on initial results in the primary experiment, heredity of resistance was investigated. TI 1311, an oriental line, and TI 1316, a cigar filler type, were used as resistant parents for stem rot resistance, with overall disease incidences of 15.3% and

12.5%, respectively. TI 1311 is highly susceptible and TI 1316 is moderately susceptible to target spot, with overall disease incidences of 54.2% and 31.2%, respectively. KY 14, a burley line, appears to have partial resistance to target spot and stem rot with disease incidences of approximately 20% for both diseases. K 326, a flue-cured line, is moderately susceptible to stem rot (36.1% overall disease incidence), but is susceptible to target spot (50.0% overall disease incidence). Hicks, another flue-cured line, is susceptible to both stem rot and target spot, with disease incidences over 50.0%. Crosses between resistant and susceptible lines were made. K 326 was crossed to TI 1311, TI 1316, and KY-14 to investigate stem rot resistance. Hicks was crossed with TI 1311, TI 1316, and KY-14 to investigate target spot resistance. The F_1 hybrid between K 326 and KY 14 was backcrossed to each parent.

The eight crosses between susceptible and resistant material parents were utilized to determine if the resistance observed in the initial screens was heritable. Eleven resistant and 10 susceptible accessions identified in the primary study were tested alongside the crosses and the parents. These accessions served as a platform to detail symptoms more closely and to confirm previous results.

Differences in stem rot incidence were observed between the crosses and parents (Table 17). The LSD of the parents and crosses evaluated for stem rot resistance was 24.6%. Hicks had a disease incidence of 69.4%, while TI 1311 had a disease incidence of 23.6%. Hicks x TI 1311 had a disease incidence equal to the resistant parent, so the resistance found in TI 1311 may be dominant. Progeny from the TI 1316 crosses had moderate to high disease incidence, similar to susceptible parents. Therefore the resistance originally found in TI 1316 is most likely to be only partially dominant or

recessive. Non-significant differences were also noted between susceptible by resistant crosses. KY 14 and K326 and the F_1 BC_1P_1 and BC_1P_2 hybrids had moderate to high disease and were not statistically different. Higher overall mean disease incidence was observed in this test compared to the primary test. TI 1311 has 23.6% disease compared to 12.5% disease in the primary study. KY 14 also previously had a relatively low disease (18%) incidence in the previous test, but was much higher (58.3%) in this test.

The 11 resistant and the 10 susceptible accessions were observed for stem rot incidence. The disease incidence ranged from 9.7 % to 79.1% for all accessions evaluated (Table 18). The accession with the least disease incidence was TI 1029 and the accessions with the highest disease incidences were TN 90 and NC 72. The majority of these results were consistent with the initial study. L8, VA 509, and TN 90 had higher disease levels than observed in the initial screen.

Stem rot lesions were measured at the end of the experiment in order to quantify the resistance or susceptibility detected. Significant differences were observed among the genotypes for stem rot lesion size (Table 19). Stem rot lesion sizes ranged from 0.37 to 1.76mm. The mean stem rot lesion size data did not correlate with the overall mean disease incidences of those evaluated. Therefore, lesion size would not be a good predictor of resistance in the accessions evaluated for stem rot resistance.

Heredity of resistance to target spot was also tested. Unlike the previous test, plant death was the only symptom observed in this test. Significant differences were observed among the parents and crosses evaluated (Table 20). Seedling death ranged from 4.2 to 20.8%, and the LSD of the parents and crosses was 13.95%. K 326 x TI 1316 had 4.6% seedling death, which is less than either parent. K 326 is significantly different

from only three other entries, but the LSD is high for this test when compared to the range.

Significant differences were observed among the accessions evaluated for target spot resistance (death) (Table 21). Seedling death ranged from 5.6 to 48.1%, and the LSD for the other accessions evaluated for target spot resistance was 13.91%. Since disease incidence was very different in this test, additional runs of this test are needed.

DISCUSSION

This paper outlines a screening procedure to evaluate tobacco seedlings for resistance to stem rot and target spot caused by different AGs of *Rhizoctonia solani*. The best way to quantify resistance to these diseases was to include seedling death as well as stem rot or target spot lesion incidence. The results demonstrated that differences in disease incidence could be observed among the accessions evaluated. These differences may be useful in future breeding programs, in which a breeder wishes to incorporate *R. solani* resistance into new cultivars.

No accession was completely free of disease, but resistant accessions were identified based on low overall mean disease incidence. Disease incidence included seedling death, as well as the classic stem rot and target spot symptoms. Seedling death occurred in the stem rot experiment until Day 17 (days after inoculation) and in the target spot experiment until Day 15. Stem rot symptoms appeared in the stem rot experiment on Day 34 and final disease incidence was determined on Day 42. Target spot lesions appeared in the target spot experiment on Day 53 and final disease incidence was determined on Day 56. Seedlings are normally transplanted at between 50 and 80 days after seeding. Therefore, the seedlings evaluated were at the proper age to be evaluated for resistance to diseases found in greenhouses, although they were not clipped.

The frequency distributions of stem rot and target spot incidences of tobacco accessions following inoculation with *R. solani* resulted in a normal distribution. Both diseases exhibited a continuous range of disease incidence with the majority of entries having a moderate incidence, and a low frequency of highly susceptible and highly resistant entries. This is characteristic of quantitative resistance and may indicate that

resistance is controlled by multiple genes. This resistance may be useful in future breeding programs, since resistant varieties would be the most effective method for control of stem rot and target spot. Geographic origin was not a common factor among the accessions found to be resistant to *R. solani*.

Two possible sources of stem rot resistance were found in TI 1311, an oriental type, and TI 1316, a cigar filler type. The oriental accessions had the least disease incidence and may be a source of stem rot resistance. In the secondary stem rot study, the genotypes were evaluated for resistance to the stem rot isolate of *R. solani*. TI 1311 and TI 1316 where shown to have stem rot resistance in the primary experiment. K 326 and Hicks had no resistance to *R. solani*. Hicks x TI 1311 is relatively tolerant of stem rot. Hicks and TI 1311 are significantly different. In this susceptible by resistant cross, it appears that the resistance found in TI 1311 is heritable and dominant. Also, TI 1316 was found to be resistant in the primary study. However, in the K 326 x TI 1316 cross, a high disease incidence was observed, possibly indicating that the resistance found in TI 1316 is recessive or further testing is needed. TN 90, KY 14, L8, and VA 509 did not appear to be resistant in this test, although they seemed to have good resistance in the first experiment. It is possible that higher disease pressure occurred in this test, resulting in the partial resistance of these lines being overcome. However, TI 1311 and TI 1316 may be a source of resistance that could be used for future breeding.

Since TI 1311 continued to display resistance to the stem rot isolate of *R. solani* in both experiments, it would most likely be the best candidate in a stem rot breeding program due to its possible dominant resistance genes. However, the recessive resistance

found in TI 1316 may also be of use in the future. Resistance to stem rot is partial because the accession was not 100% free of disease.

Lesion size was examined for several genotypes evaluated for stem rot resistance in the secondary stem rot study. Lesion size did not correlate with disease incidence. Since lesions are not the only symptom caused by the stem rot isolate of *R. solani*, lesion size alone would not be a good predictor of resistance. The best evaluation method for stem rot resistance should include both symptom incidences and should be a good predictor of resistance. Since death and/or stem rot lesions are not usually seen alone, it is best to include both symptoms in the resistance prediction.

A source of target spot resistance may have been found in the burley cultivars, due to their low mean overall disease incidence. It is possible that the burley cultivars have a common factor that results in target spot resistance. All of the burley cultivars have wildfire resistance from *N. longiflora* and black root rot resistance from *N. debneyi*. TI 1605, a burley from Japan, had the least disease incidence, and the resistance identified may be useful in the future. *Nicotiana debneyi* and *N. longiflora* are also target spot resistant.

In the heredity of target spot resistance study, a thorough evaluation of resistance could not be conducted due to the lack of secondary inoculum production (basidiospores). However, mean death due to *R. solani* was recorded and analyzed. This data is not consistent with the results obtained in the primary study, because accessions that appeared to be resistant to target spot now appear susceptible.

Resistance to stem rot and target spot was found in the wild *Nicotiana* species evaluated. *Nicotiana plumbaginifolia* and *N. rustica* had a relatively low stem rot disease

incidence, while *N. africana*, *N. nudicaulis*, *N. glutinosa*, and *N. plumbaginifolia* were found to be resistant to target spot. However, it is very difficult to transfer multigenic, partial resistance found in *Nicotiana* species to *N. tabacum*, although many disease resistances have been identified and transferred from *Nicotiana* species to tobacco, such as the wildfire resistance found in *N. longiflora*.

Dipon and Davide (1982) reported that Virginia 21 was reported to be resistant to a damping-off isolate of *R. solani*, while Coker 411, NCBY, and Harrison Special were found to be moderately resistant. In general, the researchers observed resistance to *R. solani* only in Virginia/flue-cured types, not in cigar types. Virginia 21, Coker 411, NCBY, and Harrison Special were not included in the current study. However, many flue-cured types were included. The mean disease incidence for the flue-cured accessions evaluated showed relatively high disease incidence (41.6%) in this test. The flue-cured tobacco type was not significantly different than the *Nicotiana* species, various cigar types, burley and oriental types evaluated.

Csinos and Stephenson (1999) evaluated Coker 371-Gold, Speight G-70, and K 326 seedlings for resistance to *R. solani*. The transplants were inoculated by pushing *R. solani* infested toothpicks into the stems, and stem disease ratings were taken. Eight isolates and one control of *R. solani* were used to evaluate plants and assign disease ratings. The cultivars inoculated displayed a range of susceptibility to the various isolates, but K 326 had more disease than 1071, Coker 371-Gold, and Speight G-70. This method of evaluating seedlings for resistance is different from what was used in my experiment and likely measures different response to the pathogen. Also, each cultivar-isolate combination had only 6 plants to be evaluated.

Csinos and Stephenson (1999) also evaluated isolates of stem rot and target spot for sensitivity to several fungicides. During the evaluation, Csinos and Stephenson (1999) reported that NC 72, NC 71, K 326, Coker 371-Gold, Speight G-70, Speight G-28, NC 95, NC 2326, and K 149 had low levels of *Rhizoctonia* target spot in the field. These results were the result of a natural infection during their experiment. The flue-cured varieties NC 72, NC 71, K 326, Coker 371-Gold, Speight G-70, Speight G-28, NC 95, and NC 2326 were included in the primary experiment. In contrast to the previous study, NC 72, NC 71, K326, Coker 371-Gold, Speight G-70, NC 95, and NC 2326 were all relatively susceptible to target spot in this work. Speight G-28 was less susceptible to target spot; however, differences may be due to the differences in plant stage (seedling vs. field plants) or type of inoculation.

Since many environmental concerns, such as the overuse of fungicides, would be solved, resistance breeding is highly relevant. The resistant cultivars must be durable, and must be able to substitute for well-known cultivars. Resistant cultivars are cost-effective when no known solution to a pathogen problem arises. The types of partial resistance found in the tobacco seedlings are similar to the types of resistance found in other crops evaluated for *R. solani* resistance. It is possible that the resistance found in this study may be used to produce cultivars with durable resistance in the future.

A desirable combination of characteristics is easiest to achieve among lines of the same type of tobacco, followed by combinations with different *Nicotiana* species. Possible parents were identified in the heredity study and in the accessions with the least and highest disease incidences identified in the primary study. Susceptible by resistant crosses should be made, followed by backcrossing to a desirable background for several

generations. The back cross method is a popular method used to obtain disease resistance from a species of *Nicotiana* or another class of tobacco without sacrificing quality of the tobacco. Also, the incorporation of molecular maker techniques and biotechnology are being used by tobacco breeding programs to optimize output (Rommens and Kishore, 2000).

The ultimate goal of this research was to identify sources of *R. solani* resistance in tobacco germplasm. Identification of germplasm sources with *R. solani* resistance has begun, but there are many other accessions available that were not screened in this experiment. New and better sources of resistance may be found, but the resistance found in this study could be utilized in breeding programs. The next step in a breeding program is to develop cultivars with resistance to one or both of the seedling diseases caused by *R. solani* described earlier. The new cultivar must meet the precise requirements for the many physical, agronomic, and chemical characteristics (Legg and Smeeton, 1999).

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Figure 1. Diagram of 5 x 6 polystyrene tray. There are 24 seedlings and 6 inoculation sites. S= seedling, I= inoculum.

Figure 2. Characteristic stem rot symptom. Stem rot is characterized by the presence of brown water soaked lesions near the soil surface.

Figure 3. Characteristic target spot symptom. Target spot is characterized by the classic "bulls eye", which consists of concentric necrotic rings.

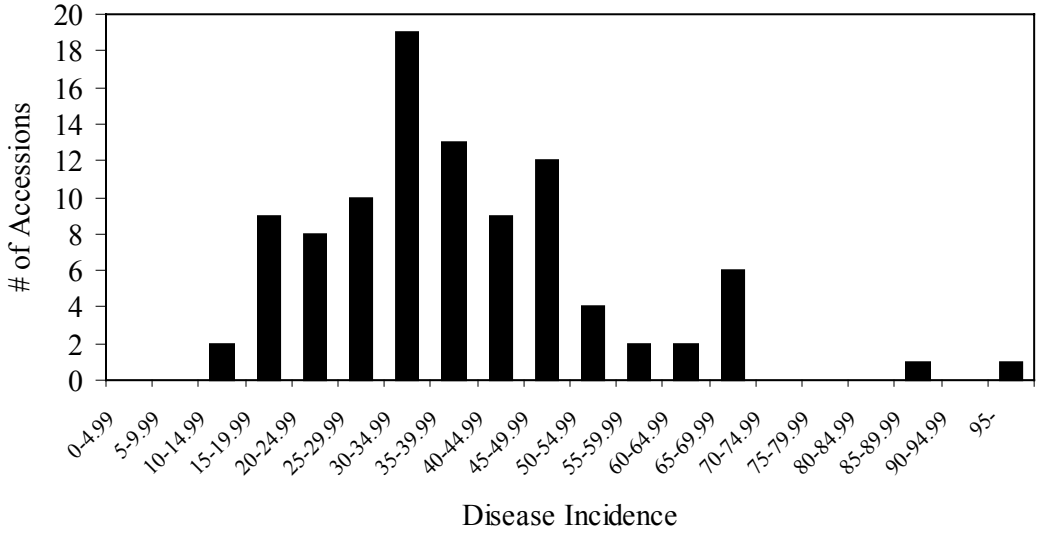

Figure 4. Frequency distribution of stem rot incidence of tobacco accessions following inoculation with *Rhizoctonia solani*. Accessions were inoculated with a stem rot isolate of *R. solani* and symptoms were observed for 42 days. Disease incidence is the percentage of plants showing disease symptoms and is an average of three replications.

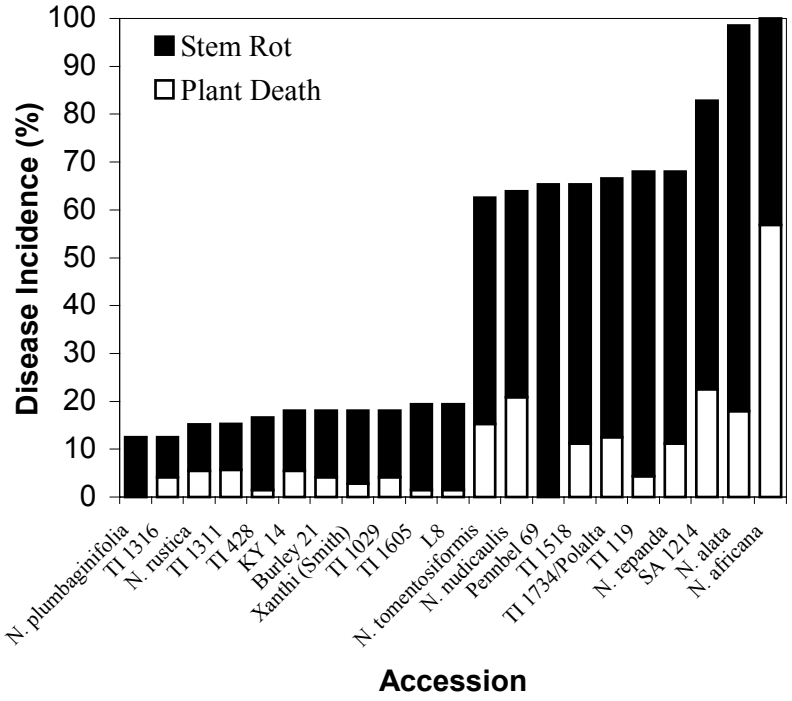

Figure 5. Comparison of the stem rot response of the 10 most resistant and 10 most susceptible accessions to a stem rot isolate of *Rhizoctonia solani*. Disease incidence of seedlings grown in float trays infested with *Rhizoctonia solani*. White bars indicate the percentage of plant death, and black bars show the percentage of plants with stem rot lesions.

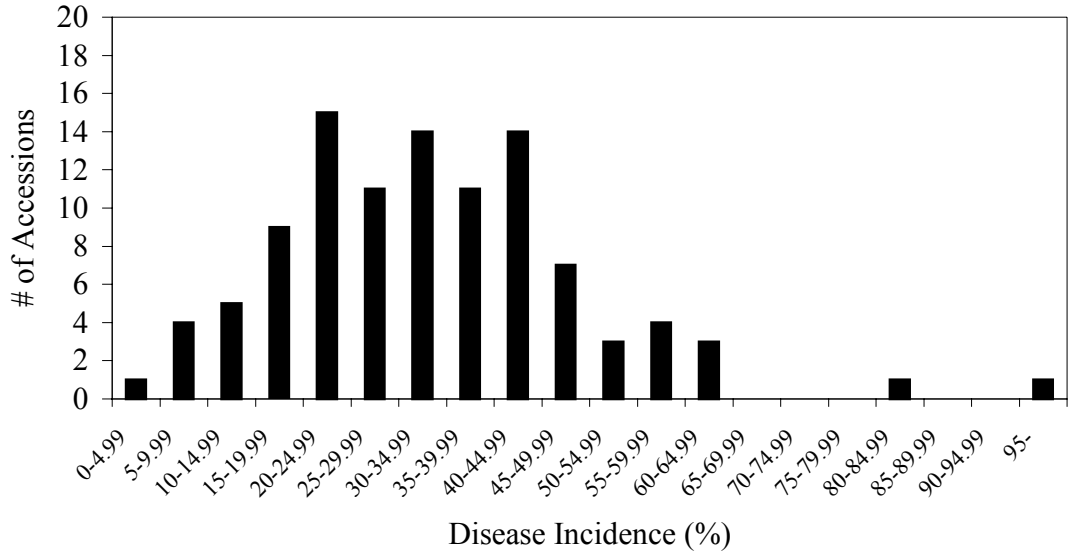

Figure 6. Frequency distribution of target spot incidence of tobacco accessions following inoculation with *Rhizoctonia solani.* Accessions were inoculated with a target spot isolate of *R. solani* and symptoms were observed for 42 days. Disease incidence is the percentage of plants showing disease symptoms and is an average of four replications.

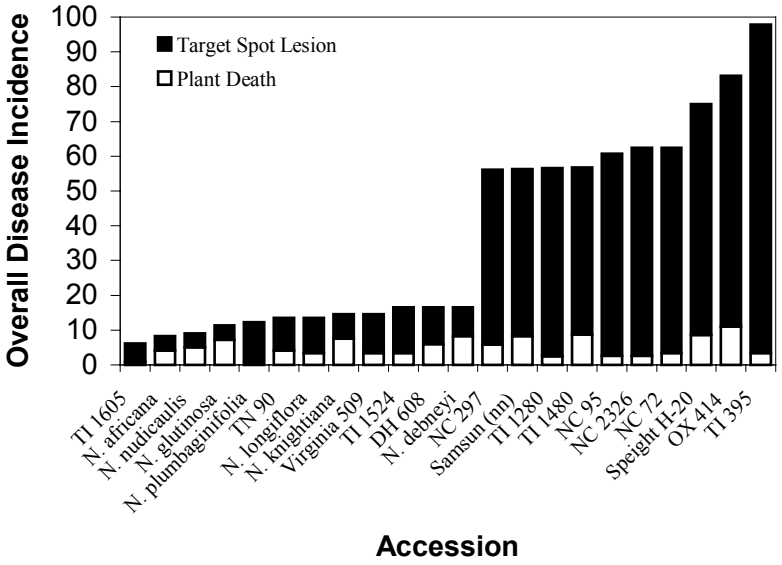

Figure 7. Comparison of the target spot response of the 12 most resistant and 10 most susceptible accessions to a target spot isolate of *Rhizoctonia solani.* Disease incidence of seedlings grown in float trays infested with *Rhizoctonia solani*. White bars indicate the percentage of plant death, and the black bars show the percentage of target spot lesions.

Table 1. Accessions evaluated for resistance to stem rot and target spot diseases caused by *Rhizoctonia solani.*

Accession Name	Accession Number	Country of Origin	Type
Burley 21	PI 552363	USA	Burley
Clay 402	-	USA	Burley
Coker 371 Gold	-	USA	Flue-cured
Connecticut	PI 552619	USA	Wrapper
DH 608/NC 2002	-	USA	Burley
Florida 301	PI 552629	USA FL	Wrapper
Havana 307	PI 552348	USA	Binder
Havana 503	PI 551281	USA	Binder
Hicks	PI 552373	USA	Flue-cured
K 326	PI 552505	USA	Flue-cured
K 346	PI 549110	USA	Flue-cured
Kavala	PI 552668	Greece	Oriental
KY-14	PI 552477	USA	Burley
L8	PI 551280	USA	Burley
McNair 944	PI 552494	USA	Flue-cured
MD 609	PI 552452	USA	Maryland
N. africana	PI 555472	Namibia	*Nicotiana*
N. alata	PI 42334	S. America	*Nicotiana*
N. debneyi	PI 503323	Australia	*Nicotiana*
N. glutinosa	PI 241768	S. America	*Nicotiana*
N. knightiana	PI 555527	Peru	*Nicotiana*
N. langsdorffii	PI 42337	S. America	*Nicotiana*
N. longiflora	PI 555531	S. America	*Nicotiana*
N. nudicaulis	PI 555540	Mexico	*Nicotiana*
N. plumbaginifolia	PI 302478	S. America	*Nicotiana*
N. repanda	PI 555552	Mexico	*Nicotiana*
N. rotundifolia	PI 555553	Australia	*Nicotiana*
N. rustica	PI 555554	S. America	*Nicotiana*
N. sylvestris	PI 555570	S. America	*Nicotiana*
N. tomentosa	TW 140	Peru	*Nicotiana*
N. tomentosiformis	PI 555572	Bolivia	*Nicotiana*
N. velutina	PI 244638	Australia	*Nicotiana*
NC 2326	PI 552453	USA	Flue-cured
NC 297	-	USA	Flue-cured
NC 4	-	USA	Burley
NC 5	-	USA	Burley

Table 1. (Continued)

NC 71	-	USA	Flue-cured
NC 72	-	USA	Flue-cured
NC 95	PI 552380	USA	Flue-cured
OX 414 NF	-	USA	Flue-cured
Pennbel 69	PI 552404	USA	Filler
Pennleaf 1	PI 552403	USA	Filler
R 7-11	-	USA	Burley
SA 1214	-	S. Africa	Flue-cured
Samsun	PI 552748	Greece	Oriental
Speight 168	-	USA	Flue-cured
Speight G-28	PI 551318	USA	Flue-cured
Speight G-70	PI 552497	USA	Flue-cured
Speight H-20	-	USA	Flue-cured
TI 1029/Habano	PI 119162	Venezuela	Wrapper
TI 109/No. 12	PI 105954	Columbia	Flue-cured
TI 119	PI 404974	China	Wrapper
TI 1222	PI 405555	Turkey	Oriental
TI 1224	PI 405557	Turkey	Flue-cured
TI 123/Cuban High Nic	PI 404976	China	Filler
TI 1241/Dubeque	PI 405571	Spain	Filler
TI 1247/Maryland	PI 405577	Spain	Oriental
TI 1269	PI 405590	Ethiopia	Oriental
TI 1279	PI 405599	S. Korea	Oriental
TI 1280	PI 405600	S. Korea	Oriental
TI 1306	PI 241414	Argentina	Oriental
TI 1311	PI 239655	Papua New Guinea	Oriental
TI 1315/Ipomopisflora	PI 405603	New Zealand	Oriental
TI 1316/Calcyiflora	PI 405604	New Zealand	Filler
TI 1330/Zrenjanin	PI 405616	Yugoslavia	Filler
TI 1379	PI 286820	Bulgaria	Oriental
TI 1414/Grand Reditto	PI 405662	Italy	Burley
TI 1449	PI 292203	France	Burley
TI 1451/Zlotolistny Ihar	PI 292205	Poland	Wrapper
TI 1462	PI 304901	Germany	Flue-cured
TI 1480/Ottawa 705	PI 405678	Canada	Filler
TI 1500/Immune 580	PI 329206	Russia	Flue-cured

Table 1. (Continued)

TI 1512/MK 94	PI 370278	S. Africa	Flue-cured
TI 1518/Golden Crest	PI 370284	New Zealand	Wrapper
TI 1524/Enshu-Ha	PI 370290	Japan	Flue-cured
TI 1555/Tirtache	PI 378072	Iran	Oriental
TI 1558/RA 821	PI 355073	Australia	Flue-cured
TI 1569/Puremozhotz 83	PI 349332	Russia	Burley
TI 1577/AZ4	PI 372918	S. Africa	Wrapper
TI 158/Kagoshima Maruba	PI 404994	Japan	Oriental
TI 1589/O-Daruma	PI 390128	Japan	Wrapper
TI 1605/Shurenshu 202	PI 415008	Japan	Burley
TI 1616/Ga-Shen 1	PI 418593	China	Flue-cured
TI 165/Hatano	PI 405002	Japan	Filler
TI 1734/Polalta	TI 1734	Poland	Air-cured
TI 186/Vena Amarilla	PI 112205	Mexico	Wrapper
TI 232	PI 112227	USA	Filler
TI 395/Arcial Chico	PI 112783	Mexico	Oriental
TI 428	PI 112830	Mexico	Wrapper
TI 501/Jaffna	PI 113985	Ceylon	Flue-cured
TI 672/Granja	PI 114634	Mexico	Filler
TI 716	PI 116144	China	Flue-cured
TI 79	PI 67720	Indonesia	Wrapper
TI 819/Samsun	PI 117674	Brazil	Burley
TI 835/Rabode Gallo	PI 118113	Venezuela	Filler
TI 88/Tykulak	PI 404956	Russia	Oriental
TN 90	PI 543792	USA	Burley
TR Madole	PI 552764	USA Cultivar	Dark
Virginia 509	CSR 468	USA	Burley
Xanthi	PI 552780	Greece	Oriental

Accession name†	Stem Rot		
	Seedling Death (%)	Lesion Incidence (%)	Disease Incidence (%)
N. plumbaginifolia	0	12.5	12.5 n-o
TI 1316	4.1	8.4	12.5 n-o
N. rustica	5.5	9.7	15.2 m-o
TI 1311	5.7	9.6	15.3 m-o
TI 428	1.4	15.2	16.6 m-o
KY 14	5.5	12.51	18.0 l-o
Burley 21	4.1	13.91	18.0 l-o
Xanthi (Smith)	2.8	15.21	18.0 l-o
TI 1029	4.1	13.91	18.0 l-o
TI 1605	1.4	18	19.4 k-o
L8	1.4	18	19.4 k-o
TI 1451	1.4	19.4	20.8 j-o
TI 1315	1.4	19.4	20.8 j-o
N. sylvestris	4.1	16.7	20.8 j-o
TI 835	7.2	13.9	21.1 j-o
TI 1555	4.1	18.1	22.2 j-o
TI 232	2.8	19.4	22.2 j-o
TI 1280	4.1	18.1	22.2 j-o
TI 109	4.2	19.4	23.6 j-o
N. knightiana	11.8	13.1	24.9 i-o
Virginia 509	1.4	23.6	25.0 i-o
NC 2326	1.4	23.6	25.0 i-o
TI 395	1.4	23.6	25.0 i-o
TI 123	2.7	23.7	26.4 i-o
TN 90	9.7	16.7	26.4 i-o
TI 1269	2.7	25	27.7 h-o
TI 165	1.4	26.4	27.8 h-o
Samsun (nn)	11.5	17.6	29.1 g-o
TI 501	5.7	23.4	29.1 g-o
Conn. Broadleaf	2.7	27	29.1 g-o
N. glutinosa	13.9	16.6	30.5 f-o
TI 1449	4.1	26.4	30.5 f-o
N. rotundifolia	15.2	15.3	30.5 f-o
TI 1524	11.1	19.4	30.5 f-o
Havana 307	4.3	26.8	31.1 f-o
Speight G-70	2.8	29	31.8 f-o
TI 88	5.6	26.3	31.9 e-o
NC 297	6.9	25	31.9 e-o
TI 716	5.5	26.4	31.9 e-o
TI 819	2.8	29.1	31.9 e-o
TI 79	2.7	29.2	31.9 e-o
Kavala	1.4	30.5	31.9 e-o
N. velutina	12.7	19.7	32.4 e-o
TI 672	9.4	23.6	33.0 e-o
MD 609	2.7	30.6	33.3 d-o
TI 1379	0	33.3	33.3 d-o
TI 1414	4.2	29.1	33.3 d-o
TI 1577	4.2	30.4	34.6 d-o
Pennleaf 1	2.7	32	34.7 d-o
TI 1589	2.8	33.3	36.1 d-n

Continued.

Florida 301	4.1	32	36.1 d-n
TI 1500	1.4	34.7	36.1 d-n
K 326	4.2	31.9	36.1 d-n
TI 1222	2.8	33.3	36.1 d-n
NC 95	4.1	32	36.1 d-n
TI 1241	0	37.4	37.4 d-n
TI 1247	2.8	34.7	37.5 d-n
Speight 168	5.6	32.7	38.3 d-n
Clay 402	9.8	29	38.8 d-n
DH 608	13.9	25	38.9 d-n
Havana 503	1.4	38	39.4 d-n
TI 158	9.7	30.5	40.2 d-n
N. longiflora	2.7	37.9	40.6 d-n
TI 186	4.1	37.5	41.6 d-n
TI 1279	1.4	40.2	41.6 d-n
TI 1462	16.6	25	41.6 d-n
N. langsdorffii	11.3	31.4	42.7 d-n
Speight H-20	4.4	38.6	43.0 d-n
TI 1569	9.7	33.3	43.0 d-n
McNair 944	9.7	34.7	44.4 d-n
NC 5	2.9	42.7	45.6 d-n
Coker 371 Gold	5.5	40.3	45.8 d-n
TI 1330	0	45.8	45.8 d-n
K 346	2.8	43	45.8 d-n
OX 414 NF	11.1	34.7	45.8 d-n
NC 4	7.1	39.5	46.6 d-n
TI 1512	4.2	43	47.2 d-n
TI 1306	6.9	40.3	47.2 d-n
TI 1480	6.9	41.7	48.6 c-m
N. debneyi	25	23.6	48.6 c-m
R 7-11	7.2	42.1	49.3 c-m
TI 1224	5.5	43.9	49.4 c-l
TI 1616	1.4	51.4	52.8 c-l
Speight G-28	5.5	48.5	54.0 c-k
Tom Rosson Madole	9.7	44.4	54.1 c-k
Hicks	2.7	51.4	54.1 c-k
NC 72	2.8	52.7	55.5 c-j
TI 1558	2.7	52.8	55.5 c-j
NC 71	2.9	56.5	59.4 c-i
N. tomentosiformis	15.3	47.2	62.5 c-h
N. nudicaulis	20.8	43.1	63.9 b-g
Pennbel 69	0	65.3	65.3 a-f
TI 1518	11.1	54.2	65.3 a-f
TI 1734/Polalta	12.5	54.1	66.6 a-e
TI 119	4.3	63.7	68.0 a-d
N. repanda	11.1	56.9	68.0 a-d
SA 1214	22.5	60.3	82.8 a-b
N. alata	18	80.6	98.6 a
N. africana	56.9	43.1	100.0 a

Table 2. Stem rot disease incidence for 99 *Nicotiana* accessions. Disease incidence, including both seedling death and lesion incidence, of seedlings grown in float trays infested with *Rhizoctonia solani*. Mean of 3 replications, with 24 plants per replication. Entries with the same letter are not significantly different. Least significant difference (LSD) = 34.8%. † TI= tobacco introduction.

Accession name†	Target Spot		
	Seedling Death (%)	Lesion Incidence (%)	Disease Incidence (%)
TI 1605	0.82	5.38	6.2 ee-ff
N. africana	4.1	4.2	8.3 dd-ff
N. nudicaulis	5.1	3.9	9.0 cc-ff
N. glutinosa	7.2	4.2	11.4 bb-ff
N. plumbaginifolia	0	12.2	12.2 aa-ff
TN 90	4.1	9.4	13.5 z-ff
N. longiflora	3.3	10.2	13.5 z-ff
N. knightiana	7.58	7.02	14.6 y-ff
Virginia 509	3.3	11.3	14.6 y-ff
TI 1524	3.3	13.3	16.6 x-ff
DH 608	6	10.6	16.6 x-ff
N. debneyi	8.3	8.3	16.6 x-ff
TI 819	1.7	15.2	16.9 x-ff
Burley 21	3.3	14.4	17.7 w-ff
Clay 402	7.6	10.1	17.7 x-ff
TI 1449	6.2	13.6	19.8 v-ff
TI 186	5.8	14	19.8 v-ff
TI 1734/Polalta	6.7	13.4	20.1 u-ff
N. repanda	5.8	15	20.8 t-ff
L8	4.1	17.8	21.9 s-ff
TI 716	0.82	21.08	21.9 s-ff
TI 165	3.3	18.6	21.9 u-ff
NC 4	7.4	14.8	22.2 r-ff
TI 1029	3.3	19.6	22.9 r-dd
KY 14	9.4	13.5	22.9 r-ee
N. rotundifolia	3.3	19.6	22.9 r-ee
N. alata	4.2	19.7	23.9 q-ee
R 7-11	3.3	20.6	23.9 u-ee
N. sylvestris	8.4	16.6	25.0 q-dd
TI 672	5.2	20.3	25.5 p-dd
TI 428	5	21	26.0 p-dd
Pennleaf 1	7.5	18.5	26.0 p-dd
TI 1569	3.3	22.7	26.0 p-dd
N. rustica	14.2	12.9	27.1 o-cc
N. tomentosiformis	13.5	13.8	27.3 o-cc
TI 835	6.7	21.4	28.1 o-bb
TI 1555	0.82	27.28	28.1 o-bb
TI 109	6.7	21.4	28.1 o-bb
TI 1518	1.7	26.4	28.1 o-bb
NC 5	7.7	20.8	28.5 n-bb
Speight 168	4.1	25	29.1 n-bb
TI 1279	3.3	25.8	29.1 n-bb
Tom Rosson Madole	5	24.1	29.1 n-bb
TI 1269	1.7	28.5	30.2 m-aa
TI 1316	0	31.2	31.2 l-z
MD 609	2.5	28.7	31.2 l-z
TI 501	4.2	27.4	31.6 k-z
Pennbel 69	8.4	23.5	31.9 k-z
TI 232	2.5	29.8	32.3 k-y
Havana 307	1.7	31.6	33.3 j-x
TI 1247	6.6	27.7	34.3 i-x
TI 1247	6.6	27.7	34.3 i-x
McNair 944	2.5	31.8	34.3 i-x
Havana 503	0.82	33.58	34.4 i-x
SA 1214	18.5	16.2	34.7 i-x
TI 158	12.1	22.9	35.0 h-x
TI 1330	1.6	33.8	35.4 h-w
Conn. Broadleaf	5	31.4	36.4 g-v
TI 1512	1.7	34.7	36.4 g-v
Speight G-28	7.5	28.9	36.4 g-v
TI 1222	0	37.5	37.5 g-v
TI 1306	2.5	35	37.5 g-v
TI 1558	0.82	36.68	37.5 g-v
TI 119	10	27.5	37.5 g-v
TI 1414	4.2	34.3	38.5 f-t
TI 123	7.5	31	38.5 f-u
TI 1315	0	39.6	39.6 f-s
TI 79	4.1	35.5	39.6 f-s
TI 1451	5.8	34.8	40.6 f-r
TI 1241	5.8	34.8	40.6 f-r
TI 1462	5.8	34.8	40.6 f-r
N. velutina	12	29.5	41.5 f-q
TI 1589	2.5	39.1	41.6 f-q
Kavala	0	42.7	42.7 e-p
N. langsdorffii	9	33.7	42.7 e-p
TI 1224	1.7	41	42.7 e-p
TI 1616	7.5	37.2	44.7 d-o
Speight G-70	0	44.8	44.8 d-o
TI 1379	1.7	43.1	44.8 d-o
Coker 371 Gold	7.5	39.4	46.9 d-n
Xanthi (Smith)	3.3	44.5	47.8 d-m
Florida 301	1.6	46.3	47.9 d-m
NC 71	8.5	39.4	47.9 d-m
K 326	2.5	46.5	49.0 d-l
TI 1577	3.3	46.7	50.0 d-k
K 346	6.7	43.3	50.0 d-k
TI 88	7.7	43.5	51.2 d-j
Hicks	8.6	43.5	52.1 d-i
TI 1500	5	48.1	53.1 d-h
TI 1311	11.6	42.6	54.2 d-g
NC 297	5.8	50.4	56.2 d-f
Samsun (nn)	8.3	48	56.3 d-f
TI 1280	2.5	54.1	56.6 d-f
TI 1480	8.8	47.9	56.7 d-f
NC 95	2.6	58.1	60.7 d-e
NC 2326	2.6	59.9	62.5 c-d
NC 72	3.4	59.1	62.5 c-d
Speight H-20	8.6	66.4	75.0 b-c
OX 414	11	72.2	83.2 a-b
TI 395	3.3	94.6	97.9 a

Continued.

Table 3. Target spot disease incidence for 99 *Nicotiana* accessions. Disease incidence, including both seedling death and lesion incidence, of seedlings grown in float trays infested with *Rhizoctonia solani*. Mean of 4 replications, with 24 plants per replication. Entries with the same letter are not significantly different. Least significant difference (LSD) = 18.4%. † TI= tobacco introduction.

Type	Stem Rot Mean	Target Spot Mean
Nicotiana sp.	45.4 a (12.50-100.0)	21.0 c (8.30-42.68)
Flue-cured	41.6 a-b (30.50-45.80)	45.4 a (16.63-83.23)
Cigar Wrapper	36.2 a-b (16.63-68.03)	36.8 b (26.00-49.98)
Cigar Binder	35.3 a-b (31.10-39.40)	33.8 b (33.30-34.40)
Cigar Filler	34.1 a-b (12.47-65.27)	33.0 b (21.85-56.65)
Burley	32.9 a-b (18.03-49.27)	20.4 c (6.23-38.53)
Oriental	30.0 b (15.33-47.17)	45.9 a (28.10-97.90)

Table 4. Stem rot and target spot disease incidence for each tobacco type.
LSD (stem rot) = 15.6%; LSD (target spot) = 7.6. Mean of 4 replications in the target spot experiment and 3 replications in the stem rot experiment, with 24 plants per replication. Entries with the same letter are not significantly different. The range of disease incidences for each disease is included in parentheses.

Name	Disease Incidence
Burley 21	18.03 b
KY 14	18.03 b
L8	19.40 b
TI 1605	19.43 b
Virginia 509	25.00 a-b
TN 90	26.37 a-b
TI 1449	30.53 a-b
TI 1414	33.30 a-b
Clay 402	38.83 a-b
DH 608	38.87 a-b
TI 186	41.67 a-b
TI 1569	43.03 a-b
NC 5	45.57 a
NC 4	46.60 a
R7-11	49.27 a

Table 5. Stem rot incidence in burley accessions. Disease incidence, including both seedling death and lesion incidence, of seedlings grown in float trays infested with *Rhizoctonia solani.* Mean of 3 replications, with 24 plants per replication. Entires with the same letter are not significantly different. LSD=25.4%. TI = tobacco introduction

Name	Disease Incidence
TI 1316	12.47 c
TI 232	22.17 b-c
TI 123	26.37 b-c
TI 165	27.77 b-c
TI 672	33.03 a-c
Pennleaf 1	34.70 a-c
TI 1241	37.37 a-c
TI 1330	45.80 a-b
TI 1480	48.60 a-b
Pennbel 69	65.27 a

Table 6. **Stem rot incidence in cigar filler accessions.** Disease incidence, including both seedling death and lesion incidence, of seedlings grown in float trays infested with *Rhizoctonia solani*. Mean of 3 replications, with 24 plants per replication. Entries with the same letter are not significantly different. LSD=35.0%. TI = tobacco introduction

Name	Disease Incidence
TI 428	16.63 c
TI 1451	20.80 c
T I835	21.10 c
Connecticut BL	29.13 c
TI 79	31.90 c
TI 1577	34.57 b-c
TI 1589	36.10 b-c
Florida 301	36.10 b-c
TI 1279	41.60 b
TI 1518	65.27 a-b
TI 119	68.03 a

Table 7. Stem rot incidence in cigar wrapper accessions. Disease incidence, including both seedling death and lesion incidence, of seedlings grown in float trays infested with *Rhizoctonia solani*. Mean of 3 replications, with 24 plants per replication. Entries with the same letter are not significantly different. LSD=31.6%. TI = tobacco introduction

Name	Disease Incidence
TI 1311	15.33 b
Xanthi	18.03 b
TI 1315	20.80 a-b
TI 1280	22.17 a-b
T I1555	22.20 a-b
TI 395	24.97 a-b
TI 1269	27.73 a-b
Samsun (nn)	29.13 a-b
Kavala	31.90 a-b
TI 88	31.93 a-b
TI 1379	33.30 a-b
TI 1222	36.07 a-b
TI 1247	37.47 a-b
TI 158	40.23 a-b
TI 1306	47.17 a

Table 8. Stem rot incidence in oriental accessions. Disease incidence, including both seedling death and lesion incidence, of seedlings grown in float trays infested with *Rhizoctonia solani*. Mean of 3 replications, with 24 plants per replication. Entries with the same letter are not significantly different. LSD=26.7%. TI = tobacco introduction

Name	Disease Incidence
N. plumbaginifolia	12.5 e
N .rustica	15.23 e
N .sylvestris	20.80 e
N .knightiana	24.87 e
N .glutinosa	30.53 d-e
N .rotundifolia	30.53 d-e
N .velutina	32.40 c-e
N .longiflora	40.60 c-e
N .langsdorffii	42.70 c-e
N .debneyi	48.57 c-e
N .tomentosiformis	62.47 b-d
N .nudicaulis	63.87 a-d
N .repanda	68.03 a-c
N .alata	98.6 a-b
N .africana	100.00 a

Table 9. Stem rot incidence in *Nicotiana* spp. accessions. Disease incidence, including both seedling death and lesion incidence, of seedlings grown in float trays infested with *Rhizoctonia solani*. Mean of 3 replications, with 24 plants per replication. Entries with the same letter are not significantly different. LSD=37.2%. TI = tobacco introduction

Name	Disease Incidence
TI 109	23.57 c
NC 2326	24.97 c
TI 501	29.13 b-c
TI 1524	30.50 b-c
Speight G-70	31.80 b-c
TI 716	31.90 b-c
NC 297	31.93 b-c
NC 95	36.07 b-c
K 326	36.10 b-c
TI 1500	36.10 b-c
Speight 168	38.27 b-c
TI 1462	41.63 b-c
Speight H-20	43.03 a-c
McNair 944	44.40 a-c
Coker 371 Gold	45.80 a-c
K 346	45.80 a-c
OX 414 NF	45.80 a-c
TI 1512	47.20 a-c
TI 1224	49.40 a-c
TI 1616	52.77 a-c
Speight G-28	54.03 a-c
Hicks	54.13 a-c
TI 1558	55.50 a-c
NC 72	55.53 a-c
NC 71	59.35 a-c
TI 1734	66.63 a-b
SA 1214	82.83 a

Table 10. Stem rot incidence in flue-cured accessions. Disease incidence, including both seedling death and lesion incidence, of seedlings grown in float trays infested with *Rhizoctonia solani*. Mean of 3 replications, with 24 plants per replication. Entries with the same letter are not significantly different. LSD=41.1%. TI = tobacco introduction

Name	Disease Incidence
TI 1605	6.23 g
TN 90	13.53 f-g
Virginia 509	14.55 e-g
DH 608	16.63 d-f
NC 4	16.69 c-f
Burley 21	17.68 c-f
Clay 402	17.68 c-f
TI 186	19.75 b-f
TI 1449	19.78 b-f
L8	21.85 b-f
KY 14	22.88 b-e
R7-11	23.93 b-d
TI 1569	26.00 b-c
NC 5	28.45 b
TI 1414	38.53 a

Table 11. Target spot incidence in burley accessions. Disease incidence, including both seedling death and lesion incidence, of seedlings grown in float trays infested with *Rhizoctonia solani*. Mean of 3 replications, with 24 plants per replication. Entries with the same letter are not significantly different. LSD=9.34%. TI = tobacco introduction

Name	Disease Incidence
TI 165	21.85 d
TI 1029	22.90 c-d
TI 672	25.53 b-d
Pennleaf 1	26.03 b-d
TI 1316	31.23 b-d
Pennbel 69	31.93 b-d
TI 232	32.25 b-d
TI 1330	35.4 b-d
TI 123	38.45 b-c
TI 1241	40.6 a-b
TI 1480	56.65 a

Table 12. Target spot incidence in cigar filler accessions. Disease incidence, including both seedling death and lesion incidence, of seedlings grown in float trays infested with *Rhizoctonia solani*. Mean of 3 replications, with 24 plants per replication. Entries with the same letter are not significantly different. LSD=16.4%. TI = tobacco introduction

Name	Disease Incidence
TI 428	26.00 c
TI 1518	28.08 c
TI 835	28.1 c
TI 1279	29.13 b-c
Connecticut BL	36.43 a-c
TI 119	37.45a-c
TI 79	39.58 a-c
TI 1451	40.58 a-c
TI 1589	41.63 a-c
Florida 301	47.9 a-b
TI 1577	49.98 a

Table 13. Target spot incidence in cigar wrapper accessions. Disease incidence, including both seedling death and lesion incidence, of seedlings grown in float trays infested with *Rhizoctonia solani*. Mean of 3 replications, with 24 plants per replication. Entries with the same letter are not significantly different. LSD=18.6%. TI = tobacco introduction

Name	Disease Incidence
N. africana	8.30 d-e
N. nudicaulis	9.00 c-e
N. glutinosa	11.43 c-e
N. plumbaginifolia	12.22 c-e
N. longiflora	13.53 c-e
N. knightiana	14.55 c-e
N. debneyi	16.63 c-e
N. repanda	20.80 c-e
N. rotundifolia	22.88 b-d
N. alata	23.90 b-d
N. sylvestris	24.98 a-d
N. rustica	27.05 a-c
N. tomentosiformis	27.25 a-c
N. velutina	41.45 a-b
N. langsdorffii	42.68 a

Table 14. **Target spot incidence in *Nicotiana* spp. accessions.** Disease incidence, including both seedling death and lesion incidence, of seedlings grown in float trays infested with *Rhizoctonia solani*. Mean of 3 replications, with 24 plants per replication. Entries with the same letter are not significantly different. LSD=18.6%. TI = tobacco introduction

Name	Disease Incidence
TI 1524	16.63 k
TI 1734	20.05 j-k
TI 716	21.85 i-k
TI 109	28.10 h-k
Speight 168	29.13 g-i
TI 501	31.63 f-k
McNair 944	34.33 e-k
SA 1214	34.70 e-k
Speight G-28	36.43 e-k
TI 1512	36.43 e-k
TI 1558	37.48 d-k
TI 1462	40.58 c-j
TI 1224	42.68 c-j
TI 1616	44.70 e-i
Speight G-70	44.75 d-i
Coker 371 Gold	46.85 c-h
NC 71	47.90 c-h
K 326	48.95 c-h
K 346	49.98 c-h
Hicks	52.10 b-g
TI 1500	53.13 b-f
NC 297	56.20 b-e
NC 95	60.73 a-d
NC 72	62.45 a-c
NC 2326	62.48 a-c
Speight H-20	74.97 a
OX 414 NF	83.23 a

Table 15. Target spot incidence in flue-cured accessions. Disease incidence, including both seedling death and lesion incidence, of seedlings grown in float trays infested with *Rhizoctonia solani*. Mean of 4 replications, with 24 plants per replication. Entries with the same letter are not significantly different. LSD=23.4%. TI = tobacco introduction

Name	Disease Incidence
TI 1555	28.10 f
TI 1269	30.18 e-f
TI 1247	34.35 d-f
TI 158	35.00 d-f
TI 1222	37.48 c-f
TI 1306	37.48 c-f
TI 1315	39.58 b-f
Kavala	42.68 b-f
TI 1379	44.75 b-f
Xanthi	47.83 b-e
TI 88	51.18 b-d
TI 1311	54.15 b-c
Samsun (nn)	56.25 b
TI 1280	56.63 b
TI 395	97.90 a

Table 16. Target spot incidence in oriental accessions. Disease incidence, including both seedling death and lesion incidence, of seedlings grown in float trays infested with *Rhizoctonia solani*. Mean of 4 replications, with 24 plants per replication. Entries with the same letter are not significantly different. LSD=18.7%. TI = tobacco introduction

Name	Disease Incidence in 1st Test	Disease Incidence (%)
Hicks x TI1311	-	22.2 c
TI 1311	15.3	23.6 c
TI 1316	12.5	27.8 c
K326 x TI 1311	-	38.9 b-c
Hicks x TI1316	-	43.1 b-c
K326 x KY14	-	44.4 b-c
(K326 x KY 14) x KY 14	-	52.8 a-b
K 326	36.1	54.2 a-b
Hicks x KY14	-	56.9 a-b
(K326 x KY 14) x KY 326	-	58.3 a-b
KY 14	18.0	58.3 a-b
K326 x TI1316	-	61.1 a-b
Hicks	54.1	69.4 a

Table 17. Inheritance of stem rot disease resistance. Accessions and crosses were infected with stem rot to determine heredity of resistance. The mean disease incidence of the parents is shown from the primary study. Means with the same letter are not significantly different. LSD=24.6%. TI = tobacco introduction

Accession Name	Classified	Seedling Death (%)	Lesion Incidence (%)	Disease Incidence (%)
TI1029	R	4.2	5.6	9.7 l
Xanthi	R	9.7	13.9	23.6 k-l
TI428	R	15.3	15.3	30.6 i-l
N. rustica	R	18	15.6	33.6 h-k
N. sylvestris	R	15.3	24.2	39.5 g-k
NC 2326	R	2.8	38.9	41.7 f-k
Burley 21	R	5.5	43.1	48.6 c-j
TI119	S	19.5	29.2	48.6 c-j
TI1734	S	11.1	43	54.2 b-i
TI1451	R	11.1	45.9	56.9 a-h
N.tomentosiformis	S	40.3	18	58.3 a-g
TR Madole	S	12.5	47.2	59.7 a-g
L8	R	26.4	36.1	62.5 a-g
TI1518	S	18	45.8	63.9 a-f
Virginia509	R	13.9	52.8	66.7 a-d
Pennbel69	S	25	43.1	68.1 a-d
R7-11	S	13.9	55.6	69.4 a-c
SA1214	S	27.8	47.2	75.0 a-b
N. alata	S	47.2	29.2	76.4 a-b
TN90	R	30.5	48.6	79.2 a
NC72	S	31.9	47.3	79.2 a

Table 18. Stem rot disease incidence of other accessions evaluated.

Some of the accessions with the lowest and highest disease incidences evaluated for target spot resistance again. The accessions were classified as R= resistant or S=susceptible in the primary study. Disease incidence includes both seedling death and lesion incidence. Entries with the same letter are not significantly different. Mean of 3 replications, with 24 plants per replication. LSD=24.1%. TI= tobacco introduction

Name	Lesion Size
TI 1029	0.37
TI 1311	0.53
N. sylvestris	0.54
Xanthi	0.62
Hicks x TI 1311	0.66
Hicks x TI 1316	0.71
SA 1214	0.8
Pennbel 69	0.83
(K326 x Ky14) x K326	0.85
TR Madole	0.86
TI 1316	0.89
Hicks x KY14	0.91
TI 119	0.91
K 326	0.94
TI 1734	0.95
(K326 x KY14) x KY14	0.99
TI 428	0.99
K326 x TI 1311	1
TI 1518	1.03
K326 x KY14	1.04
N. tomentosiformis	1.07
Virginia 509	1.07
L8	1.09
Burley21	1.1
K326 x TI 1316	1.1
TI 1451	1.11
NC 2326	1.15
R 7-11	1.16
Hicks	1.17
NC 72	1.18
TN 90	1.19
N. rustica	1.21
KY 14	1.33
N. alata	1.76

Table 19. Size of stem rot lesions on seedlings. Lesion sizes were averaged among seedling with visible stem rot only. Mean of 3 replications, with 24 plants per replication. LSD=0.23 mm. TI= tobacco introduction

Name	Death in the 1st Test	Death (%)
K326 x TI1316	-	4.2 b
K326 x KY14	-	5.6 b
Hicks x TI1311	-	5.6 b
Hicks	8.6	5.6 a-b
Hicks x TI1316	-	6.9 a-b
Hicks x KY14	-	6.9 a-b
(K326 x KY14) x K326	-	9.7 a-b
TI1316	0.0	9.7 a-b
K326 x TI1311	-	9.7 a-b
TI1311	11.6	12.5 a-b
(K326 x KY14) x KY14	-	15.3 a-b
KY14	9.4	16.7 a-b
K326	2.5	20.8 a

Table 20. Seedling death in target spot experiment. Accessions and crosses were infected with target spot to determine heredity of resistance. The mean disease incidence of the parents is shown from the primary study. Means with the same letter are not significantly different. Mean of 3 replications, with 24 plants per replication. LSD= 13.95%. TI = tobacco introduction

Accession Name	Classified	Death (%)
Ox 414	S	5.6 h-i
Speight H-20	S	6.5 g-i
NC 297	S	7.9 f-i
N. debneyi	R	7.9 f-i
NC 4	R	8.3 f-i
TI 1524	R	8.8 e-i
N. glutinosa	R	9.3 e-i
VA 509	R	9.7 e-i
TI 1280	S	9.7 e-i
NC 72	S	11.6 e-i
NC 2326	S	11.6 e-i
NC 95	S	12.0 d-i
TI 395	S	13.0 d-h
DH 608	R	13.4 d-g
TN 90	R	14.4 d-f
Burley 21	R	16.2 c-e
TI 1605	R	16.2 c-e
TI 1480	S	19.4 b-d
N. longiflora	R	26.9 b
N. plumbaginifolia	R	48.1 a

Table 21. Seedling death in target spot experiment of other accessions evaluated.
Accessions and crosses were infected with target spot to determine heredity of resistance. The accessions were classified as R= resistant or S=susceptible in the primary study. Means with the same letter are not significantly different. LSD= 13.91%. TI = tobacco introduction

LITERATURE REVIEW

Introduction

The tobacco industry is an important segment of world business. In 2002, the United States produced an estimated 345 million pounds of flue-cured tobacco and 324 million pounds of burley tobacco. North Carolina is the leading producer of flue-cured tobacco in the United States. In 2002, 169,000 acres of flue-cured and burley tobacco were harvested at a value of $650 million (*NC Annual Summary of Crop Estimates*, 2003). Therefore, tobacco is an important commodity for North Carolina.

Tobacco (*Nicotiana tabacum* L.) is an allotetraploid (2n=48) that resulted from the hybridization of *N. sylvestris* and *N. tomentosiformis* (Shew and Lucas, 1991). *Nicotiana* species have a basic chromosome number of x = 12, but many current species have a chromosome number of 2n = 2x= 24. Tobacco most likely originated in northwest Argentina or Bolivia (Goodspeed, 1954). Tobacco contains many secondary compounds such as nicotine, the compound that makes tobacco an important product.

There are many types of tobacco, including flue-cured, burley, dark, oriental, Maryland, and various cigar types, which have different properties of interest. Tobacco is usually classified based on curing method, locality of production, post-production usage, and stalk position (Tso, 1999). During the curing process, chlorophyll is degraded and carbohydrates are converted to simple sugars. The curing process is important because growers need to achieve good flavor and aroma that cigarette manufacturers are looking for.

Flue-cured tobacco, also known as “Bright” and “Virginia” tobacco, is used in cigarette blends, and has a high sugar: nitrogen ratio. Flue-cured tobacco is grown in 75

countries, including the United States. Flue curing is performed in tightly sealed barns with artificial heat that usually starts at 35 ºC and ends at 75 ºC over the course of five to seven days.

Burley tobacco is a light air-cured type, used in cigarette blends, and has low sugar: nitrogen ratio. Burley tobacco is produced in 55 countries, including the United States. Burley tobacco is cured in special air-curing barns that require an open framework where sticks of tied leaves or the whole stalk is hung and protected from the wind and sun. Barns are equipped with ventilators that can be opened or closed to control temperature and humidity. Air-curing takes approximately four to eight weeks, which is much longer than the five to seven days required for flue curing. Maryland is a light air-cured type, used in blended cigarettes, and has low nicotine content. This type of tobacco is produced in Southern Maryland in the United States, as well as in other countries.

Dark tobacco is air-cured and is used for chewing, snuff, cigar, and pipe blends. Dark tobaccos are produced world-wide, but India and China are the largest producers. Dark tobacco is used in the production of cigars. Cigars consist of three parts, each requiring tobacco with certain standards. The body of cigars is made up of cigar filler tobacco, which is enclosed in cigar binder tobacco. Finally, cigar wrapper tobacco, the leaf of the highest quality, is used to encase cigars. Cigar tobaccos can be produced in many countries, but Cuba is recognized for its production of high-quality cigars.

Oriental tobacco, also known as Turkish tobacco, is characterized by its aroma and oils, as well as its small leaf size. Oriental tobacco has low nicotine content and it used as a flavoring component of cigarettes. Oriental tobaccos are produced in Russia, Turkey, Bulgaria and Greece.

Prior to the 1900s, there was little or no distinction among types of tobacco. The types came about by farmer selection based on cultural preferences.

Seedling Diseases

Growing healthy transplants is a key step in successful tobacco production. Until recently, tobacco seedlings were grown in fumigated seedbeds in the field prior to transplanting. Today, the majority of growers in the United States produce their seedlings using greenhouse float systems. Tobacco seed is placed into polystyrene trays filled with a soilless medium and the trays are floated on a water reservoir containing nutrients for optimal growth. This produces good transplants, but is highly favorable for disease progression.

Seedling diseases can cause many problems for growers. Overall yield and quality of tobacco can be affected by many different foliar and soilborne pathogens. Three common fungi that afflict young tobacco transplants in greenhouse float systems are *Rhizoctonia solani* Kühn, *Sclerotinia sclerotiorum* (Lib.) de Bary, and *Pythium* spp. (Smith et al., 2003). *Rhizoctonia solani* causes damping-off (stem rot) and sore shin (Lucas, 1975). *Thanatephorus cucumeris,* the telomorph stage of *R. solani* causes leaf spot symptoms known as target spot (Lucas, 1975; Shew and Main, 1990). *Sclerotinia sclerotiorum* causes a symptom on the stem and/or petiole known as collar rot. *Pythium* spp. causes damping-off of seedlings at any stage of seedling growth. More information on *Sclerotinia* is included in the appendix of this thesis.

Environmental conditions, such as high humidity and high temperatures that are favorable for disease development are common in greenhouses. Once the leaves of the seedlings touch, the canopy creates an environment that maintains increased moisture

levels which can lead to increased disease (Shew and Melton, 1995). In addition, high plant densities present in greenhouse systems provide extended periods of leaf wetness that favor the development and spread of these seedling diseases. In moist environments, pre-and post-emergence damping off occurs. Both *R. solani* and *S. sclerotiorum* flourish under these greenhouse conditions, and there are no chemical controls currently registered for greenhouse use.

An epidemic within a greenhouse can be very destructive, resulting in high losses (Shew and Melton, 1995). In 2002, *Rhizoctonia* damping-off or stem rot caused an estimated $136,000 loss of flue-cured tobacco in North Carolina (Melton et al., 2002). Target spot caused an estimated $13,000 loss in 2002 (Melton et al., 2002). Also in 2002, collar rot, caused by *Sclerotinia sclerotiorum*, resulted in an estimated loss of 0.461% or $100,000 (Melton et al., 2002). Therefore, *Rhizoctonia* and *Sclerotinia* resistant tobacco lines would be highly desirable to farmers in North Carolina and possible other states, including Kentucky, Virginia and Georgia.

Biology of *Rhizoctonia solani*

Rhizoctonia solani Kühn [telomorph: *Thanatephorus cucumeris* (Frank) Donk] is a common soilborne basidiomycete fungus that occurs on many species throughout the world (Sneh et al., 1996). *Rhizoctonia solani* causes sore shin of soybean, belly rot of cucumber, damping-off of bean, and stem canker and black scurf of potatoes. In tobacco, it causes stem rot and target spot. Stem rot is characterized by a brown, water-soaked lesion on the stem near the soil surface. The target spot symptom is characterized by water-soaked spots on leaves that turn brown and halo out from the center. This is the

classic “bulls eye” symptom. Given time, the lesion center may drop out, leaving a hole in the leaf (Shew and Lucas, 1991).

Rhizoctonia solani consists of at least 12 and possibly more related strains/isolates (Anderson, 1982; Ogoshi, 1987). *Rhizoctonia* strains are distinguished from each other by the ability to anastomose, the union of a hypha with another resulting in intercommunication of their components (Sneh et al., 1991). The anastomosis grouping (AG) scheme was suggested by Schultz (1937) and later developed by Richter and Schneider (1953), Watanbe and Matsuda (1966), and Parameter et al. (1969). Hyphal anastomosis on culture media can result in one of four reactions. A CØ reaction results from no interaction between isolates. A C1 reaction is characterized by hyphal contact only. C2 or killing reaction is characterized by genetically distinct isolates of the same anastomosis groups do not fuse. A C3 or perfect fusion reaction occurs between genetically identical isolates that belong to the same AG (Cubeta and Vilgalys, 1997). The reactions are used to separate genetically distinct isolates, but reactions are not used to identify specific AGs.

The two anastomosis groups of interest in tobacco are AG-3 and AG-4. AG-3 and AG-4 have been repeatedly isolated from tobacco plants showing typical stem rot and target spot symptoms. The majority of isolates of *R. solani* associated with non-foliar symptoms are members of AG-1, AG-2-2, and AG-4 (Stevens Johnk et al., 1993). Target spot isolates are identified as *R. solani* AG-3 (Stevens Johnk et al., 1993).

Life Cycle

Rhizoctonia solani survives in soil as multinucleate hyphae within diseased host material or as sclerotia, which are irregular-shaped, brown to black hyphal structures

about 1-3 mm in diameter. Inoculum can be splashed onto transplants resulting in stem rots or a "damping-off" symptom. The fungi can persist in soil for many years, and with so many plant hosts, there is an abundance of overwintering material. The fungus attacks when the susceptible material is planted into infested soil. The fungal hyphae will come in contact with the host plant and become attached to its external surface. After attachment, the fungus will continue to grow on the external surface of the plant and will cause disease by producing appressoria that penetrate the plant cell and release nutrients for continued fungal growth and development (Ceresini, 1999). The infection process is promoted by the production of many different extra-cellular enzymes that degrade various components of plant cell walls (e.g. cellulose, cutin and pectin). As the fungus kills the plant cells, the hyphae continue to grow and colonize dead tissue, often forming sclerotia. New inoculum is produced on or in host tissue, and a new cycle is repeated when new substrates become available. Sclerotia form in diseased host tissue and remain viable in soil for years (Agrios, 1997).

The pathogen is transported in infested soil or through movement of diseased plant tissue, including seed, or infected growing containers such as float trays. In addition, haploid basidiospores, produced from the telomorph stage of *R. solani*, can be produced from sexual fruiting structures called basidia. These basidiospores are wind-borne, which results in long distance and rapid dispersal of the fungus. The basidiospores germinate to produce hyphae that infect leaves or penetrate through stomata during periods of high relative humidity and periods of extended wet weather.

Disease Control

Management of *Rhizoctonia* disease requires an integrated approach and knowledge of each stage of the disease. One of the most important initial management decisions that should be considered by growers is to purchase and plant only high quality seed material that is not infested with *Rhizoctonia*. A fungicide seed treatment, if available, may provide some relief. However, a fungicide seed treatment will usually not be beneficial if the soil or float tray is infested with high levels of the fungus.

Several cultural practices, including ventilation, have been used to control *R. solani* in greenhouses. Tobacco growers are encouraged to keep all vents open continuously unless temperatures drop below 16°C. Target spot is enhanced when growers attempt to encourage rapid growth by closing vents to elevate temperatures (Shew and Melton, 1995). Closed vents not only elevate temperatures but also greatly increase humidity and leaf wetness, which provide optimum conditions for disease initiation and development (Shew and Main, 1990). Another control measure is the avoidance of injury to transplants (Shew, 1985). Avoiding transplant injury is difficult due to mowing, a common practice that is used to maintain uniform seedling size. However, it was determined that although wounding enhanced the severity of disease, wounding was not necessary for disease to occur (Kucharek et al., 1992).

Reused float trays are a frequent source of *R. solani* inoculum. The best means of eliminating inoculum from trays is by the use of methyl bromide (Gutierrez et al., 2003) or steaming treatments. Because trays are often reused many times, management of diseases caused by *R. solani* requires the use of treatments that can eradicate survival structures of *R. solani* within the cracks of the tray walls (Gutierrez et al., 1997). Most growers wash and then dip trays in a 10% chlorine bleach solution to remove pathogen

inoculum, but this method has not been totally effective against the isolates of *R. solani* that cause stem rot or target spot. Fumigation of float trays with methyl bromide is the best control method currently available, but this chemical will be phased out of the United States in 2005 because methyl bromide is defined as a chemical that contributes to depletion of the earth's ozone layer.

Some chemicals, such as acibenzolar-S-methyl (BTH) provide some control against *R. solani* in the field in the form of reduced mycelial growth, hyphal browning and sclerotia formation (Rohilla et al., 2001). Kucharek et al. (1992) showed that iprodione reduced target spot incidence by 50% in seedbeds. Csinos et al. (1999) later supported this work by further showing that iprodione at the rate of 1.12kg ai/ha reduced incidence and severity of target spot in seedbeds. Iprodione, carboxin and flutolanil all provided good suppression of stem rot (Csinos et al., 1999). Unfortunately, none of these fungicides are registered for use in tobacco greenhouses.

The best control method is the one that is the most cost efficient as well, so sanitation is vital. The NC Cooperative Extension Service recommendations include adequate greenhouse ventilation, avoidance of over-the-top applications of water or fertilizer, and low nitrogen rates (not above 150ppm) for the production of healthy transplants (Smith et al., 2003). If float trays are re-used, they should be thoroughly sanitized with steam or methyl bromide before use.

Genetic Resistance

Genetic resistance to *Rhizoctonia* would be useful because losses would be decreased. Some examples or transgenic resistance in tobacco include transgenic tobacco plants that express the b-32 gene and transgenic tobacco expressing three proteins from

barley. b-32 is a maize gene that encodes ribosome-inactivating protein, and appears to provide increased levels of protection against infection by the fungus *Rhizoctonia solani* AG-4 (Maddaloni et al., 1997). Transgenic tobacco seedlings constitutively expressing a bean chitinase gene under control of the cauliflower mosaic virus 35S promoter showed an increased ability to survive in soil infested with *R. solani* (Broglie et al., 1991). Also, transgenic tobacco expressing three proteins from barley (*Hordeum vulgare*), a class-II chitinase, a class-II β-1,3-glucanase and a Type-1 ribosome-inactivating protein revealed significantly enhanced protection against *R. solani* (Jach et al., 1995). Unfortunately, transgenic tobacco is not currently accepted by the tobacco industry. Therefore, use in tobacco is not likely in the near future.

Evaluation of germplasm and identification of resistance to *R. solani* has been successful in several crops. Host resistance to *R. solani* is the missing component in integrated pest management systems in tobacco production. Since resistance is attainable in other crops, similar results might be found in tobacco. Several examples of screening for genetic resistance are summarized below.

Brassica. Woods et al. (2000) screened 260 *Brassica rapa* lines for resistance to *R. solani*. Seedlings were inoculated with cornmeal-sand cultures or sclerotia. The roots were rated on a scale of 0-5 where 0 was no disease and a rating of 5 indicated that the root had rotted off at or above the main lateral roots. Five lines were found to have partial resistance to brown girdling root rot caused by *R. solani*. Yang and Verma (1992) performed a screening of 122 cultivars/lines for resistance to *R. solani*. Significant differences in susceptibility were observed among and within species. Significantly higher emergence, the basis for resistance, was observed in all lines of *S. alba*; for

cultivars Arlo, Span, and Torch of *B. campestris*; for *B. napus* cultivars and lines Golden, Midas, Nugget, Target, R-533, and DM56AH and for *B. junecea* cultivars and lines Commercial Brown, Cutlass, Domo, CJ86Z, and BJB-80-1543. Keianth and Farnham (1997) evaluated 12 cultivars of *B. oleracea* crops for resistance to *R. solani*. At the four- to five-leaf stage, seedlings were inoculated with either cornmeal-sand cultures or sclerotia of *R. solani*. Disease severity was rated on a scale of 1-10, where 1 was no disease and 10 was plant death. Blue Max, a collard cultivar, had a 93.1% survival rate, indicating a possible source of resistance because it was consistently and significantly ($P \leq 0.05$) less diseased.

Carrot. One hundred twenty three lines of *Daucus carota* L. were screened for resistance to *R. solani* (Anderson et al., 1982). Colonized corn kernels served as the inoculum; one kernel was placed near the root and the roots were evaluated for disease 10 weeks later. Ten lines were found to have partial resistance, indicated by smaller lesions or no lesions, to *R. solani.* These ten lines were retested along with ten susceptible lines. W133A and W133B were found to have moderate resistance (disease index of 2.3 and 2.6, respectively), while the remaining eight had a lower level of partial resistance (disease index ranging from 3.0-3.9).

Common Bean. Five $F_{5:6}$ segregating common bean (*Phaseolus vulgaris* L.) populations were screened for resistance to *R. solani* (Montoya et al., 1997). A droplet inoculation technique in which a droplet of a *R. solani* suspension was applied to the adaxial surface of bean leaves was utilized in the experiment. The leaves were rated six days after inoculation on a 1 to 9 severity scale, where 1 = none of the leaf area infected

and 9 = greater than 25% of leaf area infected. Five lines from the MUS83 x DOR483 cross had low to moderate levels of disease in the field experiments.

Cucumber. One hundred and five accessions of *Cucumis sativus* have also been screened for resistance to *R. solani* (Uchneat and Wehner, 1998). *Rhizoctonia solani* colonized oats served as inoculum. Belly rot was rated using a disease severity index. Disease severity was rated on a scale of 1 to 9, where 1= no disease and 9=more than 30% of fruit surface with lesions. Several cucumber lines were identified as potential sources of resistance genes. 'Marketmore 76', PI 197085, PI 271328, F_1 of Gy 14 x PI 197087, and an F_4 selection of PI 197087 x PI 280096 showed resistance to *R. solani*. Resistance was defined as a severity rating $\leq$4.0.

Pea. Sixty-eight genotypes of *Pisum sativum* L. were screened for resistance to stem rot caused by *R. solani* (Shehata et al., 1981). *Rhizoctonia solani* colonized corn kernel or cornmeal was used to infest the soil and the seedlings were evaluated for resistance. Stem rot was rated using a disease severity index. Disease severity was rated on a scale of 1 to 5, where 1 = no symptoms and 5 = plant death with 100% of stem girdled. Three cultivars, four breeding lines, and one PI line were found to have partial resistance. More research is needed to determine heritability of the resistance found in several pea genotypes. High levels of resistance, defined as a disease index of 1, was not found among the genotypes tested.

Peanut. One hundred forty-one peanut (*Arachis hypogaea*) plant introductions were screened for resistance to *R. solani* (Woodard and Jones, 1980). Plants were inoculated with macerated *R. solani* colonized sorghum seed and evaluated for resistance. Differences in partial resistance were found. Resistant plants had fewer and smaller

lesions than the susceptible plants. Two plant introductions, 295724 and 296551, were found to be most resistant to *R. solani*, based on a significant (P=0.05) increase in seedling emergence and mature plant survival. Later, Franke et al. (1999) performed another screening study on peanut seedlings. The accessions were inoculated with either *R. solani* colonized oats or a soil drench method and evaluated. The seedlings were rated on a scale of 1-6, where one was slight discoloration and six was seedling death. All seedlings were compared to Georgia Browne, a commercially available peanut cultivar, which has known partial resistance to *Rhizoctonia*. Some commercial cultivars were found to have partial resistance to *R. solani*.

Pepper. Muhyi and Bosland (1995) screened 74 accessions belonging to four *Capsicum* species for resistance to *R. solani. Rhizoctonia solani* colonized corn kernels were used to infest the soil at the three to four true-leaf stage. A disease index was calculated based on phenotypic ratings of physical characteristics such as discoloration. Two accessions, 'Long Chili' and PI 167061, had 67% and 71% resistant individuals, respectively against *R. solani* (Muhyi and Bosland,1995). Nineteen accessions had ≥50% resistant individuals and could be useful in future breeding programs.

Sorghum. Pascual et al. (2000) screened 25 breeding lines and commercial varieties for resistance to *R. solani*. *Rhizoctonia solani* infested sugarcane leaves served as the inoculum. Disease reaction was measured at the soft-dough stage. Each line was compared to CS 621, a line known to have a high level of resistance to *R. solani*. To determine the heritability of the resistance, CS 621 was crossed with UPL Sg5, a susceptible variety. Additive and dominant gene effects were important in the expression of quantitative resistance to *R. solani*.

Sugar beet. A screening of sugar beet cultivars was performed to detect for resistance to *R. solani* (Campbell and Altman, 1976). Using barley grain inoculum, plants were inoculated and evaluated in a growth chamber. Differences in susceptibility were noted. Some cultivars had a greater number of seedlings survive than others. FC 702/5 and FC 701/5 are potential *Rhizoctonia*-resistant selections, because percentage seedling survival did not differ significantly (P=0.05) from resistant breeding lines. Another screening was performed for resistance to *R. solani* in sugar beet (Scholten et al., 2001). Sugar beets were inoculated with colonized millet seed and evaluated for resistance. No significant differences were observed among the accessions. Of the three accessions evaluated, FC709-2 was found to be less susceptible.

Tobacco. Dipon and Davide (1982) screened 20 tobacco varieties for resistance to damping-off caused by *R. solani*. Seedlings were grown in soil infested with *R. solani*. Evaluations were performed 45 days after sowing and were based on seedling survival. The varieties were rated from highly resistant (100-95% seedling survival) to highly susceptible (54-0% seedling survival). Lesion sizes and frequency were not reported. 'Virginia 21' was reported to be resistant to *R. solani*, while 'Coker 411', 'NCBY', and 'Harrison Special' lines were moderately resistant.

Csinos and Stephenson (1999) evaluated *R. solani* isolate virulence on commonly grown tobacco cultivars in the greenhouse and field. In the greenhouse, eight-week-old transplants were inoculated with *R. solani* infected toothpicks. The seedlings were rated on a scale of 1-10, where 1 was no disease and 10 was a completely girdled stem resulting in a dead plant. The greenhouse study was inconclusive for identifying resistant cultivars.

In another test, Csinos and Stephenson (1999) evaluated an existing field test with an incidental *R. solani* target spot infection. The cultivar evaluations were randomized with three replications. This test included 66 tobacco entries. Target spot ratings were made on 10 plants per plot. The entries were rated on a scale of 0-100, where 0 was no target spot and 100 indicated that every leaf had a minimum of 10 spots. 'NC72', 'NC71', 'K 326', 'Coker 371-Gold', 'Speight G-70', 'Speight 168', 'NC95', 'NC2326', and 'K 149' were reported to be less susceptible to *R. solani* when compared to the other 57 cultivars evaluated.

Successful screens for resistance for other tobacco diseases.

Resistance to other diseases has been detected in screening of tobacco germplasm. Resistance screens are important in the discovery of new sources of resistance. Also, screening is the first part of developing resistant cultivars. Several examples of screening for resistance to other diseases are summarized below.

Black root-rot. Black root-rot is caused by *Thielaviopsis basicola* [Berk. & Br.] Ferr. This fungus is soil-borne, thereby attacking the root system of susceptible plants. Characteristic symptoms of this disease include stunted growth and wilting. This disease affects seedlings in the greenhouses, as well as the field, but losses in greenhouses can be minimized by fumigation of the growth medium.

Genetic resistance to black root-rot was needed to help reduce costs of transplant production. Black root-rot disease resistance studies were begun by Johnson (1914). Later Clayton (1969) evaluated about 400 tobacco accessions for resistance to black root-rot. Of the accessions tested, only three were found to have high resistance to root-rot.

TI 89, a highly resistant accession identified by Clayton, was used as a parent to determine heritability of the resistance.

Two sources of black root-rot resistance have been discovered. Multigenic resistance found in *N. tabacum* is available in many cultivars with a range of levels of resistance (Shew and Lucas, 1991). Single gene black root-rot resistance can be found in *N. debneyi* (Clayton, 1969). This resistance is present in almost all recently developed burley varieties. 'Burley 21' and 'KY 14' have been developed with low to moderate levels of partial resistance to black root-rot (Heggestad and Clayton, 1955; Litton et al., 1969).

Black shank. Black shank is caused by *Phytophthora parasitica* var. *nicotianae* and is characterized by wilting followed by blackening of the roots and stem. Black shank is destructive on many types of cultivated tobacco. North Carolina lost an estimated $15 million dollars due to black shank in 2002 (Melton et al., 2002). The majority of symptoms are seen in the field on older plants, but young seedlings can be very susceptible to black shank as well (Shew and Lucas, 1991).

Management of black shank involves the use of resistant cultivars. Currently, multiple sources of resistance are available. Black shank resistance breeding began in 1922 when Tisdale crossed Big Cuba to Little Cuba, resulting in the black shank resistant line Florida 301. Genes from Florida 301, a cigar wrapper type, were moved into the first flue-cured black shank resistant cultivar, Oxford 1. Florida 301 confers resistance to both race 0 and race 1 *Phytophthora parasitica* var. *nicotianae*. A flue-cured cultivar, Coker 371-Gold, possesses the dominant *Ph* gene from *N. plumbaginifolia*, which confers resistance to race 0 of black shank (Johnson et al., 2002). Cultivar L8 possesses the

dominant monogenic black shank resistance gene from *N. longiflora* (Collins et al., 1971; Legg et al., 1982). The line does not perform well, but has been widely used in the production of burley hybrids with resistance. NC 2326, which contains a low level of partial resistance to both race 0 and 1 from *N. plumbaginifolia*, was released in 1965 (Apple, 1967).

Blue mold. Blue mold is caused by *Peronospora tabacina* Adam. This fungus produces conidia, which are responsible for the rapid airborne spread of the disease. When sporulating, this disease produces characteristic bluish, cottony-like growth on the underside of the leaf (Todd, 1981).

In 1936, Smith-White et al. evaluated 250+ genotypes of *N. tabacum*, 35 strains of *N. rustica*, a few accessions of American *Nicotiana* species and an abundant number of accessions from Australian *Nicotiana* species. The best source of blue mold resistance was found in *N. debneyi*. Clayton (1945) later confirmed this in another screen of 1000+ tobacco introductions (TI) and also found that TI 57 was resistant. Hill and Mandryk (1962) and Vinogradov et al. (1975) reported similar findings in their evaluations of seedlings for resistance to blue mold.

In the past, resistance from wild species of *Nicotiana* was incorporated into cultivated tobacco using interspecific hybridization between *N. debneyi* and an allopolyploid synthetic tobacco, but the resistance was soon overcome. Later, resistance from *N. goodspeedii* and *N. velutina* was introgressed into tobacco cultivars, and the resistance performed well in Australia. In the United States, NC-BMR-42 and NC-BMR-90 were released as improved germplasm in the 1980s with partial resistance to blue

mold. ‘Xanthi’ and NC 2002, a new cultivar that will soon be released, are two cultivars that possess resistance to blue mold.

Fusarium wilt. Fusarium wilt is caused by *Fusarium oxysporum* var. *nicotianae*. This soil-borne fungus enters the roots and attacks the vascular system (Todd, 1981). A characteristic symptom is wilting and yellowing of only one side of the plant. In 2002, North Carolina lost an estimated $61,000 due to Fusarium wilt in flue-cured tobacco (Melton et al., 2002).

The use of resistant cultivars is the primary means of control of Fusarium wilt. Resistance has been found in *N. tabacum*, TI 566, TI 55C and TI 448A, and has been incorporated into flue-cured cultivars (Lucas, 1975). ‘KY 35’ was developed using resistance derived from ‘Chileno Correntino’. KY 14 has also been developed with resistance to fusarium wilt (Litton et al., 1969).

Granville/Bacterial wilt. Granville wilt is caused by *Ralstonia solonacearum* E. F. Smith. A characteristic symptom of this disease is one-sided wilting of the plant. A diagnostic sign of Granville wilt is the appearance of ooze at the cut stem where the vascular strands were severed. North Carolina lost an estimated $9.6 million due to Granville wilt in 2002 (Melton et al., 2002). Recessive resistance to *R. solonacearum* has been found in TI 448A, and has been incorporated into several cultivars (Lucas, 1975).

Potyviruses. Potato virus Y (PVY), tobacco vein mottling virus (TVMV), and tobacco etch virus (TEV) are potyviruses that commonly afflict tobacco. PVY is vectored by aphids, and is characterized by vein banding in tobacco. TVMV is vectored by aphids and is characterized by mottling along leaf veins. TEV is transmitted by aphids, and is characterized by mottling on leaves and an etching pattern on older leaves.

Resistance to PVY, TVMV, and TEV has been found in *N. tabacum* and the Virgin A Mutant (VAM), obtained by Koelle (Lucas, 1975) through an X-ray irradiation program. The VAM tobacco cultivar contains a single recessive gene that confers tolerance to these potyviruses. The *va* gene found in VAM has been incorporated into tobacco breeding lines (Fischer and Rufty, 1993; Gooding and Kennedy, 1985). Examples of potyvirus resistant cultivars are TN 86 and TN 90.

Root-knot nematode. The root-knot nematodes (*Meloidogyne* spp.) affect tobacco-growing regions in the United States. Characteristic symptoms include stunting, yellowing, and galls or swellings (Todd, 1981). In 2002, North Carolina lost an estimated $2.3 million dollars in the flue-cured tobacco industry to root-knot nematodes (Melton et al., 2002).

Clayton discovered resistance to root-knot nematode in *N. tabacum*, and performed a cross to achieve a desirable plant with the resistance. RK 42, a root-knot resistant *N. tabacum*, was crossed with the allopolyploid of *N. slyvestris-tomentosiformis*, resulting in a resistant and desirable plant type. A selection from one of these lines was crossed with Coker 139 and Hicks and the F_6 was released as NC 95. Plants carry the *Rk* gene for nematode resistance to race 1 and 3 of *M. incognita.*

Tobacco mosaic virus. Tobacco mosaic virus (TMV) is a single-stranded, positive sense, rod-shaped RNA virus that is found worldwide. TMV reduces yield and quality. In 2002, North Carolina TMV resulted in an estimated $2.1 million dollar loss in flue-cured tobacco (Melton et al., 2002). Characteristic symptoms include light to green to dark green areas in the top plant leaves (Todd, 1981).

In 1968, Chaplin and Goodling evaluated 970 T. I. lines for resistance to TMV (1969). The lines could be separated into five classes: class 0-local lesion reaction, Class 1-no visual symptoms, Class 2-mild mosaic, Class 3-moderate mosaic, and Class 4-severe mosaic. They suggested that the 11 lines showing local lesions were of the *N. glutinosa* resistance type and the 25 Class 1 lines should be explored for resistance to TMV.

Resistance to TMV was found in *N. glutinosa* by Allard (1916). The single dominant N gene from *N. glutinosa* confers resistance to TMV (Holmes, 1938) and has been transferred to tobacco. Resistance has also been found in Ambalema tobacco, but it is inferior to the level of resistance achieved through the use of *N. glutinosa*. Flue-cured and burley tobacco resistant cultivars are available. Burley 21 and KY 14 are examples of burley tobacco resistant to TMV that have employed the N gene.

Wildfire. Wildfire is caused by *Pseudomonas syringae* pv. *tabaci* [Wolf & Foster] Stevens and is characterized by the presence of a yellow halo around leaf spots.

Since resistance to wildfire was introduced, resulting in control of the disease. Clayton (1969) transferred resistance from *N. longiflora* to *N. tabacum* breeding lines in 1938, but the first resistant cultivar was not released until 1955. Burley 21 and KY 14 have resistance to wildfire (Heggestad and Clayton, 1955; Litton et al., 1969).

Tobacco has been evaluated for wildfire resistance. Gwynn et al. (1986) examined the effect wildfire had on 22 entries. All plants were rated on a scale of 0-5 where 0= lesions and 5= 76-100% of leaf area infected. Differences were observed among the genotypes evaluated.

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APPENDIX

Sclerotinia sclerotiorum

Collar rot, also known as *Sclerotinia* rot, is caused by *Sclerotinia sclerotiorum* (Lib.) de Bary. This common soil-borne fungus attacks a wide range of hosts, has a worldwide distribution on numerous field crops, including tobacco and vegetables, and appears to be among the most nonspecific and successful of plant pathogens (Purdy, 1979). *Sclerotinia sclerotiorum* is also known as *Whetzelina sclerotiorum* (Lib.) Korf & Dumont (Korf and Dumont, 1972).

Life cycle

Sclerotinia sclerotiorum survives in the soil in the form of sclerotia, brown to black hardened compact masses of fungal tissue (Le Tourneau, 1979). Mycelium that originates from the germination of sclerotia can infect susceptible host plants (Purdy, 1979). Sclerotia can act as a direct source of inoculum or indirectly by their ability to produce ascospores (Purdy, 1958). Sclerotia produce apothecia located upon or buried in the soil, and eject ascospores, that become airborne and are deposited on plant parts where they germinate infect plant parts or colonize debris (Purdy, 1979).

Ascospores are the primary inoculum for collar rot and are produced around tobacco greenhouses throughout the transplant production period (Gutierrez and Shew, 1998). Symptoms of disease, however, are observed only after a closed canopy has formed within a seedling tray (Gutierrez and Shew, 1998). Many of the ejected ascospores become lodged within the canopy of the tobacco plants, but some escape above the canopy and are reportedly able to travel several meters to several kilometers via wind currents (Abawi and Grogan, 1979).

Following penetration, the first symptoms that often develop on leaves or young stems are water-soaked lesions that may enlarge and become a watery soft rot in most hosts (Purdy, 1979). As the lesions enlarge, the plant may become girdled. Distal leaves may become yellow and then turn brown followed by death of a portion of the plant.

Disease control

Collar rot can be controlled by the use of foliar protectants, seed treatments, sclerotial germination inhibitors, soil disinfectants, crop rotation, sanitation, moisture regulation, and microclimate regulation (Steadman, 1979). Crop rotation is routinely practiced in the field, but, unfortunately, crop rotation is not an effective control measure in greenhouses, since sclerotia are known to survive 6-8 years in the soil surrounding greenhouses (Adams and Butler, 1979; Steadman, 1979). Another cultural control measure is to limit wounding. Bruise wounds (associated with equipment damage in the greenhouse due to tobacco clipping practices) are an important factor associated with infection by *S. sclerotiorum* (Hudyncia et al., 2000).

The Flue-cured Tobacco Information (Smith et al., 2003) bulletin suggests that seeding should not occur more than 60 days before the plants are needed. Also, thoroughly ventilate and use air-circulating fans to increase air-movement. Lastly, do not dump infested soil or infected plants near greenhouses to ensure they do not served as inoculum for future crops.

Genetic resistance

Genetic resistance to *S. sclerotiorum* was observed first by Anton de Bary in 1887 when he found that *Phaseolus multiflorus* was seldom attacked whereas *Phaseolus vulgaris* (common bean) cultivars were destroyed by the fungus (Steadman and

Nickerson, 1979). Three general types of resistance reactions to *Sclerotinia* spp. are: resistance of tissue to breaks, possibly associated with nutrition of the fungus; presence of preformed antifungal materials; and phytoalexin formation (Lumsden, 1979). Genetic resistance to *S. sclerotiorum* would be useful because losses would be minimized due to the lack of labeled fungicides to treat outbreaks. Several crops have been screened for *Sclerotinia* resistance, including oilseed rape, artichoke and sunflower, and are summarized below.

Artichoke. *Helianthus tuberosus* L. (Jerusalem artichoke) has been screened for resistance (Cassells and Walsh, 1995). Thirty-four cultivars and lines were grown in field soil heavily infested with *Sclerotinia*. Differences in susceptibility were noted. More research is needed to confirm yield results and establish stability and durability of resistance. A related species, *Helianthus annuus*, (sunflower) has also been screened for resistance (Degener et al., 1999; Rodriguez et al., 2000). Degener et al. (1999) evaluated 90 inbred lines. Rodriguez et al. (2000) evaluated five varieties. Differences in susceptibility were also noted.

Bean. *Phaseolus vulgaris* L. has been screened for resistance to white mold caused by *Sclerotinia sclerotiorum*. Three-week-old seedlings were inoculated with colonized bean pods and evaluated for resistance (Dickson et al., 1982). In another screening study, bean plants were either sprayed with a suspension of ascospores or colonized celery pieces were used as inoculum (Hunter et al., 1981). A pathogen filtrate can also be used as inoculum (Miklas et al., 1992).

Field Pea. *Pisum sativum* L. has been screened for resistance to *Sclerotinia*. Fungus infested oat kernels were used to inoculate eleven-day-old seedlings (Blanchette

and Auld, 1978). Several lines were found to be more resistance than the susceptible check.

Oilseed rape. *Sclerotinia*-resistant mutants were isolated from small M_2 populations (three generations of self-pollination), obtained by ethyl methane-sulphonate (EMS)-mutagenesis of an inbred line derived from oilseed rape cultivar Linetta, of oilseed rape (Mullins et al., 1999). Mycelial plugs of *Sclerotinia* were placed onto the surface of the leaf and the resulting lesions were measured and placed into classes. Mutants with significantly greater resistance to *Sclerotinia* than the parent and other cultivars were identified.

Peanut. Peanut (*Arachis hypogaea* L.) has been screened for resistance to *S. sclerotiorum*. Twenty-five germplasm lines were inoculated using colonized bean pieces (Cruickshank et al., 2002). TxAG-4 was found to have physiological resistance to *S. sclerotiorum*.

Soybean. *Glycine max* L. has been screened for resistance to *Sclerotinia*. Excised stems from five-week-old plants were inoculated by wrapping a piece of tissue paper inoculum around each stem and evaluated for stem rot (Nelson et al., 1991). Maple Presto, Maple Ridge and Maple Ridge showed the highest levels of resistance to *S. sclerotiorum*.

Due to unforeseen circumstances, the *S. sclerotiorum* portion of the experiment was not able to be completed. Numerous attempts to produce the necessary ascospores failed. Therefore, screening of accessions could not be completed.

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