Components of Partial Resistance to Blue Mold in Six Tobacco Genotypes Under Controlled Environmental Conditions

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ABSTRACT

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Two susceptible and four partially resistant tobacco genotypes were evaluated for components of partial resistance to blue mold. Disease efficiency, incubation period, latent period, degree of colonization, and sporulation capacity were measured under high and low inoculation density in experiments conducted at the Southeastern Plant Environmental Laboratory (North Carolina State University Phytotron), Raleigh, NC. Genotypes differed significantly for all components of resistance in all trials. Commercial cultivars Speight G-70 and McNair 944 were consistently the most susceptible genotypes based on all components of partial resistance measured. Partially resistant genotypes Chemical Mutant, NC-BMR 42, and NC-BMR 90 produced fewer and smaller lesions, had lower sporulation capacity, and exhibited longer patent periods than susceptible genotypes. Breeding line NC-BMR 90 was superior to all other partially resistant genotypes in this study. The presence of multiple resistance components in NC-BMR 90 makes this line a desirable source of blue mold resistance. Results obtained in these experiments corroborated field observations and indicate that deployment of tobacco germ plasm with partial resistance to blue mold should reduce onset and progress of blue mold epidemics.

Additional keywords: *Nicotiana tabacum*, *Peronospora tabacina*.

Tobacco blue mold, also known as downy mildew of tobacco (*Nicotiana tabacum* L.), is caused by the fungus *Peronospora tabacina* Adam. Occurrence of blue mold is generally sporadic, but severe economic losses can occur when the disease reaches epidemic proportions. For example, an epidemic of blue mold in 1979 resulted in an estimated $250 million loss to U.S. and Canadian tobacco growers (12).

A high degree of resistance to *P. tabacina* has been identified in several *Nicotiana* species, primarily among those of Australian origin (1,14). *N. debneyi* Domin, *N. velutina* Wheeler, and *N. goodspeedii* Wheeler have been reported to be immune to *P. tabacina* (14). Interspecific hybridizations have been successful in transferring blue mold resistance from wild *Nicotiana* species to *N. tabacum* (2–6,15,17); however, it appears that genes that condition immunity in the alien species only provide partial resistance when incorporated into the genome of *N. tabacum* (12,13,16). It is possible that major genes for resistance have been transferred from the wild species to *N. tabacum* but that modifier genes in tobacco affect their expression (7,12,13,15,16). A moderately high level of resistance to blue mold, which behaves similarly to resistance of interspecific origin, also has been induced via chemical mutagenesis (9).

The inheritance of blue mold resistance from different sources has been studied thoroughly (2–7,12,15,17). Resistance is believed to be conditioned by relatively few genes acting in additive fashion (12). Little is known about the nature of resistance, however, and its effects on growth and reproduction of the pathogen have not been quantified. The role of partial resistance in the development of blue mold epidemics also is not clear. The present study was conducted to evaluate components of partial resistance to tobacco blue mold under controlled environmental conditions using tobacco breeding lines and cultivars possessing different levels of resistance.

MATERIALS AND METHODS

Six tobacco genotypes were selected that differ in field reaction to blue mold (12): two resistant cultivars, Ovens 62 (resistance derived from *N. velutina*) and Chemical Mutant (resistance obtained after treating seeds of flue-cured tobacco cultivar Virginia Gold with triethylene iminotriazine) (9); two resistant breeding lines, NC-BMR 42 (derived from the cross Ovens 62 × McNair 944) and NC-BMR 90 (derived from the cross Ovens 62 × KY 17) (12); and two susceptible commercial cultivars, Speight G-70 and McNair 944.

Plants of all genotypes were grown in 15-cm-diameter plastic pots containing artificial potting medium (10). At the seven- to 10-leaf stage, plants were placed in a controlled-environment growth chamber with a 9-m^2 surface area, located at the Southeastern Plant Environmental Laboratory (North Carolina State University Phytotron), Raleigh, NC (10). Plants were acclimated for 1 wk before inoculation. Environmental conditions consisted of a 9-hr light period (550 ± 50 $\mu E\ m^{-2}\ sec^{-1}$) at 22 C followed by 15 hr of darkness at 18 C with a 10-min light interruption in the middle of the dark period. Plants were watered with nutrient solution (10), as needed, using an automatic watering system.

All inoculations were performed using isolate CPT-80 of *P. tabacina* obtained from Clayton, NC (10). This isolate was chosen as representative of isolates of *P. tabacina* of diverse geographical origin. We have tested a wide collection of isolates under field, greenhouse, and laboratory conditions and never have detected host specificity; that is, partial resistance appears to be of a generalized nature (10). Inoculum of *P. tabacina* was grown on plants of susceptible tobacco cultivar McNair 944 using a day-night temperature regime of 25 and 20 C, respectively, with relative humidity at or near 100% during the night period. Sporangiospores were collected from the leaves with an artist's brush and washed with 150 ml of cold distilled water using a millipore funnel with an 8.0-μm filter (Nuclepore Corp., Pleasanton, CA) to remove germination inhibitors. The inoculum density was measured using a hemacytometer and was adjusted to number of viable sporangiospores per milliliter based on results of germination tests conducted on 1% water agar the night before inoculations. The spore suspensions were kept cold in ice baths during inoculation.

Two experiments were conducted using different methods of inoculation to provide low and high inoculum levels. In experiment one, droplets (20-μm diameter) of inoculum

containing 5×10^6 viable sporangiospores/ml of sterile distilled water were applied to the adaxial leaf surface. Six droplets were equidistantly placed on the lamina of each of four leaves per plant. In experiment two, a spore suspension containing 5×10^4 sporangiospores/ml was atomized over the entire adaxial leaf surface avoiding run-off. Approximately 4 ml of spore suspension was applied to each of four leaves per plant. After inoculation, plants were misted with a fog of atomized distilled water for 12-sec intervals four times per hour for 72 hr to provide and maintain free moisture necessary for sporangial germination on the leaf surface.

In all experiments, the experimental design was a lattice design with three replicates. The lattice design was selected to remove gradient effects within growth chambers. A replication consisted of six plants of each of the six tobacco genotypes (n = 864). Experiments were repeated twice over time; that is, there were two runs per experiment. Inoculated leaves were observed daily from the end of the incubation period (usually 7 to 8 days) until secondary infections were evident. In each run of each experiment, an analysis of variance was conducted for each character measured. An analysis across runs showed that runs were nonsignificant. In addition, Bartlett's tests for homogeneity of variance revealed no evidence of heterogeneous variances and data were pooled across runs for each experiment.

The following components of partial resistance were measured: disease efficiency, incubation period, latent period, degree of colonization, and sporulation capacity. Disease efficiency was determined by the number of lesions that developed per leaf (experiment one) or estimated by percent leaf area damaged (%LAD) (experiment two). Percent LAD was used in experiment two because of the presence of numerous, coalescent lesions, which made lesion counts difficult. In addition, there was a high, positive correlation between lesion number and lesion size, which permitted use of %LAD as an estimate of disease efficiency. Incubation period was defined as the number of days from inoculation to symptom expression. Latent period was defined as the number of days from inoculation until 50% of the lesions sporulated on a given leaf. Degree of colonization was measured by lesion diameter on days 9 and 13 after inoculation (experiment one). Sporulation capacity was measured by the number of lesions that sporulated (experiment one), or by using a sporulation index in which 1 = no sporulation, $2 = 10^3$, $3 = 10^4$, $4 = 10^5$, and $5 = 10^6$ sporangiospores/cm^2 of diseased tissue (experiment two).

RESULTS

Genotypes differed significantly ($P = 0.01$) for all components of resistance measured. Disease severity was greatest where inoculum was atomized over the entire adaxial leaf surface (experiment two) due to higher total spore deposition. Genotype reaction was nearly constant regardless of the inoculation method used.

Highly significant differences for lesion number were observed among cultivars (Table 1). Susceptible cultivars Speight G-70 and McNair 944 had the highest mean number of lesions. Resistant cultivars Ovens 62 and Chemical Mutant were intermediate in reaction. Resistant breeding line NC-BMR 42 did not differ significantly from susceptible cultivar McNair 944 in lesion number, but resistant breeding line NC-BMR 90 had the lowest lesion number among the resistant genotypes ($P = 0.01$).

Differences ($P = 0.01$) also were observed for lesion diameter with trends similar to those found with lesion number (Table 1). On both evaluation dates (days 9 and 13 after inoculation), susceptible cultivars Speight G-70 and McNair 944 had the largest lesions, followed by those of Ovens 62, Chemical Mutant, and NC-BMR 42. The smallest lesions were observed on resistant breeding line NC-BMR 90. Although resistant line NC-BMR 42 did not differ ($P = 0.01$) in lesion number from susceptible cultivar McNair 944, it differed significantly in lesion size. On day 9, lesions on NC-BMR 42 were approximately half the diameter of those present on McNair 944. A similar response was observed on day 13 after inoculation. Lesions nearly doubled in diameter in 4 days (from day 9 to day 13) in susceptible cultivars Speight G-70 and McNair 944 (Table 1). Lesion growth was moderate (40–55% increase in lesion diameter) in the resistant genotypes Ovens 62, Chemical Mutant, and NC-BMR 42. Lesions present in resistant breeding lines NC-BMR 90 had the lowest rate of expansion (approximately 12%) among all genotypes, with relatively little change in size throughout the experiment. NC-BMR 90 appears to have the highest degree of partial resistance as measured by both lesion number and lesion size.

Genotypes differed significantly for sporulation capacity in experiment one (Table 1). Sporulation was observed on nearly all lesions that developed on the susceptible cultivars Speight G-70 and McNair 944 and on the resistant cultivars Ovens 62 and Chemical Mutant. In contrast, only about 50% of the lesions on NC-BMR 42 and NC-BMR 90 sporulated. NC-BMR 90 differed significantly from all other genotypes in number of sporulating lesions. There also were significant differences among genotypes for sporulation index on day 9, although these differences were not of a high magnitude (Table 1). Differences in sporulation index were much greater on day 13 when susceptible cultivars doubled their sporulation capacity. Sporulation increased slightly among most resistant genotypes. There was almost no increase in sporulation from day 9 to day 13 after inoculation in lesions present on NC-BMR 90 (Table 1).

In experiment two, genotypes differed significantly for both disease efficiency (%LAD) and sporulation capacity (Table 2). Percent LAD of all cultivars was significantly higher in experiment two than in experiment one. Nevertheless, ranking of cultivars was similar in the two experiments for all components of resistance. Some rank differences occurred in moderately resistant genotypes, but reaction of susceptible cultivars and highly resistant breeding line NC-BMR 90 remained constant in all trials. Plants of the susceptible cultivars McNair 944 and Speight G-70 had the highest percentage of LAD, followed by Chemical Mutant, NC-BMR 42, Ovens 62, and NC-BMR 90. There was almost twice as much damage on the susceptible cultivars as on the highly resistant breeding line NC-BMR 90 on both evaluation dates (Table 2). In most cultivars, %LAD nearly doubled in 5 days (from day 8 to day

TABLE 1. Disease efficiency (mean number of lesions per leaf), degree of colonization (mean lesion diameter), and sporulation capacity (mean number of sporulating lesions and mean sporulation index) of *Peronospora tabacina* on resistant and susceptible tobacco genotypes in experiment one[a]

Genotype	Mean number of lesions	Mean lesion diameter (mm)		Mean number of sporulating lesions	Mean sporulation index[b]	
		Day 9	Day 13		Day 9	Day 13
Speight G-70	10.1	20.1	35.1	10.1	2.3	3.6
McNair 944	10.0	18.9	35.1	9.9	2.1	3.6
Ovens 62	8.3	15.5	24.1	8.0	2.1	3.1
Chemical Mutant	6.9	10.7	16.1	5.7	2.0	2.6
NC-BMR 42	9.0	10.3	14.5	4.9	2.0	2.4
NC-BMR 90	5.1	7.2	8.1	2.3	2.0	2.1
Least significant difference$_{0.01}$	1.1	1.3	2.4	1.1	0.1	0.2

[a] Data represent results of two trials (runs) each replicated three times ($n = 864$).
[b] Sporulation index: 1 = no sporulation; $2 = 10^3$, $3 = 10^4$, $4 = 10^5$, and $5 = 10^6$ sporangiospores/cm^2 of diseased tissue.

TABLE 2. Disease efficiency measured as percent leaf area damaged (%LAD) and sporulation index of *Peronospora tabacina* on resistant and susceptible tobacco genotypes in experiment two[a]

Gentoype	%LAD		Mean sporulation index[b]	
	Day 8	Day 13	Day 8	Day 13
McNair 944	52.1	87.5	2.9	4.0
Speight G-70	44.7	90.6	2.8	3.8
Chemical Mutant	40.2	81.1	2.3	3.4
NC-BMR 42	35.4	79.6	2.2	3.0
Ovens 62	29.6	74.3	2.3	3.4
NC-BMR 90	27.9	48.9	2.1	2.4
Least significant difference$_{0.01}$	7.2	6.0	0.2	0.2

[a]Data represent results of two trials (runs) each replicated three times ($n = 864$).

[b]Sporulation index: 1 = no sporulation, 2 = 10^3, 3 = 10^4, 4 = 10^5, and 5 = 10^6 sporangiospores/cm^2 of diseased tissue.

TABLE 3. Mean incubation (days from inoculation to symptom expression) and latent periods (days from inoculation to sporulation) of *Peronospora tabacina* on resistant and susceptible tobacco genotypes[a]

Entry	Mean incubation period (days)	Mean latent period (days)
Speight G-70	7.8	11.6
McNair 944	7.3	12.2
Ovens 62	7.5	13.4
Chemical Mutant	8.3	15.0
NC-BMR 42	7.2	15.3
NC-BMR 90	7.8	18.5
Least significant difference$_{0.01}$	0.6	1.0

[a]Data represent results of two experiments each with two runs and three replications per run ($n = 1,728$).

13) due to conditions highly favorable to disease development.

For experiment two, mean sporulation indexes for all cultivars (Table 2) on both evaluation dates also were similar to those for experiment one (Table 1). The highest degree of sporulation was observed in the two susceptible cultivars (Speight G-70 and McNair 944) and the lowest in resistant breeding line NC-BMR 90. As before, a large increase in the degree of sporulation was observed in the susceptible cultivars over the experimental period, but sporulation remained relatively low and constant in resistant genotype NC-BMR 90.

Mean incubation and latent periods were almost identical in the two experiments. Thus, data for these parameters from the two experiments were combined and are presented in Table 3. There were significant differences among genotypes for incubation period, but these differences were not large. The largest difference in incubation period was only 1 day and was observed between NC-BMR 42 (7.2 days) and Chemical Mutant (8.3 days). In contrast, differences among cultivars in latent periods were not only highly significant, but of a greater magnitude. Latent periods for susceptible cultivars were approximately 12 days. Resistant genotypes Ovens 62, Chemical Mutant, and NC-BMR 42 exhibited latent periods ranging from 13 to 15 days. Breeding line NC-BMR 90 had the longest latent period (18.5 days). NC-BMR 90 was consistently the most resistant genotype based on all components of resistance, with the exception of incubation period.

DISCUSSION

The six tobacco genotypes evaluated in controlled environments could be differentiated for partial resistance to *P. tabacina*. Disease reactions under controlled conditions, particularly under relatively low inoculum density (experiment one), were similar to those observed by the authors under field conditions (*unpublished*). Partial resistance was expressed as reductions in components of the infection cycle related to growth and reproduction of the pathogen (number and size of lesions to develop, number of lesions to sporulate, and degree of sporulation). Time-related parameters (incubation and latent period) also were significantly different among genotypes. Thus, differences in components of partial resistance are reflected over various developmental stages in the pathogen's life cycle, from the establishment phase just after penetration to the late phases of colonization terminating in spore production (11). The length of the infectious period among genotypes was not determined because experimental conditions highly conducive to blue mold development resulted in collapse of susceptible tissue approximately 3 wk after inoculation.

Results obtained in these experiments indicate that deployment of cultivars with partial resistance to blue mold should result in a delay in the onset of epidemics due to a significant reduction in disease efficiency. Furthermore, resistant genotypes would be expected to delay the progress of blue mold epidemics due to reduced spore production and fewer cycles of the pathogen's reproduction (increased latent period) in a given amount of time. The importance of increased latent periods has been discussed by Leonard and Mundt (8) who indicated that, for pathogens with high rates of reproduction (as is the case with tobacco blue mold), increases in latent period would result in greater decreases in the rate of the pathogen's population growth than changes of equal magnitude in other components of resistance.

For some plant diseases, length of latent period has been reported to be affected by the density of infections (8). Plants with higher lesion densities generally exhibit shorter latent periods. For any given genotype, we did not observe changes in duration of latent period regardless of the level of disease that developed using high or low inoculum densities. It appears, therefore, that expression of some components of resistance, such as incubation and latent periods, in blue mold-resistant genotypes is not dependent on inoculum density. We have observed similar responses under field conditions (*unpublished*).

Partial resistance to tobacco blue mold is greatly influenced by the age of the plant. Generally, partial resistance is better expressed in adult plants (at the six-leaf stage or older) than in seedlings (12). In addition, plants of both partially resistant and susceptible genotypes become less susceptible as they enter the flowering stage (12).

A high degree of association was found among the various resistance components, particularly in breeding line NC-BMR 90. This association makes deployment of resistant germ plasm a viable option for control of tobacco blue mold. Furthermore, the presence of multiple components of blue mold resistance in some genotypes makes them attractive as sources of resistance. Selection among genotypes could be performed initially on the basis of longer latent periods, and this would require very little effort in comparison to measuring other components of resistance. Detailed evaluations on disease efficiency and sporulation capacity could be performed subsequently on elite germ plasm previously selected on the basis of latent period.

Some breeding lines, such as NC-BMR 90 (derived from the cross Ovens 62 × Ky 17), appear to have an enhanced level of partial resistance relative to the resistant progenitor, which suggests transgressive segregation. Enhanced expression of resistance may be due to regulation of structural genes derived from the wild *Nicotiana* species by so-called modifying factors from the tobacco genome.

The inheritance of partial resistance to tobacco blue mold in *N. tabacum* is believed to be additive and controlled by a few genes (12). Therefore, the probability of rapid development of new races of *P. tabacina* may be reduced due to the quantitative nature of resistance and its apparent interference with several stages of the pathogen's development. Nevertheless, the durability of the resistance described herein remains to be determined.

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