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Genetics of Burley and Flue-Cured Tobacco Resistance to *Globodera tabacum tabacum*

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Abstract: Genotypes of burley (cultivars B-21 and B-49), flue-cured (line VA-81 and cultivar PD-4), and Connecticut broadleaf (cultivar C9) tobacco (*Nicotiana tabacum*) resistant (R) or susceptible (S) to the tobacco cyst nematode *Globodera tabacum tabacum* were crossed. F1 progeny of burley and susceptible broadleaf were selfed and backcrossed to produce additional progeny for evaluation of resistance in greenhouse experiments. Plants without adult female nematodes visible (×10 magnification) on the root surface 6 weeks after inoculation were classified as resistant, whereas those plants in which one or more females were evident were classified as susceptible. Segregation ratios for progeny of resistant and susceptible plants were not different from 3:1 and 1:1 for F2 (F1×F1) and BC1 (F1×S) lines, respectively, indicating that resistance in burley to *G. t. tabacum* is conferred by a single, dominant gene. Segregation ratios for resistance in crosses between nematode-resistant burley and flue-cured tobacco (F1 and F2 progeny) and between burley-flue-cured hybrids and broadleaf BC1 (F1×S) and BC2 (BC1×S) progeny were consistent with the assumption that resistance to *G. t. tabacum* in burley and flue-cured tobacco is conferred by the same or closely linked single, dominant gene(s).

Key words: breeding, *Globodera tabacum solanacearum,* nematode, *Nicotiana tabacum,* resistance, tobacco cyst nematode.

The tobacco cyst nematodes *Globodera tabacum tabacum* (Lownsbery and Lownsbery) Stone and *Globodera tabacum solanacearum* (Miller and Gray, 1972) Stone, 1983 are important pathogens of tobacco (*Nicotiana tabacum* L.). *Globodera t. tabacum* affects shade-grown cigar wrapper and field-grown broadleaf cigar tobaccos in the Connecticut River Valley. It reduces the growth and yield of shade tobacco by up to 45% (LaMondia, 1995; Lownsbery and Peters, 1955), directly reduces broadleaf yields (LaMondia, unpubl., and indirectly increases the incidence and severity of Fusarium wilt of broadleaf tobacco (LaMondia and Taylor, 1987). *Globodera t. solanacearum* suppresses the growth and yield of flue-cured tobacco in Virginia (Komm et al., 1983). *Globodera t. tabacum* and *G. t. solanacearum* appear to be closely related to each other and to another subspecies described on horsenettle (*Solanum carolinense* L.), *G. tabacum virginiae* (Miller and Gray) Stone (Miller and Gray, 1968). The three subspecies all reproduce on tobacco and common horsenettle but can be distinguished morphologically and by host preference (Harrison and Miller, 1969; Miller and Gray, 1968, 1972).

Resistance to *G. t. tabacum* and *G. t. solanacearum* has been identified in various *Nicotiana* species (Baalawy and Fox, 1971; Gwynn et al., 1986) and, in some cases, resistance has been transferred to cultivated tobacco (Hayes et al., 1995; Herrero et al., 1996). A number of flue-cured and burley cultivars have been reported to be resistant to tobacco cyst nematode (Fox and Spasoff, 1976; LaMondia, 1991; Spasoff et al., 1971). Recently, two shade tobacco cultivars were released with resistance to *G. t. tabacum*. These two cultivars obtained their resistance from the flue-cured breeding line VA-81 (LaMondia, 2000a, 2000b). Resistance to *G. t. tabacum* in the two shade tobacco cultivars and the flue-cured line VA-81 and cultivar PD-4 segregates as a single dominant gene (LaMondia, 1991).

Reliance on single-gene resistance as a primary means of managing plant-parasitic nematodes may lead to selection for ability to overcome the resistance gene. Therefore, it would be desirable to identify one or more potentially different resistance genes in suitable adapted tobacco cultivars or lines for use in a breeding program. Resistance to *G. t. solanacearum* in tobacco was described as multigenically inherited for an advanced germplasm line (Elliot et al., 1986) and two burley and dark-fired breeding lines (BVA 523 and DVA 606) (Miller et al., 1972; Spasoff et al., 1971). The objectives of this report were to determine: (i) the number of genes responsible for cyst nematode resistance in two burley cultivars, B-21 and B-49, and (ii) whether the gene(s) responsible were different from those in flue-cured VA-81 and PD-4 tobacco.

Materials and Methods

To determine the number of *G. t. tabacum* resistance gene(s) in burley tobacco, B-21 and B-49 burley tobacco plants resistant to *G. t. tabacum* were used as controls and as either male or female parents in crosses with susceptible Connecticut shade or broadleaf cigar wrapper tobacco lines. F1 hybrids of Connecticut × burley types were either selfed to produce F2 progeny or backcrossed to susceptible Connecticut types with desirable cigar wrapper characteristics (BC1). The Connecticut broadleaf cultivar C9 was planted as a susceptible control in all experiments.

Two resistant burley lines (B-21 and B-49), six F1 hybrids between the resistant burley lines and susceptible Connecticut shade and broadleaf types, six F2 lines (F1 × F1 plants), and four BC1 lines (F1 backcrossed to the susceptible Connecticut parent) were each evaluated for resistance to *G. t. tabacum* on two to three occasions from 1998 to 2000.

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Crosses were also made and evaluated to determine whether the *G. t. tabacum* resistance gene(s) in burley tobacco were different than the gene in flue-cured tobacco. B-21 and B-49 burley tobacco (Heggestad, 1966) and VA-81 and PD-4 flue-cured plants resistant to *G. t. tabacum* (Currin et al., 1980) were evaluated as controls and as either male or female parents in F1 crosses between burley and flue-cured types. F1 hybrids of flue-cured × burley types were either selfed to produce F2 progeny or backcrossed once or twice to the susceptible Connecticut broadleaf cultivar C9 (BC1 or BC2). Two lines of F2, BC1, and BC2 tobacco were each evaluated for resistance to *G. t. tabacum* on two to three occasions from 1998 to 2000. C9 was planted as a susceptible control.

Plant resistance to *G. t. tabacum* was evaluated in greenhouse tests. Appropriate cultivars, lines, or progeny from crosses were directly seeded to two rows (32 cells) in 16 × 8 rows, 128-cell seedling trays containing 20 cm^3 of Sunshine (Sun Gro Horticulture, Bellevue, WA) potting mix per cell on 16 March 1998, 9 April 1999, and 19 April 2000. Unseeded border rows were left between lines to reduce the potential for cross-contamination. Plants were thinned to one seedling per cell after emergence (about 3 weeks after seeding) and inoculated approximately 6 weeks after seeding with 5,000 second-stage juveniles in eggs per plant. The *G. t. tabacum* population used in these experiments was a composite collected from shade and broadleaf tobacco types. Seedlings were grown in the greenhouse at 18 to 30 °C. Approximately 6 weeks after inoculation, the plants were removed from the trays and the numbers of white, developing females of *G. t. tabacum* visible on the root system on all sides of the root ball were determined at ×10 magnification. Plants without visible females were classified as resistant, and those with one or more females visible were considered susceptible. Roots of known susceptible control plants were stained in acid fuschin (Byrd et al., 1983) to determine the optimum time for root examination for presence of visible developing females. Data on the frequency of resistance and susceptibility to *G. t. tabacum* were subjected to chi-square analysis. The experiment was performed three times with similar results, and the data were combined for analyses.

Results

The ratios of transplants resistant or susceptible to *G. t. tabacum* were similar for all three experiments. The Connecticut broadleaf tobacco cultivar C9 was a susceptible host of *G. t. tabacum*, with a range of 0 to 51 white females visible on the root system (mean = 23.0; s.d. = 10.4, data not shown). Resistant burley parental lines and F1 progeny of homozygous resistant burley × homozygous susceptible Connecticut shade or broadleaf parent lines were overwhelmingly resistant (161 of 161 and 198 of 201 plants classified as resistant, respectively) (Table 1). The segregation of resistant to susceptible phenotypes in the F2 was consistent with a 3:1 ratio, and progeny of F1 plants backcrossed to homozygous susceptible lines segregated with a 1:1 resistant: susceptible ratio. Reciprocal crosses had no effect on resistance-segregation ratios. There were no differences between segregation ratios for B-21 or B-49. Segregation ratios of resistant to susceptible phenotypes are consistent with a proposed model for a single dominant major effect gene in burley tobacco conferring resistance to *G. t. tabacum*.

Crosses between nematode-resistant burley and flue-cured tobacco were evaluated to determine segregation ratios. Resistant parental lines and F1 progeny of homozygous resistant burley × homozygous resistant flue-cured parent lines were uniformly resistant (320 of 320 parent and 122 of 122 F1 plants classified as resistant, respectively) (Table 2). F2 and BC1 progeny were overwhelmingly resistant. The segregation of resistant to susceptible phenotypes in the BC2 was consistent with a 1:1 ratio for resistance and susceptibility. There were no differences between segregation ratios for B-21 or B-49. Segregation ratios of resistant to susceptible phenotypes are consistent with a proposed model that the same or closely linked dominant major effect gene(s) for resistance to *G. t. tabacum* occurs in burley and flue-cured tobacco.

Table 1. Phenotypic ratios of burley, Connecticut broadleaf, and progeny tobacco lines resistant to *Globodera tabacum tabacum* based on the presence or absence of developing females on roots.

	Ratio resistant/susceptible plants			
Tobacco seedlings	Sum	Test ratio[a]	χ^{2b}	P value[c]
Resistant (R) burley (B-21 and B-49)	161/0	1:0	–	–
Susceptible CT cultivar (S)	5/217	0:1	–	–
R × S: F1	198/3	1:0	–	–
F1 × F1: F2	170/48	3:1	1.03	>0.30
F1 × S: BC1	53/38	1:1	2.47	>0.15

[a] Test ratio of resistant: susceptible phenotypes consistent with a single dominant gene for resistance to *G. t. tabacum*.
[b] Chi-square value for data combined over experiments.
[c] Probability of obtaining a greater chi-square value.

TABLE 2. Phenotypic ratios of flue-cured, burley, and progeny tobacco lines resistant to *Globodera tabacum tabacum* based on the presence or absence of developing females on roots.

	Ratio resistant/susceptible plants			
Tobacco seedlings	Sum	Test ratio[a]	χ^{2b}	P value[c]
Resistant burley (B-21)	46/0	1:0	–	–
Resistant burley (B-49)	100/0	1:0	–	–
Resistant flue-cured (VA-81)	82/0	1:0	–	–
Resistant flue-cured (PD-4)	92/0	1:0	–	–
Susceptible CT cultivar	5/98	0:1	–	–
Burley × flue-cured: F1	122/0	1:0	–	–
Burley × flue-cured: F2	311/0	1:0	–	–
Burley × flue-cured F1x S: BC1	276/2	1:0	–	–
Burley × flue-cured BC1x S: BC2	29/30	1:1	0.02	0.90

[a] Test ratio of resistant: susceptible phenotypes consistent with the same single dominant gene for resistance to *G. t. tabacum* in both flue-cured and burley tobacco cultivars.
[b] Chi-square value for data combined over experiments.
[c] Probability of obtaining a greater chi-square value.

DISCUSSION

Resistance to tobacco cyst nematodes has been identified in a number of *Nicotiana* species, including *N. repanda* (Gwynn et al., 1986), *N. longiflora, N. glutinosa, N. plumbaginifolia* (Baalawy and Fox, 1971), and *N. miersii* (Hayes et al., 1997). *Nicotiana longiflora* was the most resistant of the species tested and did not allow female development (Baalawy and Fox, 1971). Although Baalawy and Fox (1971) were unable to obtain fertile hybrids of *N. longiflora* and *N. tabacum,* Clayton (1947) transferred a single dominant gene for resistance to wildfire, caused by *Pseudomonas syringae* pv. *tabaci,* from *N. longiflora* to *N. tabacum.* In doing so, resistance to tobacco cyst nematodes was also apparently transferred to the burley breeding line TL 106. Linkage between resistance to wildfire and cyst nematodes is common (Gwynn et al., 1986; Spasoff et al., 1971) but not absolute. Spasoff et al. (1971) reported that linkage was broken in the F3, and while burley lines with resistance to wildfire were resistant to cyst nematodes 40% of nematode resistant lines were susceptible to wildfire (Gwynn et al., 1986). Hayes et al. (1997) found that wildfire resistance was highly correlated with tobacco cyst nematode resistance, but some tobacco accessions tested had no resistance relationship to the two pathogens.

The flue-cured lines VA-81 and Clemson PD-4 appear to possess a single gene for resistance to *G. t. tabacum.* This single dominant gene segregates in a diploid manner in the allotetraploid tobacco genome (LaMondia, 1991). Cyst nematode resistance in VA-81 was presumably conferred from Burley 523. Resistance in PD-4 was obtained from *N. longiflora* and TL-106 through B-21 (Currin et al., 1980).

VA-81 and PD-4 have previously been reported to possess multigenic resistance to *G. t. solanacearum* (Elliot et al., 1986). Spasoff et al. (1971) and Miller et al. (1972) determined that cyst nematode resistance in Burley 523 and DVA 606 (a dark-fired line) was multigenic due to intermediate resistance in the F1 and a continuous range of resistance in the F2 inconsistent with the range of females produced on a susceptible cultivar.

The results of the current experiments do not indicate either intermediate resistance in the F1 or a continuous range of resistance in the F2 inconsistent with the range of females produced on a susceptible cultivar. Both conditions have been cited in determining the multigenic nature of resistance to *G. t. solanacearum* (Spasoff et al., 1971).

It is not inconsistent that the genetics of resistance to *G. t. tabacum* may differ from reports of multigenic resistance to *G. t. solanacearum* for a number of reasons. First, it is possible that a single gene for resistance was selected from several in the development of VA-81 and PD-4. To partially test this, two burley lines, B-21 and B-49, were evaluated against *G. t. tabacum* in these experiments. Segregation ratios indicated that resistance to *G. t. tabacum* in the burley cultivars was inherited as a single dominant gene segregating in a diploid manner. Further, it appears as if the same or closely linked gene is responsible for cyst nematode resistace in each of the lines or cultivars tested. Diallel analyses of tobacco resistance to *G. t. solanacearum* showed that resistance is additive and probably multigenic (Hayes et al., 1995). Tobacco resistance to the two different nematode subspecies may be somewhat different. Resistance to *G. t. tabacum* may involve some of the same genes, but additional genes may be required for the expression of resistance to *G. t. solanacearum.* It is possible that a single gene for resistance among several may confer resistance to *G. t. tabacum.* Finally, differences in methods may have influenced the interpretation of results. The visual observation of roots for developing females and classification into resistant or susceptible categories based on presence or absence of females is quite different from counting numbers of cysts produced per root system (Miller et al., 1972; Spasoff et al., 1971).

The number of females visible on the root system was quite variable in our experiments. Nematode reproduction reported in the diallel analyses was extremely low and may have included males (Hayes et al., 1995). Differences in total swollen or pyriform juveniles, females, or cysts per plant may be due to variation such as that seen in susceptible checks in this experiment. Finally, additional modifier genes may condition different levels of susceptibility.

If multiple genes for resistance to *G. t. tabacum* cannot be identified in commercially accepted tobacco genotypes, the search for additional and different genes for resistance to tobacco cyst nematodes may have to be expanded from the original *N. longiflora* and TL-106 source. Advanced breeding lines with resistance from *N. repanda* may be the next logical source of additional genes in adapted tobacco types (Gwynn et al., 1986; Herrero et al., 1996).

The long-term effectiveness of a single dominant gene for resistance against *G. t. tabacum* or *G. t. solanacearum* is unknown and remains to be determined. However, genotypes varied in response to *G. t. solanacearum* between evaluations in North Carolina (Herrero et al., 1996) and Virginia (Gwynn et al., 1986). These differences may be due to selection of the tobacco lines over time, experimental technique, or differences in *G. t. solanacearum* isolates. However, Rideout et al. (2000) determined that different *G. t. solanacearum* isolates from different geographic areas responded similarly to resistant and susceptible tobacco lines. The *G. t. tabacum* population used in these experiments was a composite from shade and broadleaf tobacco collected at the Valley Laboratory in Windsor, Connecticut. The potential variability of *G. t. tabacum* in Connecticut and Massachusetts is unknown, but tobacco cyst nematode collections from various locations and small numbers of cysts produced on resistant tobacco cultivars are currently being increased for future studies.

Currently, *G. t. tabacum* is managed by fumigation or rotation with nonhost crops. The availability of resistant cultivars as a means of reducing population densities by up to 80% (LaMondia, 1988, 2000a, 2000b) will be an important management tool in an integrated nematode management program.

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