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Tobacco Resistance to *Globodera tabacum*

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Abstract: Two flue-cured tobacco cultivars, VA-81 and PD-4, resistant to *Globodera tabacum solanacearum* were tested for resistance to *G. tabacum* in field and greenhouse experiments. A *G. tabacum*-susceptible broadleaf cultivar CT86-4 was used for comparison. In a greenhouse screening procedure, VA-81 and PD-4 had, respectively, 1 of 24 and 0 of 24 plants with any cysts visible on the root systems. All 24 plants of CT86-4 had at least four *G. tabacum* cysts visible per plant. Up to 80% of juveniles emerged from cysts in response to all tobacco cultivars transplanted into pots. Subsequent reproduction was much less on VA-81 and PD-4 than on CT86-4. Staining nematodes in roots 5 weeks after inoculation indicated that fewer nematodes had developed in VA-81 and PD-4 than in CT86-4. During one growing season, population densities of *G. tabacum* in naturally infested field soil declined by 72–80% under VA-81 and PD-4 and increased by 144% under CT86-4.

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

The tobacco cyst nematode, *Globodera tabacum* (Lowns and Lowns), suppresses the growth of shade tobacco *Nicotiana tabacum* L. (10), and increases the incidence and severity of Fusarium wilt of broadleaf tobacco (9). Despite treatment of nematode-infested land with nematicides, losses are estimated at $50,000 annually (11). Connecticut shade and broadleaf tobacco varieties tested are susceptible to *G. tabacum* (7).

Another tobacco cyst nematode, *Globodera tabacum solanacearum* (Miller and Gray) Stone, suppresses the growth and yield of flue-cured tobacco in Virginia (8). *Globodera tabacum* and *G. tabacum solanacearum* appear to be closely related to each other and to the horsenettle cyst nematode, *G. tabacum virginiae* (Miller and Gray) Stone (14). All three species reproduce on common horsenettle, *Solanum carolinense* L., but can be distinguished morphologically and by host preference (7,13).

Flue-cured tobacco cultivars with resistance to *G. tabacum solanacearum* have been developed (5,6,8). This resistance is multigenically inherited (12,15), and loss of resistance was not demonstrated in 3 years of continuous cropping with resistant cultivars (5).

The objective of this study was to identify a source of resistance to *G. tabacum* that could be used to breed for nematode resistance in Connecticut broadleaf tobacco. Preliminary experiments on the effects of these cultivars on *G. tabacum* population densities also are reported.

Materials and Methods

Plant resistance to *G. tabacum* was evaluated in greenhouse tests. A Connecticut broadleaf type, CT86-4, and the *G. tabacum solanacearum*-resistant flue-cured tobacco cultivars VA-81 and PD-4 were each directly seeded to 96-cavity seedling trays containing a potting mix previously inoculated with at least 100 *G. tabacum* field cysts (4,000 encysted juveniles in eggs) per 30-cm^3 cavity. Two rows of 12 plants each of each cultivar were thinned to one seedling per cavity after emergence. Six weeks after emergence the plants were removed from the trays and the roots on the surface of the root ball were scored visually for the presence of *G. tabacum* females. Plants were classified into categories of 0, 1–5, or > 5 developing females visible with the aid of a hand lens (10× magnification) on the root system (2).

The ability of *G. tabacum* to hatch, invade, and develop in the roots of CT86-4, VA-81, and PD-4 tobacco cultivars was also examined. Nylon mesh bags (2 cm^2, mesh

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TABLE 1. Residual *Globodera tabacum* second-stage juveniles (J2) per inoculum cyst and subsequent reproduction after 10 weeks under three tobacco cultivars in the greenhouse.

Cultivar	Pi†	Unemerged J2/cyst	New cysts/plant	J2/new cyst	Pf/Pi (J2/pot)
CT86-4	25	90.0	271.7	127.9	4.1
CT86-4	50	99.1	282.4	126.7	2.2
VA-81	25	78.6	1.0	51.8	0.2
VA-81	50	70.1	0.6	45.0	0.2
PD-4	25	66.1	0.7	48.5	0.2
PD-4	50	62.7	2.1	69.7	0.2
LSD ($P = 0.05$)		24	84	32	

† Initial population density 25 or 50 cysts/pot, 364 juveniles/cyst.

opening 210 μm) were placed in the center of 200-cm³ pots containing a Merrimac fine sandy loam field soil (72% sand, 23% silt, 5% clay, and 2% organic matter; pH 6.2). The soil had been previously fumigated with methyl bromide (1 kg/20 m² soil) and was free of *G. tabacum* cysts. Each bag contained 25 or 50 *G. tabacum* cysts of uniform size, age, and juvenile content (0.45–0.84 mm d, 1 year old, stored air dried at 20 C, mean content 364 juveniles in eggs per cyst determined by crushing 10 replications of 10 cysts each). One plant of each tobacco cultivar was transplanted to each inoculum level for six treatments with 10 replications. Five weeks after transplanting, three plants of each cultivar (50-cyst inoculum) were destructively sampled. Two 1-g representative root samples were selected from each plant and stained for nematodes (4). All remaining plants were harvested 10 weeks after transplanting. Nylon mesh bags containing inoculum cysts were removed, the cysts were crushed to determine the number of residual juveniles remaining, and the entire soil mass was processed to quantify numbers of new cysts produced and juveniles per cyst. Differences between means were determined by analysis of variance and least significant differences.

Changes in population densities of *G. tabacum* in field plots planted to each of the described tobacco cultivars were studied in a Merrimac fine sandy loam soil naturally infested with *G. tabacum*. Initial nematode population densities were estimated in one-row plots (1 × 15 m) at planting by means of two sets of 25-core samples (1.5 cm d × 15 cm deep) per plot. The three tobacco cultivars were transplanted to two plots of 25 plants each on 6 June 1987 and harvested on 1 August 1987. Plant spacing was 1 m between rows and 0.6 m within 15-m rows. Two soil samples per plot were taken at harvest using the method described. *Globodera tabacum* cysts were extracted from air dried soil by means of a modified Fenwick can (16), and cysts were collected on a 250-μm sieve nested under an 850-μm sieve. All flotsam retained on the 250-μm sieve was dried, and cysts were further extracted in acetone in a milk-filter sock (3). Cysts were then crushed in water, and the released viable juveniles in eggs were counted to determine the change in viable juveniles per cubic centimeter of soil under each tobacco cultivar. Data were subjected to analysis of variance and single degree of freedom mean comparisons.

RESULTS AND DISCUSSION

The Connecticut broadleaf tobacco cultivar CT86-4 was a susceptible host of *G. tabacum*. Most plants of CT86-4 had many more than five developing females per root system. The cultivar PD-4 had only one female visible on the roots of one plant, and VA-81 had no females on any of the 24 plants. This experiment was repeated once with similar results.

The number of unemerged juveniles remaining in inoculum cysts was not different ($P = 0.05$) for the two initial nematode densities (Table 1). Juveniles emerging and leaving cysts were fewer in CT86-4 than in the other cultivars. Few of the emerged juveniles developed to adults in roots of VA-81 and PD-4 tobacco. More new cysts were produced on CT86-4 than on VA-81 or PD-4 ($P = 0.05$). In addition, fewer encysted juveniles in eggs per cyst were produced on the roots of VA-81 and PD-4 than on CT86-4 ($P = 0.05$). Numbers of new cysts produced and juveniles per cyst

were not affected by initial nematode density. The ratio of final to initial nematode densities per cubic centimeter of soil was 3.2 for CT86-4 and 0.2 for VA-81 and PD-4.

Tobacco roots stained 5 weeks after inoculation with cysts of *G. tabacum* showed juveniles had invaded both VA-81 and PD-4 (Table 2). Nematode development in these cultivars was much less than in CT86-4. All juveniles that developed into adults in PD-4 were males.

Globodera tabacum population densities increased under CT86-4 and decreased under the cultivars VA-81 and PD-4 in field plots. Ratios of final populations to initial populations (Pf/Pi) were 2.4 for CT86-4, 0.2 for VA-81, and 0.3 for PD-4. Population changes under VA-81 and PD-4 were not different from each other.

A broad base of resistance to *G. tabacum solanacearum* in several *Nicotiana* species had been described (1). Juveniles invaded resistant plants but failed to develop beyond the second stage on some species or developed only into adult males in other species. Interspecific crosses between these species and *N. tabacum* were intermediate in resistance. The *G. tabacum solanacearum*-resistant tobacco cultivars VA-81 and PD-4 in our study were also resistant to the Connecticut tobacco cyst nematode, *G. tabacum*, as reported here. That tobacco plants resistant to *G. tabacum solanacearum* also are resistant to *G. tabacum* is further evidence that these nematodes are closely related. Further studies concerning the mechanism of this resistance as well as the phylogeny of the two species are warranted.

Approximately 80% of *G. tabacum* juveniles in cysts may hatch and leave the cysts to invade tobacco roots. Fewer *G. tabacum* juveniles were present in the roots of VA-81 and PD-4 than in CT86-4 roots 5 weeks after transplanting. Those juveniles that invade VA-81 and PD-4 either leave roots, die, or have little chance of developing into adult females.

Resistance to *G. tabacum*, and the resulting change in nematode population densities, were consistent under both field and greenhouse conditions. The number of juveniles per cubic centimeter of soil declined 78–82% after 10 weeks under VA-81 and PD-4 in the greenhouse. Field populations under these cultivars declined 73–80% after 8 weeks.

TABLE 2. *Globodera tabacum* juveniles and adults per gram root for three tobacco cultivars 5 weeks after inoculation.

Cultivar	Adults	J3–J4	J2
CT86-4	19.7	26.7	14.0
VA-81	0	0	1.0
PD-4	3.7†	0.5	7.3

Each observation was the mean of three replications each consisting of two 1-g samples.
† Adults on PD-4 were all males.

The multigenic nature of this resistance and the stability of these cultivars to *G. tabacum solanacearum* in Virginia (5) indicates that although selection of nematode races may occur, this resistance may be useful for several years.

The two flue-cured tobacco cultivars resistant to *G. tabacum* are agronomically distinct from Connecticut broadleaf and shade tobacco cultivars. Neither PD-4 nor VA-81 is suitable for production in Connecticut. Still, these cultivars will be useful as a source of resistance in a backcross breeding program to incorporate resistance into locally adapted and commercially acceptable tobacco lines.

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