Journal of Nematology 31(3):272–282. 1999.

Identification of Sources of Resistance to Four Species of Root-knot Nematodes in Tobacco

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Abstract: Resistance to the southern root-knot nematode, *Meloidogyne incognita* races 1 and 3, has been identified, incorporated, and deployed into commercial cultivars of tobacco, *Nicotiana tabacum*. Cultivars with resistance to other economically important root-knot nematode species attacking tobacco, *M. arenaria, M. hapla, M. javanica,* and other host-specific races of *M. incognita,* are not available in the United States. Twenty-eight tobacco genotypes of diverse origin and two standard cultivars, NC 2326 (susceptible) and Speight G 28 (resistant to *M. incognita* races 1 and 3), were screened for resistance to eight root-knot nematode populations of North Carolina origin. Based on root gall indices at 8 to 12 weeks after inoculation, all genotypes except NC 2326 and Okinawa were resistant to *M. arenaria* race 1, and races 1 and 3 of *M. incognita*. Except for slight root galling, genotypes resistant to *M. arenaria* race 1 responded similarly to races 1 and 3 of *M. incognita*. All genotypes except NC 2326, Okinawa, and Speight G 28 showed resistance to *M. javanica*. Okinawa, while supporting lower reproduction of *M. javanica* than NC 2326, was rated as moderately susceptible. Tobacco breeding lines 81-R-617A, 81-RL-2K, SA 1213, SA 1214, SA 1223, and SA 1224 were resistant to *M. arenaria* race 2, and thus may be used as sources of resistance to this pathogen. No resistance to *M. hapla* and only moderate resistance to races 2 and 4 of *M. incognita* were found in any of the tobacco genotypes. Under natural field infestations of *M. arenaria* race 2, nematode development on resistant tobacco breeding lines 81-RL-2K, SA 1214, and SA 1215 was similar to a susceptible cultivar with some nematicide treatments; however, quantity and quality of yield were inferior compared to K 326 plus nematicides.

Key words: Javanese root-knot nematode, *Meloidogyne* species, nematode, resistance, southern root-knot nematode, tobacco.

Root knot, caused by several species of root-knot nematodes (*Meloidogyne* Goeldi), is a serious disease of tobacco (*Nicotiana tabacum* L.) in most tobacco-growing regions of the world (Shepherd and Barker, 1990). Affected plants become stunted, develop a yellowish color with nitrogen and potassium deficiencies, show a tendency to wilt during hot days, and develop galls on the roots (Shepherd and Barker, 1990). This disease has been estimated to cause yield losses of approximately 15% annually in the world's tobacco crop, but losses in the United States generally are much lower (Mackenzie et al., 1986; Sasser and Freckman, 1987; Shew and Lucas, 1991). Annual losses due to this disease in flue-cured tobacco are estimated to range from 0.1% to 5% in the southeastern United States and about 1% or more in North Carolina (Johnson, 1989; Melton et al., 1992).

Currently, four major species of root-knot nematodes, *M. arenaria* (Neal) Chitwood (Ma), *M. hapla* Chitwood (Mh), *M. incognita* (Kofoid and White) Chitwood (Mi), and *M. javanica* (Treub) Chitwood (Mj), are responsible for root-knot disease in the southeastern United States (Barker and Lucas, 1984; Barker et al., 1986; Lucas, 1975; Ng'ambi, 1998), with Mi being the predominant species. Recently, shifts have occurred in populations resulting in the prevalence of other species or races (Barker, 1989; Fortnum et al., 1984; Schmitt and Barker, 1988; Sosa-Moss et al., 1983).

Control of root-knot nematodes is accomplished through the use of nematicides, crop rotation, resistant cultivars, and destruction of residual crop roots followed with a rye or other cover crop (Melton et al., 1992; Shepherd and Barker, 1990). The use of resistant cultivars is the preferable economic management tactic for these pathogens in less-developed countries in tropical and subtropical areas of the world (Roberts, 1992). Because of economics, effectiveness,

Received for publication 14 December 1998.
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This paper was edited by L. D. Young.

and loss of highly effective nematicides such as ethylene dibromide, resistant cultivars may play an even more important role in future crop-production systems in developed countries (Roberts, 1992).

For many years, plant breeders in the United States have been working to develop tobacco cultivars resistant to root-knot nematodes (Clayton et al., 1958; Gwynn and Powell, 1966; Reed and Schneider, 1992; Stavely, 1979). The first known root-knot resistance gene, designated as *Rk* (Yi et al., 1998), was derived from *N. tomentosa* as first suggested by Slana et al. (1977) and was recently confirmed by Yi et al. (1998) using molecular markers. This gene confers resistance to races 1 and 3 of Mi and is inherited in a dominant fashion (Drolsom et al., 1958). Tobacco plants possessing this gene exhibit a hypersensitive response to nematode infection, and usually no galls or a few small galls are produced (Lucas, 1975). The first tobacco cultivar (NC 95) with the *Rk* gene was released in 1961 (Moore et al., 1962), and all root-knot resistant flue-cured tobacco cultivars released since then carry this gene.

No tobacco cultivars with resistance to all four species have been developed. Although populations have shifted from races 1 and 3 of Mi to other species or races (Barker, 1989; Fortnum et al., 1984; Sosa-Moss et al., 1983), resistance to these other *Meloidogyne* species has been identified in related *Nicotiana* species (Davis et al., 1988; Milne et al., 1965; Schweppenhauser, 1975a, 1975b; Slana and Stavely, 1980; Stavely et al., 1973). A number of interspecific breeding lines have been developed (Gwynn et al., 1986; Schweppenhauser, 1975a; Schweppenhauser, 1975b; Stavely et al., 1973), and some exhibit high levels of resistance to Ma race 1, Mi races 2 and 4, and Mj (Cornelissen and Van Wyk, 1987; Davis et al., 1988; Mackenzie et al., 1986). Recently, new commercial tobacco cultivars (Kutsaga RK 1, RK 3, RK 6, RK 8, and T 14) with resistance to Mj were released in Zimbabwe (Jack, 1995). All five cultivars are male-sterile hybrids with moderate to high resistance.

Information is unavailable on the effectiveness of resistance in these breeding lines to the four common species and host races of the root-knot nematode of North Carolina origin. Therefore, the objectives of this study were to (i) evaluate 28 available tobacco genotypes for greenhouse-test reactions to North Carolina populations of *Meloidogyne* species and races, and (ii) compare the performance of three resistant tobacco breeding lines with that of *M. arenaria* race 2-susceptible cultivar K 326 plus selected chemical soil treatments under field conditions.

Materials and Methods

Greenhouse Experiments

Nematode populations and inoculum preparation: Nematode populations of the *Meloidogyne* spp. (Ma, Mh, Mi, and Mj) used in this study were of North Carolina origin. *Meloidogyne arenaria* race 1, Mh, Mi races 1 and 3, and Mj were maintained on tomato (*Lycopersicon esculentum* Mill cv. Rutgers). To minimize risks of contamination with other populations, Ma race 2 and Mi races 2 and 4 were maintained on Mi-resistant tobacco cv. NC 95 in greenhouse-pot cultures. Eggs for inocula were extracted from galled roots of the respective host plants with a 0.5% sodium hypochlorite (NaOCl) technique (Hussey and Barker, 1973). Egg suspensions were diluted to inoculate plants with a 30-ml egg suspension of each nematode population for a total of 10,000 eggs/pot or plant.

A total of 28 entries were evaluated for resistance to eight different *Meloidogyne* spp. Nine tobacco breeding lines were from Zimbabwe (81-RL-2B, 81-RL-2F, 81-RL-2G, 81-RL-2H, 81-RL-2J, 81-RL-2K, 81-R-617A, ms Speight G 28 × STNCB, and RK 3), two lines were from the United States (81-RL-2B × NC 82 and 81-RL-2B × NC 4001), one was from Japan (Okinawa), and 16 were from South Africa (SA 1210, SA 1211, SA 1212, SA 1213, SA 1214, SA 1215, SA 1216, SA 1217, SA 1218, SA 1219, SA 1220, SA 1221, SA 1222, SA 1223, SA 1224, and SA 1225). Control cultivars were NC 2326 (universal suscep-

tible) and Speight G 28 (resistant to Mi races 1 and 3). The breeding lines obtained from Zimbabwe were derived from *N. repanda* or *N. longiflora* × *N. tabacum* crosses. The 16 lines from South Africa were provided by the Tobacco and Cotton Research Institute and are selections from the cross (R 83 × NC 95) × TL 33 (Cornelissen and Van Wyk, 1987).

Seeds of all entries were germinated in seedling trays containing Metro-Mix 220 (Grace-Sierra Horticultural Products, Milpitas, CA), covered with a clear polyethylene film, and placed in a growth chamber at 25 °C with a 16-hour photoperiod having a light intensity of 400 ± 50 $\mu Em^{-2}s^{-1}$. Eighty 4-week-old seedlings of each genotype were transplanted singly into sterilized 10-cm-diam. clay pots containing a 1:1 mixture of steam-sterilized sand and sandy loam soil (vol/vol; 85% sand, 10% silt, 5% clay) for each experiment. Seedlings were transferred to a greenhouse and allowed to recover from transplanting shock for 1 week prior to inoculation.

Ten plants of each genotype per nematode population were individually inoculated with 10,000 eggs/plant as described above. Extra plants of NC 2326 also were inoculated with each nematode population to permit periodic determinations of relative root-knot nematode reproduction and disease development over time before terminating the experiment. Uninoculated plants of the two commercial cultivars NC 2326 (susceptible) and Speight G 28 (resistant to Mi races 1 and 2) served as standard controls for any contamination due to extraneous sources of inoculum. Plants were fertilized as needed with either 20:20:20 Peter's (Grace-Sierra) at 741 μg/ml nitrogen or Hoagland's solution (Hoagland and Arnon, 1950).

Eight to 12 weeks after inoculation, all plants were assessed for percentage of root area galled and root necrosis with 0 = healthy roots and 100 = entire root galled or necrotic (Barker et al., 1986). Roots were washed and rated for galling and root necrosis. The root system of each plant then was cut into 2 to 4-cm segments, and eggs were extracted from a representative 5-g sample with an NaOCl extraction technique (Hussey and Barker, 1973).

Experimental design and statistical analysis: Each nematode population was tested as a randomized complete block design with five replications. A replication consisted of two plants per entry, and all plants were arranged on a greenhouse bench in a block. Nematode populations were not randomized, and each population was treated as a separate experiment. Each experiment was conducted twice, with the first running from 5 March to 11 May 1993 and the second from 6 August to 27 October 1993. Daily mean temperature in the greenhouse in the first experiment was 27 ± 2 °C and 30 ± 3 °C in the second.

All data were first analyzed using the univariate procedure of the Statistical Analysis System (SAS Institute, Cary, NC). Nematode reproduction and root-necrosis data were subjected to logarithmic ($\log_{10}$[eggs/g root + 1]) and arcsin (arcsin [%root necrosis × 0.01]) transformations, respectively, to normalize variance (Steel and Torrie, 1960) for further analysis. All data then were analyzed with the correlation (PROC CORR) and general linear models (PROC GLM) procedures of SAS and Tukey's Studentized Range mean-separation test (Steel and Torrie, 1960).

Designation of resistance: Due to the inherent nature of variability in egg densities, and the associated differential root necrosis (severe on susceptible cultivars), only root-gall indices were used to designate resistance. Tobacco germplasm lines were classified according to the following scale: A root-knot gall index of 0 to 20% of roots galled = high resistance, 21 to 39% galled = moderate resistance, 40 to 59% galled = moderate susceptibility, and ≥60% galled = high susceptibility.

Field Experiment

Three *M. arenaria* race 2-resistant tobacco germplasm lines, 81-RL-2K, SA 1214, and SA 1215, were evaluated under field tests conducted on the Jimmy Smith Farm in Ala-

mance County, North Carolina, in 1994. The soil was an Appling sandy loam soil with pH = 6.2 (79% sand, 15% clay, 6% silt) that was naturally infested with Ma race 2. Tobacco cultivar K 326 (resistant to Mi races 1 and 3) was used as a standard control because it is susceptible to the Ma race 2 infesting the test site.

The field used in this experiment was selected because substantial losses to root knot due to Ma race 2 had occurred in 1993 even though fenamiphos (Nemacur) had been used. Tobacco had been grown in this field in 1991, but the field was fallow in 1992. The field was plowed on 10 April 1994. Metalaxyl (Ridomil 2E) was applied (broadcast) at 5.7 liters/ha as a general pretransplant treatment for black shank (*Phytophthora parasitica* var. *nicotianae*) control. Five additional chemical soil treatments were provided for nematode or fungus-pathogen control on the standard cultivar K 326 (Table 1). Seedlings were transplanted on 10 May 1994 and fertilized with 787.5 kg/ha of 8:8:24, followed, 2 weeks later, by 180 kg/ha of 16:0:0. Plots were four rows, each 13.5 m long with an inter- and intra-row spacing of 121.9 and 58.4 cm, respectively, and arranged in a randomized complete block design with four replications.

Three months after transplanting, crop vigor ratings were determined on a scale of 1 to 100, where 1 = poor and 100 = excellent vigor. Soil samples comprised of 20 2.5-cm-diam. cores from two center rows of each plot were collected on 10 August 1994 to determine numbers of root-knot eggs and juveniles that developed in each treatment. Root-gall and root-necrosis indices were assessed visually (0 = no galls; 100 = 100% of root area galled) on a scale of 0 to 100 on 4 October 1994 from six plants from the middle two rows of each plot; the resulting data were averaged. Cured leaf from each plot was weighed and graded. Prices were assigned based on average price for the grade during the past two seasons. Yield (kilograms per hectare) and revenue (U.S. dollars per hectare) were computed. All data were analyzed with the general linear models (PROC GLM) procedure of SAS and Fisher's LSD mean separation test.

TABLE 1. Response of selected tobacco germplasm lines to *Meloidogyne arenaria* race 2 under natural field infestations at Alamance County, North Carolina, in 1994.[a]

Material/rate per hectare and application	Yield (Kg per ha)	Value ($ per ha)	Crop vigor (1–100)	Root-knot nematode eggs + juveniles/500 cm^3	Root-knot gall index (0–100)[b]	Root-necrosis index (0–100)[c]
K 326 untreated	1,487 bcdf	5,463 bcd	50 ab	2,005 a	31	14
K 326 + 1,3-D+chloropicrin, 99.2 liters, row	2,354 a	8,665 a	59 a	83 b	12	7
K 326 + 1,3-D, 56.7 liters, row	2,378 a	8,865 a	58 ab	1,125 ab	16	7
K 326 + 1,3-D, 113.4 liters, broadcast	2,255 ab	8,390 a	51 ab	85 b	8	2
K 326 + chloropicrin 100, 28.4 liters, row	2,233 ab	8,225 ab	61 a	540 ab	24	5
K 326 + chloropicrin, 28.4 liters, row + fenamiphos 9.5 liters, 61-cm band	2,097 abc	7,673 abc	59 a	890 ab	34	12
81-RL-2K untreated	1,457 cd	5,168 cd	45 ab	128 b	0	5
SA 1214 untreated	1,126 d	3,820 d	44 ab	145 b	6	2
SA 1215 untreated	1,207 d	4,223 d	38 b	130 b	10	4

[a] Numbers are means of four replicates. Column means followed by the same letter are not significantly different according to least significant difference (LSD), $P = 0.05$. Numbers in columns without letters are not significantly different according to Fisher's LSD ($P = 0.05$).

[b] Root-gall ratings. 0 to 20% of root galled = high resistance; 21 to 39% = moderate resistance; 40 to 59% = moderate susceptibility; and ≥60% galled = high susceptibility.

[c] The root-necrosis index reflects the degree of decay (necrosis) of the root system. The index ranges from 0 to 100 as follows: 0 = trace; 25 = 25% decay; 50 = 50% decay; 100 = root system dead.

Results

Greenhouse experiments: Analyses of variance revealed genotype × run (spring vs. fall planting dates) interaction effects for three nematode populations: Ma race 1 and Mi races 1 and 2 with *P* values of 0.0008, 0.0001, and 0.0214, respectively. Since these interactions were due to rank order changes in response of germplasm lines to the individual nematode populations rather than to change in variation in the magnitude of the root-gall indices, data for these nematode populations were considered separately between the runs. Data for Ma race 2, Mh, and Mi races 3 and 4 were pooled over runs.

Based on root-gall indices, all genotypes, except NC 2326 and Okinawa, exhibited very high levels of resistance to Ma race 1 (Table 2). All germplasm lines had root-gall indices less than 18, with SA 1214 having the lowest gall index (root-gall indices of 10 and 2, respectively, in each of the two runs). NC 2326 had root-gall indices of 48 and 66 for runs 1 and 2, respectively. Okinawa was moderately susceptible to moderately resistant (root-gall indices of 42 and 36 for runs 1 and 2, respectively).

A moderate level of resistance to Ma race 2 was found in SA 1214 (root-gall index of 22) and in 81-RL-2B, 81-RL-2J, 81-RL-2K, 81-R-617A, SA 1213, SA 1214, SA 1215, SA

Table 2. Root-gall development in tobacco genotypes of diverse origin inoculated with *Meloidogyne* races 1 or 2, *M. hapla,* or *M. javanica* in greenhouse tests.[a]

	M. arenaria				
	Race 1				
Genotypes	Run 1	Run 2	Race 2	*M. hapla*	*M. javanica*
NC 2326	48	66	72	56	65
Speight G 28	10	8	70	53	50
81-RL-2B	12	10	47	41	17
81-RL-2F	16	8	34	53	10
81-RL-2G	18	10	42	56	12
81-RL-2H	14	8	44	46	10
81-RL-2J	14	10	38	50	11
81-RL-2K	10	4	26	52	10
81-RL-2B×NC82	12	4	43	53	12
81-RL-2B×NC4001	18	8	58	53	16
81-R-617A	16	12	31	48	8
ms Speight G 28×STNCB	18	12	55	55	18
Okinawa	42	36	56	48	46
RK 3	16	10	56	56	17
SA 1210	8	4	54	51	9
SA 1211	8	8	61	52	8
SA 1212	10	6	48	55	15
SA 1213	10	6	32	53	9
SA 1214	10	2	22	54	5
SA 1215	18	8	38	53	8
SA 1216	14	12	36	52	7
SA 1217	14	8	48	54	9
SA 1218	12	6	41	52	6
SA 1219	12	12	38	54	10
SA 1220	14	12	39	52	9
SA 1221	16	8	43	49	8
SA 1222	16	14	36	49	10
SA 1223	12	14	34	47	8
SA 1224	12	6	34	52	10
SA 1225	12	6	38	52	10
Tukey's Test ($P = 0.01$)	16	14	25	22 NS	18

[a] Root-gall ratings: 0 to 20% of root galled = high resistance; 21 to 39% = moderate resistance; 40 to 59% = moderate susceptibility; and ≥60% galled = high susceptibility.

All data except for *M. arenaria* race 1 were pooled across two runs.

1216, SA 1219, SA 1220, SA 1222, SA 1223, SA 1224, and SA 1225 (Table 2). Both NC 2326 and Speight G 28 had the highest gall indices, 72 and 70, respectively. Okinawa, RK 3, SA 1210, and SA 1211 were moderately high to highly susceptible. The remaining germplasm lines were moderately susceptible to Ma race 2.

All tobacco genotypes included in this study were moderately susceptible to Mh (Table 2). Mean root-gall indices ranged from 41 for 81-RL-2B to 56 for NC 2326, 81-RL-2G, and RK 3.

High levels of resistance to Mj were found in all germplasm lines with root-knot indices less than 18 (Table 2). As with Ma races 1 and 2, SA 1214 had the lowest nematode infection with Mj (root-gall index of 5). In contrast, Okinawa, Speight G 28, and NC 2326 were moderately to highly susceptible, with root-knot gall indices of 46, 50, and 65, respectively.

All germplasm lines were almost as resistant to Mi races 1 and 3 as was the Mi-resistant commercial cultivar Speight G 28 (Table 3). Root-knot gall indices with Mi race 1 ranged from 0 to 20 for the resistant germplasm lines and from 1 to 21 with Mi race 3. Cultivars NC 2326 and Okinawa had root-knot indices of 48 or greater for both races. Most entries were found to be moderately susceptible to Mi races 2 and 4 (Table 3). Although all entries exhibited susceptibility to Mi race 2 in run 2, several genotypes

TABLE 3. Root-gall development in tobacco genotypes of diverse origin after inoculation with four races of *Meloidogyne incognita* under greenhouse conditions.[a]

	Race 1		Race 2			
Genotypes	Run 1	Run 2	Run 1	Run 2	Race 3	Race 4
NC 2326	66	70	62	74	64	68
Speight G 28	0	2	58	76	1	70
81-RL-2B	4	2	60	64	3	57
81-RL-2F	2	0	40	68	2	47
81-RL-2G	0	4	50	68	5	51
81-RL-2H	6	0	34	64	5	51
81-RL-2J	0	4	38	62	3	52
81-RL-2K	0	0	32	64	1	48
81-RL-2B×NC82	0	0	46	68	3	56
81-RL-2B×NC4001	10	2	48	60	4	55
81-R-617A	20	42	32	50	21	42
ms Speight G 28×STNCB	2	2	60	72	5	61
Okinawa	48	60	64	76	54	61
RK 3	2	0	56	63	5	59
SA 1210	4	0	48	66	6	48
SA 1211	0	2	46	56	1	48
SA 1212	0	2	48	66	2	53
SA 1213	4	0	30	50	7	48
SA 1214	2	0	28	48	2	35
SA 1215	4	0	30	60	5	45
SA 1216	4	0	62	70	3	55
SA 1217	4	2	38	72	4	48
SA 1218	4	0	46	56	6	51
SA 1219	2	0	36	62	4	50
SA 1220	4	0	42	64	4	52
SA 1221	10	2	36	62	5	47
SA 1222	0	0	46	56	3	47
SA 1223	0	0	46	62	3	56
SA 1224	4	2	36	64	7	48
SA 1225	2	0	48	70	2	52
Tukey's Test ($P = 0.01$)	19	11	29	24	13	22

[a] Root-gall ratings: 0 to 20% of root galled = high resistance; 21 to 39% = moderate resistance; 40 to 59% = moderate susceptibility; and ≥60% galled = high susceptibility.

Data for races 3 and 4 were pooled across two runs.

were classified as moderately resistant in run 1. Root-knot indices for Mi race 2 ranged from 28 (run 1 for Mi 4) and 35 (Mi 4) for SA 1214 to 76 for Speight G 28 and Okinawa. For Mi race 4, root-galling indices ranged from 35 for SA 1214 to 70 with Speight G 28 (Table 3).

Egg densities at termination of the tests generally paralleled root-gall development (Table 4). The susceptible cultivar NC 2326 showed the highest levels of nematode reproduction (eggs per gram of root) when inoculated with all species or races except *M. hapla*. In general, line SA 1214 had the lowest reproductive values. As compared to NC 2326, Okinawa, and Speight G-28, all test genotypes supported low reproduction (egg numbers) of Ma race 2 and Mj. Except for Okinawa and NC 2326, all genotypes also supported low reproduction of Ma-1 (Table 4). Although not assigned any level of resistance, Okinawa supported a lower level of reproduction of *M. javanica* than NC 2326.

Number of eggs per gram of root also confirmed the high level of resistance in most genotypes to Mi-1 and Mi-3 as compared to root-gall indices (egg data not included). SA 1214 again supported the lowest egg reproduction (only 2 eggs/g of root for both Mi races). This genotype also supported the lowest egg number for Mi-2 (4,024 eggs) and Mi-4 (6,448 eggs), but the latter was not significantly different from the eggs (20,306) extracted from the susceptible control, NC 2326.

Correlations between root-knot index values and nematode reproduction ranged from as low as 0.31 ($P = 0.01$) for Mh to as high as 0.76 ($P = 0.01$) for Mi race 3. Other *Meloidogyne* species populations had correla-

TABLE 4. Nematode reproduction (eggs per gram of root) in tobacco genotypes of diverse origin inoculated with *Meloidogyne* species under greenhouse conditions across two runs.

	M. arenaria			
Genotypes	Race 1	Race 2	*M. hapla*	*M. javanica*
NC 2326	25,731	34,474	7,283	33,046
Speight G 28	1,144	26,693	10,633	36,461
81-RL-2B	3,873	15,420	5,224	6,946
81-RL-2F	1,792	11,246	12,919	182
81-RL-2G	3,539	10,862	9,242	6,100
81-RL-2H	1,638	10,118	7,634	258
81-RL-2J	754	11,007	6,170	1,558
81-RL-2K	123	3,260	11,660	57
81-RL-2B×NC82	1,939	15,940	7,242	4,750
81-RL-2B×NC4001	251	13,097	11,720	1,271
81-R-617A	4,245	7,483	8,557	427
ms Speight G 28×STNCB	2,880	25,330	6,454	5,767
Okinawa	15,992	31,552	6,589	22,349
RK 3	3,943	14,915	10,891	4,714
SA 1210	210	14,679	6,222	1,025
SA 1211	333	15,020	12,859	1,468
SA 1212	1,582	11,454	8,221	704
SA 1213	2,079	10,241	9,435	309
SA 1214	55	2,518	8,696	20
SA 1215	1,612	7,297	13,557	226
SA 1216	2,293	10,433	6,169	488
SA 1217	3,266	10,210	12,380	707
SA 1218	1,715	8,538	7,599	394
SA 1219	1,011	10,651	11,319	362
SA 1220	1,483	6,974	6,813	322
SA 1221	1,822	8,672	6,705	543
SA 1222	2,503	8,977	7,076	362
SA 1223	1,638	7,137	5,696	375
SA 1224	1,132	7,429	8,884	137
Tukey's Test ($P = 0.01$)	8,899	30,979	12,551	8,261

tions of 0.50 ($P = 0.01$) for Ma race 1, 0.38 ($P = 0.01$) for Ma race 2, 0.72 ($P = 0.01$) for Mi race 1, 0.44 ($P = 0.01$) for Mi race 2, 0.37 ($P = 0.01$) for Mi race 3, and 0.60 ($P = 0.01$) for Mi race 4 and Mj for the two variables. Correlations among the three variables of root-knot indices, root-necrosis, and nematode reproduction were positive in all nematode populations and were significant ($P = 0.01$) (root-necrosis data not included).

Field experiment: The test field had a low to moderate infestation of Ma race 2 typical of North Carolina tobacco fields. Within this population range (ca. 2,000 J2/500 cm^3 soil in susceptible control to 100 J2/500 cm^3 soil), few differences were observed among the three germplasm lines and K 326 alone or in combination with one or two nematicides.

The three germplasm lines performed as well as K 326 alone with respect to yield and crop value when K 326 was grown in untreated plots (Table 1). However, these treatments had the lowest yields in the test. Tobacco breeding line 81-RL-2K also gave yields and crop values that did not differ ($P = 0.05$) from those of K 326 grown in plots treated with a combination of the nematicide-fungicide chloropicrin (Chlor-O-Pic 100) and fenamiphos.

Plants of all breeding lines were as vigorous as K 326 alone or in combination with one or two nematicides with the exception of SA 1215 plants, which were less vigorous ($P = 0.05$) than those of K 326 grown in plots treated with either 1,3-D + chloropicrin at 99.2 liters/ha or chloropicrin at 113.4 liters/ha or a combination of chloropicrin at 28.4 liters/ha and fenamiphos at 9.5 liters/ha (Table 1).

The germplasm lines and K 326 grown in plots treated with either 1,3-D + chloropicrin at 99.2 liters/ha or dichloropropene (Telone II) at 113.4 liters/ha supported ($P = 0.05$) lower nematode numbers (root-knot nematode eggs + juveniles in 500 cm^3 soil) than K 326 grown in untreated plots (Table 4). However, the breeding lines performed as well as all other treatment combinations.

All germplasm lines supported lower nematode parasitism (egg numbers and root-gall indices) than K 326 when grown in untreated plots or in plots treated with one or a combination of nematicides, except where dichloropropene at 113.4 liters/ha was used, but the differences for gall indices were not significant. K 326 had a gall index of 31 in untreated plots and a gall index of 34 when grown in plots treated with a combination of chloropicrin at 28.4 liters/ha and fenamiphos at 9.5 liters/ha (Table 1). The degree of decay of the root systems was extremely low, with root-necrosis indices ranging from 2 to 14. Differences among treatments were not significant.

Discussion

Based on root-gall indices, high levels of resistance to Ma race 1, Mi races 1 and 3, and Mj were identified in some breeding lines that are cross-compatible with cultivated tobacco. Resistance to Ma race 1 was found in all breeding lines evaluated in this study. Our data confirm previous reports of resistance to Ma race 1 in the Zimbabwean lines (Davis et al., 1988). Resistance to Ma race 1 also was found in the commercial flue-cured tobacco Speight G 28, confirming a previous report that Mi-resistant cultivars also exhibit resistance to this pathogen (Barker and Melton, 1990).

Resistance to Mi races 1 and 3 likewise was found in all tobacco breeding lines from Zimbabwe, the Republic of South Africa, and the United States. This resistance is presumed to be that derived from *Nicotiana tomentosa* due to the fact that Mi-resistant tobacco cultivars form part of the pedigrees of all these lines (Cornelissen and Van Wyk, 1987; Mackenzie et al., 1986) and that the reaction of these lines to Mi races 1 and 3 in our study was almost the same as that of the Mi-resistant commercial cv. Speight G 28.

Resistance to Mj in the Zimbabwean and the Republic of South African breeding lines was reported earlier (Cornelissen and Van Wyk, 1987; Mackenzie et al., 1986). Our data confirm these reports with the North Carolina population of Mj. The two lines 81-RL-2B × NC 82 and 81-RL-2G × NC 4001 from the United States were resistant to Mj.

However, our experiments failed to confirm an acceptable level of resistance to Mj in Okinawa as reported earlier (Fukudome and Kamagama, 1982; Fukudome and Yamaguchi, 1976; Ohashi, 1977). Although Okinawa supported lower reproduction (egg numbers) and root-gall development than NC 2326, it exhibited considerable susceptibility to all eight North Carolina root-knot nematode populations used in this study. Also, contrary to previous reports (Barker and Melton, 1990; Cornelissen and Van Wyk, 1987; Mackenzie et al., 1986), our results provided little evidence of resistance to Mj in Speight G 28, an Mi-resistant flue-cured tobacco cultivar carrying the *Rk* gene (Yi et al., 1998). In our experiments, Speight G 28 was almost as susceptible to Mj as the universal susceptible check cv. NC 2326. Possible differences in the aggressiveness of the Mj populations or inoculum as well as test conditions could be responsible for the varied plant responses.

A high level of resistance to Ma race 2 was found in the breeding line SA 1214, and a moderate level of resistance was found in two of the Zimbabwean breeding lines, 81-RL-2K and 81-R-617A, and three South African breeding lines, SA 1213, SA 1223, and SA 1224. This finding is the first report of resistance to Ma race 2 in breeding lines that are cross-compatible with cultivated tobacco.

All germplasm lines evaluated were susceptible to Mh and supported some reproduction of Mi races 2 and 4. The breeding lines were either rated as moderately resistant to highly susceptible to these three nematode populations. Resistance to Mi races 2 and 4 in the South African lines was reported earlier (Cornelissen and Van Wyk, 1987). Our results, possibly due to the high inocula used, did not consistently confirm resistance to the North Carolina populations of either Mi race 2 or race 4. However, SA 1214 and a number of other lines exhibited moderate resistance to Mi-2 in one run but did not in a second run. SA 1214 also was the most resistant (moderate) test genotype to Mi-4.

Under natural field infestations, the germplasm lines 81-RL-2K, SA 1214, and SA 1215 compared favorably with some of the best K 326 plus nematicide treatments for numbers of nematodes at harvest; however, yield and crop values of the breeding lines were inferior. Backcrossing these breeding lines with commercial cultivars possessing desirable yield and quality characteristics, with selection for resistance to the root-knot nematode, could be useful in cultivar improvement. The test site at Alamance County was infested with mostly *M. arenaria* race 2, against which germplasm lines 81-RL-2K and SA 1214 showed resistance under greenhouse conditions.

It has been suggested that resistance to Ma, Mi, and Mj would be effective against 90% of economically important field populations of root-knot nematodes on a wide range of vegetable and field crops throughout the world (Fassuliotis, 1979). Therefore, adding resistance to Ma races 1 and 2 and Mj in cultivated tobacco reported herein could greatly increase the utilization of commercial tobacco cultivars already resistant to Mi races 1 and 3. The availability of resistance to Ma races 1 and 2, and Mj, in breeding lines of cultivated tobacco should expedite the breeding work required to transfer the resistance genes to agronomically important tobacco cultivars. Resistance to Ma race 1 already is available in commercial tobacco cultivars that possess the *Rk* gene for resistance to races 1 and 3 of Mi (Barker, 1989; Barker and Melton, 1990; Sosa-Moss et al., 1983).

Owing to the increasing importance of Mh, Ma races 1 and 2, Mj, and races 2 and 4 of Mi in North Carolina and the entire southeastern United States (Graham, 1969; Schmitt and Barker, 1988; Walters and Barker, 1994), host resistance to these pathogens is urgently needed. Any breeding effort will need to address ongoing shifts in the genetic diversity encountered in various cropping systems involving flue-cured tobacco (*M. incognita*-resistant and susceptible) as well as other crops (Barker, 1989; Schmitt and Barker, 1988; Shepherd and Barker, 1990).

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