

## Evaluation of *Nicotiana otophora* as a Source of Resistance to *Meloidogyne incognita* Race 4 for Tobacco

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**Abstract:** No currently available tobacco cultivar possesses resistance to *Meloidogyne incognita* race 4, nor has any source of resistance been reported within *Nicotiana tabacum*. The purpose of this study was to evaluate *N. otophora* acc. La Quinta as a source of resistance to this pathogen. Plants of tobacco cvs. NC 95 and NC 2326, *N. otophora* La Quinta and *N. repanda* were inoculated with second-stage juveniles of *M. incognita* race 4. Gall indices and egg-mass ratings were assessed at 4 and 8 weeks after inoculation. The two *N. tabacum* cultivars were heavily galled and had numerous egg masses at both rating periods. *Nicotiana repanda* was only weakly resistant. The galls on this species were very small and present at a low to moderate level; however, egg-mass ratings approaching those of the tobacco cultivars were observed 8 weeks after inoculation. In contrast, low gall indices and egg-mass ratings were found for *N. otophora* La Quinta at both the 4- and 8-week rating periods. In addition, little variability was observed within this species for either disease rating. Therefore, it appears that the La Quinta accession of *N. otophora* is a very promising source of *M. incognita* race 4 resistance for transfer to *N. tabacum*.

**Key words:** breeding, *Meloidogyne incognita* race 4, nematode, *Nicotiana tabacum*, *N. otophora*, resistance, tobacco.

Root-knot nematodes (*Meloidogyne* spp.) have been a major cause of disease losses to flue-cured tobacco (*Nicotiana tabacum* L.) in the southeastern United States for many years. The first root-knot-resistant cultivar released was NC 95 (7), which originated from breeding lines developed using interspecific sources of resistance (5,10). Today many cultivars with this single dominant gene for resistance are available and provide protection against the common races of *M. incognita* Chitwood. However, shortly after the release of NC 95, a new race of *M. incognita* was identified (6). This race, designated as race 4, is pathogenic on cultivars having the NC 95 form of resistance. No other flue-cured tobacco cultivar possesses resistance to *M. incognita* race 4, nor have any sources of resistance within *N. tabacum* been reported.

La Quinta, an accession of *N. otophora* Griesb., was reported to have moderate resistance to *M. incognita* race 4 (10), al-

though individual plants varied in their disease reaction, with 12% being severely damaged by the nematodes. Subsequently, La Quinta and four other accessions of *N. otophora* were evaluated for resistance to *M. incognita* race 4 (1); La Quinta was the most resistant of the five accessions. Considerable variability in galling index between individual La Quinta plants was also reported. This variability was interpreted as evidence of genetic heterogeneity within the La Quinta population for resistance to race 4. *Nicotiana repanda* Will. ex Leh, *N. glauca* Graham, *N. paniculata* L., and *N. nudicaulis* Watson have also been reported as resistant to race 4 (11), but data indicating levels of resistance have not been presented.

The increasing occurrence of *M. incognita* race 4 in North Carolina tobacco fields, combined with the possible loss of chemical root-knot control measures, has created a strong need for tobacco cultivars with resistance to this organism. In terms of achieving an interspecific gene transfer, *N. otophora* is the most promising source of resistance to this pathogen because of its close relationship to *N. tabacum*. The primary objective of this study was to determine if *N. otophora* possesses a sufficiently high level of resistance to warrant its use in an interspecific gene transfer program

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aimed at developing *M. incognita* race 4 resistant tobacco germplasm. In addition, we wished to address the problem of variability in disease readings found in earlier studies involving *N. otophora* La Quinta (1,10) and to determine if this variability truly reflects population heterogeneity.

#### MATERIALS AND METHODS

Seeds of *N. tabacum* cvs. NC 95 and NC 2326, *N. otophora* acc. La Quinta and *N. repanda* were germinated in sterile vermiculite. Six weeks after sowing, seedlings were transplanted to 15-cm clay pots filled with a steamed loamy sand (89% sand, 10% clay, 1% silt) and randomly arranged on benches. For the first test, ten plants of each of these four genotypes were screened. Ten plants of NC 95 and 20 plants each of La Quinta and *N. repanda* were evaluated in the second test. NC 2326, which is susceptible to the common races of *M. incognita*, was omitted from the second screening.

A population of *M. incognita* race 4 (M4NC23) was obtained from K. R. Barker, North Carolina State University. Race designation was verified with the North Carolina Differential Host Test (12) before the start of multiplication of this inoculum. The race 4 population was increased in the greenhouse on 'Rutgers' tomato (*Lycopersicon esculentum* Mill.) plants. Inoculum was collected using the sodium hypochlorite and hatching chamber extraction method described by Barker (2). Juveniles collected in the first 24 hours were discarded. Freshly hatched second-stage juveniles (J2) were collected and inoculum was calibrated.

Plants were inoculated 2 weeks after transplanting. Inoculum was placed into two holes, 3 cm deep and halfway between plant and pot wall. Inoculum contained approximately 1,500 J2 in 3–4 ml water per hole. Inoculation dates for the two tests were July 5 and November 2, 1990. During test 1 air temperature ranged from 22.8–28.9 C with a mean of 26.6 C, and soil temperature ranged from 22.5–30.6 C with a mean of 28.1 C. During test 2 air

temperatures ranged from 22.8–28.3 C with a mean of 26.2 C and soil temperature ranged from 18.3–23.6 C with a mean of 20.2 C.

Four weeks after inoculation, half of the plants of each genotype were randomly selected for sampling, roots were carefully rinsed free of soil, and a gall index was determined for each plant. Four weeks later, gall indices for the remaining plants were determined. The gall index, which ranged from 0 to 5, was based on percentage of galled roots (13). The roots were then stained with phloxine B (8), and egg-mass ratings were assigned. The egg-mass rating was based on a 0 to 5 scale, where 0 = no egg masses, 1 = 1 or 2, 2 = 3 to 10, 3 = 11 to 30, 4 = 31 to 100, and 5 = >100 egg masses per root system (9).

#### RESULTS

In test 1, NC 95 and NC 2326 showed moderate galling 4 weeks after inoculation (Table 1). Eight weeks after inoculation, the gall index had increased to nearly 5, the maximum possible value, in both cultivars. NC 95 reacted similarly in test 2 (Table 1), although the increase in gall index at 8 weeks was not as dramatic as in the first test. In the two tests, the egg-mass rating was at or near the maximum for the susceptible control(s) at both the 4- and 8-week readings. As indicated by the ranges of disease ratings in both experiments, little variability was seen for gall index or egg-mass rating among the individual plants at any rating period.

In the first test, *N. repanda* had an average gall index of 1.2 four weeks after inoculation. This rating increased to 2.8 at the 8-week reading. A similar 4-week gall index occurred in test 2 but did not increase by the 8-week reading. In both tests, the galls on *N. repanda* were extremely small and could easily escape detection. They became readily apparent only after the roots were stained with phloxine B. The egg-mass rating for *N. repanda* 4 weeks after inoculation in experiment 1 was 3.4, but only 0.8 in experiment 2. In both tests, however, high egg-mass ratings

TABLE 1. Gall indices† and egg mass ratings‡ of four *Nicotiana* genotypes 4 and 8 weeks after inoculation with *Meloidogyne incognita* race 4.

Genotype	Gall index		Egg-mass rating	
	4 weeks	8 weeks	4 weeks	8 weeks
Experiment 1				
NC 95	2.8 (2-3)	4.6 (4-5)	4.6 (4-5)	5.0 (5)
NC 2326	3.0 (3)	4.2 (4-5)	5.0 (5)	5.0 (5)
<i>N. repanda</i>	1.2 (1-2)	2.8 (2-3)	3.4 (3-4)	4.0 (2-5)
<i>N. otophora</i> 'La Quinta'	1.0 (1)	1.2 (1-2)	0.8 (0-1)	0.6 (0-2)
LSD .05	0.4	0.7	0.6	1.0
Experiment 2				
NC 95	3.0 (3)	3.8 (3-4)	3.6 (3-4)	5.0 (5)
<i>N. repanda</i>	1.3 (1-2)	1.0 (1)	0.8 (0-2)	3.4 (2-4)
<i>N. otophora</i> 'La Quinta'	1.4 (1-2)	1.2 (1-2)	0 (0)	0.6 (0-1)
LSD .05	0.4	0.4	0.5	0.6

Data represent mean disease ratings; range of ratings is indicated in parentheses.

† Gall index ranged from 0 to 5 and was based on percentage of galled roots.

‡ Egg-mass ratings of 0 = 0, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100 and 5 = >100 egg masses per root system.

were assigned to *N. repanda* by 8 weeks after inoculation. With the exception of the 8-week egg-mass ratings, there was little variability within a single evaluation for either disease criterion for *N. repanda*.

Low gall indices and egg-mass ratings were found for *N. otophora* La Quinta in each experiment at both 4 and 8 weeks after inoculation. The galls were generally localized, presumably near the original infection site. The maximum number of egg masses on a single La Quinta plant was 3. Both gall index and egg-mass ratings for La Quinta were very consistent within any particular rating period.

### DISCUSSION

Reaction to *M. incognita* race 4, as assessed by gall index and egg-mass rating, was evaluated in two susceptible and two presumed resistant genotypes. The two susceptible *N. tabacum* cultivars gave the expected high disease ratings. These cultivars had numerous galls, which produced large numbers of egg masses. Because the egg-mass rating scale peaked at 101 egg masses/plant root system, the increase in number of egg masses 8 weeks after inoculation on NC 95 and NC 2326 is not reflected in the egg-mass ratings assigned at that time. However, both galling and egg-mass production continued to increase in

the tobacco cultivars throughout the course of the study.

The experiment was performed twice under somewhat different environmental conditions to insure the validity of the results. The only major differences between the results of the two experiments involved *N. repanda*. The gall index 8 weeks after inoculation and the egg-mass rating 4 weeks after inoculation for this species were much less in test 2 than in test 1. Gall development in NC 95 at 8 weeks in test 2 also lagged somewhat behind that in the first test. These differences were likely the result of longer nematode generation time resulting from the lower soil temperatures in the second test.

*Nicotiana repanda* was included in this study to provide a highly resistant control with which to compare *N. otophora* La Quinta. Although *N. repanda* has been described as resistant to race 4, no data indicate the level of resistance in this species (11). Because *N. repanda* has a high level of resistance to many tobacco diseases (3), we presumed that it would also have a high level of resistance to *M. incognita* race 4. In contrast, *N. repanda* was only weakly resistant to this pathogen. Although galling was not as severe in this species as it was in the tobacco cultivars, egg-mass ratings at 8 weeks after inoculation approached those of NC 95 and NC 2326. The small size of

the galls in *N. repanda* may have led to its initial misclassification as being resistant to *M. incognita* race 4.

Low gall indices and egg-mass ratings were assigned to *N. otophora* La Quinta at the 4-week readings. Although these galls were much larger than those in *N. repanda*-infected roots, only a few had egg masses. The limited reproduction of *M. incognita* race 4 on La Quinta after 4 weeks severely restricted the number of second-generation nematodes available for reinfection and thereby resulted in low gall indices at the 8-week reading.

In contrast to previous evaluations of race 4 resistance in *N. otophora* (1,10), very consistent disease ratings were obtained in this study. This indicates that the La Quinta population is not highly heterogeneous for genes conferring resistance to *M. incognita* race 4. Instead, we believe that the variability observed in the other investigations was a result of the use of eggs or chopped roots as inoculum. Such inoculum is not as uniform as the infective juveniles used in this study, and may contribute to variability between plants for disease reaction. Because early generations of a tobacco breeding program using alien sources of germplasm must be screened on a plant-by-plant basis, use of a highly precise disease screening procedure is essential. By use of J2 as inoculum, segregating populations developed from an *N. tabacum* × *N. otophora* La Quinta hybridization can be screened with confidence.

At this time, *N. otophora* La Quinta is a very promising source of resistance to *M. incognita* race 4. *Nicotiana tabacum* and *N. otophora* hybridize easily, and pairing occurs between the chromosomes of *N. otophora* and half of the *N. tabacum* chromosomes (4). Future studies will concentrate on determining whether the *M. incognita* race 4 resistance found in *N. otophora* La Quinta is conditioned by dominant factors,

and if it can be expressed stably in *N. tabacum*. If so, efforts will be made to transfer this resistance into flue-cured tobacco via interspecific hybridization and gene introgression.

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