Resistance to Osborne's Cyst Nematode in Selected *Nicotiana* Species[1]

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Abstract: Resistance to an undescribed species of *Heterodera*, Osborne's cyst nematode, was compared in *Nicotiana glutinosa, N. paniculata, N. plumbaginifolia* and *N. longiflora*. These species were differentially resistant in greenhouse tests as shown by nematode development, the reaction of the invaded roots, and the expression of resistance in interspecific hybrids. *Key Words:* Sesquidiploid hybrids, Hypersensitivity.

An undescribed *Heterodera* sp., Osborne's cyst nematode (OCN) (6), was found parasitizing *Nicotiana tabacum* L. (var. 'Hicks') in Amelia County, Virginia (8). Heavily parasitized tobacco plants were severely stunted, had greatly reduced root systems, and tended to wilt in the hottest part of the day. Although chemical control of this nematode is feasible (9), resistant varieties are needed for a balanced control program.

L. I. Miller (personal communication) reported *N. paniculata* L., *N. glutinosa* L., *N. longiflora* Cav., and *N. plumbaginifolia* Viv. resistant to OCN. Since interspecific character transfer from these species to *N. tabacum* L. is possible by the sesquidiploid method described by Chaplin and Mann (1), the present studies were conducted to determine the relative resistance of these species and of the sesquidiploid hybrids derived from crosses with a susceptible autotetraploid of *N. tabacum* ('SC58').

MATERIALS AND METHODS

Six seedlings each of *N. paniculata* (N = 12), *N. glutinosa* (N = 12), *N. plumbaginifolia* (N = 10), *N. longiflora* (N = 10), *N. tabacum* 'SC58' (4N = 96) and *N. tabacum* var. 'NC95' (N = 24), the latter a susceptible variety, were transplanted into 4-inch pots containing eggs and larvae from 30 OCN cysts (Watkins' isolate from Amelia Co., Virginia) mixed with steam sterilized soil. The plants were maintained in the greenhouse for 9 weeks. The roots and soil in each pot were washed carefully to remove the females and cysts.

Resistance of each plant to OCN was determined by counting the number of females recovered from each plant. Plants with no females or cysts were repotted and used as the male parent in crosses with the autotetraploid *N. tabacum* 'SC58'.

Seed from interspecific crosses, designated F_1, were germinated and inoculated by the procedure used for the parental materials. 'NC95' was used as a check for inoculum viability.

In order to determine the penetration of roots by the nematode, three seedlings of each species or variety were inoculated with a mixture of 1,000 eggs and larvae using the following procedure. Each seedling was placed in a plastic petri dish, eggs and larvae were added, and the root system covered with Weblite® (Weblite Corporation, Roanoke, Virginia). Plants were fertilized with Hoagland's solution (4). All seedlings were placed under Gro-Lux® (Sylvania) fluorescent lights, 1400 ft-c on a 14-hr day length

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TABLE 1. Penetration of *Nicotiana* spp. roots by Osborne's cyst nematode and the development of females on *Nicotiana* spp. and their interspecific hybrids.

Species and hybrids	Larvae per plant[a]	Females per plant[b]
N. tabacum 'NC95'	260	285.0 ± 21.7
N. tabacum (tetraploid) 'SC58'	—	335.0 ± 71.7
N. paniculata	180	0.8 ± 0.8
N. glutinosa	159	0.7 ± 0.8
N. plumbaginifolia	276	1.0 ± 0.8
N. longiflora	303	0.0 ± 0.0
N. tabacum 'SC58' × *N. paniculata*	—	121.0 ± 67.0
N. tabacum 'SC58' × *N. glutinosa*	—	22.0 ± 9.5
N. tabacum 'SC58' × *N. plumbaginifolia*	—	5.0 ± 2.0

[a] Mean of three replications; differences not statistically significant.
[b] Mean of six replications and confidence interval (95%).

darkness cycle. After one week, plants were washed free of Weblite and inoculum and the roots were fixed and stained in ethanol-acetic acid (1:1, v/v) with acid fuchsin as described by Southards (12). The roots were cleared in saturated chloral hydrate and mounted in lactophenol and the nematodes that had penetrated each root system were counted.

Development of OCN in roots was studied by inoculating nine seedlings of each species with a mixture of 2,000 eggs and larvae. After one week, the inoculum and Weblite were removed by gently washing the roots, and the plants were then grown in distilled water. The roots of three plants of each of the species or variety were fixed, stained, and mounted at 17, 22, and 26 days after inoculation. The water in which the plants had been growing was examined for emerged male nematodes. One hundred nematodes in each root system were grouped by gross morphology into three stages of development. Second-stage larvae were those larvae which had not molted. Third and fourth-stage larvae were grouped together as those larvae that were not second stage and not yet adults. Fully developed males that had not emerged from the cuticle and young females were considered adults. Some males emerged earlier and were counted separately from those that were still in the roots.

Only two groups of hybrids were tested for the development of the nematode because the "longiflora" hybrids did not produce viable seedlings and there was a limited amount of seed of the "paniculata" hybrid. Six seedlings of "glutinosa" hybrids and "plumbaginifolia" hybrids were inoculated by the method described above and 'NC95' was the susceptible check.

RESULTS

When the parental materials were examined, it was found that only *N. longiflora* did not allow females to develop (Table 1). The number of females which developed on *N. paniculata*, *N. glutinosa*, and *N. plumbaginifolia* varied from zero to three per plant but the average of all species was less than 0.5% of the check.

Of all the hybrids tested, the "plumbaginifolia" hybrids were the most resistant and had the most extensive and succulent root systems. "Paniculata" hybrids had small root systems but produced the highest population of females. Resistance in "paniculata" hybrids was variable ranging from a plant which supported 27 females to that which supported 200 females. "Glutinosa" hybrids were more resistant than "paniculata" hybrids, but were not as resistant as "plumbaginifolia" hybrids (Table 1).

There was no apparent difference in penetration in 'NC95', the susceptible check, and the resistant *Nicotiana* spp. (Table 1). However, there was an apparent difference in the

TABLE 2. Maturation of Osborne's cyst nematode in roots of selected *Nicotiana* species and their interspecific hybrids 26 days after inoculation.

Species and hybrids	Percentage of nemas in different stages[a]				Emerged males[b]
	Second stage	Stages three and four	Adult females	Adult males	
N. tabacum 'NC95'	1	9	68	22	237
N. paniculata	34	7	0	59	21
N. glutinosa	90	6	0	4	1
N. plumbaginifolia	99	0	0	1	0
N. longiflora	100	0	0	0	0
N. tabacum 'SC58' × *N. glutinosa*	53	22	4	21	26
N. tabacum 'SC58' × *N. plumbaginifolia*	80	17	0	3	0

[a] Based on 100 nematodes.
[b] Average number of emerged males per plant.

rate of development. Adult males and females were found in roots of 'NC95' but not in roots of the resistant species after 17 days. OCN on 'NC95' required 26 days following inoculation for 90% of the larvae to develop to maturity but for the other host species, development of the nematode was either slow or did not occur (Table 2). While four males were counted on *N. glutinosa*, 59 males were found in *N. paniculata*. The root system of *N. glutinosa* was severely necrotic which made it difficult to find 100 nematodes to characterize by the 26th day following inoculation. *N. longiflora* and *N. plumbaginifolia* were not visibly affected by the presence of the nematode in their roots. Since males emerged earlier than females, they were counted separately from the nematodes that were still in the roots. The average number of emerged males per plant is given in Table 2. Although one male nematode had developed on *N. plumbaginifolia*, no other advanced stages of OCN were observed. No development beyond second-stage was observed on *N. longiflora*.

DISCUSSION

When *N. glutinosa*, *N. paniculata*, *N. plumbaginifolia*, and *N. longiflora* were tested in the greenhouse, very few females were recovered from the roots of any species indicating that all species were resistant to OCN. The most resistant of the species were invaded by second-stage larvae to the same degree as the most susceptible, indicating the lack of a mechanical barrier to the entry of nematodes. This has been generally noted among endoparasitic nematodes and has been shown by different workers (2, 3, 5, 10). It also substantiates the statement by Rohde (10) that resistance to nematodes usually occurs after the plant has been invaded.

Each *Nicotiana* sp. tested reacted differently to OCN:

(i) *N. longiflora* was the most resistant. The nematodes exhibited no evidence of feeding and the root system was not visibly affected. The "longiflora" hybrids were nonviable, therefore, no information is available on the inheritance of resistance.

(ii) *N. plumbaginifolia* matured some females in the pot tests but most larvae showed

no indication of feeding or developing. The root system was not visibly affected by the nematode. A high degree of resistance was expressed in the "plumbaginifolia" hybrids.

(iii) *N. glutinosa* allowed few females to develop but larvae showed more evidence of feeding. The root systems exhibited severe necrosis and appeared to have responded to infection similarly to soybeans resistant to *H. glycines* (2) and for *N. repanda* resistant to root knot nematode (7). The "glutinosa" hybrids were not as resistant as *N. glutinosa* or the "plumbaginifolia" hybrids, possibly indicating incomplete dominance of the factors controlling resistance.

(iv) *N. paniculata* allowed few females to develop but did allow many males to develop. The "paniculata" hybrids were also the most susceptible of the hybrids tested.

These differential reactions indicate that there is potentially a broad base of resistance to OCN that may be utilized in tobacco breeding programs. *N. plumbaginifolia* appears to be the most promising as it apparently combines a high degree of resistance with low hypersensitivity and is relatively easy to hybridize with *N. tabacum.*

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