

The Inheritance of Resistance to the Southern Root-knot Nematode in 'Carolina Hot' Cayenne Pepper

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Abstract. Greenhouse experiments were conducted to determine the inheritance of the high level of southern root-knot nematode [*Meloidogyne incognita* (Kofoid & White) Chitwood] resistance exhibited by 'Carolina Hot' cayenne pepper (*Capsicum annuum* L.) and to compare the genetic nature of this resistance to that exhibited by 'Mississippi Nemaheart.' Evaluation of parental, F₁, F₂, and backcross generations of the cross 'Mississippi Nemaheart' × 'California Wonder' confirmed an earlier published report that the 'Mississippi Nemaheart' resistance is conditioned by a single dominant gene. Evaluation of parental, F₁, F₂, and backcross generations of a cross between highly resistant and highly susceptible lines selected from a heterogeneous 'Carolina Hot' population indicated that the resistance exhibited by 'Carolina Hot' is conditioned by two genes, one dominant and one recessive. Evaluation of the parental and F₂ populations of a cross between 'Mississippi Nemaheart' and the highly resistant 'Carolina Hot' line indicated that the dominant resistance gene in 'Mississippi Nemaheart' is allelic to the dominant resistance gene in 'Carolina Hot.' Comparison of the data that were collected on the parental lines in the latter cross demonstrated the superior nature of the resistance exhibited by 'Carolina Hot.' The presence of the second resistance gene in 'Carolina Hot' probably accounted for the higher level of resistance. The ease and reliability of evaluating plants for resistance to root-knot nematodes and the availability of a simply inherited source of resistance makes breeding for southern root-knot nematode resistance a viable objective in pepper breeding programs. This objective should be readily obtainable by the application of conventional plant breeding methodologies.

The southern root-knot nematode (*Meloidogyne incognita*) is a major pest of pepper (*Capsicum annuum*) in the United States. Parasitism of susceptible cultivars results in severely stunted plants and significantly reduced yields (Di Vito et al., 1992; Fery and Dukes, 1984; Lindsey and Clayshulte, 1982). The primary control method is soil fumigation, and the principal fumigant used at present is methyl bromide. The pending withdrawal of methyl bromide from the U.S. market (U.S. Dept. of Agriculture, 1993) has focused considerable interest on host plant resistance as a control measure. Resistant cultivars of bell-type peppers are badly needed by the fresh-market industry.

Martin's report (1948) of resistance in a pungent pepper was one of the first reports of resistance in *C. annuum* to root-knot nematodes. Martin and Crawford (1958) subsequently released the root-knot nematode-resistant cayenne pepper 'Carolina Hot.' Hare (1957) reported that resistance to *M. incognita* is conditioned by a single, dominant gene. He proposed that this gene be symbolized *N*. Hare (1966) later released the resistant pimiento-type pepper 'Mississippi Nemaheart.' The *M. incognita* resistant peppers 'Carolina Cayenne' and 'Charleston Hot' were selected from a 'Carolina Hot' population heterogeneous for many traits, including resistance to *M. incognita* (Fery et al., 1986; U.S. Dept. of Agriculture, 1992).

Observations of many greenhouse and field tests at our laboratory recently suggest that the resistance derived from 'Carolina Hot' is superior to the resistance exhibited by 'Mississippi Nemaheart.' For example, most 'Carolina Cayenne' and 'Charleston Hot' plants removed from *M. incognita* infested soils do not exhibit any symptoms or signs (egg masses) of *M. incognita* infection, whereas most 'Mississippi Nemaheart' plants usually

exhibit a limited galling reaction and obvious signs of parasite reproduction. Fery et al. (1986) noted that roots of 'Carolina Cayenne' plants removed from a naturally infested field yielded 99.7% fewer *M. incognita* eggs than roots of a composite of susceptible lines selected from 'Carolina Hot'; postharvest soil samples from plots with resistant plants yielded 99.9% fewer *M. incognita* larvae than samples from plots with susceptible plants. Results of a recent study by Zamora et al. (1994) indicated that 'Carolina Cayenne' is resistant to all four known races of *M. incognita*. Zamora et al. (1994) also reported that 'Carolina Cayenne' roots yielded 99.9% fewer *M. incognita* eggs than roots of the susceptible 'NuMex R Naky.'

Cultivars with *M. incognita* resistance derived from 'Carolina Hot' hold considerable promise for use as parental materials in pepper breeding programs. The breeding value of any pest resistant germplasm would be greatly enhanced if the mode of inheritance was understood. Additionally, many modern bell pepper cultivars are hybrids, and the usefulness of specific genes in the development of such cultivars depends on the availability of detailed information about gene action. These needs prompted us to determine the inheritance of resistance to *M. incognita* exhibited by 'Carolina Hot,' and compare the genetic nature of this resistance to that exhibited by 'Mississippi Nemaheart.'

Materials and Methods

The data reported here are from a series of greenhouse experiments conducted at the U.S. Vegetable Laboratory, Charleston, S.C. Seeds of all parental, F₁, F₂, and backcross generations were produced in the greenhouse using standard crossing and selfing procedures. The tests were conducted in 4.1 × 1.7 × 0.2-m benches containing a steam-sterilized mixture of about 6 soil : 3 sand : 1 peatmoss (by volume). The reaction of the mixture was maintained at pH 6.0. Seedlings were started in flats containing a sterilized artificial growth medium, and transplanted into the benches after true leaves had expanded. After the plants were established and growing, each plant was inoculated with about 2100 *M. incognita*

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eggs. The egg inocula for all tests were extracted from cowpea, sweetpotato, or pepper roots infected with *M. incognita* using 0.525% sodium hypochlorite in a procedure described by Hussey and Barker (1973). All *M. incognita* populations were Race 3.

Completely randomized experimental designs were used and the planting arrangement was a 10 × 12-cm rectangular pattern. Each plot contained five plants. To minimize the effects of moisture and temperature stress, the outmost two rows around each bench were used as buffers. Greenhouse temperature was maintained between 24 and 32 °C and all plants were evaluated 10 to 12 weeks after inoculation. Each plant received two subjective scores, one for the prevalence of root galling and another for the prevalence of egg masses. The following scale was used to score the severity of galling: 1 = no galls; 2 = light galling, 1% to 25% of root system galled; 3 = moderate galling, 26% to 50% of root system galled; 4 = heavy galling, 51% to 75% of root system galled; and 5 = severe galling, 76% to 100% of root system galled. The number of egg masses per root system was scored as follows: 1 = no egg masses evident, 2 = scattered egg masses covering 1% to 25% of the root system, 3 = moderate number of egg masses covering 26% to 50% of root system, 4 = numerous egg masses covering 51% to 75% of root system, and 5 = extremely large numbers of egg masses covering 76% to 100% of root system. All plants with gall and egg mass indices of 1 to 3 were classified as root knot resistant; plants rated 4 or 5 for either galls or egg masses were classified as susceptible. Chi-square tests for goodness of fit were used in testing all genetic hypotheses.

Experiment I. Plants of the parental, F₁, F₂, and backcross generations of the cross 'Mississippi Nemaheart' × 'California Wonder' were tested for resistance to *M. incognita*. 'Mississippi Nemaheart' is homozygous for the *N* gene governing resistance to *M. incognita*. 'California Wonder' is highly susceptible. The test contained 10 plots of 'Mississippi Nemaheart' plants, 7 plots of 'California Wonder' plants, 3 plots of F₁ plants, 8 plots of F₂ plants, 18 plots of F₁ × 'Mississippi Nemaheart' backcross plants, and 7 plots of F₁ × 'California Wonder' backcross plants. The seeds were planted on 18 Nov., the seedlings were transplanted on 15 Dec., established plants were inoculated on 12 Jan., and the roots of each plant were evaluated on 25 Mar.

Experiment II. Plants of the parental, F₁, F₂, and backcross generations of the cross PA-135 × PA-136 were tested for resistance to *M. incognita*. The PA-135 and PA-136 lines resulted from single plant selections from a 'Carolina Hot' population that was heterogeneous for many traits, including resistance to *M. incognita*. PA-135 is highly resistant to *M. incognita*, and it is one of the five horticulturally superior lines selected from a 'Carolina Hot' population that were composited to create 'Carolina Cayenne' (Fery et al., 1986). PA-136 is highly susceptible to *M. incognita*

(Dukes and Fery, 1992). Except for its reaction to *M. incognita*, the PA-136 phenotype is quite similar to the phenotype of PA-135. Two greenhouse tests were conducted to evaluate the PA-135 × PA-136 cross. The first test contained 6 plots of PA-135 plants, 6 plots of PA-136 plants, 6 plots of F₁ plants, and 20 plots of F₂ plants. The seeds for this test were planted on 18 Nov., the seedlings were transplanted on 15 Dec., established plants were inoculated on 12 Jan. and the roots of each plant were evaluated on 25 Mar. The second test contained 10 plots of PA-135 plants, 10 plots of PA-136 plants, 30 plots of F₁ × PA-135 backcross plants, and 20 plots of F₁ × PA-136 backcross plants. The seeds for this test were planted on 2 Sept., the seedlings were transplanted on 14 Sept., established plants were inoculated on 27 Sept., and the roots of each plant were evaluated on 6 Dec.

Experiment III. Plants of the parental and F₂ generations of the cross 'Mississippi Nemaheart' × PA-135 were tested for resistance to *M. incognita*. The test contained 6 plots of PA-135 plants, 6 plots of 'Mississippi Nemaheart' plants, and 20 plots of the F₂ plants. Additionally, 6 plots of highly susceptible 'Sweet Banana' plants were included as checks. The seeds for this test were planted on 2 Sept., the seedlings were transplanted on 14 Sept., established plants were inoculated on 27 Sept., and the roots of each plant were evaluated on 6 Dec.

Results and Discussion

The procedures used to infest the established plants in the growing medium with *M. incognita* eggs were effective and reliable. All homozygous lines with known reactions to the parasite reacted as expected. Preliminary analyses revealed no significant differences between either reciprocal crosses or the two tests conducted for Expt. II. As a result, reciprocals and the Expt. II tests were pooled for genetic analyses.

Experiment I. 'Mississippi Nemaheart' and 'California Wonder' plants reacted to *M. incognita* as expected (Table 1). 'Mississippi Nemaheart' exhibited a high level of resistance. The roots of most of the 'California Wonder' plants exhibited heavy to severe galling and egg masses were numerous. The frequency of phenotypes in the progeny generations indicated that the *M. incognita* resistance in 'Mississippi Nemaheart' is conditioned by a single dominant gene. All 9 F₁ plants were resistant and all but one of the 89 F₁ × 'Mississippi Nemaheart' backcross plants were of the expected resistant phenotype. The F₂ segregated 3 resistant : 1 susceptible and the F₁ × 'California Wonder' backcross segregated 1 resistant : 1 susceptible. These results confirm Hare's (1957) conclusion that *M. incognita* resistance is conditioned by a single dominant gene.

Experiment II. The PA-135 and PA-136 plants reacted as

Table 1. Segregation for reaction to the southern root-knot nematode (*Meloidogyne incognita*) in parental, F₁, F₂, and backcross generations of the cross 'Mississippi Nemaheart' × 'California Wonder' (Expt. I).

| Generation | No. of plants | | Expected ratios (R:S) ² | χ ² | P |
|----------------------------------|---------------|-------------|------------------------------------|----------------|---------|
| | Resistant | Susceptible | | | |
| Mississippi Nemaheart | 49 | 0 | All R | --- | --- |
| California Wonder | 1 | 29 | All S | --- | --- |
| F ₁ | 9 | 0 | All R | --- | --- |
| F ₂ | 27 | 9 | 3R:1S | 0.00 | 1.00 |
| F ₁ × Miss. Nemaheart | 88 | 1 | All R | --- | --- |
| F ₁ × Calif. Wonder | 15 | 10 | 1R:1S | 1.00 | 0.5–0.2 |

²R = resistant, S = susceptible.

Table 2. Segregation for reaction to the southern root-knot nematode (*Meloidogyne incognita*) in parental, F₁, F₂, and backcross generations of the cross PA-135 x PA-136 (Expt. II).

| Generation | No. of plants | | Expected ratios (R:S) ^z | χ^2 | P |
|-------------------------|---------------|-------------|------------------------------------|----------|---------|
| | Resistant | Susceptible | | | |
| PA-135 ^y | 79 | 0 | All R | --- | --- |
| PA-136 ^x | 0 | 80 | All S | --- | --- |
| F ₁ | 28 | 0 | All R | --- | --- |
| F ₂ | 86 | 15 | 13R:3S | 1.01 | 0.5–0.2 |
| F ₁ x PA-135 | 150 | 0 | All R | --- | --- |
| F ₁ x PA-136 | 37 | 42 | 1R:1S | 0.32 | 0.8–0.5 |

^zR = resistant, S = susceptible.

^yHomozygous resistant selection from a 'Carolina Hot' population heterogeneous for resistance to *M. incognita*.

^xHomozygous susceptible selection from a 'Carolina Hot' population heterogeneous for resistance to *M. incognita*.

expected (Table 2). The roots of all PA-135 plants exhibited a high level of resistance. The roots of all PA-136 plants exhibited heavy to severe galling and supported numerous egg masses. The frequency of phenotypes in the progeny generations of the PA-135 x PA-136 cross indicates that the *M. incognita* resistance in PA-135 is inherited in a more complex manner than it is in 'Mississippi Nemaheart.' Although all of the F₁ and F₁ x PA-135 backcross plants were resistant and the F₁ x PA-136 backcross segregated 1 resistant : 1 susceptible, the F₂ segregated in a 13 resistant : 3 susceptible rather than the 3 resistant : 1 susceptible ratio observed in the earlier cross. These results indicate that *M. incognita* resistance in the PA-135 line extracted from 'Carolina Hot' is conditioned by two genes, one dominant and one recessive.

Experiment III. All of the plants of the parental lines of the PA-135 x 'Mississippi Nemaheart' cross were resistant and all of the 'Sweet Banana' check plants were susceptible (Table 3). The lack of segregation in the F₂ generation of this cross indicates that the dominant resistance gene in 'Mississippi Nemaheart' is allelic to the dominant resistance gene in PA-135. Thus, the gene (N) reported by Hare in 1957 to condition resistance to *M. incognita* is probably the same gene as the dominant resistance gene carried by PA-135.

Examination of the actual gall ratings of the parental lines of the PA-135 x 'Mississippi Nemaheart' cross illustrates the differences in resistance levels between these lines that we have observed in previous tests (Table 3). Although quite resistant, all of the 'Mississippi Nemaheart' plants exhibited limited galling, i.e., gall index rating of 1+ or 2. Examination of the data for the PA-135 plants, however, shows that 26 of 28 plants did not exhibit even limited galling. Similar results were observed when egg mass

ratings were evaluated (data not presented). The presence of the second resistance gene in PA-135 probably accounts for the higher level of resistance.

Our results showing the existence of multiple genes in pepper conditioning root-knot nematode resistance and the presence of dominant and recessive gene action are in agreement with findings reported by others. Hendy et al. (1985b) evaluated homozygous progenies of two *C. annuum* lines obtained by androgenesis and identified five genes that each conditioned resistance to one or more *Meloidogyne* species. Hendy et al. (1985a) studied *Meloidogyne* resistances in the same lines, and they observed the presence of additive effects of multiple genes conditioning resistances to various *Meloidogyne* species. Di Vito et al. (1985) studied lines obtained by interspecific hybridization between *C. annuum* and *C. frutescens* L. and they concluded that resistance exhibited by *C. frutescens* is monogenic dominant and the resistance exhibited by *C. annuum* is monogenic recessive. Data published by Peter et al. (1984) suggested that resistances to *M. incognita* exhibited by Indian hot peppers are recessive. Recent work published by Di Vito et al. (1993) demonstrated the complex nature of the resistance to root-knot nematodes in *Capsicum*: a single dominant gene in *C. frutescens* controlled resistances to *M. incognita*, *M. javanica* (Treub) Chitwood, and *M. arenaria* (Neal) Chitwood; a single dominant gene in *C. chacoense* Hunz. controlled resistance to *M. incognita* and *M. arenaria*, but two dominant genes controlled resistance to *M. javanica*; and a single dominant gene in *C. chinense* Jacq. controlled resistance to *M. incognita* and *M. javanica*, but two duplicate genes controlled resistance to *M. arenaria*.

There is ample evidence that 'Carolina Hot' has resistance to *Meloidogyne* species other than *M. incognita*. Hare (1957) noted

Table 3. Segregation for reaction to the southern root-knot nematode (*Meloidogyne incognita*) in parental and F₂ generations of the cross 'Mississippi Nemaheart' x PA-135 (Expt. III).

| Generation | Gall index ^z | | | | | | | | |
|---------------------------|-------------------------|-----------------|----|----|---|----|----|----|----|
| | 1 | 1+ ^y | 2 | 2+ | 3 | 3+ | 4 | 4+ | 5 |
| Miss. Nemaheart | | 20 | 9 | | | | | | |
| PA-135 ^x | 26 | 2 | | | | | | | |
| F ₂ | 59 | 29 | 11 | | | | | | |
| Sweet Banana ^w | | | | | | | 15 | 2 | 13 |

^zGall index: resistant ≤3+, susceptible ≥4.

^yA + indicates a score intermediate between two reaction classes.

^xHomozygous resistant selection from 'Carolina Hot' population heterogeneous for resistance to *M. incognita*.

^wSusceptible check.

that lines homozygous for the *N* gene, a gene apparently carried by 'Carolina Hot,' also exhibit resistances to the peanut root-knot nematode (*M. arenaria*) and the tropical root-knot nematode (*M. javanica*). Noe (1992) recently reported that 'Carolina Cayenne,' a southern root-knot resistant cultivar selected from a heterogeneous 'Carolina Hot' population, is highly resistant to the two known races of *M. arenaria*. 'Charleston Hot,' a cultivar also selected from a heterogeneous 'Carolina Hot' population, is resistant not only to all four known races of *M. incognita*, but also to *M. hapla* Chitwood (the northern root-knot nematode), *M. javanica*, and *M. arenaria* (U.S. Dept. of Agriculture, 1992). Additional studies are needed to determine whether the two genes shown in this study to condition the *M. incognita* resistance in 'Carolina Hot' also condition the resistances to other *Meloidogyne* species.

Conclusions

This study indicated that two genes, one dominant and one recessive, condition the high level of resistance to the southern root-knot nematode exhibited by 'Carolina Hot' cayenne pepper. The ease and reliability of evaluating plants for resistance to root-knot nematodes and the availability of a simply inherited source of outstanding resistance makes breeding for southern root-knot nematode resistance a viable objective in pepper breeding programs. This objective should be readily obtained by the application of conventional plant breeding methodologies.

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