Journal of Nematology 32(4):356–361. 2000.


# Heat Stability of Resistance to *Meloidogyne incognita* in Scotch Bonnet Peppers (*Capsicum chinense* Jacq.)[1]

Judy A. Thies[2] and Richard L. Fery[2]

*Abstract:* Stability of resistance to *Meloidogyne incognita* (Kofoid & White) Chitwood was determined in pepper (*Capsicum chinense* Jacq. and *C. annuum* L.) at 24, 28, and 32 °C. Reactions of the *C. annuum* cultivars Charleston Belle and Keystone Resistant Giant and the *C. chinense* cultigens PA-426 and PA-350 to *M. incognita* were compared. Charleston Belle is homozygous for the *N* gene that confers resistance to *M. incognita* in *C. annuum,* and Keystone Resistant Giant is the susceptible recurrent parent of Charleston Belle. PA-426 is homozygous for a single dominant resistance gene that is allelic to the *N* gene, and PA-350 is susceptible. Root galling, egg-mass production, numbers of eggs per g fresh root, and reproductive factor of *M. incognita* increased for all pepper genotypes as temperature increased. Severity of root galling and nematode reproduction were less for PA-426 and Charleston Belle compared to PA-350 and Keystone Resistant Giant at all temperatures. However, both PA-426 and Charleston Belle exhibited a partial loss of resistance at the higher temperatures. For example, at 32 °C, the numbers of *M. incognita* eggs per g fresh root and the reproductive index for PA-426 and Charleston Belle were in the susceptible range. Nevertheless, the gall index for both cultivars was still within the resistant range. Both PA-350 and Keystone Resistant Giant exhibited highly susceptible reactions at 28 and 32 °C. Although the resistances of PA-426 and Charleston Belle were somewhat compromised at high temperatures, cultivars possessing these resistances will still be useful for managing *M. incognita* under high soil temperatures.

*Key words:* *Capsicum annuum* L., *C. chinense* Jacq., habanero, heat stability, *Meloidogyne incognita,* resistance, root-knot nematode, Scotch Bonnet pepper, soilborne pathogen, soil temperature, vegetable breeding.

The southern root-knot nematode *Meloidogyne incognita* (Kofoid & White) Chitwood is a major constraint to pepper (*Capsicum* spp.) production throughout the world (Di Vito et al., 1992; Lindsey and Clayshulte, 1982; Thies et al., 1997; Thies et al., 1998; Thomas, 1994). Currently, pre-plant fumigation of soil with methyl bromide or other fumigant nematicides is the primary method used to control root-knot nematodes in peppers in the United States and worldwide. However, there is growing public uncertainty about the routine use of pesticides, including nematicides, in global agriculture. Concern about pesticide use has stimulated interest in the development of alternative pest management strategies with increased emphasis on the development of resistant cultivars. Several sources of resistance to *M. incognita* have been reported in pepper including *C. annuum* L., *C. chinense* Jacq., *C. chacoense* Hunz., and *C. frutescens* L. (Di Vito and Saccardo, 1986; Di Vito et al., 1989; Di Vito et al., 1993; Fery et al., 1986; Fery et al., 1998; Fery and Thies, 1997; Hare, 1957; Hendy et al., 1985).

A major portion of the world's pepper production occurs in hot climates where root-knot nematodes are a severe pest; however, little is known about the heat stability of root-knot resistance in pepper. The breakdown in expression of resistance to root-knot nematodes at high temperatures is widely recognized in tomato (Dropkin, 1969; Holtzmann, 1965) and also has been reported in cotton, alfalfa, sweetpotato, and bean (Carter, 1982; Griffin, 1969; Jatala and Russell, 1972; Mullin et al., 1991; Omwega et al., 1990). Recently, Thies and Fery (1998) reported that expression of the *N* gene, which controls resistance to *M. incognita* in pepper (*C. annuum*), is modified at high temperatures (28 and 32 °C). In contrast, Di

Received for publication 11 August 2000.

[1] This work was supported, in part, by the Integrated Pest Management Collaborative Research Support Program (IPMCRSP), U.S. Agency for International Development (USAID), under grant LAG-4196-G-00-3053-00.

[2] Research Plant Pathologist and Research Geneticist, respectively. U.S. Vegetable Laboratory, USDA, ARS, 2875 Savannah Highway, Charleston, SC 29414.

E-mail: jthies@awod.com

The authors thank E. L. Corley, Jr., S. W. Miller, and R. Reeves for technical assistance in conducting the experiments. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

This paper was edited by Lawrence Young.

Vito et al. (1995) observed stability of resistance in *C. annuum, C. chacoense, C. chinense,* and *C. frutescens* to *M. incognita* race 1 from 26 to 38 °C. Likewise, Djian-Caporalino et al. (1999) reported stability of resistance in *C. annuum* at high temperatures (32 and 42 °C).

Primary areas of global pepper production occur in temperate, sub-tropical, and tropical areas where root-knot nematodes complete several life cycles annually and soil temperatures during the growing season often reach or exceed those at which expression of resistance to *M. incognita* may be compromised. Thus, knowledge about the heat stability of root-knot-nematode resistance controlled by various genetic systems in pepper is essential to the development of useful root-knot-resistant cultivars and also to the development of vegetable-cropping schemes for hot climates. Our objectives were to (i) determine the heat stability of a new source of resistance to *M. incognita* in *C. chinense* and (ii) confirm the modified expression of the *N* gene in *C. annuum* at high temperatures.

## Materials and Methods

*Nematode inoculum: Meloidogyne incognita* race 3, obtained from S. A. Lewis, Clemson University, Clemson, South Carolina, was cultured on tomato (*Lycopersicon esculentum* Mill. 'Rutgers') in an isolated greenhouse bench. Egg inocula for all experiments were extracted from *M. incognita*-infected tomato roots using 0.5% NaOCl (Hussey and Barker, 1973).

*Pepper genotypes:* Two *C. chinense* cultigens, PA-426 and PA-350, and two *C. annuum* cultivars, Charleston Belle and Keystone Resistant Giant, were used in this experiment. PA-426 is a Scotch Bonnet-type cultigen released by USDA, ARS that is resistant to *M. incognita* (Fery and Thies, 1998a). Resistance of PA-426 is conditioned by a single dominant gene that is allelic to the *N* root-knot resistance gene in *C. annuum* (Fery and Thies, 1998b). PA-350 is a susceptible habanero-type cultigen (Fery and Thies, 1997). Charleston Belle is a root-knot-nematode resistant, open-pollinated bell pepper released by USDA, ARS (Fery et al., 1998). Charleston Belle is homozygous for the *N* root-knot nematode resistance gene. Keystone Resistant Giant is the susceptible recurrent parental cultivar used in the backcross breeding procedure (six backcrosses) used to develop Charleston Belle.

*Controlled temperature experiments:* Reactions of the two resistant and two susceptible pepper genotypes to *M. incognita* race 3 were compared in controlled temperature environments. The experiment was a split-plot design with three replicates conducted at different times. Whole plots were three temperatures (24, 28, and 32 °C) that were randomly assigned to three growth chambers before each replicate was initiated. Sub-plots were the four pepper genotypes (PA-426, PA-350, Charleston Belle, and Keystone Resistant Giant) arranged in randomized complete blocks within each growth chamber. Each replicate consisted of 18 subsamples (plants) per temperature × pepper genotype treatment combination.

Seedlings of each genotype were started in the greenhouse in plastic growing trays containing 50 individual 5.5 × 5.5 × 7.0-cm cells filled with Metro-Mix 360 (The Scotts Company, Marysville, OH). Twenty-one days later, single seedlings were transplanted into individual 10 × 10 × 10-cm pots filled with a pasteurized planting medium of 2 sandy loam soil: 1 washed river sand (by volume). The soil surrounding the roots of each plant was infested with approximately 5,000 *M. incognita* eggs in 5 ml tap $H_2O$ at transplanting. Eighteen pots of each genotype were placed in each of three growth chambers that had been programmed to maintain a temperature of 24, 28, or 32 °C, with a 16-hour/8 hour (light/dark) cycle. Visible radiation was 258 $\mu mol \cdot s^{-1} \cdot m^{-1}$ during the light cycle. Nine weeks later, the plants were removed from the pots, and roots were carefully washed. Roots were rated for severity of galling and for egg-mass production using a 1-to-5 scale, where 1 = 0 to 3% root system galled or covered with egg masses; 2 = 4 to 25%, 3 = 26 to 50%, 4 = 51 to 80%, and 5 = greater than 80% root system galled or cov-

ered with egg masses. Root-gall and egg-mass indices ≥3 were considered susceptible reactions. All fibrous roots were clipped from the tap root, cut into 1 to 2-cm pieces, and root fresh weight recorded. Eggs were extracted from the roots of each plant using 1% NaOCl (Hussey and Barker, 1973). Eggs were counted using a stereomicroscope. Nematode reproduction was assessed by calculating a reproductive index R = $P_f/P_i$, where $P_i$ = initial inoculum level and $P_f$ = final egg recovery (Sasser et al., 1984).

*Data analysis:* Egg-count data were transformed with $\log_{10}(x + 1)$ before analysis to normalize data. Temperature was considered a discrete variable. Data were analyzed using the GLM procedure of SAS for Windows System Version 6.12 (SAS Institute, Inc., Cary, NC). Because there was a temperature × pepper genotype interaction ($P < 0.001$), the data were analyzed by temperature (using GLM) and means were separated within temperatures using Duncan's multiple-range test.

## Results

Both the *C. chinense* cultigen PA-426 and the *C. annuum* cultivar Charleston Belle were more resistant to *M. incognita* than the *C. chinense* cultigen PA-350 and the *C. annuum* cultivar Keystone Resistant Giant at the three temperatures tested (24, 28, and 32 °C). Severity of root galling, egg-mass production, numbers of *M. incognita* eggs per g fresh root, and reproductive index of *M. incognita* were less ($P \leq 0.05$) for PA-426 and Charleston Belle than for PA-350 and Keystone Resistant Giant at all three temperatures (Fig. 1A–D), except at 28 °C Charleston Belle and Keystone Resistant Giant did not differ in eggs per g fresh root.

At 24 °C, both PA-426 and Charleston Belle exhibited high resistance, i.e., root galling and reproduction of *M. incognita* were minimal (Fig. 1A–D). In contrast, PA-350 and Keystone Resistant Giant were susceptible; both genotypes exhibited moderate root galling and permitted substantial reproduction of *M. incognita*. Severity of root galling for PA-350 was 2.5 × greater ($P \leq 0.05$) than for PA-426, and 2.6 × greater ($P \leq 0.05$) for Keystone Resistant Giant than for Charleston Belle. Egg-mass indices were similar to gall indices for all four genotypes. PA-426 and Charleston Belle supported 95 and 97% fewer ($P \leq 0.05$) eggs per g fresh root than PA-350. The reproductive indices of *M. incognita* for PA-426 and Charleston Belle were 96 and 98% lower ($P \leq 0.05$) than for PA-350 and Keystone Resistant Giant, respectively.

At 28 °C, both PA-426 and Charleston Belle exhibited a partial loss of resistance to *M. incognita*. Root-gall symptoms were within the moderately resistant range for both PA-426 and Charleston Belle (gall indices = 1.7 and 1.7, respectively). However, the degree of reproduction of *M. incognita* on both resistant cultivars at 28 °C would be considered susceptible because the numbers of eggs per g fresh root (10,163 and 20,809 for PA-426 and Charleston Belle, respectively) and reproductive indices (18.6 and 17.2 for PA-426 and Charleston Belle, respectively) were comparable to those for the susceptible genotypes PA-350 and Keystone Resistant Giant when grown at 24 °C. Both PA-350 and Keystone Resistant Giant exhibited highly susceptible reactions for root galling (gall indices = 3.7 and 4.5, respectively) and nematode reproduction (59,813 and 32,969 eggs per g fresh root, respectively). The reproductive indices of *M. incognita* for PA-426 and Charleston Belle were 39 and 62% lower ($P \leq 0.05$) than for PA-350 and Keystone Resistant Giant, respectively.

At 32 °C, resistance of PA-426 and Charleston Belle to *M. incognita* was compromised. Although root-gall severity was still within the moderately resistant range for both PA-426 and Charleston Belle (gall indices = 1.9 and 2.2, respectively), reproduction of *M. incognita* on both resistant cultivars was in the susceptible range (21,733 and 26,580 eggs per g fresh root for PA-426 and Charleston Belle, respectively). Likewise, the reproductive indices of 18.1 and 35.7 for PA-426 and Charleston Belle, respectively, were in the susceptible range. It should be noted that Charleston Belle supported greater ($P \leq 0.05$) numbers of *M.*

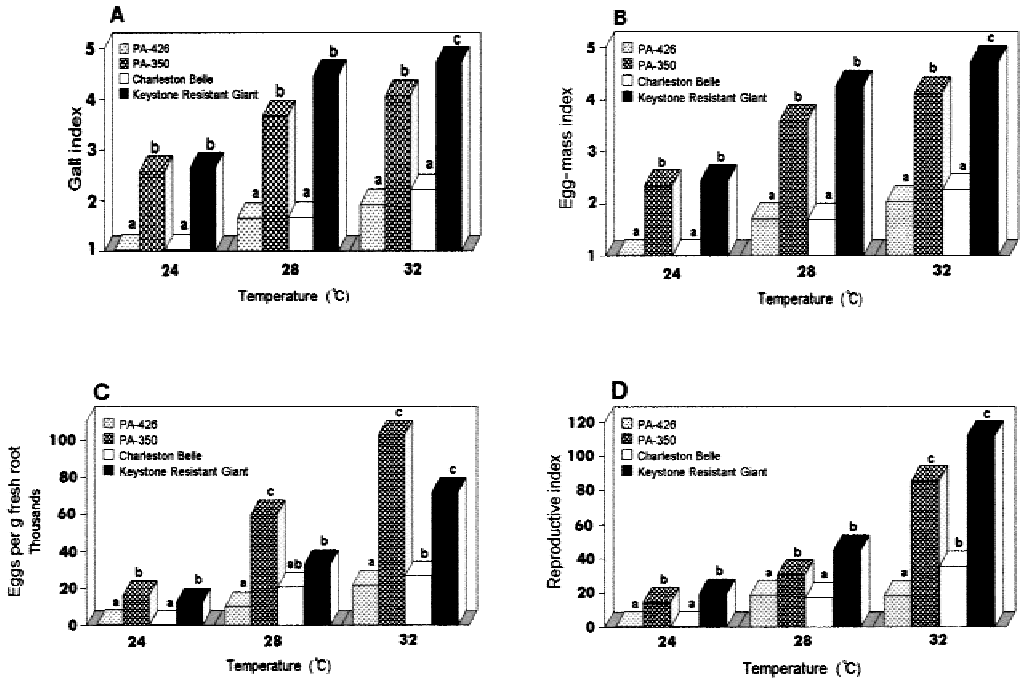


FIG. 1. Comparison of two *Capsicum chinense* cultigens (PA-426 and PA-350) and two *C. annuum* cultivars (Charleston Belle and Keystone Resistant Giant) differing in resistance to *Meloidogyne incognita* grown at 24, 28, or 32 °C for root-gall severity (A), egg-mass production (B), numbers of *M. incognita* eggs/g fresh root (C), and reproductive index of *M. incognita* (D) 9 weeks after inoculation with 5,000 eggs of *M. incognita*. Root galling and egg-mass production were rated on a 1-to-5 index, where 1 = 0 to 3% root system galled or covered with egg masses, 2 = 4 to 25%, 3 = 26 to 50%, 4 = 51 to 79%, and 5 = 80% or more root system galled or covered with egg masses. Reproductive index of *M. incognita* = $P_f/P_i$, where $P_i$ = initial inoculum level and $P_f$ = final egg recovery. Values are untransformed means of three tests ($n$ = 54 plants). Egg-count data were transformed with $\log_{10}$ (x + 1) before analysis. Means were compared within temperatures; bars with the same letters are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

*incognita* eggs per g fresh root than PA-426 and that the reproductive index for Charleston Belle was nearly 2 × greater ($P \leq 0.05$) than for PA-426. Both PA-350 and Keystone Resistant Giant exhibited highly susceptible reactions for root galling (gall indices = 4.0 and 4.7, respectively) and reproduction of *M. incognita* (numbers of eggs per g fresh root = 102,641 and 71,627, respectively; reproductive indices = 85.6 and 112.0, respectively).

## DISCUSSION

Our results differ from those of Di Vito et al. (1995) and Djian-Caporalino et al. (1999), who observed that resistance to *M. incognita* in pepper was stable at high temperatures. The contrast in results may be attributed to several factors, including differences in: (i) isolates and races of *M. incognita* used in the respective studies, (ii) the genetic systems controlling resistances in the pepper genotypes studied, (iii) temperature regime treatments, and (iv) life stages and levels of *M. incognita* inocula used. Di Vito and co-workers observed heat stability of resistance to an Italian isolate of *M. incognita* race 1 in germplasm lines of *C. annuum, C. chacoense,* and *C. frutescens* up to 38 °C; the genetic system controlling resistance was not identified for any of the *Capsicum* spp. studied. Di Vito inoculated individual pepper plants with 2,000 *M. incognita* J2 and then held the plants for 28 days at 34 °C or 24 days at 38 °C. Djian-Caporalino and colleagues (1999) observed stability of resistance to the *M. incognita* Calissane Fr. population in *C. annuum* at 32 and 42 °C; resistance was conditioned by a single dominant gene and possibly additional minor genes. Djian-Caporalino treated pepper plants at

32 °C for various periods before and (or) after infestation with 500 *M. incognita* J2 and then held the plants at 22 °C to allow completion of one nematode generation. For example, Djian-Caporalino held pepper plants a maximum of 28 days at 32 °C (7 days before nematode infestation plus 21 days after infestation) followed by 28 additional days at 22 °C. Results of the present studies are based on interaction of an isolate of *M. incognita* race 3 collected in South Carolina, with isogenic lines of *C. annuum* that differ for presence or absence of the *N* gene (Charleston Belle and Keystone Resistant Giant, respectively) (Fery et al., 1998) and with *C. chinense* cultigens that differ in resistance or susceptibility to *M. incognita* conditioned by a single dominant gene that is allelic to the *N* gene (Fery and Thies, 1998b). Pepper plants in the present experiments were held for 10 weeks at constant temperatures of 24, 28, or 32 °C immediately after inoculation with 5,000 *M. incognita* eggs.

The results of the present studies also confirm the partial loss of resistance conditioned by the *N* gene in *C. annuum* that we reported in earlier studies (Thies and Fery, 1998). The similarity in the expression of the *N* gene in *C. annuum* and a gene allelic to the *N* gene in *C. chinense* at high temperatures is not unexpected. However, because reproduction of *M. incognita* was lower ($P \leq 0.05$) for PA-426 than Charleston Belle at 32 °C, gene expression may differ in PA-426 and Charleston Belle or additional genes may control resistance in PA-426 at high temperatures.

Resistance to *M. incognita* in PA-426 was compromised at 28 and 32 °C; however, at 32 °C, the gall index for PA-426 was still within the resistant range and the reproductive index of *M. incognita* for PA-426 was only 21% that of the susceptible PA-350. Thus, cultivars possessing this resistance should be useful in temperate, sub-tropical, and tropical areas where high soil temperatures occur. However, soil populations of *M. incognita* may build up on PA-426 under high soil temperatures, limiting the usefulness of such resistant cultivars as rotational crops for reducing root-knot nematode populations in the soil. To determine the actual usefulness of resistant *C. chinense* cultivars, it will be necessary to evaluate their performance in root-knot nematode-infested fields in hot climates.

## Literature Cited


Carter, W. W. 1982. Influence of soil temperature on *Meloidogyne incognita* resistant and susceptible cotton, *Gossypium hirsutum*. Journal of Nematology 14:343–346.

Djian-Caporalino, C., L. Pijarowski, A. Januel, V. Lefebvre, A. Daubèze, A. Palloix, A. Dalmasso, and P. Abad. 1999. Spectrum of resistance to root-knot nematodes and inheritance of heat-stable resistance in pepper (*Capsicum annuum* L.). Theoretical and Applied Genetics 99:496–502.

Di Vito, M., V. Cianciotta, and G. Zaccheo. 1992. Yield of susceptible and resistant pepper in microplots infested with *Meloidogyne incognita*. Nematropica 22:1–6.

Di Vito, M., and F. Saccardo. 1986. Response of inbred lines of *Capsicum* to root-knot nematode (*Meloidogyne* spp.). Pp. 119–123 *in* Proceedings of the VIth EUCARPIA Meeting on Genetics and Breeding on *Capsicum* and Eggplant. Zaragoza, Spain.

Di Vito, M., F. Saccardo, and N. Sasanelli. 1989. Reaction of lines of *Capsicum* spp. to root-knot nematodes (*Meloidogyne* spp.). Pp. 171–175 *in* N. Marinkovic, Z. Miladinovic, D. Stevanovic, Dj. Gvozdenovic, and D. Jankulovski, eds. EUCARPIA VIIth meeting on genetics and breeding on *Capsicum* and eggplant. Kragujevac, Yugoslavia.

Di Vito, M., F. Saccardo, and G. Zaccheo. 1993. Response of new lines of pepper to *Meloidogyne incognita, M. javanica, M. arenaria,* and *M. hapla*. Afro-Asian Journal of Nematology 3:135–138.

Di Vito, M., G. Zaccheo, F. Catalano, and G. Oreste. 1995. Effect of temperature on stability of resistance to root-knot nematode (*Meloidogyne* spp.). Pp. 230–232 *in* EUCARPIA IXth Meeting on Genetics and Breeding on *Capsicum* and Eggplant. Budapest, Hungary.

Dropkin, V. H. 1969. The necrotic reaction of tomatoes and other hosts resistant to *Meloidogyne:* Reversal by temperature. Phytopathology 59:1632–1637.

Fery, R. L., P. D. Dukes, and W. L. Ogle. 1986. 'Carolina Cayenne' pepper. HortScience 21:330.

Fery, R. L., P. D. Dukes, Sr., and J. A. Thies. 1998. 'Carolina Wonder' and 'Charleston Belle': Southern root-knot nematode resistant bell peppers. HortScience 33:900–902.

Fery, R. L., and J. A. Thies. 1997. Evaluation of *Capsicum chinense* Jacq. cultigens for resistance to the southern root-knot nematode. HortScience 32:923–926.

Fery, R. L., and J. A. Thies. 1998a. PA-353, PA-398, and PA-426: Southern root-knot nematode resistant *Capsicum chinense* Jacq. germplasm lines. HortScience 33:760–761.

Fery, R. L., and J. A. Thies. 1998b. Genetic analysis of resistance to the southern root-knot nematode in *Capsicum chinense* Jacq. Journal of the American Society for Horticultural Science. 123:1008–1011.

Griffin, G. D. 1969. Effects of temperature on *Meloidogyne hapla* in alfalfa. Phytopathology 59:599–602.

Hare, W. W. 1957. Inheritance of resistance to root-knot nematodes in pepper. Phytopathology 47:455–459.

Hendy, H., A. Dalmasso, and M. C. Cardin. 1985. Differences in resistant *Capsicum annuum* attacked by different *Meloidogyne* species. Nematologica 31:72–78.

Holtzmann, O. V. 1965. Effect of soil temperature on resistance of tomato to root-knot nematode (*Meloidogyne incognita*). Phytopathology 55:990–992.

Hussey, R. S., and K. R. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Disease Reporter 57: 1025–1028.

Jatala, P., and C. C. Russell. 1972. Nature of sweet potato resistance to *Meloidogyne incognita* and the effects of temperature on parasitism. Journal of Nematology 4:1–7.

Lindsey, D. L., and M. S. Clayshulte. 1982. Influence of initial population densities of *Meloidogyne incognita* on three chile cultivars. Journal of Nematology 14:353–358.

Mullin, B. A., G. S. Abawi, and M. A. Pastor-Corrales. 1991. Modification of resistance expression of *Phaseolus vulgaris* to *Meloidogyne incognita* by elevated soil temperatures. Journal of Nematology 23:182–187.

Omwega, C. O., I. J. Thomason, and P. A. Roberts. 1990. Effect of temperature on expression of resistance to *Meloidogyne* spp. in common bean (*Phaseolus vulgaris*). Journal of Nematology 22:446–451.

Sasser, J. N., C. C. Carter, and K. M. Hartman. 1984. Standardization of host suitability studies and reporting of resistance to root-knot nematodes. Crop Nematode Research and Control Project. Cooperative Publication, Dept. of Plant Pathology, N.C. State University and U.S. Agency for International Development. Raleigh, N. C.: Dept. of Plant Pathology, N.C. State University.

Thies, J. A., and R. L. Fery. 1998. Modified expression of the *N* gene for southern root-knot nematode resistance in pepper at high soil temperatures. Journal of the American Society of Horticultural Science 123: 1012–1015.

Thies, J. A., J. D. Mueller, and R. L. Fery. 1997. Effectiveness of resistance to southern root-knot nematode in 'Carolina Cayenne' pepper in greenhouse, microplot, and field tests. Journal of the American Society of Horticultural Science 122:200–204.

Thies, J. A., J. D. Mueller, and R. L. Fery. 1998. Use of a resistant pepper as a rotational crop to manage southern root-knot nematode. HortScience 33:716–718.

Thomas, S. H. 1994. Influence of 1,3-dichloropropene, fenamiphos, and carbofuran on *Meloidogyne incognita* populations and yield of chile peppers. Supplement to the Journal of Nematology 26(4S):683–689.