Modified Expression of the *N* Gene for Southern Root-knot Nematode Resistance in Pepper at High Soil Temperatures

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ABSTRACT. Heat stability of the *N* gene that confers resistance to the southern root-knot nematode, *Meloidogyne incognita* (Kofoed & White) Chitwood in pepper (*Capsicum annuum* L.), was determined at 24, 28, and 32 °C. Responses of resistant bell pepper cultivars Charleston Belle and Carolina Wonder (homozygous for the *N* gene) and their respective susceptible recurrent backcross parents, 'Keystone Resistant Giant' and 'Yolo Wonder B', to *M. incognita* were compared. Numbers of eggs/g fresh root, reproductive factor of *M. incognita*, numbers of second-stage juveniles in soil, egg mass production, and root galling increased ($P < 0.05$) for all cultivars as temperature increased. The response of the resistant cultivars to temperature increase was less dramatic than the response of the susceptible cultivars. Both 'Charleston Belle' and 'Carolina Wonder' exhibited a partial loss of resistance at 28 and 32 °C. Reproduction of *M. incognita* was minimal on the resistant cultivars at 24 °C, but increased at higher temperatures. However, at 32 °C reproduction of *M. incognita* on the resistant cultivars was only 20% of that on the susceptible cultivars and root gall indices were within the range considered moderately resistant. Unlike the susceptible cultivars, the shoot dry weights of the resistant cultivars were not suppressed at 32 °C. This suggests that 'Charleston Belle' and 'Carolina Wonder' may be somewhat tolerant to *M. incognita* at high soil temperatures. Although results indicate a partial loss of resistance occurred in 'Charleston Belle' and 'Carolina Wonder' under high soil temperatures, resistant cultivars may be a useful component of cropping systems designed to manage *M. incognita* in hot climates.

The southern root-knot nematode, *Meloidogyne incognita* (Kofoed & White) Chitwood, is a major pest in pepper (*Capsicum annuum*) growing areas of the United States and throughout the world (DiVito et al., 1985; Lamberti, 1979; Sasser and Freckman, 1987; Thies et al., 1997; Thomas et al., 1995). Preplant soil fumigation with methyl bromide is the principal control measure, but the proposed removal of this fumigant from the U.S. market in 2001 has focused considerable interest on host plant resistance. Two root-knot nematode resistant, open-pollinated bell pepper cultivars, Charleston Belle and Carolina Wonder, were released in April 1997 by the U.S. Vegetable Laboratory, Charleston, S.C. (Fery et al., 1998). Both are homozygous for the *N* gene, which confers resistance to *M. incognita* (Fery and Dukes, 1996; Hare, 1957), and should provide an alternative to fumigation with methyl bromide for the management of southern root-knot nematodes in bell pepper plantings.

Expression of resistance to root-knot nematodes (*Meloidogyne* spp.) is heat sensitive in cotton, tomato, alfalfa, sweetpotato, and bean (Carter, 1982; Dropkin, 1969; Griffin, 1969; Griffin and Elgin, 1977; Jatala and Russell, 1972; Mullin et al., 1991; Omwega et al., 1990). A large portion of U.S. pepper production occurs in hot climates where root-knot nematodes are a severe pest. Therefore, knowledge about the expression of resistance under high temperatures is essential for pepper resistance breeding programs and for recommending cultivars for high temperature production regimes.

Root-knot nematode resistant tomato cultivars have limited use in

areas of the world where soil temperatures commonly reach or exceed 28 °C (Fassuliotis, 1982; Roberts, 1992). Break-down of root-knot resistance encoded by the *Mi-1* gene, which confers resistance to *M. incognita*, *Meloidogyne arenaria* (Neal) Chitwood, and *Meloidogyne javanica* (Treub) Chitwood in tomato (*Lycopersicon esculentum* Mill.), occurs at soil temperatures ≥ 28 °C (Dropkin, 1969; Holtzmann, 1965). Excised roots of tomato genotypes homozygous or heterozygous for *Mi-1* were resistant to *M. incognita* and *M. arenaria* at 31 °C, partially resistant at 34 °C, and susceptible at 37 °C (Abdul-Baki and Haroon, 1996). Genotypes of *L. peruvianum* (L.) Mill. and *L. glandulosum* C. H. Mull. have been identified that have heat stable resistance to *M. incognita* (Ammati et al., 1986); this resistance is conditioned by the *Mi-2* locus (Cap et al., 1993).

Partial loss of resistance to *Meloidogyne hapla* Chitwood occurred in alfalfa clones grown at 32 °C (Griffin and Elgin, 1977). Resistance to *M. incognita* was partially repressed at 35 °C in resistant cotton (Carter, 1982), and at 24 and 32 °C, but not at 16 °C in a resistant bean line (Mullin et al., 1991). Resistance to *M. incognita* in two bean lines was lost at 30 °C, but in other lines was stable from 24 to 30 °C (Omwega et al., 1990).

Information about the heat stability of resistance in pepper (*Capsicum* spp.) to *M. incognita* is limited. Resistance of breeding lines of *C. annuum*, *C. chacoense*, *C. chinense* Jacq., and *C. frutescens* L. to *M. incognita* race 1 was stable at 26, 30, 34, and 38 °C (DiVito et al., 1995) with minimal root galling and nematode reproduction at all temperatures. However, the heat stability of the *N* gene for resistance to *M. incognita* in pepper has not been investigated. This paper investigates the heat stability of the *N* gene in Charleston Belle and Carolina Wonder bell pepper cultivars.

Materials and Methods

NEMATODE INOCULUM. A *Meloidogyne incognita* race 3 isolate (obtained from S. L. Lewis, Clemson University, Clemson, S.C.) was increased on 'Rutgers' tomato in the greenhouse and used for

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all experiments. Egg inocula were extracted from *M. incognita*-infected tomato roots by using 0.5% NaOCl (Hussey and Barker, 1973).

PEPPER CULTIVARS. The bell pepper cultivars used in all experiments were 'Charleston Belle', 'Carolina Wonder', 'Keystone Resistant Giant', and 'Yolo Wonder B'. 'Charleston Belle' and 'Carolina Wonder' are root-knot nematode resistant, open-pollinated bell pepper cultivars released by USDA-ARS (Fery et al., 1998). Both cultivars are homozygous for the *N* gene that confers resistance to *M. incognita*. 'Keystone Resistant Giant' and 'Yolo Wonder B' are susceptible to *M. incognita*, and are the respective recurrent parental cultivars in the backcross breeding procedure (six backcrosses) used to develop 'Charleston Belle' and 'Carolina Wonder', respectively (Fery et al., 1998). 'Charleston Belle' and 'Carolina Wonder' will be hereinafter referenced as "resistant isolines" and 'Keystone Resistant Giant' and 'Yolo Wonder B' as "susceptible parents".

HEAT STABILITY EXPERIMENTS. The response of the resistant isolines and the susceptible parents to *M. incognita* were compared in controlled temperature experiments. The experimental design was a split-plot with three replications in time. Whole plots were three temperatures (24, 28, and 32 °C) randomly assigned to three growth chambers before each replication was initiated. Sub-plots were the four cultivars: Charleston Belle, Carolina Wonder, Keystone Resistant Giant, and Yolo Wonder B. All plants were completely randomized within each growth chamber. There were 8, 12, and 17 plants per cultivar in each of three replicates, respectively.

Seedlings of each cultivar were started in the greenhouse in plastic growing trays containing 40 individual 5.5 × 5.5 × 7.0-cm-deep cells filled with Jiffy Mix (Jiffy Products of America, Inc., Batavia, Ill.), as previously described (Thies et al., 1997). Twenty-one days after seeding, single plants of each cultivar were transplanted into 10 × 10 × 10-cm plastic pots containing a pasteurized mix of 2 sandy loam soil : 1 fine washed river sand (by volume). About 5000 eggs of *M. incognita* suspended in 5 mL H₂O were dispensed into the soil surrounding the root system of each plant, and the pots were placed in growth chambers programmed to maintain a temperature of 24, 28, or 32 °C, with a 16 h/8 h (light/dark) cycle. Visible radiation was 258 μmol·m⁻²·s⁻¹ at the top of the plant canopy during the light cycle. Fifty-eight days after transplanting, at the initiation of fruit set, the plant shoots were excised at the base of the stem, dried at 40 °C in a forced air oven, and shoot dry mass recorded.

The roots were carefully removed from the soil, washed, and scored for severity of root galling and for egg mass production using a 1 to 9 scale, where 1 = no galling or visible egg masses present, 2 = 1% to 3% roots galled or covered with egg masses, 3 = 4% to 10%, 4 = 11% to 25%, 5 = 26% to 35%, 6 = 36% to 50%, 7 = 51% to 65%, 8 = 66% to 80%, and 9 = >80% (Thies et al., 1997). The fibrous roots were excised from the tap root, cut into 1- to 2-cm pieces, and root fresh mass recorded. Eggs were extracted from the entire root sample by using 1.0% NaOCl (Hussey and Barker, 1973). Second-stage juveniles (J2) were extracted from a 100 cm³ sample of soil from each pot using the centrifugal-flotation method (Jenkins, 1964). Numbers of eggs and J2 were counted using a stereomicroscope. Nematode reproduction was assessed by calculating the reproduction factor $R = P_f/P_i$, where P_i = the initial inoculum level and P_f = the final inoculum level (Sasser et al., 1984).

DATA ANALYSIS. Nematode egg and J2 data were log₁₀ (x+1) transformed before analysis. Temperature was considered a discrete variable. Data were analyzed using the GLM procedure of SAS for Windows System Version 6.12 (SAS Institute, Cary, N.C.) and means were separated using Duncan's multiple range test.

Results

Susceptibility of 'Charleston Belle', 'Carolina Wonder', 'Keystone Resistant Giant', and 'Yolo Wonder B' to *M. incognita* increased as temperature increased from 24 to 32 °C. Numbers of eggs/g fresh root, reproductive factor of *M. incognita*, numbers of J2 in soil, egg mass production, and root galling increased ($P < 0.05$) for all cultivars as temperature increased (Fig. 1 A–E). Plant size and thriftiness of the susceptible parents (but not the resistant isolines) were generally reduced at the higher temperatures, as evidenced by the reduction in shoot dry mass at 28 and 32 °C compared to 24 °C (Fig. 1F). Overall, reproduction of *M. incognita* and severity of root galling on the resistant isolines 'Charleston Belle' and 'Carolina Wonder' were less ($P < 0.05$) than on susceptible 'Keystone Resistant Giant' and 'Yolo Wonder B', and the two cultivars within each group did not differ. However, temperature × cultivar interaction was found ($P < 0.05$) for numbers of eggs/g fresh root, reproductive index, numbers of J2/100 cm³ soil, egg mass production, and root galling.

Both resistant isolines 'Charleston Belle' and 'Carolina Wonder' exhibited a partial loss of resistance at 28 and 32 °C. Reproduction of *M. incognita* was minimal on the resistant isolines at 24 °C, but increased ($P < 0.05$) at 32 °C (Fig. 1 A–C). For example, numbers of eggs/g fresh root for the resistant isolate, 'Charleston Belle', were 190, 2890, and 15010 at 24, 28, and 32 °C, respectively (Fig. 1A). In comparison, numbers of eggs/g fresh root mass for the susceptible backcross parent, 'Keystone Resistant Giant', were 4190, 27660, and 91160 at 24, 28, and 32 °C, respectively. The increase ($P < 0.05$) in the reproductive factor of *M. incognita* for the resistant isolines as temperature increased from 24 to 32 °C also demonstrates the partial loss of resistance under high temperatures (Fig. 1B). Reproductive indices of *M. incognita* for 'Charleston Belle' were 0.3, 4.9, and 24.7 at 24, 28, and 32 °C, respectively; whereas reproductive indices for the susceptible backcross parent, 'Keystone Resistant Giant', were 6.1, 32.2, and 129.9. The 22-fold increase ($P < 0.05$) in numbers of *M. incognita* J2 present in soil associated with roots of 'Charleston Belle' at 32 vs. 24 °C also indicates a breakdown in expression of the *N* gene (Fig. 1C). Although egg mass production and severity of root galling increased on the resistant isolines 'Charleston Belle' and 'Carolina Wonder' as temperatures increased from 24 to 32 °C, the scores for these traits were still within the resistant range (≤ 4.0) (Fig. 1 D and E). In contrast, 'Keystone Resistant Giant' and 'Yolo Wonder B' exhibited susceptible reactions for egg mass production and root galling severity at 24, 28, and 32 °C. Egg mass indices for the resistant isolate, 'Charleston Belle', were 1.3, 2.1, and 3.7 at 24, 28, and 32 °C, respectively, vs. 4.6, 7.7, and 8.9 at 24, 28, and 32 °C, respectively, for the susceptible recurrent backcross parent, 'Keystone Resistant Giant' (Fig. 1D). Similarly, both resistant isolines exhibited resistant reactions for severity of root galling at 24, 28, and 32 °C; and their susceptible recurrent backcross parents were susceptible at all three temperatures (Fig. 1E). Root gall severity indices for the resistant 'Charleston Belle' were 1.5, 2.5, and 3.6 at 24, 28, and 32 °C, respectively compared to 4.8, 7.6, and 8.7 for the susceptible backcross parent, 'Keystone Resistant Giant'. Furthermore, galls on the roots of the resistant isolines were much smaller than those produced on the roots of the susceptible parents.

Shoot dry weights of the resistant isolines were ≈160% of those of the susceptible backcross parents at 32 °C (Fig. 1F). For example, shoot dry weights for 'Charleston Belle' were 2.0, 2.1, and 2.3 g at 24, 28, and 32 °C, respectively compared to 1.9, 1.6, and 1.4 at 24, 28, and 32 °C, respectively for 'Keystone Resistant Giant'. Dry shoot weights of the resistant isolines were not suppressed by *M. incognita* at 28 or 32 °C.

Fig. 1. Comparison of four pepper cultivars differing in resistance to southern root-knot nematode grown at 24, 28, or 32 °C for numbers of *Meloidogyne incognita* eggs per g fresh root (A), reproductive index of *M. incognita* = P_f/P_i , where P_i = initial inoculum level and P_f = final inoculum level (B), numbers of *M. incognita* second stage juveniles (J2) per 100 cm³ soil (C), egg mass production (rated on a 1 to 9 index, where 1 = no egg masses and 9 = greater than 80% of root system covered with egg masses) (D), root galling (rated on a 1 to 9 index, where 1 = no galling and 9 = greater than 80% of root system galled) (E), and shoot dry mass (F), 8 weeks after inoculation with 5000 *M. incognita* eggs. Bars with the same letters are not significantly different according to Duncan's multiple range test ($P < 0.05$). Means were compared across all temperature and cultivar combinations.

Discussion

Breakdown of resistance to *M. incognita* under high soil temperatures has been documented in several horticultural and field crop species (Carter, 1982; Holtzmann, 1965; Griffin and Elgin, 1977; Jatala and Russell, 1972; Mullin et al., 1991; Omwega et al., 1990). However, this is the first report of heat instability of resistance to *M. incognita* conditioned by the *N* gene in bell pepper (*C. annuum*). Our results contrast with those of DiVito et al. (1995), who reported heat stability of resistance to *M. incognita* race 1 in *C. annuum*, *C. chacoense*, *C. chinense*, and *C. frutescens*. These workers observed minimal reproduction of *M. incognita*, egg mass production, and root galling in all resistant genotypes evaluated at 26, 30, 34, and 38 °C, whereas, we observed significant increases ($P < 0.05$) in these criteria in resistant 'Charleston Belle' and 'Carolina Wonder' at 28 and 32 °C, compared to 24 °C. DiVito and coworkers did not identify the gene(s) conditioning resistance to *M. incognita* in the germplasm they evaluated. Variability in heat stability of resistance conditioned by different genes would not be unexpected. For example, in tomato, heat stable resistance to *M. incognita* is conditioned by the *Mi-2* locus, whereas resistance conditioned by the *Mi-1* locus is not heat stable (Ammati et al., 1986; Cap et al., 1993).

Our results indicate that susceptibility of both the resistant and susceptible cultivars increased as soil temperatures increased from 24 to 32 °C. The cultivar × temperature interactions suggest a differential rate of increase. However, root gall indices of the resistant cultivars at 32 °C were similar to those of the susceptible cultivars at 24 °C; and root gall indices of the resistant cultivars at 32 °C were within the range considered moderately resistant. Although nematode reproduction on the resistant cultivars was greater ($P < 0.05$) at 32 °C than at lower temperatures, reproduction was only 20% of that on the susceptible cultivars. The suppression of shoot dry weights of the susceptible parental cultivars compared to the resistant isolines at 32 °C, also suggests that 'Charleston Belle' and 'Carolina Wonder' may be tolerant to *M. incognita* at high soil temperatures.

Although our results indicate that a partial loss of resistance occurred in 'Charleston Belle' and 'Carolina Wonder' under high soil temperatures, bell pepper cultivars possessing resistance conditioned by the *N* gene may be a useful component of integrated systems designed to manage soilborne pests in hot climates. However, the utility of root-knot resistant pepper cultivars (possessing the *N* gene) as rotational crops may be limited in hot climates because soil populations of root-knot nematodes probably would increase under such cultivars. Nevertheless, because reproduction of *M. incognita* on the resistant isolines at 32 °C was only 20% of that on the susceptible backcross parents and plant growth was not suppressed, planting resistant cultivars should be advantageous. Field evaluations in areas where high soil temperatures normally occur will be necessary to determine the usefulness of *N* root-knot resistant bell pepper cultivars under high soil temperatures.

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