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Stability of Resistance to Root-knot Nematodes in ‘Charleston Belle’ and ‘Carolina Wonder’ Bell Peppers in a Sub-tropical Environment

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Abstract**. Two root-knot nematode-resistant bell pepper cultivars, ‘Charleston Belle’ and ‘Carolina Wonder’ (*Capsicum annuum* L. var. *annuum*], and their susceptible parents, ‘Keystone Resistant Giant’ and ‘Yolo Wonder B’, were compared for managing the southern root-knot nematode [*Meloidogyne incognita* (Chitwood) Kofoid and White] in fall and spring tests at Citra, FL. In the fall test, ‘Charleston Belle’ and ‘Carolina Wonder’ exhibited minimal root galling and nematode reproduction, and ‘Keystone Resistant Giant’ and ‘Yolo Wonder B’ exhibited severe root galling and high nematode reproduction. Fruit yield of ‘Charleston Belle’ was 97% greater than yields of the two susceptible cultivars ($P \leq 0.006$). In the spring test, one-half of the plots were treated with methyl bromide/chloropicrin before planting the same four bell pepper cultivars. ‘Keystone Resistant Giant’ and ‘Yolo Wonder B’ grown in untreated control plots exhibited severe root galling and high nematode reproduction, but the other six cultivar × methyl bromide combinations exhibited minimal root galling and nematode reproduction. Although soil temperatures (10-cm depth) were greater than 30 °C on 78 days and 57 days during the Fall 2002 and Spring 2003 trials, respectively, resistance did not break in ‘Charleston Belle’ and ‘Carolina Wonder’ in either test. These results demonstrate that root-knot nematode-resistant cultivars such as Charleston Belle and Carolina Wonder are viable alternatives to methyl bromide for managing southern root-knot nematode in bell pepper in sub-tropical environments.**

The southern root-knot nematode, *Meloidogyne incognita* (Chitwood) Kofoid and White, causes severe yield losses to pepper production in sub-tropical climates throughout the world (DiVito et al., 1985, 1992; Sasser and Freckman, 1987). In the United States, pepper ranks third among crops for use of methyl bromide for preplant fumigation of planting beds to control root-knot nematodes, soilborne diseases, and weeds (U.S. Department of Agriculture, 1998). In accordance with the Montreal Protocol and the U.S. Clean Air Act, methyl bromide has been phased out effective 1 Jan. 2005 (Rich and Olson, 2004; U. S. Environmental Protection Agency, 2000); however, under the U.S. nomination for critical use exemption program, growers continue to use specified allocations (U.S. Environmental Protection Agency, 2006). Other nematocides used for controlling root-knot nematodes in vegetable crops are also being lost from the U.S. market because of human health risks and groundwater contamination. Thus, there is significant interest in the development of alternative methods for managing root-knot nematodes in vegetable crops.

‘Charleston Belle’ and ‘Carolina Wonder’ are the only root-knot nematode-resistant bell pepper cultivars available to commercial growers and home gardeners (Fery et al., 1998). Both of these cultivars are homozygous for the dominant *N* gene that controls resistance to *M. incognita*, *M. arenaria* races 1 and 2, and *M. javanica* (Thies and Fery, 1998, 2000). Although ‘Charleston Belle’ and ‘Carolina Wonder’ exhibited partial loss of resistance when grown at 32 °C for 8 weeks after inoculation with *M. incognita*, these cultivars had root gall severity indices that were still in the low resistance range, and the number of *M. incognita* eggs per gram of fresh root was 95% less for ‘Charleston Belle’ than its susceptible parent, ‘Keystone Resistant Giant’ (Thies and Fery, 2002); similarly, the number of *M. incognita* eggs per gram of fresh root was 80% less for ‘Carolina Wonder’ than its susceptible parent, ‘Yolo Wonder B’ (Thies and Fery, 1998). Bell peppers are a very important crop in sub-tropical areas of the United States, in particular Florida, where root-knot nematodes are a primary pathogen of vegetable crops. The objective of these studies was to evaluate the effectiveness of the *N* gene in bell pepper in *M. incognita*-infested fields under Florida conditions.

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Materials and Methods

These studies were conducted at the Citra Agricultural Experiment Station, University of Florida, Plant Science Research and Education Unit, Citra, FL, in a field infested with *M. incognita* race 4. The *M. incognita* isolate was identified using the North Carolina differential host test (Taylor and Sasser, 1978) and esterase and malate dehydrogenase phenotyping (Ebenshade and Triantaphyllou, 1985, 1990). A fall and a spring evaluation of bell peppers were conducted in adjacent sections of the same field block in 2002 and 2003, respectively. The pepper genotypes used in these studies were the bell pepper cultivars, ‘Charleston Belle’ (*NN*), ‘Keystone Resistant Giant’ (*nn*), ‘Carolina Wonder’ (*NN*), and ‘Yolo Wonder B’ (*nn*) that differ in resistance to root-knot nematodes controlled by the *N* gene.

Fall 2002 bell pepper test. The field was planted with ‘Clemson Spineless’ okra to increase the population of *M. incognita* during Spring 2002, before the fall pepper test. Seeds of the four bell pepper cultivars were planted in the greenhouse on 1 July 2002. On 23 July 2002, the beds were formed, covered with 3-mL white-on-black polyethylene film mulch, and the seedlings were transplanted into single-row plots on beds that were 91 cm wide and 1.8 m apart. Each plot contained a single row of 40 plants spaced 30 cm apart. The experimental design was a randomized complete block with four pepper cultivars replicated eight times. Insect pests and foliar fungal pathogens were controlled according to standard practices (Olson and Simonne, 2005) and plots were subsurface drip-irrigated. Soil temperatures were recorded at 10-minute intervals at a 10-cm depth during the entire experiment. Fruits were harvested from all plants in each plot on 31 Oct. 2002. On the same day, root systems of all pepper plants were dug from each plot and scored for root galling using a scale of 1 to 5 in which 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% to 100% root system galled (Thies and Fery, 2002). *Meloidogyne incognita* eggs were extracted from a 20-g subsample of roots from each plot using 1% NaOCl (Hussey and Barker, 1973).

Spring 2003 bell pepper test. The field was planted with 'Clemson Spineless' okra to increase the population of *M. incognita* during Summer and Fall 2002, preceding the spring test.

Pepper seed was planted on 20 Feb. 2003. On 19 Mar. 2003, the planting beds were formed, covered with 3-mL black polyethylene film mulch, and one-half of the plots were fumigated with 80% methyl bromide:20% chloropicrin broadcast at 448 kg·ha. Each plot contained a double row of 15 plants per row with 30-cm spacing between plants. The experimental design was a randomized complete block with four pepper cultivars × two treatments (untreated control and methyl bromide) replicated seven times. Twelve cores 2.5-cm diameter × 30-cm deep were collected for nematode extractions from each plot immediately before fumigation with methyl bromide. Second-stage juveniles (J2) of *M. incognita* were extracted from 113 cm^3 soil from each sample using the centrifugal flotation method (Jenkins, 1964). The pepper seedlings were transplanted into the field on 9 Apr. 2003. Soil temperatures were recorded during the experiment as previously described. Fruits were harvested from all plants in each plot on 19 June 2003. On 26 June 2003, the root systems of all plants were dug from each plot and scored for root galling using a scale of 1 to 5 as previously described. *Meloidogyne incognita* eggs were extracted from a 20-g subsample of roots from each plot using 1% NaOCl (Hussey and Barker, 1973).

Data analysis. Nematode egg and J2 data were $\log_{10}(x + 1)$ transformed to normalize the error variance before analysis (Noe, 1985). Data were analyzed using the GLM procedure of SAS for Windows System Version 6.12 (SAS Institute, Cary, NC). Means were separated using Duncan's multiple range test when the *F* test was significant ($P \leq 0.05$) for a test factor.

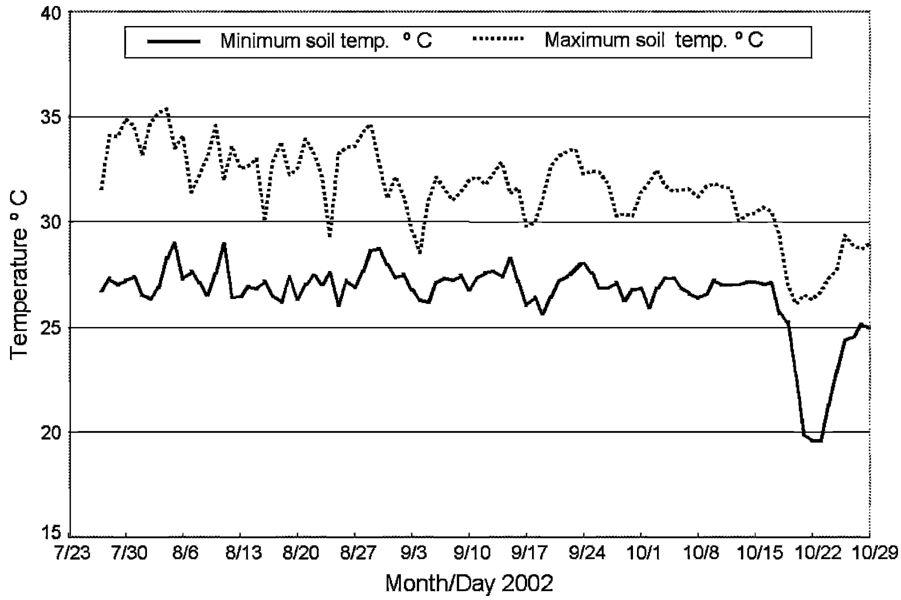

Fig. 1. Minimum and maximum temperatures at 10-cm soil depth, Fall test, 2002, Citra, FL.

Table 1. Gall index, numbers of *Meloidogyne incognita* eggs per gram of fresh root, total fruit yield, and total fruit numbers of Charleston Belle, Keystone Resistant Giant, Carolina Wonder, and Yolo Wonder B bell peppers, Fall test, 2002.

Pepper cultivar	Resistance classification[z]	Gall index[y]	Eggs/g fresh root[x]	Yield (kg/plot)	No. fruit
Charleston Belle	Resistant	1.0 a[w]	3,391 b	33.8 b	232 b
Keystone Resistant Giant	Susceptible	4.5 b	84,508 c	17.0 a	121 a
Carolina Wonder	Resistant	1.0 a	550 a	—	—
Yolo Wonder B	Susceptible	4.5 b	72,560 c	17.3 a	127 a

[z]Classification of cultivar reaction to *M. incognita*.
[y]Gall index: 1 = 0% to 3% root system galled, 2 = 4% to 25%, 3 = 26% to 50%, 4 = 51% to 79%, and 5 = 80% to 100% root system galled.
[x]Data were $\log_{10}(x + 1)$ transformed before analysis. Nontransformed data are shown.
[w]Means were separated using Duncan's multiple range test ($P \leq 0.05$). Differences among values with the same letter within a column were not detected.

Results and Discussion

Fall 2002 bell pepper test. Soil temperatures ranged from 19.6 to 35.5 °C at a 10-cm depth during the fall test (Fig. 1). Minimum soil temperatures were 25 °C or greater for 91 of 101 d during the test; the lowest minimum soil temperatures occurred near the end of the test (from 16 to 25 Oct. 2002). Maximum soil temperatures were greater than 30 °C on 78 d during the test. 'Charleston Belle' exhibited minimal root galling and *M. incognita* reproduction; the gall index (GI) was 1.0 with 3391 eggs per gram of fresh root (Table 1). 'Keystone Resistant Giant' exhibited severe root galling and very high *M. incognita* reproduction: GI = 4.5 with 84,508 eggs per gram of fresh root. 'Carolina Wonder' exhibited high resistance: GI = 1.0 with 550 eggs per gram of fresh root. 'Yolo Wonder B' was susceptible: GI = 4.5 with 72,560 eggs per gram of fresh root. 'Charleston Belle' produced 98.8% heavier ($P \leq 0.05$) fruit yield and 91.7% greater ($P \leq 0.05$) numbers of fruit than its parent, 'Keystone Resistant Giant'. Yield data were not reported for 'Carolina Wonder' because of very low plant numbers resulting from poor seed germination. Both the resistant and susceptible pepper cultivars reacted as expected. 'Charleston Belle' and 'Carolina Wonder' exhibited high resistance and their respective susceptible parents, 'Keystone Resistant Giant' and 'Yolo Wonder B', were highly susceptible. Fruit yield and fruit numbers of 'Charleston Belle' were nearly twice as large as those of 'Keystone Resistant Giant'.

Spring 2003 bell pepper test. Soil temperatures ranged from 17.4 to 37.4 °C at a 10-cm depth during the spring test (Fig. 2). Minimum soil temperatures were 25 °C or greater for 46 of 79 d during the spring test. Maximum temperatures were greater than 30 °C on 57 d during the test. Soil density of *M. incognita* J2 before fumigation with methyl bromide/chloropicrin was less than 1 per cm^3 on 19 Mar. 2003. In the untreated plots, 'Charleston Belle' exhibited minimal root galling and *M. incognita* reproduction; the GI was 1.0 with 390 eggs per gram of fresh root (Table 2). 'Keystone Resistant Giant' exhibited severe root galling (GI = 4.5) and very high *M. incognita* reproduction (48,184 eggs per gram of fresh root). 'Carolina Wonder' exhibited high resistance: GI = 1.0 with 73 eggs per gram of fresh root. 'Yolo Wonder B' was susceptible: GI = 4.3 with 51,964 eggs per gram of fresh root. There was no evidence of root galling in any of the cultivars in the methyl bromide-treated plots and numbers of eggs per gram fresh root ranged from 10 for 'Charleston Belle' to 70 for 'Keystone Resistant Giant'. No differences were detected for fruit yield or fruit number among any of the treatments.

In the Spring 2003 test, both the resistant and susceptible pepper cultivars reacted as expected in the untreated control plots; 'Charleston Belle' and 'Carolina Wonder' exhibited high resistance and 'Keystone Resistant Giant' and 'Yolo Wonder B' were susceptible. Root gall severities of the susceptible cultivars grown in the untreated plots were similar in the fall and spring tests (mean GI = 4.5 and 4.4, respectively). However, *M. incognita* reproduction was 39.6% and 75.4% greater in the fall test than the spring test for 'Yolo Wonder B' and 'Keystone Resistant Giant', respectively, in the untreated plots. The higher average soil temperatures during the fall test than the spring test probably contributed to the greater reproduction of *M. incognita* in the fall test. For example, reproduction of *M. incognita* on wheat grown at 30 °C was ≈35% greater than *M. incognita* reproduction on wheat grown at 26 °C

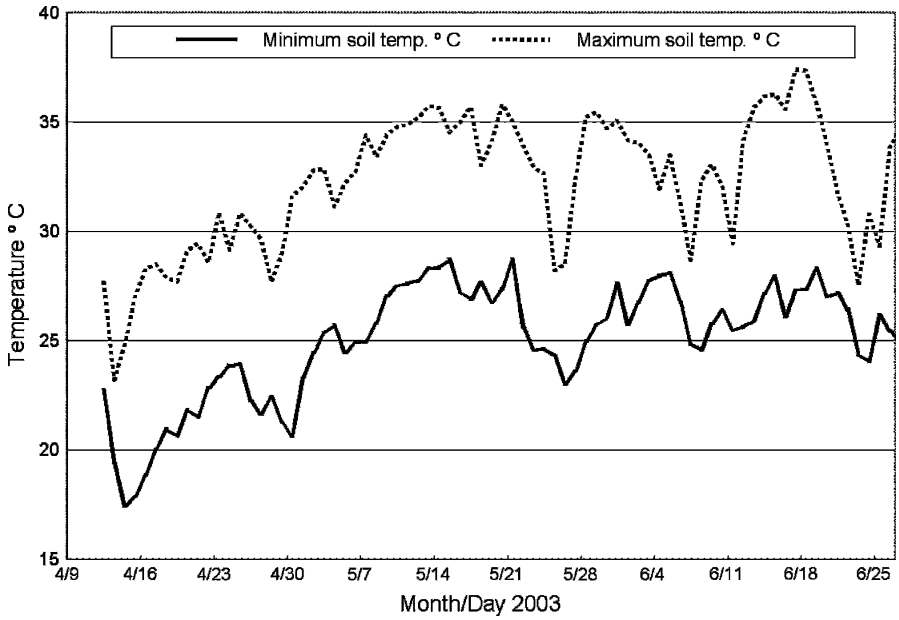

Fig. 2. Minimum and maximum temperatures at 10-cm soil depth, Spring test, 2003, Citra, FL.

Table 2. Gall index, numbers of *Meloidogyne incognita* eggs per gram of fresh root, total fruit yield, and total fruit numbers of Charleston Belle, Keystone Resistant Giant, Carolina Wonder, and Yolo Wonder B bell peppers, Spring test, 2003.

Pepper cultivar[z]	Methyl bromide	Gall index[y]	Eggs/g fresh root[x]	Yield	No. fruit (kg/plot)
Charleston Belle	0	1.0 a[w]	390 c	16.9 a	90 a
	+	1.0 a	10 a	16.1 a	107 a
Keystone Resistant	0	4.5 c	48,184 d	10.1 a	59 a
Giant	+	1.0 a	70 b	10.9 a	73 a
Carolina Wonder	0	1.0 a	73 b	11.9 a	73 a
	+	1.0 a	15 a	8.6 a	62 a
Yolo Wonder B	0	4.3 b	51,964 d	13.9 a	74 a
	+	1.0 a	61 b	12.2 a	88 a

[z]Charleston Belle and Carolina Wonder are resistant and Keystone Resistant Giant and Yolo Wonder B are susceptible to *M. incognita*.
[y]Gall index: 1 = 0% to 3% root system galled, 2 = 4% to 25%, 3 = 26% to 50%, 4 = 51% to 79%, and 5 = 80% to 100% root system galled.
[x]Data were $\log_{10}(x + 1)$ transformed before analysis. Nontransformed data are shown.
[w]Means were separated using Duncan's multiple range test ($P \leq 0.05$). Differences among values with the same letter within a column were not detected.

(Roberts and Van Gundy, 1981). Also, greater reproduction of the *M. incognita* population may have been the result of buildup of nematodes on the preceding okra crop in the spring and early summer.

In the Spring 2003 test, methyl bromide was highly effective in controlling *M. incognita* in the two susceptible cultivars, 'Keystone Resistant Giant' and 'Yolo Wonder B'. Although both of the resistant cultivars, 'Charleston Belle' and 'Carolina Wonder', had significantly greater numbers of *M. incognita* eggs per gram of fresh root in the untreated plots than in the methyl bromide-treated plots, these differences are probably not biologically significant and the numbers of eggs observed were very low (less than 400 eggs per gram of fresh root).

Resistance conditioned by the *N* gene in both 'Charleston Belle' and 'Carolina Wonder' was stable under the high soil temperatures in the present studies. In contrast, resistance conditioned by the *N* gene was compromised in 'Charleston Belle' and 'Carolina Wonder' when these cultivars were grown in growth chambers maintained at 32 °C for 8 weeks after infestation of the soil with *M. incognita* (Thies and Fery, 2002). Although soil temperatures reached 35.5 and 37.4 °C in the Fall 2002 and Spring 2003 trials, respectively, soil temperatures fluctuated diurnally and did not remain at the constant high temperatures that were maintained in the growth chamber tests in which expression of the *N* gene was compromised. Root-knot nematode-resistant bell peppers should provide economical and environmentally compatible alternatives to methyl bromide and other nematicides for managing *M. incognita* in sub-tropical environments.

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