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Response of Bell Pepper Cultivars Near-isogenic for the *N* Gene to *Meloidogyne incognita* in Field Trials

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***Abstract*. Resistance of two sets of bell pepper [(*Capsicum annuum* L. var. *annuum* (Grossum Group)] cultivars near-isogenic for the *N* gene that conditions resistance to root-knot nematodes [*Meloidogyne incognita* (Chitwood) Kofoid and White, *M. arenaria* (Neal) Chitwood races 1 and 2, and *M. javanica* (Treub) Chitwood] was evaluated in field tests at Blackville, S.C. and Charleston, S.C. The isogenic bell pepper sets were 'Charleston Belle' (*NN*) and 'Keystone Resistant Giant' (*nn*), and 'Carolina Wonder' (*NN*) and 'Yolo Wonder B' (*nn*). The resistant cultivars Charleston Belle and Carolina Wonder were highly resistant; root galling was minimal for both cultivars at both test sites. The susceptible cultivars Keystone Resistant Giant and Yolo Wonder B were highly susceptible; root galling was severe at both test sites. 'Charleston Belle' had 96.9% fewer eggs per g fresh root than 'Keystone Resistant Giant', and 'Carolina Wonder' had 98.3% fewer eggs per g fresh root than 'Yolo Wonder B' (averaged over both test sites). 'Charleston Belle' and 'Carolina Wonder' exhibited a high level of resistance in field studies at both sites. These results demonstrate that resistance conferred by the *N* gene for root-knot nematode resistance is effective in field-planted bell pepper. Root-knot nematode resistant bell peppers should provide economical and environmentally compatible alternatives to methyl bromide and other nematicides for managing *M. incognita*.**

The southern root-knot nematode, *Meloidogyne incognita* (Chitwood) Kofoid and White, is a major limiting factor to bell pepper (*Capsicum annuum* L. var. *annuum*) production in the United States and worldwide (Di Vito et al., 1985, 1992; Sasser and Freckman, 1987; Thies et al., 1997). In the United States, methyl bromide is currently the primary method for controlling root-knot nematodes in bell peppers and accounts for ≈12% of methyl bromide used for pre-plant fumigation of all crops in the country [U.S. Department of Agriculture (USDA), 1993]. However, because of the ozone-depleting properties of methyl bromide, its production in the United States is scheduled to cease by 1 Jan 2005. Although other soil fumigants and nematicides may have varying degrees of usefulness for controlling root-knot nematodes in vegetable crops, environmental concerns and high costs of reregistration may limit these pesticides as feasible alternatives to methyl bromide. Resistant cultivars may provide the most economical and environmentally benign alternative to pesticides for managing root-knot nematodes.

The U.S. Vegetable Laboratory, USDA, Agricultural Research Service (ARS), Charleston, S.C., released the root-knot nematode resistant bell pepper cultivars Charleston Belle and Carolina Wonder in 1997 (Fery et. al., 1998). Charleston Belle and Carolina Wonder are the only root-knot nematode resistant bell pepper cultivars available to commercial growers and home gardeners. Both of these open-pollinated cultivars are homozygous for the dominant *N* gene that controls resistance to *M. incognita* (Fery et al., 1998), *M. arenaria* races 1 and 2, and *M. javanica* (Thies and Fery, 2000). Although the reactions to root-knot nematodes of 'Charleston Belle', 'Carolina Wonder', and their respective near-isogenic susceptible parents, 'Keystone Resistant Giant' and 'Yolo Wonder B', have been characterized in controlled greenhouse and growth chamber environments (Thies and Fery, 1998; Thies and Fery, 2000; Thies and Fery, 2002), these types of studies have not been conducted under actual field conditions. The objective of the studies reported here was to use these two near-isogenic sets of bell pepper cultivars to evaluate the effectiveness of the *N* gene in *M. incognita*-infested fields.

Materials and Methods

These field studies were conducted at the U.S. Vegetable Laboratory, USDA, ARS, Charleston, S.C., and the Clemson Univ. Edisto Research and Education Center, Blackville, S.C. Egg inocula of *M. incognita* race 3 for all experiments was maintained on 'Rutgers' tomato (*Lycopersicon esculentum* Mill.) and 'Kentucky Wonder 191' pole bean (*Phaseolus vulgaris* L.) in isolated greenhouse benches. *Meloidogyne incognita* egg inocula were extracted from infected tomato and bean roots using 0.5% NaOCl (Hussey and Barker, 1973). Pepper genotypes used in these studies were two sets of near-isogenic bell pepper cultivars that differ for resistance to root-knot nematodes conditioned by the *N* gene. The bell pepper genotypes used in all experiments were Isogenic Set I: 'Charleston Belle' (*NN*) and 'Keystone Resistant Giant' (*nn*) and Isogenic Set II: 'Carolina Wonder' (*NN*) and 'Yolo Wonder B' (*nn*). 'Keystone Resistant Giant' and 'Yolo Wonder B' are the susceptible recurrent parental cultivars used in the backcross breeding procedure (six backcrosses) to develop 'Charleston Belle' and 'Carolina Wonder', respectively (Fery et al., 1998).

Blackville study. The seeds of all of the entries were planted in the greenhouse on 30 Mar. 2001 in 25 × 51 × 4.5-cm-deep plastic flats containing MetroMix 360 (The Scotts Co., Marysville, Ohio) growing medium. Ten days later, seedlings were transplanted into plastic growing trays containing 50 individual 0.2-L plastic cells (Growing Systems, Milwaukee) filled with MetroMix 360. On 2 May 2001, the field was tilled, beds were formed, and covered with 3-mL black plastic. On 9 May 2001, the pepper seedlings were inoculated with ≈5000 *M. incognita* race 3 eggs per seedling, and on 10 May 2001, the seedlings were transplanted into single-row plots on beds 2-m apart. Each plot contained 20 plants spaced 30-cm apart. The experimental design was a randomized complete block with 10 replications. At planting, ten cores of soil were collected from each plot. Second-stage juveniles (J2) were extracted from 400 cm^3 soil using the centrifugal flotation method (Jenkins, 1964). Standard cultural and insect control practices were followed and plots were irrigated with drip irrigation. Fruits were harvested weekly from 28 July through 9 Aug. 2001. On 15 Aug. 2001, the root systems of 10 plants in the center of each plot were dug, roots were washed and rated for gall severity and egg mass production using a scale of 1 to 5, 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% root system galled or covered with egg masses (Thies et al., 1998). *Meloidogyne incognita* eggs were extracted from a bulked 20-g subsample of roots from each plot using 1% NaOCl (Hussey and Barker, 1973).

Charleston study. The seeds of all of the

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entries were planted in the greenhouse on 9 Apr. 2001 and grown as previously described. On 15 May 2001, the field was tilled and beds were formed. On 16 May 2001, 6-week-old pepper seedlings were inoculated with ≈5000 *M. incognita* race 3 eggs per seedling, and on 17 May 2001, the seedlings were transplanted in single rows on beds 1-m apart with 30-cm in-row plant spacing and 20 plants of one cultivar per row. The experimental design was a randomized complete block with 10 replications. Each block was considered a replication. At planting, soil samples were collected from each plot and J2 were extracted as previously described. On 21 May 2001, the soil surrounding each plant was infested with ≈10,000 eggs of *M. incognita*. Standard cultural and insect control practices were followed and plots were irrigated with overhead irrigation as needed. Fruits were harvested weekly from 24 Jul. through 17 Aug. 2001. On 12 Sept. 2001, all remaining fruit was harvested from each row of plants. The shoots of 10 plants from the center of each plot were severed at the crown and fresh weight recorded, and the 10 root systems were dug, washed, and scored for gall severity and egg mass production as described above. *Meloidogyne incognita* eggs were extracted from a bulked 20-g subsample of roots from each plot using 1% NaOCl (Hussey and Barker, 1973).

Data analysis. Nematode egg data were $\log_{10}(x+1)$ transformed to normalize the data before analysis (Noe, 1985). Data were analyzed using the general linear models (GLM) procedure of SAS for Windows (Statistical Analysis System, Version 6.12, SAS Inst., Cary, N.C.) and means were separated using Duncan's multiple range test at $P \leq 0.05$.

Table 1. Gall indices, egg mass indices, numbers of *Meloidogyne incognita* eggs per g fresh root, numbers of eggs per plant, and marketable fruit yields for two sets of *Capsicum annuum* cultivars (differing for the *N* gene) evaluated in *M. incognita*-infested fields at Blackville, S.C., and Charleston, S.C., 2001.[z]

Pepper genotype	Gall index[y]	Egg mass index[y]	Eggs/g fresh root[x]	Eggs/plant[x]	Marketable fruit yield (g/plant)
Blackville, S.C. Test					
Isogenic set I					
Charleston Belle[w]	1.2 a[v]	1.2 a	287 a	2,567 a	558 a
Keystone Resistant Giant[u]	4.5 b	4.4 b	11,930 b	109,303 b	401 a
Isogenic set II					
Carolina Wonder[w]	1.2 a	1.2 a	148 a	866 a	382 a
Yolo Wonder B[u]	4.3 b	4.3 b	11,279 b	80,468 b	398 a
Charleston, S.C. Test					
Isogenic set I					
Charleston Belle	1.0 a[v]	1.0 a	730 a	10,974 a	346 a
Keystone Resistant Giant	4.8 b	4.8 b	18,973 b	349,945 b	323 a
Isogenic set II					
Carolina Wonder	1.0 a	1.0 a	247 a	3,420 a	262 a
Yolo Wonder B	4.8 b	4.5 b	12,082 b	135,358 b	389 a
Combined analysis of both tests					
Isogenic set I					
Charleston Belle	1.1 a[v**]	1.1 a[NS]	509 a[NS]	6,770 a[NS]	452 b[NS]
Keystone Resistant Giant	4.7 b	4.6 c	15,637 b	235,956 c	362 ab
Isogenic set II					
Carolina Wonder	1.1 a	1.1 a	197 a	2,143 a	322 a
Yolo Wonder B	4.5 b	4.4 b	11,681 b	107,913 b	393 ab

[z]Blackville test: transplanted 10 May 2001; final harvest and root samples collected 15 Aug. 2001. Charleston test: transplanted 17 May 2001; final harvest and root samples collected 12 Sept. 2001. Data for each test are means of 10 replications. Results of combined analyses of both tests indicated a significant cultivar × test interaction for gall indices. Therefore, results are presented for both single test and combined analyses to aid interpretation.

[y]Scale of 1 to 5, 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 = 81% to 100% root system galled or covered with egg masses.

[x]Data were $\log_{10}(x+1)$ transformed before analysis.

[w]Resistant to *M. incognita*.

[v]Mean separation within a column and test by Duncan's multiple range test, $P \leq 0.05$.

[u]Susceptible to *M. incognita*.

[NS, **]Nonsignificant or significant interaction between cultivar and test at $P \leq 0.01$.

Results

Blackville study. Numbers of *M. incognita* J2 were <1 per cm^3 soil at planting. The resistant cultivars Charleston Belle and Carolina Wonder were highly resistant; root gall severity indices were 1.2 for both cultivars (Table 1). In contrast, the susceptible cultivars Keystone Resistant Giant and Yolo Wonder B were highly susceptible; root gall severity indices were 4.5 and 4.3 for 'Keystone Resistant Giant' and 'Yolo Wonder B', respectively. 'Charleston Belle' had 97.6% fewer eggs per g fresh root than its susceptible isoline 'Keystone Resistant Giant' and 'Carolina Wonder' had 98.7% fewer eggs per g fresh root than its susceptible isoline 'Yolo Wonder B'. Likewise, 'Charleston Belle' had 97.6% fewer eggs per plant than 'Keystone Resistant Giant' and 'Carolina Wonder' had 98.9% fewer eggs per plant than 'Yolo Wonder B'. 'Charleston Belle' produced greater marketable fruit yield ($P \leq 0.06$) than the other cultivars.

Charleston study. Numbers of *M. incognita* J2 were ≈2 per cm^3 soil at planting. The resistant cultivars Charleston Belle and Carolina Wonder were both highly resistant; root gall severity indices were 1.0 for both cultivars (Table 1). The susceptible cultivars Keystone Resistant Giant and Yolo Wonder B were highly susceptible; root gall severity indices were 4.8 for both cultivars. 'Charleston Belle' had 96.2% fewer eggs per g fresh root than its susceptible isoline 'Keystone Resistant Giant' and 'Carolina Wonder' had 98.0% fewer eggs per g fresh root than its susceptible isoline 'Yolo Wonder B'. Similarly, 'Charleston Belle' had 96.9% fewer eggs per plant than 'Keystone Resistant Giant', and 'Carolina Wonder' had 97.5% fewer eggs per plant than 'Yolo Wonder B'. 'Charleston Belle' produced 25.6% and 13.3% heavier ($P \leq 0.05$) top fresh weights than 'Keystone Resistant Giant' and 'Carolina Wonder', respectively (data not shown). Differences in marketable fruit yield were not detected among any of the cultivars.

Discussion

'Charleston Belle' and 'Carolina Wonder' exhibited high resistance in both the Blackville and Charleston studies, confirming results of previous controlled-environment experiments where root galling, egg mass production, and numbers of eggs per g fresh root were minimal for both cultivars (Thies and Fery, 1998; Thies and Fery, 2000; Thies and Fery, 2002). 'Keystone Resistant Giant' and 'Yolo Wonder B' were highly susceptible at both locations. The numbers of eggs per plant for 'Charleston Belle' were somewhat higher than expected in the Charleston study (10,974 eggs per plant). The greater numbers of eggs per plant in the Charleston study were primarily due to a small number of plants that had large numbers of small egg masses on the roots, suggesting the presence of a highly virulent isolate of *Meloidogyne* spp. Similar susceptible reactions have been noted for the root-knot nematode resistant 'Carolina Cayenne' in past field studies (J.A. Thies and R.L. Fery, unpublished data).

'Charleston Belle' produced a significantly greater marketable yield than it's susceptible isoline 'Keystone Resistant Giant' in the Blackville study. Similarly, 'Charleston Belle' produced greater top fresh weight than 'Keystone Resistant Giant' in the Charleston study. In previous studies, the resistant 'Carolina Cayenne' pepper produced significantly greater yields than its susceptible near-isoline PA-136 when grown in *M. incognita*-infested fields (Thies et al., 1997).

The results of the tests at Blackville and Charleston were quite similar. The only significant test × cultivar interaction was for the root gall index. The root gall indices of the resistant cultivars ('Charleston Belle' and 'Carolina Wonder') were slightly lower and the root gall indices of the susceptible cultivars ('Keystone Resistant Giant' and 'Yolo Wonder B') were slightly higher for the Charleston test than the Blackville test, respectively. Although the interaction was statistically significant, it does not appear to be biologically significant.

'Charleston Belle' and 'Carolina Wonder' exhibited a high level of resistance to *M. incognita* in field studies at two sites in South Carolina. These results demonstrate that resistance to root-knot nematodes conferred by the *N* gene is effective in field planted bell pepper. Thus, these resistant cultivars should provide a useful source of root-knot nematode resistance for the development of root-knot nematode resistant hybrid bell pepper cultivars. Root-knot nematode resistant bell peppers should provide economical and environmentally compatible alternatives to methyl bromide and other nematicides for managing *M. incognita*. Such resistant bell pepper cultivars may also be useful as rotation crops in the development of strategies for managing southern root-knot nematode in susceptible vegetable crops.

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