

Virulence of *Meloidogyne incognita* to expression of *N* gene in pepper

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Abstract: Four pepper genotypes classified as resistant and four pepper genotypes classified as susceptible to several avirulent populations of *M. incognita* were compared for their reactions against a population of *Meloidogyne incognita* (Chitwood) Kofoid and White which had been shown to be virulent to resistant bell pepper (*Capsicum annuum*) in preliminary tests. The virulent population of *M. incognita* originated from a commercial bell pepper field in California. The resistant pepper genotypes used in all experiments were the *Capsicum annuum* cultivars Charleston Belle, Carolina Wonder, and Carolina Cayenne, and the *C. chinense* cultigen PA-426. The susceptible pepper genotypes used in the experiments were the *C. annuum* cultivars Keystone Resistant Giant, Yolo Wonder B, California Wonder, and the *C. chinense* cultigen PA-350. Root gall indices (GI) were ≥ 3.0 for all genotypes in both tests except for PA-426 (GI=2.57) in test 1 and 'Carolina Cayenne' (GI=2.83) in test 2. Numbers of eggs per gram fresh root weight ranged from 20,635 to 141,319 and reproductive indices ranged from 1.20 to 27.2 for the pepper genotypes in both tests, indicating that all eight pepper genotypes tested were susceptible to the *M. incognita* population used in these tests. The *M. incognita* population used in these studies overcame resistance conferred by the *N* gene in all resistant genotypes of both *C. annuum* and *C. chinense*.

Key words: *Capsicum annuum* var. *annuum*, *Capsicum chinense*, *Meloidogyne incognita*, methyl bromide alternatives, nematode resistance.

Root-knot nematodes (*Meloidogyne* spp.) are a major pest of bell pepper (*Capsicum annuum* L.) in the U.S. and world-wide (Sasser and Freckman, 1987; Di Vito et al., 1992). Methyl bromide, 1,3-dichloropropene/chloropicrin, and other nematicides are currently used to control root-knot nematodes in peppers (Olson and Simone, 2005). However, methyl bromide is being withdrawn from use in the U.S., and environmental concerns about water and air quality may limit future use of other fumigants and nematicides such as 1,3-dichloropropene/chloropicrin and metam sodium (U.S. Environmental Protection Agency, 2000). Thus, attention has focused on the development of alternative methods for managing plant-parasitic nematodes in peppers, in particular, host plant resistance. The *N* gene and alleles of the *N* gene confer resistance to *M. incognita* (Kofoid and White) Chitwood, *M. javanica* (Treib) Chitwood, and *M. arenaria* (Neal) Chitwood races 1 and 2 in several pepper cultivars and cultigens (Hare, 1956; Fery and Dukes, 1996; Fery and Thies, 1998a and 1998b; Fery et al., 1998; Thies and Fery, 2000a and 2000b; Thies and Fery, 2001). Two root-knot nematode resistant, open-pollinated bell pepper (*C. annuum*) cultivars Charleston Belle and Carolina Wonder are homozygous for the *N* gene (Fery et al., 1998). Resistance to *M. incognita* conditioned by the *N* gene in both 'Charleston Belle' and 'Carolina Wonder' was stable against different *M. incognita* isolates in field studies in Florida, Georgia, and South Carolina (Thies et al., 2005; Thies et al., 2008). 'Charleston Belle' and 'Carolina Wonder' exhibited high resistance to *M. incognita* in both spring and fall field tests in Florida (Thies et al., 2008). The resistant

'Charleston Belle' and 'Carolina Wonder' exhibited no visible root galling and low nematode reproduction, and their near-isogenic susceptible parents, 'Keystone Resistant Giant' and 'Yolo Wonder B', exhibited severe root galling and high nematode reproduction in the Florida field tests (Thies et al., 2008). For example, the average root gall indices of both 'Charleston Belle' and 'Carolina Wonder' were 1.0 (no visible root galling) and the average root gall indices of the susceptible parental cultivars 'Keystone Resistant Giant' and 'Yolo Wonder B' were 4.5 (on a 1.0 to 5.0 rating scale). Resistance of 'Carolina Cayenne' (*C. annuum*) is conditioned by a gene that is allelic to the *N* gene and an additional recessive gene (Fery and Dukes, 1996). Resistance of three Scotch Bonnet-type pepper (*C. chinense* Jacq.) cultigens is controlled by a gene that is allelic to the *N* gene (Fery and Thies, 1998b). Several of these *C. annuum* and *C. chinense* cultivars and cultigens are being used as sources of root-knot nematode resistance for the development of hybrid pepper. 'Charleston Belle', 'Carolina Wonder', 'Carolina Cayenne', and PA-426 have been evaluated against several avirulent populations of *M. incognita* at the U.S. Vegetable Laboratory, USDA, ARS, Charleston, SC and were never observed to be susceptible to any of the isolates tested (J.A. Thies, unpublished data). Although resistance conditioned by the *N* gene has been reported stable against different isolates of *M. incognita* in Florida, Georgia, and South Carolina, more information is needed about the utility of the *N* gene against different *M. incognita* populations. Furthermore, these pepper cultivars and cultigens have been tested in many geographical locations throughout the U.S. by pepper breeders and plant pathologists and have been found to exhibit high resistance to *M. incognita*.

The objective of this study was to determine the effectiveness of the root-knot nematode resistance *N* gene against an isolate of *M. incognita* which we had observed to be virulent to root-knot nematode resistant pepper in preliminary tests under greenhouse conditions.

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MATERIALS AND METHODS

Pepper genotypes: The pepper genotypes used in all experiments were the *Capsicum annuum* cultivars: Charleston Belle, Keystone Resistant Giant, Carolina Wonder, Yolo Wonder B, California Wonder, Carolina Cayenne, and the *C. chinense* cultivars: PA-426, and PA-350 (Table 1). Typically, 'Charleston Belle', 'Carolina Wonder', 'Carolina Cayenne', and PA-426 exhibit high resistance to *M. incognita* with root gall indices equal to 1.0 on a 1.0 to 5.0 rating scale, where 1.0 = no visible galling and 5.0 = greater than 80% of root system galled, and reproductive indices are < 0.5 (Thies and Fery, 1998; Thies and Fery, 2000a). Two near-isogenic sets of bell pepper differing in presence or absence of the *N* gene were included in order to determine the effectiveness of the *N* gene in conferring resistance to the *M. incognita* isolate; Set I: 'Charleston Belle' (*NN*, resistant) and 'Keystone Resistant Giant' (*nn*, susceptible); and Set II: 'Carolina Wonder' (*NN*, resistant) and 'Yolo Wonder B' (*nn*, susceptible). 'Charleston Belle' and 'Carolina Wonder' are root-knot nematode-resistant, open-pollinated bell pepper cultivars developed by USDA, ARS (Fery et al., 1998). 'Charleston Belle' and 'Carolina Wonder' are both homozygous for the *N* gene (Fery et al., 1998). 'Keystone Resistant Giant' (*nn*) and 'Yolo Wonder B' (*nn*) are the susceptible recurrent parental cultivars used in the backcross breeding procedure to develop 'Charleston Belle' and 'Carolina Wonder', respectively (Fery et al., 1998). 'Carolina Cayenne' is a highly resistant cayenne cultivar that contains both the *N* gene and a recessive gene that control resistance to root-knot nematodes (Fery and Dukes, 1996). For example, 'Carolina Cayenne' exhibited high resistance (GI=1.0 on a 1.0 to 5.0 scale and the root system supported an average of 496 eggs of *M. incognita* per gram root) in a multi-year field rotation test in Blackville, SC (Thies et al., 1998).

'California Wonder' is an open-pollinated standard bell pepper cultivar that is highly susceptible to avirulent populations of *M. incognita* (Thies et al., 1998). PA-426 and PA-350 are *C. chinense* cultivars. PA-426 is a Scotch Bonnet-type that possesses resistance to root-knot nematodes conferred by a gene that is allelic to the *N* gene (Fery and Thies, 1998b). PA-426 exhibited high resistance to *M. incognita* in greenhouse tests; root gall index = 1.3 and numbers of *M. incognita* eggs per gram root = 1473 (Fery and Thies, 1998a and 1998b). PA-350 is a habanero-type pepper that is highly susceptible to root-knot nematodes (Fery and Thies, 1998b).

Nematode inoculum: *Meloidogyne incognita* was increased on 'Rutgers' tomato (*Solanum lycopersicum*) in a separate soil bench in the greenhouse. The nematode species was identified using perineal patterns, esterase and malate dehydrogenase phenotypes (Esbenshade and Triantaphyllou, 1985), and the North Carolina host test (Taylor and Sasser, 1978). The isolate of *M. incognita* used in these experiments originated from a commercial bell pepper (*C. annuum*) field in California. Roots of pepper plants collected from this field were heavily galled. Egg inocula were extracted from infected tomato roots using the 0.5% NaOCl method (Hussey and Barker, 1973).

Experimental design and data analysis: The eight pepper genotypes were evaluated for reactions to *M. incognita* in two separate experiments. The experimental design for all experiments was a randomized complete block with 4 replicates. Each replicate consisted of 5 plants. Nematode egg data were $\log_{10}(x+1)$ transformed to normalize the data before analysis (Noe, 1985). Data were analyzed using the GLM procedure of SAS for Windows System Version 8.1 (SAS Institute, Cary, N.C.) and means were separated using Fisher's protected least significant difference (L.S.D.) test. Differences reported in the text were significant at the $P < 0.05$ level.

TABLE 1. Gall indices, numbers of *Meloidogyne incognita* egg masses per root system and eggs per g fresh root, and reproductive indices of eight pepper genotypes differing in root-knot resistance in a greenhouse test.^a

| Phenotype for resistance to avirulent <i>M. incognita</i> populations / Pepper Genotype | Nematode resistance genes | Gall index ^b | No. egg masses/root system | Eggs/g fresh root | Reproductive index ^c |
|---|---|-------------------------|----------------------------|------------------------|---------------------------------|
| Resistant/ | | | | | |
| Carolina Wonder | <i>N</i> | 3.35 bcd ^d | 19.0 bc | 44,957 ab ^e | 1.8 bc ^e |
| Charleston Belle | <i>N</i> | 3.57 d | 24.0 c | 31,412 a | 1.9 d |
| Carolina Cayenne | Dominant gene (allelic to <i>N</i> gene) + a recessive gene | 2.95 ab | 22.3 | 32,411 a | 2.0 d |
| PA-426 | Dominant gene (allelic to <i>N</i> gene) | 2.57 a | 7.1 a | 31,710 a | 0.5 a |
| Susceptible/ | | | | | |
| California Wonder | <i>n</i> | 3.47 cd | 16.3 b | 41,658 ab | 1.0 bc |
| Yolo Wonder B | <i>n</i> | 3.55 cd | 21.7 bc | 64,224 b | 1.5 bcd |
| Keystone Res. Giant | <i>n</i> | 3.61 cd | 22.2 c | 52,966 ab | 2.0 d |
| PA-350 | <i>n</i> | 3.20 bc | 16.2 b | 65,433 b | 0.9 ab |

^aFour replicates of five plants per replicate ($n=20$) arranged in a randomized complete block design.

^bGall 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.

^cReproductive index = P_1/P_2 .

^dValues in a column followed by the same letter are not significantly different according to Fisher's Least Significant Difference ($P \leq 0.05$).

^eData were $\log_{10}(x+1)$ transformed before analysis. Back transformed data are shown.

Greenhouse test I: Seeds of each pepper cultivar were planted in flats containing a pasteurized artificial growth medium. After seedlings developed two sets of true leaves, five seedlings per replicate of each pepper genotype were planted 10-cm apart in a single row in a greenhouse bench containing a steam-pasteurized mixture of 2 fine washed river sand : 1 sandy loam soil (by volume). Each seedling was inoculated at planting with 5,000 eggs of *M. incognita* suspended in 5 mL tap water. Approx. 10 weeks later, the roots were lifted and washed, and egg masses were stained by submersing in red food color (Thies et al., 2002) for approximately 15 minutes to aid in visualizing the egg masses. All egg masses were counted on the entire root system of each plant and recorded, and roots were scored for root galling using a 1 to 5 scale where 1 = 0 to 3% of 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% of root system galled. A gall index (GI) < 3.00 was considered resistant and GI \geq 3.00 was susceptible. The entire root system was weighed and root fresh weights recorded. Nematode eggs were extracted from the entire fibrous root sample from each five-plant plot using the NaOCl method (Hussey and Barker, 1973). Eggs were counted using a stereomicroscope. Nematode reproduction was assessed by calculating a nematode reproduction factor (R) where $R = P_f/P_i$, with P_i = the initial inoculum level and P_f = the final egg recovery (Sasser et al., 1984).

Greenhouse test II: Experimental procedures were performed as previously described except that percentages of egg masses covering the entire root system of each plant were estimated using the procedure for root gall ratings as described for Greenhouse Test I.

RESULTS AND DISCUSSION

Greenhouse test I: All eight pepper genotypes were susceptible to *M. incognita* (Table 1). Root gall indices (GI) ranged from 2.57 for PA-426 to 3.61 for 'Keystone Resistant Giant'. Numbers of egg masses per root system ranged from 7.1 for PA-426 to 24.0 for 'Charleston Belle'. Numbers of eggs per gram fresh root ranged from 31,412 for 'Charleston Belle' to 65,433 for PA-350. Reproduction factors ranged from 0.5 for PA-426 to 2.0 for 'Keystone Resistant Giant'. These results indicate the importance of using more than one criterion for assessing RKN resistance. Clearly, numbers of eggs per gram fresh root indicated that all of the genotypes tested were susceptible, since numbers of eggs per gram fresh root ranged from >31,000 to >65,000. Thus, although PA-426 would be classified in the low resistance category according to root gall index (GI=2.57) and classified in the resistant category according to the reproductive index (0.5), the numbers of eggs per gram fresh root (31,710) indicate that this genotype was very susceptible. Likewise, although the known susceptible *C. chinense* genotype PA-350 had a reproduction

factor = 0.9, it is clearly not resistant because the GI=3.20 and the root system supported >65,000 eggs per gram fresh root.

Greenhouse test II: All eight pepper genotypes were susceptible to *M. incognita* (Table 2). Root gall indices (GI) ranged from 2.83 for 'Carolina Cayenne' to 4.97 for 'California Wonder'. Egg mass indices ranged from 2.32 for 'Carolina Cayenne' to 4.97 for 'California Wonder'. Numbers of eggs per gram fresh root ranged from 20,635 for 'Carolina Cayenne' to 141,319 for 'California Wonder'. Reproductive indices ranged from 1.2 for 'Carolina Cayenne' to 27.2 for 'California Wonder'.

Resistance of pepper cultivars controlled by the *N* gene has been effective against several avirulent isolates of *M. incognita* (Hare, 1956; Kokalis-Burelle, 2009; Thies et al., 1997; Thies et al., 1998). Typically, the five resistant pepper genotypes exhibit high resistance to avirulent isolates of *M. incognita* with root gall indices of 1.0 to 1.3 and reproductive indices \leq 0.5 (Thies et al., 1997; Thies and Fery, 2000b; Thies and Fery, 2002). However, all of the root-knot resistant *C. annuum* cultivars and the resistant *C. chinense* cultigen were susceptible to the isolate of *M. incognita* used in the present studies. These results differ from previous tests where 'Charleston Belle' and 'California Wonder' were highly resistant to *M. incognita* with no or very minimal root galling and very low nematode reproduction in greenhouse, growth chamber, and field studies (Fery et al., 1998; Thies et al., 1998; Thies and Fery, 2002). 'Charleston Belle' exhibited high resistance to *M. incognita* in Tifton, GA where root gall indices averaged 1.3 and numbers of *M. incognita* eggs per gram fresh root were 1,179 (Thies et al., 2005). In comparison, 'Keystone Resistant Giant', the susceptible near-isogenic recurrent parent of 'Charleston Belle', had root gall indices of 3.9 and numbers of *M. incognita* eggs per gram fresh root were 11,632. 'California Wonder' exhibited high resistance to *M. incognita* in Blackville, SC where root gall indices averaged 1.2 and numbers of *M. incognita* eggs per gram fresh root were 148, compared to its near-isogenic susceptible parent 'Yolo Wonder', which had root gall indices of 4.3 and 80,468 *M. incognita* eggs per gram fresh root (Thies et al., 2003). 'Charleston Belle' and 'California Wonder' exhibited high resistance to *M. incognita* in field tests in Florida, where root gall indices averaged 1.0 for both cultivars in spring and fall tests (Thies et al., 2008). 'Carolina Cayenne' was susceptible in the present studies, compared to previous studies where it had exhibited high resistance to *M. incognita* (Thies et al., 1997; Thies et al., 1998). In microplot and field studies, 'Carolina Cayenne' exhibited minimal root galling (gall index = 1.4 on a 1.0 to 9.0 rating scale and supported 90 *M. incognita* eggs per gram fresh root (Thies et al., 1997). In comparison, PA-136, a near-isogenic sister line of 'Carolina Cayenne', exhibited severe root galling (gall index = 9.0 and supported 3,322 eggs per gram fresh root) (Thies et al., 1997). Both *C. chinense* cultigens PA-426 and PA-350

TABLE 2. Gall indices, egg mass indices, numbers of *Meloidogyne incognita* eggs per g fresh root, and reproductive index of eight pepper genotypes differing in root-knot resistance in a greenhouse test.^a

| Phenotype for resistance to avirulent <i>M. incognita</i> populations / Pepper Genotype | Gall index ^b | Egg mass index ^b | Eggs/g fresh root ^c | Reproductive index ^c |
|---|-------------------------|-----------------------------|--------------------------------|---------------------------------|
| Resistant/ | | | | |
| Carolina Wonder | 4.05 ccd ^d | 4.05 c | 76,4542 bc ^e | 5.7 c ^e |
| Charleston Belle | 3.57 b | 3.55 b | 89,187 bcd | 4.2 b |
| Carolina Cayenne | 2.83 a | 2.32 a | 20,635 a | 1.2 a |
| PA-426 | 4.61 d | 4.59 d | 135,020 cd | 4.4 b |
| Susceptible/ | | | | |
| California Wonder | 4.97 d | 4.97 d | 141,319 d | 27.2 d |
| Yolo Wonder B | 4.03 c | 4.03 c | 140,734 d | 7.2 bc |
| Keystone Res. Giant | 3.93 bc | 3.92 bc | 72,577 b | 5.9 bc |
| PA-350 | 4.12 c | 4.08 c | 111,506 bcd | 9.3 c |

^aFour replicates of five plants per replicate ($n=20$) arranged in a randomized complete block design.

^bGall and egg mass indices: 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 or covered with egg masses.

^cReproductive index = P_T/P_1 .

^dValues in a column followed by the same letter are not significantly different according to Fisher's Least Significant Difference ($P \leq 0.05$).

^eData were $\log_{10}(x+1)$ transformed before analysis. Back transformed data are shown.

were susceptible to *M. incognita* in the present test, whereas PA-426 exhibited high resistance and PA-350 was susceptible to avirulent isolates of *M. incognita* in previous tests (Fery and Thies, 1998a and 1998b). The results of these studies have shown that the isolate of *M. incognita* tested in the present studies overcame resistance conferred by the *N* gene in resistant genotypes of both *C. annuum* and *C. chinense*. Although isolates of *M. incognita* have been reported to overcome resistance in the pepper line HDA149 containing the *Me3* gene in France (Castagnone-Serono et al. 2001), this is the first report of an isolate of *M. incognita* virulent to root-knot resistant pepper in the U.S. Thus, it will be important to continue evaluating the current resistant pepper genotypes against different isolates of *M. incognita* and searching for new sources of resistance to virulent *M. incognita* isolates.

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