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Evaluation of *Capsicum chinense* Jacq. Cultigens for Resistance to the Southern Root-knot Nematode

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Abstract. **Scotch Bonnet and Habanero peppers, extremely pungent cultivar classes of *Capsicum chinense* Jacq., are increasing in popularity in the United States. Because the southern root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood, is a major pest of many *C. annuum* cultivars, a series of greenhouse and field experiments was conducted to determine if Scotch Bonnet and Habanero peppers from available commercial and private sources also are vulnerable to the pest. In an initial greenhouse test, a collection of 59 *C. chinense* cultigens was evaluated for reaction to *M. incognita* race 3. All cultigens obtained from commercial sources were moderately susceptible or susceptible. However, four accessions obtained through Seed Savers Exchange listings exhibited high levels of resistance. Three of these cultigens (PA-353, PA-398, and PA-426) were studied in subsequent greenhouse and field plantings, and each was confirmed to have a level of resistance similar to that available in *C. annuum*. All three of the resistant cultigens are well-adapted and each is potentially useful in commercial production without further development. None of the Habanero cultigens was resistant to the southern root-knot nematode. The resistant Scotch Bonnet cultigens may serve as sources of resistance for development of root-knot nematode–resistant Habanero peppers.**

Although most peppers cultivated in the United States belong to the species *Capsicum annuum* L. (Bosland, 1992), the increasing popularity of hot peppers has created an intense interest in two cultivar classes of another domesticated *Capsicum* species, *C. chinense*. These cultivar classes, Habanero and Scotch Bonnet, contain some of the world's most pungent peppers (Hazen-Hammond, 1993). The southern root-knot nematode is a major pest of pepper in the United States. Parasitism of susceptible plants can result in severe yield losses (Di Vito et al., 1992; Fery and Dukes, 1984; Lindsey and Clayshutte, 1982; Thies et al., 1997). Although resistance to *M. incognita* has been reported in *C. chinense* (Di Vito and Saccardo, 1979; Di Vito et al., 1989, 1993), the reactions of Habanero and Scotch Bonnet peppers to this parasite are unknown. The objectives of this study were 2-fold: 1) to evaluate available *C. chinense* cultigens for reaction to *M. incognita*, and 2) to identify potentially useful germplasm for use as parents in breeding programs to develop improved Habanero and Scotch Bonnet cultivars.

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Materials and Methods

The data reported herein are from a series of greenhouse and field experiments conducted at the U.S. Vegetable Laboratory, Charleston, S.C. The original seeds of all *C. chinense* accessions were obtained from commercial seed companies, pepper researchers employed by public agencies, or private individuals with listings in the Seed Savers Yearbook (Seed Savers Exchange, 1993). Most of the *C. chinense* lines tested were described by the offerer as either a type of Habanero or Scotch Bonnet. The most readily distinguishable difference between the Habanero and Scotch Bonnet is fruit shape; the Habanero bears lantern-shaped fruits, while the Scotch Bonnet bears bonnet-shaped fruits.

Initial screening (Greenhouse Expt. I). A total of 59 *C. chinense* accessions and 10 *C. annuum* controls were evaluated in a greenhouse experiment for resistance to *M. incognita*. The *C. annuum* controls included the highly resistant cvs. Carolina Cayenne (Fery et al., 1986; Fery and Dukes, 1996) and Charleston Hot (U.S. Dept. of Agriculture, 1992), the resistant cv. Mississippi Nemaheart (Hare, 1966), six susceptible cultivars, and the susceptible germplasm line PA-136 (Dukes et al., 1997). The test was conducted on 4.1 × 1.7 × 0.2-m benches containing a steam-pasteurized mixture of soil, sand, and peat moss (6:3:1, by volume). The pH of the mixture was maintained at 6.0. Seeds of each accession were planted in flats containing a pasteurized artificial growth medium on 6 May 1994, and the resulting seedlings were transplanted into the benches on 27 May 1994. After the seedlings were established and growing, each plant was inoculated with ≈3000 *M. incognita* race 3 eggs on 23 June 1994. The inoculum used for the test was extracted from PA-136 pepper roots infected with *M. incognita* using 0.525% NaOCl (Hussey and Barker, 1973).

A completely randomized design was used and the planting arrangement for the single-plant plots was a 10 × 12-cm rectangular pattern. Ten plants per accession were planted. To minimize the effects of moisture and temperature stress, the outer two rows around each bench were used as buffers. The greenhouse air was maintained between 24 and 32 °C, and all plants were evaluated on 23 Aug. 1994. Each plant was rated on a scale of 1 (no galling) to 5 (severe galling, >80% root system galled) for prevalence of root galling.

Characterization of resistance (Greenhouse Expt. II). This experiment was conducted to further characterize the resistance of *C. chinense* cultigens identified in Greenhouse Expt. I as being resistant to *M. incognita*. The experiment included three resistant *C. chinense* cultigens (PA-398, PA-426, and PA-353), three susceptible *C. chinense* cultigens (PA-350, PA-343, and PA-388), two resistant *C. annuum* controls ('Carolina Cayenne' and 'Mississippi Nemaheart'), and two susceptible *C. annuum* controls ('Sweet Banana' and PA-136). Plants of the 10 cultigens were evaluated for reaction to *M. incognita* using the general procedures outlined for Greenhouse Expt. I. The experiment design was a randomized complete block with six replications. Each replication contained a single, five-plant plot of each cultigen. Seeds were planted on 24 Jan. 1995, the seedlings were transplanted into soil benches on 14 Feb. 1995, and each plant was inoculated with ≈3000 *M. incognita* race 3 eggs on 8 Mar. 1995. All plants were removed from the soil benches on 15 May 1995, and the roots of each plant were scored for galling and egg mass production using a 1 to 9 scale (1 = no galls or egg masses; 9 = galls or egg masses covering at least 80% of root system). The 0.525% NaOCl procedure was used to extract eggs from a bulked fibrous root sample of each five-plant plot (Hussey and Barker, 1973).

Verification of resistance under field conditions (Field Expt. I). The resistant *C. chinense* cultigens PA-398, PA-426, and PA-353, the susceptible *C. chinense* cultigens PA-350, PA-343, and PA- 388, the resistant *C. annuum* cvs. Carolina Cayenne and Mississippi Nemaheart, the intermediate resistant *C. annuum* cv. Keystone Resistant Giant, and the susceptible *C. annuum* cv. California Wonder were evaluated in a field naturally infested with *M. incognita*. Seeds of each accession were planted in flats containing a pasteurized artificial growth medium on 13 Apr. 1995, and the resulting greenhouse-produced seedlings (≈15 cm tall) were transplanted to the field on 31 May 1995. The experiment design was a randomized complete block with nine replications. Each plot consisted of a single row of five plants spaced

Table 1. Gall indices of 59 *Capsicum chinense* cultigens and 10 *C. annuum* controls inoculated with the southern root-knot nematode, *Meloidogyne incognita* race 3 (Greenhouse Expt. I).

Pepper species/ accession	Cultigen	Seed source	Gall index[z]
C. chinense			
PA-426	Yellow Scotch Bonnet	SSE member[y]	1.0
PA-427[x]	Scotch Bonnet x Equadoran Aji I	SSE member	1.0
PA-353	Red Habanero	SSE member	1.2
PA-398	Jamaica Scotch Bonnet	SSE member	1.2
PA-376	Red Scotch Bonnet #2	SSE member	2.2
PA-368	Barbados #2	SSE member	2.3
PA-365	Harold's St. Barts #2	SEE member	2.3
PA-373	Jamaica Small Red #1	SEE member	2.3
PA-367	Barbados #1	SEE member	2.4
PA-375	Red Scotch Bonnet #1	SSE member	2.4
PA-374	Jamaica Small Red #2	SSE member	2.5
PA-364	Harold's St. Barts #1	SSE member	2.5
PA-380	Red Scotch Bonnet #6	SSE member	2.5
PA-387	Yellow Scotch Bonnet #5	SSE member	2.5
PA-394	Scotch Bonnet	Lincoln Univ.	2.5
PA-423	Habanero	SSE member	2.5
PA-371	Jamaican Orange #2	SSE member	2.5
PA-395	Habanero	Pinetree Garden Seeds	2.5
PA-366	Harold's St. Barts #2	SSE member	2.6
PA-382	Red Scotch Bonnet #8	SSE member	2.6
PA-359	Habanero	Park Seed Co.	2.6
PA-347	Habanero	SSE member	2.6
PA-350	Habanero	SSE member	2.6
PA-356	Habanero	Nickles Garden Nursery	2.6
PA-385	Yellow Scotch Bonnet #3	SSE member	2.6
PA-355	Habanero	Redwood City Seed	2.7
PA-349	Habanero	SSE member	2.7
PA-420	Habanero	SSE member	2.7
PA-372	Jamaica Large Red	SSE member	2.7
PA-399	Habanero	Hastings	2.7
PA-402	Habanero	SSE member	2.7
PA-274	Habanero	Shepherd's Garden Seeds	2.7
PA-361	Jamaican Hot Chocolate #1	SSE member	2.7
PA-386	Yellow Scotch Bonnet #4	SSE member	2.7
PA-362	Jamaican Hot Chocolate #2	SSE member	2.7
PA-225	Habanero Hot	Porter and Sons	2.7
PA-396	Habanero	SSE member	2.8
PA-337	Habanero	D.V. Burrell Seed Growers	2.8
PA-295	Habanero Hot	Stokes Seeds	2.8
PA-360	Habanero	Bill's Exotic Seeds	2.8
PA-351	Habanero	SSE member	2.8
PA-352	Habanero	SSE member	2.8
PA-400	Jamaica Scotch Bonnet (Yellow)	SSE member	2.8
PA-378	Red Scotch Bonnet #4	SSE member	2.9
PA-397	Chocolate Congo Habanero	SSE member	2.9
PA-384	Yellow Scotch Bonnet #2	SSE member	2.9
PA-358	Habanero	Tomato Growers Supply	2.9
PA-390	Yellow Scotch Bonnet #8	SSE member	2.9
PA-343	Habanero	Jordan Seeds	2.9
PA-388	Yellow Scotch Bonnet #6	SSE member	2.9
PA-338	Habanero	Mixon Seed	3.0
PA-377	Red Scotch Bonnet #3	SSE member	3.0
PA-354	Habanero	Totally Tomatoes	3.0
PA-381	Red Scotch Bonnet #7	SSE member	3.0
PA-370	Jamaican Orange #7	SSE member	3.0
PA-383	Yellow Scotch Bonnet #1	SSE member	3.0
PA-357	Habanero	The Cook's Garden	3.0
PA-389	Yellow Scotch Bonnet #7	SSE member	3.0
PA-379	Red Scotch Bonnet #5	SSE member	3.2
C. annuum			
PA-149	Carolina Cayenne[w]	USDA, Charleston, S.C.	1.0
PA-195B	Charleston Hot[w]	USDA, Charleston, S.C.	1.0
PA-003	Mississippi Nemaheart[w]	Mississippi Agr. Expt. Sta.	1.0
PA-001	Yolo Wonder[v]	Stokes Seeds	2.6
PA-209	Sweet Banana[v]	PetoSeed	2.8
PA-105	California Wonder[v]	Stokes Seeds	2.9
PA-336	TAM Mild Chili[v]	S.C. Foundation Seed	3.0
PA-335	TAM Mild Jalapeno[v]	S.C. Foundation Seed	3.0
PA-256	Early Calwonder[v]	Holmes Seed	3.4
PA-136	PA-136[v]	USDA, Charleston, S.C.	3.5
$LSD_{0.05}$	---	---	0.5

[z]Rated on a scale of 1 to 5: 1 = no galling and 5 = severe galling.
[y]Listed member, Seed Savers Exchange.
[x]Population heterogeneous for fruit shape and mature fruit color.
[w]Resistant control.
[v]Susceptible control.

76 cm apart on beds 1 m apart. Standard cultural and insect control practices were followed, and the plots were irrigated with an overhead system, as needed. Fruit were harvested weekly from 16 Aug. 1995 through 20 Sept. 1995. Each plant was removed from the field on 10 Oct. 1995, the roots were evaluated for galling response using the 1 to 5 scale described for Greenhouse Expt. I, and eggs were extracted from samples of fibrous root tissue using 0.525% NaOCl (Hussey and Barker, 1973). Ten cores of soil were collected from each plot on 12 June 1995 and before plants were removed from the field on 10 Oct. 1995; the cores were bulked and *M. incognita* second-stage juveniles (J2) were extracted from a 500 cm^3 subsample by elutriation (Byrd et al., 1976) and centrifugal flotation (Jenkins, 1964).

Fruit and yield characteristics of resistant cultigens (Field Expt. II). This experiment was conducted to evaluate the fruit and yield characteristics of three root-knot nematode–resistant *C. chinense* cultigens studied in the experiments discussed above. The experiment included the resistant cultigens PA-398, PA-426, and PA-353, and the susceptible cultigens PA-274, PA-295, PA-337, PA-338, PA-343, PA-350, PA-356, and PA-395. Except for PA-350, all of the susceptible cultigens were Habanero-type cultivars obtained from commercial seed companies. The times of seeding and transplanting, the experiment design, the plot size, and fruit harvest times were identical to those described for Field Expt. I. Data were collected on fruit shape, fruit size, mature fruit color, number of locules per fruit, total number of fruit per plant, and total mass of fruit per plant. The field had no history of infestation by root-knot nematodes.

Results and Discussion

Initial screening (Greenhouse Expt. I). All *C. annuum* controls reacted to *M. incognita* as expected (Table 1). All plants of the resistant cultivars Carolina Cayenne, Charleston Hot, and Mississippi Nemaheart exhibited highly resistant reactions, while the plants of the seven susceptible controls exhibited moderately susceptible to susceptible reactions. A significant amount of genetic variability for resistance to *M. incognita* was observed among the *C. chinese* cultigens. Although most of the accessions were moderately susceptible or susceptible, four accessions (PA-398, PA-426, PA-353, and PA-427) exhibited a high level of resistance. Interestingly, none of the *C. chinense* cultigens obtained from commercial seed companies was resistant; each of the four resistant accessions was obtained from heirloom collections maintained by members of the Seed Savers Exchange.

Characterization of resistance (Greenhouse Expt. II). The results of this test confirmed that the *C. chinense* cultigens PA-353, PA-398, and PA-426 are resistant to *M. incognita* (Table 2). Few galls or egg masses were produced on the roots of resistant *C. chinense* plants, and the average number of *M. incognita* eggs extracted from fresh roots of resistant *C. chinense* plants was only 4.5% of the average number extracted from susceptible *C. chinense* plants. The level of resistance in *C. chinense* was similar to that in *C. annuum*; there were no significant differences between the gall indices, egg mass indices, or the numbers of eggs extracted from fresh root tissue. Roots of the susceptible *C. chinense* plants exhibited slightly less galling and *M. incognita* egg production than those of the susceptible *C. annuum* plants.

Verification of resistance under field conditions (Field Expt. I). Preliminary examination of the galling and egg extraction data for the resistant *C. annuum* and *C. chinense* cultigens suggested the presence of a resistance-breaking strain of *M. incognita* or another *Meloidogyne* spp. in replications V through IX. For example, we have used 'Carolina Cayenne' as a resistant control in numerous field and greenhouse studies in recent years and have never obtained a susceptible reaction to any *Meloidogyne incognita* isolate prior to this study. Since an occasional susceptible reaction has been observed in populations of resistant cowpea [*Vigna unguiculata* (L.) Walp.] cultivars grown in these plots in recent years (R.L. Fery and P.D. Dukes, unpublished data), the presence of a resistance-breaking *M. incognita* strain or an unknown *Meloidogyne* spp. in some of the test plots was not totally unexpected. Since all of the resistant *C. annuum* controls responded as expected in replications I through IV, we proceeded under the assumption that the resistance-breaking strain of *M. incognita* or unknown *Meloidogyne* spp. was not prevalent in the portion of the field containing these replications, and executed all planned analyses using only the data from the first four replications.

The *C. chinense* cultigens PA-398, PA-426, and PA-353 were resistant to *M. incognita* under field conditions (Table 3). The roots of the resistant *C. chinense* plants produced few galls, and the number of eggs extracted from the fresh root tissue was similar to that extracted from the resistant *C. annuum* controls. The reaction of the susceptible *C. chinense* cultigens tended to be intermediate between the reactions of the intermediate resistant *C. annuum* cv. Keystone Resistant Giant and the susceptible *C. annuum* cv. California Wonder. Counts of J2 *M. incognita* were relatively low for preplant and postharvest soil samples, and this probably accounted for the similar fruit yields of the resistant and susceptible *C. chinense* cultigens. Root-knot nematode damage was probably not responsible for the low yield of fruit harvested from the susceptible PA-388 cultigen; this cultigen produced a luxuriant vine and did not appear to be adapted for culture in coastal South Carolina.

Fruit and yield characteristics of resistant cultigens (Field Expt. II). The fruit size and yield potential of each of the root-knot nematode–resistant cultigens were similar to the those of commercial *C. chinense* cultivars (Table 4). Each of the resistant cultigens was a Scotch Bonnet–type pepper. PA-398 and PA-426 produce typical Scotch Bonnet type fruit, i.e., a bonnet shape and a yellow mature

Table 2. Gall indices, egg mass indices, and number of eggs per gram fresh root tissue of six *Capsicum chinense* cultigens and four *C. annuum* controls inoculated with the southern root-knot nematode, *Meloidogyne incognita* race 3 (Greenhouse Expt. II).

Pepper species/ accession	Gall index[z]	Egg mass index[y]	No. eggs/g fresh root tissue
C. chinense			
PA-353	1.7	1.8	3068
PA-398	1.3	1.2	2020
PA-426	1.3	1.3	1473
PA-343	5.5	5.4	45350
PA-350	5.5	5.4	52352
PA-388	6.5	6.1	45556
C. annuum			
Carolina Cayenne[x]	1.3	1.4	935
Mississippi Nemaheart[x]	1.5	1.5	1313
PA-136[w]	7.5	7.6	65391
Sweet Banana[w]	6.5	6.7	70659
$LSD_{0.05}$	0.5	0.5	12816

[z]Rated on a scale of 1 to 9; 1 = no galls and 9 = galls covering more than 80% of root system.
[y]Rated on a scale of 1 to 9; 1 = no egg masses and 9 = egg masses covering more than 80% of root system.
[x]Resistant control.
[w]Susceptible control.

Table 3. Number of *Meloidogyne incognita* second-stage juveniles (J2) in preplant and postharvest soil samples, gall indices, number of eggs per gram fresh root tissue, and fruit yield from plots of six *Capsicum chinense* cultigens and four *C. annuum* controls grown in a field naturally infested with a population of the southern root-knot nematode, *Meloidogyne incognita* (Field Expt. I).

Pepper species/	No. J2/100 cm^3 soil		Gall	No. eggs/g fresh root	Fruit yield/
accession	Preplant	Harvest	index[z]	tissue	plant (g)
C. chinense					
PA-353	120	240	1.24	1796	522
PA-398	98	166	1.30	2701	572
PA-426	120	114	1.05	443	570
PA-343	112	158	2.81	8118	508
PA-350	86	180	3.44	10134	511
PA-388	114	116	3.62	4287	167
C. annuum					
California Wonder[y]	122	192	4.74	25880	464
Carolina Cayenne[x]	90	112	1.00	30	580
Keystone Resistant Giant[w]	112	210	2.42	5167	742
Mississippi Nemaheart[x]	72	168	1.46	464	459
$LSD_{0.05}$	NS	NS	0.69	4430	158

[z]Rated on a scale of 1 to 5; 1 =no galling and 5 = severe galling.
[y]Susceptible control.
[x]Resistant control.
[w]Intermediate resistant control.

Table 4. Comparison of the fruit and yield characteristics of root-knot nematode resistant and susceptible *Capsicum chinense* cultigens (Field Expt. II).

Nematode	Fruit characteristic					
reaction[z]/ accession	Fruit shape	Dimension (cm) (width × depth)[y]	Color (mature)	Locules (no.)	Mean mass (g)	Yield/ plant (g)
Resistant						
PA-353	Bonnet	6.0 × 3.5	Red	3	7.6	329
PA-398	Bonnet	6.0 × 3.5	Yellow	3	10.0	377
PA-426	Bonnet	6.0 × 2.5	Yellow	3	7.6	301
Susceptible						
PA-274[x]	Lantern	3.0 × 5.0	Orange	3	6.7	368
PA-295[x]	Lantern	3.0 × 6.0	Orange	3	7.2	378
PA-337[x]	Lantern	3.0 × 6.0	Orange	3–4	6.1	256
PA-338[x]	Lantern	3.0 × 6.0	Orange	3	7.0	316
PA-343[x]	Lantern	3.5 × 6.0	Orange	3	6.9	285
PA-350	Lantern	3.5 × 5.0	Orange	2–4	8.3	452
PA-356[x]	Lantern	3.0 × 5.0	Orange	2–3	7.0	332
PA-395[x]	Lantern	3.0 × 5.0	Orange	3–4	7.4	331
$LSD_{0.05}$	---	---	---	---	1.8	NS

[z]Based on results of Greenhouse Expt. I.
[y]Typical fruit.
[x]Commercial Habanero-type cultivar.

fruit. Although the offerer identified the PA-353 cultigen as a Red Habanero, this description is not totally accurate. The color of the mature fruit is indeed red, but the fruit were unquestionably bonnet-shaped.

Conclusions

These greenhouse and field experiments document the existence of useful levels of resistance to the southern root-knot nematode in *C. chinense* cultigens. All of the cultigens obtained from commercial seed companies were susceptible, and each of the resistant cultigens was obtained from heirloom collections. PA-353, PA-398, and PA-426, the three root-knot nematode–resistant *C. chinense* cultigens studied in detail in these studies, are all well-adapted, Scotch Bonnet–type peppers, and each is potentially useful in commercial production without further development. None of the Habanero peppers obtained from either commercial sources or heirloom collections were resistant to the southern root-knot nematode. The root-knot nematode–resistant Scotch Bonnet cultigens likely will be excellent sources of resistance for development of root-knot nematode–resistant Habanero peppers.

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