Journal of Nematology 34(4):374–377. 2002.

Host Plant Resistance as an Alternative to Methyl Bromide for Managing *Meloidogyne incognita* in Pepper

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Abstract: Pre-plant soil fumigation with methyl bromide and host resistance were compared for managing the southern root-knot nematode (*Meloidogyne incognita*) in pepper. Three pepper cultivars (Carolina Cayenne, Keystone Resistant Giant, and California Wonder) that differed in resistance to *M. incognita* were grown in field plots that had been fumigated with methyl bromide (98% CH_3Br : 2% CCl_3NO_2 [w/w]) before planting or left untreated. Carolina Cayenne is a well-adapted cayenne-type pepper that is highly resistant to *M. incognita*. The bell-type peppers Keystone Resistant Giant and California Wonder are intermediate to susceptible and susceptible, respectively. None of the cultivars exhibited root galling in the methyl bromide fumigated plots and nematode reproduction was minimal (<250 eggs/g fresh root), indicating that the fumigation treatment was highly effective in controlling *M. incognita*. Root galling of Carolina Cayenne and nematode reproduction were minimal, and fruit yields were not reduced in the untreated plots. The root-galling reaction for Keystone Resistant Giant was intermediate (gall index = 2.9, on a scale of 1 to 5), and nematode reproduction was moderately high. However, yields of Keystone Resistant Giant were not reduced in untreated plots. Root galling was severe (gall index = 4.3) on susceptible California Wonder, nematode reproduction was high, and fruit yields were reduced ($P \leq 0.05$) in untreated plots. The resistance exhibited by Carolina Cayenne and Keystone Resistant Giant provides an alternative to methyl bromide for reducing yield losses by southern root-knot nematodes in pepper. The high level of resistance of Carolina Cayenne also suppresses population densities of *M. incognita*.

Key words: Capsicum annuum, Meloidogyne incognita, methyl bromide alternatives, nematode management, nematode resistance, pepper, root-knot nematodes.

The root-knot nematodes *Meloidogyne incognita* Kofoid and White (Chitwood), *M. arenaria* (Neal) Chitwood races 1 and 2, *M. javanica* (Treub) Chitwood, and *M. hapla* Chitwood are harmful pathogens of sweet and hot peppers (*Capsicum* spp.) in the United States (Thies et al., 1997; Thomas et al., 1995). Currently, methyl bromide is used as a pre-plant soil fumigant for controlling plant-parasitic nematodes, weeds, and soilborne pathogens on approximately 40% of the U.S. pepper crop. However, signatory countries of the Montreal Protocol, an international treaty to protect the Earth from effects of ozone depletion, agreed to completely phase out production and use of methyl bromide and other ozone-depleting substances by 1 January 2005 (U.S. Department of Agriculture, 2001). This pending loss of methyl bromide from the U.S. market by 2005 is increasing the urgency of developing alternative strategies for managing nematodes in high-value vegetable crops such as pepper. Nematode-resistant cultivars, if available, provide one of the most cost-effective and environmentally safe methods for managing plant-parasitic nematodes in food crops.

Major vegetable seed companies are currently developing root-knot-resistant sweet and hot hybrid pepper cultivars. However, no root-knot-resistant pepper hybrids are available yet. Carolina Cayenne, a cayenne pepper (*Capsicum annuum* L.) developed by the U.S. Vegetable Laboratory (USDA, ARS, Charleston, SC) and Clemson University (Clemson, SC), is highly resistant to *M. incognita, M arenaria* races 1 and 2, and *M. javanica* (Fery et al., 1986; Thies and Fery, 2000). Carolina Cayenne exhibited exceptionally high resistance to *M. incognita* in field and microplot tests, but the effectiveness of its resistance has not been compared to preplant soil fumigation treatment with methyl bromide (Thies et al., 1997; 1998). Keystone Resistant Giant is a bell-type pepper cultivar that is intermediate to susceptible in reaction to *M. incognita*, relative to the highly susceptible California Wonder and Early CalWonder (Thies et al., 1997, 1998). The objective of this study was to compare pre-plant fumigation with methyl bromide and differing levels of host plant resistance for managing southern root-knot nematodes in pepper.

Materials and Methods

This field study was conducted in 1995 at the U.S. Vegetable Laboratory, Charleston, South Carolina, on a Yonges loamy sand (fine loamy mixed, thermal Albaqualfs; 80% sand, 11% silt, 9% clay, <1% organic matter) soil. In July 1994, root-knot susceptible PA-136 cayenne pepper seedlings were transplanted into the field site and inoculated with approximately 5,000 eggs of *M. incognita* race 3 per seedling (Dukes et al., 1997). A cover crop of hairy vetch (*Vicia villosa* Roth) was sown over the pepper plants in December 1994 to aid in maintaining the nematode population over the winter.

The pepper cultivars used were Carolina Cayenne, Keystone Resistant Giant, and California Wonder. Carolina Cayenne is a well-adapted cayenne cultivar that is highly resistant to *M. incognita* (Fery et al., 1986). Keystone Resistant Giant and California Wonder are bell-type peppers; Keystone Resistant Giant is intermediate to susceptible in reaction to *M. incognita* and California Won-

Received for publication 28 March 2002.

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The authors thank E. L. Corley, Jr., and F. P. Maguire for technical assistance in conducting the experiments.

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This paper was edited by J. L. Starr.

der is highly susceptible (Thies et al., 1997; Thies et al., 1998). The seeds of all of the entries were planted in the greenhouse in 25-×-51-×-4.5-cm-deep plastic flats filled with Metro-Mix 360 (The Scotts Co., Marysville, OH) on 13 April to 18 April 1995. Approximately 10 days later, the seedlings were transplanted into plastic Pro-Trays (Growing Systems Inc., Milwaukee, WI) containing 50 individual 0.2-l plastic cells filled with MetroMix.

The experimental design was a split-plot with treatments arranged in a randomized complete-block with six replications. The whole plots were pre-plant soil fumigation with methyl bromide and an untreated control. The sub-plots were the three pepper cultivars. On 18 May 1995, treated main plots were covered with 3-mil black plastic and fumigated with methyl bromide [98% CH_3Br : 2% CCl_3NO_2 (w/w)] at 651 kg/ha. The plastic was removed on 25 May 1995, and the pepper seedlings were transplanted in single rows on beds 1 m apart with 30-cm in-row plant spacing and 15 plants per row on 26 May 1995. Mean pre-plant densities of *M. incognita* in the untreated plots were 28 ± 4 second-stage juveniles (J2) per 100 cm^3 soil. Standard cultural practices and insect control procedures for pepper were followed, and plots were watered with overhead irrigation as needed. Mature pepper fruit from Keystone Resistant Giant and California Wonder were harvested weekly from 1 August to 29 August 1995; mature red fruit from Carolina Cayenne were harvested from 25 August to 27 September 1995. The root systems of 10 plants from the center of each plot were dug on 13 September 1995 (Keystone Resistant Giant and California Wonder) and 28 September 1995 (Carolina Cayenne). Each root system was rated for severity of galling where 1 = 0 to 3% root system galled, 2 = 4 to 25%, 3 = 26 to 50%, 4 = 51 to 79%, and 5 = ≥80% root system galled (Thies et al., 1998). *Meloidogyne incognita* eggs were extracted from a 20-g bulked subsample of roots from each plot using 1% NaOCl (Hussey and Barker, 1973), and eggs were counted using a stereomicroscope.

Data analysis: Data were analyzed using the general linear models (GLM) procedure of SAS for Windows, v. 6.12 (SAS Institute, Cary, NC), and means were compared using Duncan's multiple-range test.

Results

Carolina Cayenne, Keystone Resistant Giant, and California Wonder showed no evidence of root-galling and had extremely low nematode reproduction in the roots (<250 *M. incognita* eggs/g fresh root) in the methyl bromide fumigated plots (Table 1). Plants grown in plots fumigated with methyl bromide had lower gall indices and fewer *M. incognita* eggs per g fresh root ($P \leq 0.05$) than plants grown in untreated plots (averaged over all cultivars). Fumigation treatment x cultivar interactions were observed for all parameters except total numbers of fruit pods. Carolina Cayenne had a root gall severity index of 1.2 and supported 4,339 *M. incognita* eggs/g fresh root in the untreated plots. Fruit yields and fruit numbers of Carolina Cayenne plants grown in the methyl bromide fumigated plots were similar to those of plants grown in the untreated control plots (Table 1). Keystone Resistant Giant had a root gall index of 2.9 and supported 26,505 *M. incognita* eggs/g fresh root in the untreated plots. Root systems of Keystone Resistant Giant plants grown in untreated plots had similar amounts of fibrous roots as those of

Table 1. Gall index, numbers of *Meloidogyne incognita* eggs extracted from roots, marketable and total fruit yield, and marketable and total fruit number of three pepper cultivars grown in methyl bromide fumigated or untreated soil.

Soil fumigation treatment/ pepper cultivar[a]	Gall index[b]	No eggs/g fresh root	Marketable fruit yield (kg/ha)	Total fruit yield (kg/ha)	Marketable fruit (no./ha)	Total fruit (no./ha)
Methyl bromide						
Carolina Cayenne	1.0 a[c]	38 a	16,511 A[d]	17,188 A[d]	104,204 A[d]	176,888 A[d]
Keystone Resistant Giant	1.0 a	231 a	10,507 ab	15,984 ab	13,408 ab	33,637 a
California Wonder	1.0 a	221 a	13,777 b	19,337 b	17,407 b	31,990 b
Untreated control						
Carolina Cayenne	1.2 a	4,339 a	20,484 A	20,950 A	125,139 A	187,473 A
Keystone Resistant Giant	2.9 b	26,505 b	10,351 ab	14,801 ab	14,349 ab	29,403 a
California Wonder	4.3 c	48,569 c	6,209 a	9,950 a	8,233 a	20,700 a
			F ratios for main effects and interaction effects[e]			
Fumigation (Fum)	197.50****[h]	141.72****	12.46***	16.84**	11.73***	6.11**
Block	0.87 ns	3.18*	13.65***	12.41***	10.32**	21.57***
Fum × block	0.62 ns	3.18 ns	3.99*	4.11*	3.87*	4.35*
Cultivar (Cvr)	48.45***	33.66****	0.16 ns	0.34 ns	0.82 ns	9.59*
Fum × Cvr	48.26****	33.11****	11.47**	10.15**	15.69**	4.07 ns

[a] Plots were fumigated with 651 kg/ha (w/w) 98% CH_3Br:2%CCl_3NO_2 before planting or left untreated.
[b] Gall index: 1 = 0 to 3% root system galled; 2 = 4 to 25%, 3 = 26 to 50%, 4 = 51 to 79%, and 5 = ≥ 80% root system galled.
[c] Mean separation within columns by Duncan's multiple-range test ($P < 0.05$).
[d] Means for Carolina Cayenne were compared separately from those of the two bell pepper cultivars because of the difference in fruit types.
[e] F ratios of effects for gall index and numbers of eggs per g fresh root are from the analysis of all three cultivars; F ratios for marketable fruit yields, total fruit yields, marketable fruit numbers, and total fruit numbers are from the analysis of the two bell cultivars only. Fruit yield and fruit numbers data were analyzed separately for Carolina Cayenne because of the difference in fruit types.
[h] *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, ****$P \leq 0.0001$, ns = non-significant.

plants of this cultivar grown in methyl bromide fumigated plots (Fig. 1A, B). Fruit yields and fruit numbers of Keystone Resistant Giant plants grown in the methyl bromide fumigated plots were similar to that of plants grown in the untreated control plots (Table 1). California Wonder had a root gall index of 4.3 and supported 48,569 *M. incognita* eggs/g fresh root. In untreated plots, the root systems of California Wonder had more severe root galling ($P \leq 0.05$) and fewer fibrous roots than root systems of Keystone Resistant Giant (Table 1; Fig. 1B). Marketable and total fruit yields of California Wonder plants grown in methyl bromide fumigated plots were approximately twice as large ($P \leq 0.05$) as yields from plants grown in untreated plots. Numbers of marketable fruit produced by California Wonder plants grown in methyl bromide fumigated plots were nearly four times ($P \leq 0.05$) the numbers produced by plants grown in untreated plots.

In the untreated control plots, the root gall severity index for Carolina Cayenne was 58.6% less ($P \leq 0.05$) than the root gall severity index for Keystone Resistant Giant and 72.1% less ($P \leq 0.05$) than that for California Wonder. Likewise, numbers of eggs per g fresh root for Carolina Cayenne were 86.6% less ($P \leq 0.05$) than the numbers of eggs per g fresh root for Keystone Resistant Giant and 91.1% less ($P \leq 0.05$) than for California Wonder. Fruit yields and fruit numbers of Carolina Cayenne were not compared with those of the two bell-type cultivars because of differences in types of fruit. However, differences in fruit yields and fruit numbers were not detectable between Keystone Resistant Giant and California Wonder within the soil fumigation treatments.

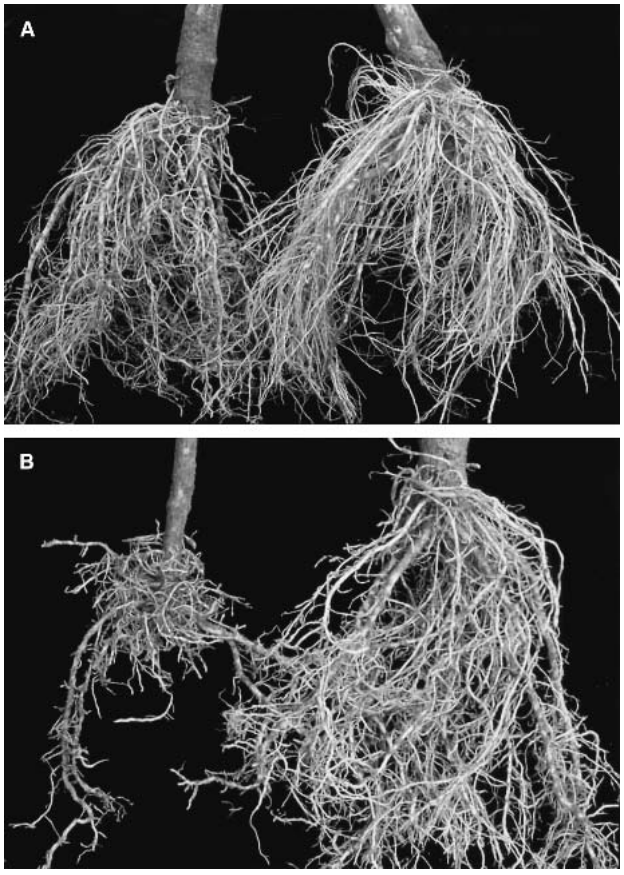

FIG. 1. A) Roots of Keystone Resistant Giant from untreated control plots (left) and from plots fumigated with methyl bromide (right). B) Roots of California Wonder (left) and Keystone Resistant Giant (right) from untreated plots.

DISCUSSION

The methyl bromide fumigation treatment gave a high level of nematode control as evidenced by the lack of root galling and extremely low nematode reproduction (<250 *M. incognita* eggs/g fresh root tissue) on Carolina Cayenne, Keystone Resistant Giant, and California Wonder. Carolina Cayenne exhibited high resistance to *M. incognita* in the untreated plots. The performance of Carolina Cayenne in both the methyl bromide fumigated plots and the untreated plots demonstrated the effectiveness of its resistance in soils that are infested with damaging population densities of *M. incognita*. For example, Carolina Cayenne sustained minimal root galling, supported by low reproduction of *M. incognita*, and produced similar fruit yields in both the methyl bromide fumigated plots and the untreated plots. These results confirm those of previous tests where Carolina Cayenne exhibited high resistance to *M. incognita*, both in field tests where plants were inoculated with *M. incognita* at planting and in tests conducted in fields that were highly infested with *M. incognita* (Thies et al., 1997, 1998). Fruit yields of Carolina Cayenne in the present study were similar to those of previous experiments conducted at the U.S. Vegetable Laboratory, Charleston, South Carolina, and at the Edisto Research and Education Center, Clemson University, Blackville, South Carolina (Thies et al., 1997).

Keystone Resistant Giant exhibited an intermediate reaction to *M. incognita* in the untreated plots, i.e., root galling and nematode reproduction were intermediate between those for the resistant Carolina Cayenne and the susceptible California Wonder. This reaction to *M. incognita* suggests that Keystone Resistant Giant may have a minor gene that conditions an intermediate level of resistance to *M. incognita*. Keystone Resistant Giant exhibited a less severe root-galling reaction and supported fewer eggs per g fresh root than California Wonder in the untreated plots, confirming results of a previous field study in which Keystone Resistant Giant had less severe root galling ($P \leq 0.05$) and supported fewer eggs per g fresh root ($P \leq 0.05$) than California Wonder (Thies et al., 1998). The authors of the present study have observed similar intermediate reactions of Keystone Resistant Giant in several greenhouse and field tests (Thies and Fery, unpubl. data). The moderately high reproduction of *M. incognita* (approximately 26,500 eggs/g fresh root) in the untreated plots and

the lack of difference in fruit yields between the methyl bromide fumigated and untreated plots of the present study suggest that this cultivar may be tolerant to *M. incognita*.

California Wonder exhibited a highly susceptible reaction to *M. incognita* in the untreated plots of the present study. These results confirm those of a previous field study, in which California Wonder had a root gall severity index of 3.5 and supported approximately 18,800 *M. incognita* eggs/g fresh root (Thies et al., 1998). In the present study, marketable fruit yields and fruit numbers of California Wonder were approximately doubled when grown in methyl bromide fumigated plots compared to untreated plots. Likewise, when California Wonder was grown in rotation following Carolina Cayenne, fruit yields were approximately 4.5-fold greater than when California Wonder was grown following PA-136, a highly susceptible cayenne pepper line that enhanced the build-up of soil populations of *M. incognita* (Thies et al., 1998).

As Carolina Cayenne exhibited a high level of resistance to *M. incognita* in both the methyl bromide fumigated and untreated plots, host plant resistance of this type appears to be sufficiently high that pre-plant soil fumigation with methyl bromide should not be necessary to control *M. incognita*, even in highly infested fields. Resistant pepper cultivars such as Carolina Cayenne and the recently released bell-type cultivars Charleston Belle and Carolina Wonder (Fery et al., 1998) also should be valuable as rotation crops in developing alternative strategies for managing southern root-knot nematode in susceptible vegetable crops.

Literature Cited

Dukes, P. D., R. L. Fery, and J. A. Thies. 1997. PA-136 pepper, an exceptional host for production of southern root-knot nematode inoculum. HortScience 32:335.

Fery, R. L., P. D. Dukes, and W. L. Ogle. 1986. 'Carolina Cayenne' pepper. HortScience 21:330.

Fery, R. L., P. D. Dukes, Sr., and J. A. Thies. 1998. 'Carolina Wonder' and 'Charleston Belle': Southern root-knot nematode resistant bell peppers. HortScience 33:900–902.

Hussey, R. S., and K. R. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Disease Reporter 57:1025–1028.

Thies, J. A., and R. L. Fery. 2000. Characterization of resistance conferred by the *N* gene to *Meloidogyne arenaria* races 1 and 2, *M. hapla,* and *M. javanica* in two sets of isogenic lines of *Capsicum annuum*. Journal of American Society of Horticultural Science 125:71–75.

Thies, J. A., J. D. Mueller, and R. L. Fery. 1997. Effectiveness of resistance to southern root-knot nematode in 'Carolina Cayenne' pepper in greenhouse, microplot, and field tests. Journal of American Society of Horticultural Science 122:200–204.

Thies, J. A., J. D. Mueller, and R. L. Fery. 1998. Use of a resistant pepper as a rotational crop to manage southern root-knot nematode. HortScience 33:716–718.

Thomas, S. H., L. W. Murray, and M. Cardenas. 1995. Relationship of preplant population densities of *Meloidogyne incognita* to damage in three chile pepper cultivars. Plant Disease 79:557–559.

U.S. Department of Agriculture. 2001. Atmospheric impact of agricultural use of methyl bromide. Methyl Bromide Alternatives 7:1–2.