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Greenhouse and field resistance in cucumber to root-knot nematodes

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Summary – Ten cultigens were evaluated for resistance to *Meloidogyne arenaria* races 1 and 2, and *M. javanica* under greenhouse and field conditions. Resistance to *M. arenaria* races 1 and 2, and *M. javanica* was verified in *Cucumis sativus* var. *hardwickii* line LJ 90430 and to *M. arenaria* race 2 in *C. sativus* var. *sativus* Southern Pickler and Mincu in a greenhouse test. Another cultigen of *C. sativus* var. *hardwickii* (PI 215589) was found to be resistant to *M. arenaria* race 2 but not to other root-knot nematode species tested. LJ 90430 is the cultigen of choice to develop root-knot nematode resistant cucumbers, since it has multiple root-knot nematode resistance and is cross-compatible with cucumber. Greenhouse and field data were positively correlated ($r = 0.74$) over both years. Experiment repeatabilities were calculated from the cultigens infected with root-knot nematodes under both greenhouse and field conditions. Four environments (greenhouse and field over 2 years) were used in the analysis. Repeatabilities were high in all instances (ranging from 0.83-0.99) and indicated that the environment (field or greenhouse) was not an important factor in assessing root-knot nematode resistance for the cultigens evaluated.

Zusammenfassung – ***Resistenz von Gurken gegen Wurzelgallennematoden im Gewächshaus und im Freiland*** – Unter Gewächshaus- und Freilandbedingungen wurden zehn Cultigene auf ihre Resistenz gegen *Meloidogyne arenaria* Rassen 1 und 2 und gegen *M. javanica* geprüft. Bei *Cucumis sativus* var. *hardwickii* Linie LJ 90430 wurde im Gewächshausversuch Resistenz gegen *M. arenaria* Rassen 1 und 2 sowie gegen *M. javanica* nachgewiesen, und in *C. sativus* var. *sativus* "Southern Pickler" und "Mincu" Resistenz gegen *M. arenaria* Rasse 2. Cultigen *C. sativus* var. *hardwickii* (PI 215589) war resistent gegen *M. arenaria* Rasse 2 aber nicht gegen die anderen geprüften Arten von Wurzelgallennematoden. LJ 90430 ist das Cultigen der Wahl bei der Entwicklung von Gurken, die gegen Wurzelgallennematoden resistent sind, da es multiple Resistenzen gegen Wurzelgallennematoden besitzt und kreuzungsverträglich mit Gurke ist. Die Ergebnisse der Gewächshaus- und Feldversuche waren über beide Versuchsjahre hin positiv korreliert ($r = 0,74$). Ausgehend von den Cultigenen, die im Gewächshaus und im Freiland mit Wurzelgallennematoden infiziert waren, wurden die Wiederholbarkeiten der Versuche berechnet. Dabei wurden vier verschiedene Umweltbedingungen (Gewächshaus und Freiland über zwei Jahre) verwendet. Die Wiederholbarkeiten waren in allen Fällen hoch (0,83-0,99) und zeigten an, dass die Umwelt (Freiland oder Gewächshaus) kein wichtiger Faktor bei der Bestimmung der Resistenz gegen Wurzelgallennematoden bei den geprüften Cultigenen war.

Keywords: *Cucumis sativus*, cucurbit, disease resistance, *Meloidogyne arenaria*, *M. hapla*, *M. incognita*, *M. javanica*, vegetable breeding.

Root knot, caused by *Meloidogyne* spp. is one of the most important diseases in tropical and sub-tropical areas of the world where cucumber (*Cucumis sativus* L.) is grown (Netscher & Sikora, 1990). Three root-knot nematode species (*M. arenaria*, *M. incognita*, and *M. javanica*) are of primary importance in the southeastern U.S. Cultivars resistant to one or more of these species would be useful to cucumber growers, providing disease control with minimal use of nematicides. Many advances have been made in breeding for root-knot nematode resistance in several important horticultural crops, but little progress has been made in cucumber (Fassuliotis, 1979). Recently, resistance to *M. arenaria* races 1 and 2, and *M. javanica* was found in *C. sativus* var. *hardwickii* (R.) Alef. line LJ 90430 (Walters *et al.*, 1996); and resistance to *M. arenaria* race 2 was found in *C. sativus* Southern Pickler, Mincu, Producer, and Poinsett (Walters *et al.*, 1993). The purpose of this study was to verify the resistance in these cultivars, breeding lines, and accessions (hereafter collectively referred to as cultigens) as well as to determine if greenhouse resistance was effective under field conditions.

Materials and methods

Verification of greenhouse resistance

Four cucumber (*C. sativus* var. *sativus*) cultigens, Southern Pickler, Mincu, Sumter, and Wisconsin SMR-18, two cultigens of *C. sativus* var. *hardwickii* (PI 215589 and line LJ 90430), and two cultigens of *C. metuliferus* (PI 482443 and PI 482454) were evaluated for resistance in a greenhouse to six root-knot nematodes (*M. arenaria* races 1 and 2, *M. incognita* races 1 and 3, *M. javanica*, and a Wisconsin population of *M. hapla*) to verify the results of an earlier study (Walters *et al.*, 1993).

Plants were grown from seed in 15 cm diam. (1750 cm^3 volume) clay pots that contained a moist, steam-sterilized loamy sand (85% sand, 10% silt, and 5% clay). At the two-leaf stage, plants were thinned to 1/pot. Greenhouse temperatures averaged 24 to 32°C (day) and 20 to 24°C (night). The experiment was a split-plot treatment arrangement in a randomized complete block design with 4 replications. Whole plots were the six nematodes and subplots were the eight cultigens.

Each pot was infested with 5000 root-knot nematode eggs two weeks after planting. Eggs were extracted from infected Rutgers tomato (*Lycopersicon esculentum* Mill.) roots using a 1% NaOCl solution stirring for 4 min (Byrd *et al.*, 1972). Pots were irrigated twice daily using drip irrigation with fertilizer injection.

Ten weeks after soil infestation, plants were harvested and rated for the percentage (0 to 100%) of the roots galled (Barker *et al.*, 1986). Roots were weighed and cut into 1 to 3 cm long segments, and eggs were extracted from a 5 g subsample of roots (Byrd *et al.*, 1972). Number of eggs per root system was counted and used to calculate reproduction factor.

Cultigen means for gall index and reproduction factor [$Rf = Pf$ (final egg number per root system)/Pi (initial inoculum level)] were compared using Fisher's LSD at $P \leqslant 0.05$. Data were analyzed utilizing the GLM procedure of SAS (Anon., 1989).

Correlation of greenhouse with field resistance

A second experiment was run in the greenhouse and field in 1994 and 1995. The treatments were ten cultigens and three nematodes (*M. javanica*, and *M. arenaria* races 1 and 2). Field sites were infested with *M. javanica*, *M. arenaria* race 1, or *M. arenaria* race 2, and planted with the same cultigens.

Cultigens were chosen based on resistance from previous tests. LJ 90430 was reported to have resistance to all three nematodes. Southern Pickler and Mincu have resistance to *M. arenaria* race 2, and Producer is a parent of Southern Pickler. Susceptible checks included in this experiment were Straight 8, Coolgreen, WI 2757, Poinsett, Sumter, and Wisconsin SMR-18.

Greenhouse test. Plants were grown from seed in 15 cm diam. (1750 cm^3 volume) clay pots filled with a moist, steam-sterilized loamy sand (85% sand, 10% silt, and 5% clay). At the two-leaf stage, plants were thinned to 1/pot. Temperatures averaged 35°C days and 24°C nights. Plants were irrigated twice daily, and fertilized once a week with Peter's® 20-20-20 (N-P-K) (W.R. Grace & Co., Fogelsville, PA, USA).

The experiment was a split-plot treatment arrangement in a randomized complete block design with four replications. Whole plots were the three nematodes and subplots were the ten cultigens.

Root-knot nematode populations were maintained on Rutgers tomato in the greenhouse for use as inoculum. Inoculum was prepared by extracting eggs from infected roots using a 1% NaOCl solution and stirring for 4 min (Byrd *et al.*, 1972). Pots were infested 2 weeks after seeding with 5000 root-knot nematode eggs. Plants were evaluated 12 weeks after planting (10 weeks after inoculation), using the gall-index system described previously.

Field test. Three fields at the Central Crops Research Station, Clayton, North Carolina, were each infested on 10 May 1994 with one of three root-knot nematodes: *M. javanica*, *M. arenaria* race 1, or *M. arenaria* race 2. Nematodes were allowed to increase on a tobacco (*Nicotiana tabacum* L.) crop grown in the field; *Meloidogyne arenaria* race 1 numbers were increased on Coker 371-Gold, and *M. javanica* and *M. arenaria* race 2 were increased on Speight G-70. Eggs of each root-knot nematode were extracted from Rutgers tomato using a 1% NaOCl solution and stirring for 4 min (Byrd *et al.*, 1972). Initial field infestation was accomplished by inoculating each plant with 2500 eggs utilizing the technique described by Fortnum *et al.* (1987).

Plants were transplanted at an in-row spacing of 55 cm with rows 1.2 m apart and 600 m long. For the second year of the test, tobacco plants were transplanted the second week of May and were not inoculated (assuming that the inoculum would carry over from the previous year). Approximately 2 months after planting, all fields were sampled to determine the root-knot population density. Fifteen cores were taken randomly from each infested row,

and numbers of nematodes were determined. Soil samples were processed by elutriation and centrifugation to extract juveniles (Byrd *et al.*, 1976), and roots were collected during elutriation for counting of eggs in roots (Byrd *et al.*, 1972). All rows had more than 250 nematodes/500 cm^3 soil. After an estimation of root-knot nematode population densities, the tobacco was mowed at soil level, and the field was disked so that beds could be formed on 1.2 m centers. The cucumber test was established during the first week of August for both years.

Three field experiments were conducted to evaluate the resistance of the 10 cultigens (listed previously in the greenhouse test) to *M. javanica*, *M. arenaria* race 1, or *M. arenaria* race 2. Experiments were designed as a randomized complete block with four replications. Two plants were grown in each field plot. Plots were 1.5 m long and were planted on raised, shaped beds 1.2 m apart (centre to centre), and separated at each end by 1.5 m alleys. Guard rows of susceptible Sumter surrounded each test.

Plants were harvested 10 weeks after planting (mid-October) and root-gall index data were obtained. Gall-index data were analyzed using the GLM, CORR, and VARCOMP procedures of SAS (Anon., 1989).

The greenhouse and field data were used to calculate an experiment repeatability. Repeatability was estimated from cultigen gall-index data for 1994, 1995, and both years for each root-knot nematode as well as over all three nematodes as follows: $(R) = \sigma^2G/(\sigma^2G + \sigma^2GE/e + \sigma^2\,error/re)$ where R = repeatability, σ^2G = genotypic variance, σ^2GE = genotype-environment interaction variance, σ^2error = experimental error variance, e = number of environments, r = number of replications within environments (Halluer & Miranda, 1981). This formula was used because it contains the genotype × environment variance component and allows one to observe the effects of the environment on cultigens infected with root-knot nematodes.

Results

Verification of greenhouse resistance

Accessions (PI 482443 and PI 482454) of *C. metuliferus* were resistant to all root-knot nematodes evaluated (gall indices < 20% and reproduction factors of 1 or less in all instances). These accessions were the only cultigens tested that were resistant to *M. incognita* races 1 and 3; all others were susceptible (Table 1). A cultigen is considered highly resistant if it has a gall-index rating < 10% and resistant if < 20%. *Cucumis sativus* var. *hardwickii* PI 215589 and line LJ 90430 showed some resistance to *M. incognita* race 3 (Table 1). Previously, we did not find this resistance in LJ 90430 (Walters *et al.*, 1993). PI 215589 had a slightly higher gall index than did LJ 90430, but supported less nematode reproduction than did LJ 90430. However, additional testing of LJ 90430 (data not shown) revealed that it was not resistant to *M. incognita* race 3. Based on gall indices, resistance to *M. incognita* race 3 in the *C. sativus* var. *hardwickii* cultigens was less ($P \leqslant 0.05$) than what was found in the *C. metuliferus* accessions. However, reproduction factors did not differ between the *C. sativus* var. *hardwickii* cultigens and the *C. metuliferus* cultigens (Table 1). LJ 90430 and the accessions of *C. metuliferus* were resistant to *M. arenaria* race 1. Several cultigens were resistant to *M. arenaria* race 2 and expressed little galling and no reproduction (Table 1). Only LJ 90430 and the *C. metuliferus* cultigens were highly resistant to *M. arenaria* race 1 and *M. javanica*. Sumter and Wisconsin SMR-18, the two susceptible controls, showed susceptibility to *M. incognita* races 1 and 3, *M. arenaria* races 1 and 2, and *M. javanica*.

Zimmer and Walkof (1968) reported that *M. hapla* was a problem of cucumber mainly in northern areas of North America. A population of *M. hapla* was obtained from Wisconsin, and all cultigens tested were found to be resistant with no reproduction occurring on any of the cultigens (Table 1). Sumter had a gall index of 14 which was the highest of all cultigens infected with *M. hapla*, but was not statistically different ($P \leqslant 0.05$) from most of the other cutigens tested. Thus, all cultigens of cucumber appear to be resistant to *M. hapla*, confirming an earlier study using a North Carolina population of *M. hapla* (Walters *et al.*, 1990).

Correlation of greenhouse with field resistance

The greenhouse test was run to verify resistance of LJ 90430 to *M. javanica*, and *M. arenaria* races 1 and 2, and of Southern Pickler and Mincu to *M. arenaria* race 2 (Table 2). All other cultigens tested were susceptible to all three root-knot nematodes. Cultigens found resistant in greenhouse tests were also resistant under field conditions (Table 2). However, the resistance of LJ 90430 to *M. arenaria* race 2 was of a higher level than that found in either Southern Pickler or Mincu in both greenhouse and field tests (Table 2).

Table 1. *Resistance of* Cucumis sativus, C. s. *var.* hardwickii, *and* C. metuliferus *to all major species of root-knot nematodes in a greenhouse test*

Cultigen	Mi1		Mi3		Ma1		Ma2		Mj		Mh	
	Gi	*Rf*	*Gi*	*Rf*	*Gi*	*Rf*	*Gi*	*Rf*	*Gi*	*Rf*	*Gi*	*Rf*
LJ 90430[1]	53	18	26	2	11	0	8	0	10	0	9	0
Mincu	55	39	45	13	31	5	14	0	41	3	10	0
Southern Pickler	63	28	35	6	33	3	13	0	30	6	12	0
PI 215589[1]	48	15	31	1	23	6	17	0	46	19	9	0
PI 482443[2]	10	0	7	1	9	0	6	0	7	0	2	0
PI 482454[2]	7	0	12	0	15	0	8	0	4	1	6	0
Sumter[3]	52	29	43	15	43	10	33	2	48	25	14	0
Wis. SMR-18[3]	57	40	38	7	29	13	23	2	40	33	7	0
Mean	43	21	28	6	24	5	15	1	28	11	9	0
LSD ($P \leqslant 0.05$)	18	43	14	9	11	9	4	1	15	24	9	0

Data presented are means of 4 replications. Plants were rated for resistance using the gall-index (*Gi*) system (0-100% of roots galled) 10 weeks after planting (8 weeks after inoculation). $Rf = Pf$ (final nematode density)/*Pi* (initial nematode density) and is calculated as final number of eggs in roots/5000 (number of eggs that each pot was initially inoculated with). Mi1 = *Meloidogyne incognita* race 1, Mi3 = *M. incognita* race 3, Ma1 = *M. arenaria* race 1, Ma2 = *M. arenaria* race 2, Mj = *M. javanica*, and Mh = *M. hapla*, Wisconsin population.

[1] *Cucumis sativus* var. *hardwickii*.

[2] *Cucumis metuliferus*.

[3] Susceptible controls.

Table 2. *Root-knot nematode gall indices for* Cucumis *cultigens tested in greenhouse and field for 1994 and 1995*

Cultigen	Field				Greenhouse			
	Mean	Ma1	Ma2	Mj	Mean	Ma1	Ma2	Mj
Southern Pickler	53	70	24	68	54	51	33	78
Mincu	50	67	26	60	54	51	33	78
LJ 90430[1]	13	18	8	13	8	6	11	6
Poinsett	64	69	50	74	66	58	67	73
Producer	62	72	48	66	76	68	78	84
Coolgreen	63	66	58	64	75	69	78	77
Straight 8	67	69	64	69	80	71	86	83
WI 2757	66	67	67	65	65	60	62	75
Wis. SMR-18	68	71	64	69	74	64	78	81
Sumter	65	70	56	69	69	58	68	82
Mean	57	64	46	62	62	55	60	71
LSD ($P \leqslant 0.05$)	18	13	8	11	17	12	7	12

Data for each root-knot nematode are means of eight replications (four replications/year) of one plant (greenhouse) or two plants (field). The gall index system rates the percentage of the roots that are galled, ranging from 0 to 100%. Ma1 = *Meloidogyne arenaria* race 1, Ma2 = *M. arenaria* race 2, and Mj = *M. javanica*.

[1] *Cucumis sativus* var. *hardwickii*.

Table 3. *Correlations (r) of root-knot nematode gall-index data for* Cucumis *cultigens in greenhouse versus field over two years*

Year	Correlations			
	Overall	Ma1	Ma2	Mj
1994	0.72	0.70	0.79	0.83
1995	0.76	0.81	0.91	0.87
Both Years	0.74	0.75	0.85	0.84

All correlations are significant at $P \leq 0.0001$. Ma1 = *Meloidogyne arenaria* race 1, Ma2 = *M. arenaria* race 2, and Mj = *M. javanica*.

Table 4. *Greenhouse versus field repeatability of root-knot nematode root-gall development for* Cucumis *cultigens tested in 1994 and 1995*

Cultigens[2]	Experiment Repeatability[1]			
	Overall	Ma1	Ma2	Mj
Field-Greenhouse 1994				
Ten	0.90	0.91	0.90	0.92
Six	0.92	0.95	0.83	0.92
Three	0.97	0.98	0.98	0.94
Field-Greenhouse 1995				
Ten	0.94	0.97	0.98	0.95
Six	0.95	0.99	0.99	0.97
Three	0.99	0.99	0.99	0.98
Both Years				
Ten	0.97	0.98	0.98	0.97
Six	0.97	0.99	0.98	0.98
Three	0.99	0.99	0.99	0.99

[1] Experiment repeatability: $R = \sigma^2G/(\sigma^2G + \sigma^2GE/e + \sigma^2error/re)$, where σ^2G = genotypic variance, σ^2GE = genotype-environment interaction variance, σ^2error = experimental error variance, e = number of environments, and r = number of replications within environments. Ma1 = *Meloidogyne arenaria* race 1, Ma2 = *M. arenaria* race 2, and Mj = *M. javanica*.

[2] ten = all cultigens evaluated; six = Southern Pickler, Mincu, Producer, LJ 9040, Wis. SMR-18, and Sumter; three = LJ 90430, Wis. SMR-18, and Sumter.

Greenhouse and field data were highly correlated for root galling in both 1994 and 1995 for all nematodes (Table 3). There was a significant correlation ($r = 0.74$, $P = 0.0001$) between field and greenhouse for all nematodes over both years. Some of the variability observed between greenhouse and field for gall-index was due to susceptible cultigens. Depending on the environment, these cultigens often developed fewer or more root galls. Most important, however, was that LJ 90430 was consistently resistant in the field and greenhouse tests (Table 2).

Repeatabilities were calculated based on two environments (greenhouse and field for 1994 and 1995), and on four environments (greenhouse and field over years). In analyzing data, three cultigen combinations were used: *i*) all cultigens, *ii*) resistant cultigens (LJ 90430, Southern Pickler, and Mincu) plus check cultigens (Sumter, Producer, and Wisconsin SMR-18), and *iii*) one highly resistant cultigen (LJ 90430) plus two susceptible checks (Sumter and Wisconsin SMR-18). In all instances, repeatabilities were high, ranging from 0.83 to 0.99 (Table 4). Repeatabilities were essentially the same for all three nematodes and were slightly higher for 1995 compared to 1994. The repeatabilities calculated based on three cultigens (LJ 90430 and two susceptible checks) were slightly higher than the other two cultigen combinations for both years. This difference can be explained by

the ratings for these three cultigens being more consistent compared to the ratings of the cultigens within the other two combinations. The experiment repeatabilities calculated indicated that the environment (field *vs* greenhouse or 1994 *vs*. 1995) was not an important factor in evaluating root-knot nematode resistance.

Discussion

The tests conducted indicated that the environment (field or greenhouse) was not an important factor in assessing root-knot nematode resistance for the cultigens evaluated. Cultigens found to be resistant under greenhouse conditions were also resistant in the field. This is important since cucumbers can be developed under greenhouse conditions for root-knot nematode resistance without the possibility of the resistance breaking down in the field after resistant cultivars have been developed.

The two *C. metuliferus* accessions evaluated (PI 482443 and PI 482454) were resistant to all root-knot nematodes evaluated under both greenhouse and field conditions. However, the genes in *C. metuliferus* controlling root-knot nematode resistance cannot be readily used in developing root-knot nematode resistant cucumber cultivars, since *C. metuliferus* is not cross-compatible with cucumber (*C. sativus*). This is primarily due to the difference in chromosome number between the two species, and all attempts to produce interspecific hybrids have failed (Deakin *et al.*, 1971). LJ 90430 is resistant to *M. arenaria* races 1 and 2, and *M. javanica*, and the resistance was shown to be high enough to control nematodes under field conditions. LJ 90430 is cross compatible with the cultivated cucumber and should be used to develop root-knot nematode resistant cucumber cultivars, since it is the most resistant cultigen in the species of *C. sativus* having multiple root-knot nematode resistance.

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