

A Single Recessive Gene for Resistance to the Root-Knot Nematode (*Meloidogyne javanica*) in *Cucumis sativus* var. *hardwickii*

S. A. Walters, T. C. Wehner, and K. R. Barker

Resistance to the root-knot nematode (*Meloidogyne javanica*) was identified in the *Cucumis sativus* var. *hardwickii* line LJ 90430. Parents, F_1 , F_2 , and BC_1 to both parents of a cross between Sumter (*Cucumis sativus* var. *sativus*) and LJ 90430 were evaluated in two greenhouse experiments to determine genetics of resistance to *M. javanica*. All F_1 progeny were susceptible, and segregation ratios in the F_2 resulted in 1 resistant:3 susceptible. Backcross progeny to the susceptible parent were susceptible, and the BC_1 to the resistant parent segregated 1 resistant:1 susceptible. Reciprocal crosses did not differ and therefore showed no evidence of maternal or cytoplasmic effects. Results from the crosses of several inbreds (Addis, Gy 14, Gy 57u, Poinsett 87, and Sumter) with LJ 90430 indicated that use of those five different genetic backgrounds had no influence on gene expression. The five F_2 families segregated in a 1 resistant:3 susceptible ratio. The five BC_1 (to LJ 90430) families segregated in a 1 resistant:1 susceptible ratio. F_3 families were developed from resistant and susceptible F_2 plants using the five inbreds. Resistant $F_{2,3}$ families produced progeny that were all resistant, and susceptible $F_{2,3}$ families fit a ratio of 1 susceptible:2 segregating. Thus, resistance to *M. javanica* is conferred by a single recessive gene for which we propose the symbol *mj*.

Root knot, caused by several species of root-knot nematodes (*Meloidogyne* spp.), is an important disease of cucumber (*Cucumis sativus* var. *sativus* L.) in areas of the world where it is grown (Netscher and Sikora 1990). North Carolina is a leading producer of field-grown cucumbers in the United States, ranking second in the production of pickling cucumbers (USDA 1993) and fourth in the production of slicing cucumbers (Jewell 1987). Economically, root knot is the most damaging disease of cucumbers in North Carolina (St. Amand and Wehner 1991). The use of resistant cultivars would be the most economical and environmentally acceptable way to control this disease. However, no

cultivars are available that have resistance to the most important root-knot nematodes in cucumber producing areas: *M. incognita* (Kofoid and White) Chitwood and *M. arenaria* (Neal) Chitwood in subtropical areas, and *M. javanica* (Treub) Chitwood in tropical areas. Walters et al. (1993) evaluated the U.S. cucumber germplasm collection and found only the *Cucumis sativus* var. *hardwickii* (R.) Alef. line LJ 90430 to be highly resistant to *M. javanica*.

An understanding of the inheritance of resistance to *M. javanica* in LJ 90430 is essential for the development of resistant cultivars. The mode of inheritance of resistance in cucumber to any species of root-knot nematode is not known. Thus, the objective of this study was to determine the genetics of resistance to *M. javanica* in LJ 90430.

Material and Methods

Germplasm

Cucumis sativus var. *hardwickii* line LJ 90430 was used as the resistant parent in crosses with susceptible *Cucumis sativus* var. *sativus* Sumter. Sumter, an inbred developed at Clemson University (Clemson, South Carolina), was a commonly used pickling cucumber in the previous two decades in the United States. LJ 90430 is difficult to work with because seeds often germinate slowly and at a low percentage. However, two treatments were used to overcome seed coat dormancy and improve germination: soaking the seeds overnight in acetone (Amritphale et al. 1993), or soaking the seeds in water overnight, peeling off and discarding the seed coats, and planting the seeds (Weston et al. 1992).

Resistance to *M. javanica* in LJ 90430 was reported in a previous study (Walters et al. 1993). Reciprocal F_1 , F_2 , and BC_1 (to the susceptible and resistant parents) crosses between Sumter and LJ 90430 were made in the greenhouse using standard pollination techniques for cucumber (Whitaker and Davis 1962). Crosses between LJ 90430 and five cucumber inbreds (Addis, Sumter, Poinsett 87, Gy 57u, and Gy 14) were made in the greenhouse to produce seeds of the F_2 and BC_1 (to LJ 90430) generations.

Inoculation and Rating of Resistance

The following methods were used for nematode inoculation: (1) two seeds were sown in a 15 cm diameter (1,750 cm³ volume) clay pot containing a sterile, moist

loamy soil (80% sand, 15% silt, and 5% clay); (2) plants were thinned to one per pot at the second true leaf stage; (3) *Meloidogyne javanica* inoculum was produced in the greenhouse on Rutgers tomato (*Lycopersicon esculentum* Mill.); (4) nematode eggs were extracted from roots for 4 min using a 1% NaOCl solution according to the method of Byrd et al. (1972); and (5) each plant was inoculated 2 weeks after planting (second true leaf stage) with 5,000 eggs that were applied to roots using a suspension of eggs in water poured onto the soil. The first two methods were different for the split-root test and are explained under the materials and methods for that test.

Plants were harvested 10 weeks after inoculation (12 weeks after planting) and rated for the percentage (0–100%) of the root system galled (Barker et al. 1986). Plants were classified as resistant ($\leq 35\%$ of root system galled) or susceptible ($> 35\%$ of root system galled) based upon the distribution of F_2 plants infected with *M. javanica* (Figure 1). For the split-root test, the numbers of egg masses on roots from each pot were counted using the method of Hadisoeganda and Sasser (1982), and plants were rated as either resistant (egg masses ≤ 15 per root system) or susceptible (egg masses > 15 per root system). Goodness-of-fit of observed to expected segregation ratios in the F_2 and backcross progeny were determined by chi-square tests.

Inheritance

The F_1 , F_2 , and BC_1 (to the susceptible and resistant parents) of the cross Sumter \times LJ 90430 (no reciprocals), as well as the parents, were evaluated in a split-root test. Seeds were initially sown in flats containing vermiculite, and at the appearance of the first true leaf, the root system of each plant was split into three separate 10 cm diameter (450 cm³ volume) plastic pots that contained a sterile, moist loamy sand soil (80% sand, 15% silt, and 5% clay). The three pots per plant as well as the single plant plots were arranged in a completely random design. Each pot containing one-third of the root system of a plant was inoculated with one of three root-knot nematodes, but only the *M. javanica* data will be presented here. Plants were watered and fertilized twice daily using drip irrigation with fertilizer injection.

The number of egg masses was used to confirm segregation ratios obtained from the gall index rating for the split-root greenhouse test. After rating for gall index

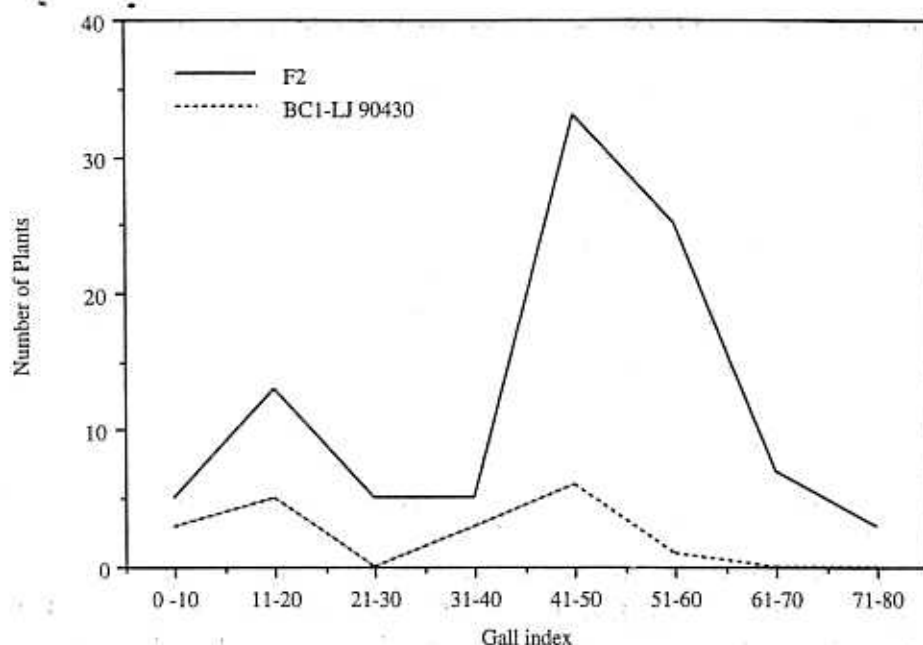


Figure 1. Distribution of F_2 and BC_1 to LJ 90430 plants, based on gall indices, from the cross of Sumter \times LJ 90430 infected with *Meloidogyne javanica*.

(percentage of roots galled), roots were placed in a solution of tap water and 0.15 g/l of Phloxine B (Sigma Chemical Company, St. Louis, Missouri) for 20 min, then rinsed with tap water to remove residual stain (Hartman and Sasser 1985). The numbers of egg masses on roots from each pot were counted using the method of Hadisoeganda and Sasser (1982), and plants were rated as either resistant (egg masses \leq 15 per root system) or susceptible (egg masses $>$ 15 per root system). The numbers of egg masses were transformed using \log_{10} (number of egg masses + 1) and correlated with the gall index rating (%). Chi-square tests were used to de-

termine goodness-of-fit from observed to expected segregation ratios in the F_2 and BC_1 generations for gall index and number of egg masses.

Cytoplasmic/Maternal

Parents, F_1 , F_{1R} , F_2 , F_{2R} , BC_1 (to each parent) and BC_{1R} (to each parent) were evaluated in a greenhouse test to determine if any paternal, maternal, or cytoplasmic effects were present for *M. javanica* resistance. Plants were grown in 15 cm diameter (1,750 cm³ volume) clay pots containing the same soil mixture as used in the previous test. Pots were arranged in a completely random design. Plants were wa-

tered twice daily and fertilized once weekly using Peter's[®] 20-20-20 (N-P-K) (W. R. Grace & Co., Fogelsville, Pennsylvania).

Effect of Genetic Background

A greenhouse test was conducted to determine whether genetic background affected the expression of resistance. Forty F_2 plants from each of five families (Addis \times LJ 90430, Sumter \times LJ 90430, Poinsett 87 \times LJ 90430, Gy 57u \times LJ 90430, and Gy 14 \times LJ 90430) and 20 BC_1 (to LJ 90430) were evaluated for resistance to *M. javanica*. Families were arranged in the greenhouse in a completely random design. Individual F_2 plants from the five families were self-pollinated to obtain seeds of the F_3 generation, which were needed to complete the next test.

Fifteen F_3 families were developed by self-pollinating 5 resistant and 10 susceptible F_2 plants in five genetic backgrounds: Addis \times LJ 90430, Sumter \times LJ 90430, Poinsett 87 \times LJ 90430, Gy 57u \times LJ 90430, and Gy 14 \times LJ 90430. Eight plants from each F_3 family were evaluated for *M. javanica* resistance. F_3 families from each cross were arranged in a completely random design in the greenhouse.

If a single recessive gene is responsible for the resistance, all progeny from a self-pollinated, resistant F_2 plant (F_{2R} resistant family) should be entirely resistant. Progeny from a self-pollinated susceptible F_2 plant (F_{2S} susceptible family) should be either entirely susceptible or segregating (1 resistant:3 susceptible). F_3 families developed from susceptible F_2 plants should be present in a 1 susceptible:2 segregating ratio.

Results and Discussion

Inheritance

The susceptible parent Sumter had a gall index rating (percentage of roots galled) that averaged 58. The resistant parent, LJ 90430, had a mean gall index rating of 5. The cross of resistant LJ 90430 with susceptible Sumter produced F_1 progeny that were susceptible to *M. javanica* (Table 1), indicating that all F_1 plants had more than 35% of their roots galled. The segregation observed in the F_2 generation indicated simple inheritance with 1 resistant:3 susceptible, with resistance determined by a single recessive gene. To verify the F_2 ratios, F_1 plants were backcrossed to Sumter or LJ 90430. The BC_1 progeny to Sumter were all susceptible (all had more than 35% of their roots galled) and BC_1 to LJ 90430 progeny segregated 1 resistant:1

Table 1. Segregation for resistance (utilizing both gall indices and numbers of egg masses) to *Meloidogyne javanica* in progenies from crosses (no reciprocals) between susceptible *Cucumis sativus* var. *sativus* Sumter and resistant *Cucumis sativus* var. *hardwickii* line LJ 90430^a

Generation	No. observed (R:S)	No. expected (R:S)	Fitted ratio (R:S)	χ^2	P
Gall index					
Sumter (P_S)	0:10	0:10	All S	—	—
LJ 90430 (P_R)	9:0	9:0	All R	—	—
F_1	0:18	0:18	All S	—	—
F_2	23:73	24:72	1:3	0.06	.82
$BC_1 \times P_S$	0:19	0:19	All S	—	—
$BC_1 \times P_R$	8:10	9:9	1:1	0.22	.66
Egg mass number					
Sumter (P_S)	0:10	0:10	All S	—	—
LJ 90430 (P_R)	9:0	9:0	All R	—	—
F_1	0:18	0:18	All S	—	—
F_2	23:73	24:72	1:3	0.06	.82
$BC_1 \times P_S$	0:19	0:19	All S	—	—
$BC_1 \times P_R$	8:10	9:9	1:1	0.22	.66

^a Gall index: resistant \leq 35% of roots galled, susceptible $>$ 35% of roots galled. Egg mass number: resistant \leq 15 egg masses/root system, susceptible $>$ 15 egg masses/root system. P_S = susceptible parent and P_R = resistant parent.

Table 2. Segregation in progenies from crosses between susceptible *C. sativus* var. *sativus* Sumter and resistant *Cucumis sativus* var. *hardwickii* line LJ 90430 and their reciprocal crosses after inoculation with *Meloidogyne javanica**

Generation	No. observed (R:S)	No. expected (R:S)	Fitted ratio (R:S)	χ^2	P
Sumter (P_s)	0:50	0:50	All S	—	—
LJ 90430 (P_r)	50:0	50:0	All R	—	—
$F_1 (P_s \times P_r)$	0:75	0:75	All S	—	—
$F_1 (P_r \times P_s)$	0:75	0:75	All S	—	—
$F_2 (P_s \times P_r)$	60:173	58:175	1:3	0.09	.77
$F_2 (P_r \times P_s)$	53:151	51:153	1:3	0.10	.75
F_2 (pooled)	113:324	109:328	1:3	0.20	.68
$BC_1 (P_s \times P_r) \times P_s$	0:62	0:62	All S	—	—
$BC_1 (P_r \times P_s) \times P_s$	0:73	0:73	All S	—	—
$BC_1 (P_s \times P_r) \times P_r$	36:32	34:34	1:1	0.24	.65
$BC_1 (P_r \times P_s) \times P_r$	35:37	36:36	1:1	0.06	.82
BC_1 to P_r (pooled)	71:69	70:70	1:1	0.03	.88

* Gall index: resistant $\leq 35\%$ of roots galled, susceptible $> 35\%$ of roots galled. P_s = susceptible parent and P_r = resistant parent.

susceptible (with similar numbers of plants found in the $< 20\%$ and $> 35\%$ ranges, and none found in the 21–35% range). The two BC_1 families reacted as expected if resistance were determined by a single recessive gene. Figure 1 shows the distribution of progeny infected with *M. javanica* from the F_2 and BC_1 to LJ 90430 generations. For F_2 plants, two distinct classes were formed with plants segregating in a 1 resistant:3 susceptible ratio. Observing plants of the BC_1 to LJ 90430 generation, two classes are formed with equal numbers of plants in each class.

Plants of parental, F_1 , F_2 , and BC_1 generations of Sumter \times LJ 90430 were classified similarly using egg masses as they were using the gall index rating (Table 1). For numbers of egg masses, Sumter

ranged from 75 to 175 with an average of 104. The resistant parent, LJ 90430, ranged from 0 to 6 with a mean of 2. All F_1 plants had egg mass numbers greater than 25. F_2 plants segregated in a 1 resistant:3 susceptible ratio using 15 egg masses as the cut-off point between the two classes. The backcross to Sumter produced plants that had egg masses ranging from 18 to 925. The plants from the backcross to LJ 90430 all had low numbers of egg masses, ranging from 0 to 10.

The correlation between gall index and number of egg masses was high ($r = 0.73$, $P = .0001$) when the numbers of egg masses were transformed using \log_{10} (number of egg masses + 1). Therefore, the number of egg masses would be an excellent measure of resistance. The criterion we used

of ≤ 15 and > 15 egg masses for designating resistant and susceptible classes, respectively, was valid since it produced results similar to the gall index data.

Cytoplasmic/Maternal

Progeny of reciprocal crosses reacted similarly in the F_1 and F_2 generations, so there were no maternal, paternal, or cytoplasmic effects for *M. javanica* resistance (Table 2). Data from the reciprocal crosses were pooled for the F_2 and BC_1 to LJ 90430 and are shown in Figure 2. The data indicated that resistant and susceptible F_2 plants were in two distinct classes, with the resistant plants in the smaller class. Also, the BC_1 to LJ 90430 plants form two distinct classes with similar numbers of plants in each class. This again indicated that a single recessive gene was responsible for the resistance.

Effect of Genetic Background

Crosses of five cucumber inbreds (Addis, Poinsett 87, Gy 14, Gy 57u, and Sumter) with LJ 90430 confirmed that the inheritance of resistance to *M. javanica* was conditioned by a single recessive gene. F_2 from all crosses segregated in a ratio of 1 resistant:3 susceptible, and progeny of the BC_1 (to LJ 90430) segregated in a ratio of 1 resistant:1 susceptible (Table 3). Genetic background had no influence on the inheritance of resistance, and thus the gene was expressed in a stable manner and was not affected by epistatic interactions.

Resistant F_2 plants that were self-pollinated to make F_3 families ($F_{2,3}$ resistant families) produced progeny that were entirely resistant (Table 4), with gall indices ranging from 0 to 25%. Susceptible $F_{2,3}$ families fit the expected ratio of 1 susceptible:2 segregating (Table 4). The five crosses had the same pattern (Table 4), indicating that genetic background had no influence on the expression of the gene. It was apparent from F_3 family data that resistance to *M. javanica* in the *C. sativus* var. *hardwickii* line LJ 90430 was consistent with the hypothesis that resistance was controlled by a single recessive gene.

New Gene for Resistance

The results obtained from all tests indicated that resistance to *M. javanica* in LJ 90430 was controlled by a single recessive gene. The pooled F_2 data from all experiments showed 183 plants resistant and 550 susceptible (conforming exactly to the expected numbers for a 3:1 ratio; $\chi^2 = 0.0$, $P = 1.0$). The pooled data for the BC_1 to LJ 90430 had 130 resistant and 127 suscep-

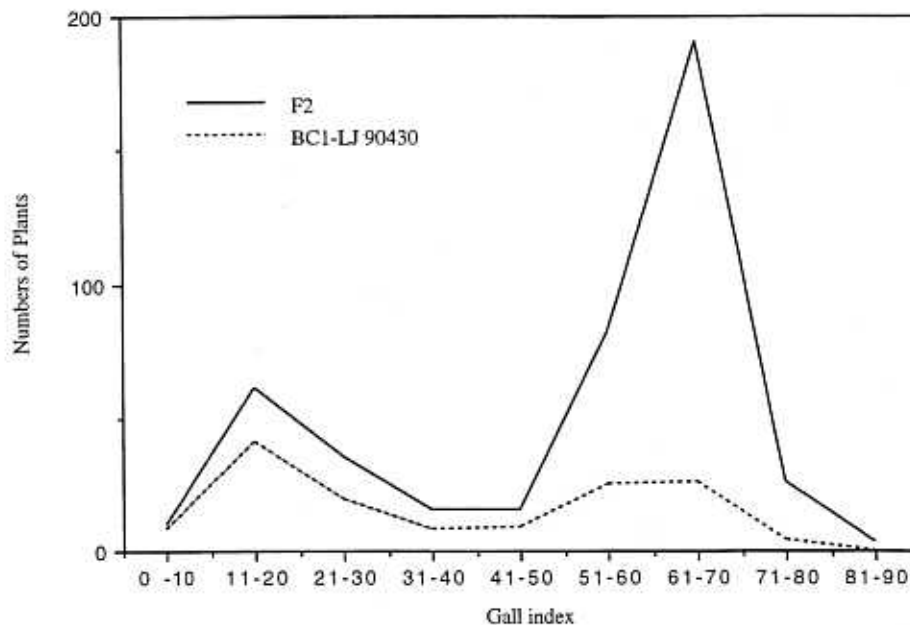


Figure 2. Distribution of F_2 and BC_1 to LJ 90430 plants, based on gall indices, from the reciprocal cross Sumter \times LJ 90430 and LJ 90430 \times Sumter infected with *Meloidogyne javanica*.

Table 3. Evaluation of the *mj* gene in five cucumber backgrounds utilizing segregation ratios in the F₂ and BC₁ to LJ 90430 generations*

Generation	No. observed (R:S)	No. expected (R:S)	Fitted ratio (R:S)	χ^2	P
F₂					
(Addis × LJ 90430)	8:32	10:30	1:3	0.53	.48
(Sumter × LJ 90430)	10:30	10:30	1:3	0.00	1.00
(Gy 14 × LJ 90430)	8:32	10:30	1:3	0.53	.48
(Poinsett 87 × LJ 90430)	13:27	10:30	1:3	1.20	.28
(Gy 57u × LJ 90430)	8:32	10:30	1:3	0.53	.48
Pooled	47:153	50:150	1:3	0.24	.65
BC₁ to recessive parent					
(Addis × LJ 90430)	11:9	10:10	1:1	0.20	.68
(Sumter × LJ 90430)	9:11	10:10	1:1	0.20	.68
(Gy 14 × LJ 90430)	12:8	10:10	1:1	0.80	.40
(Poinsett 87 × LJ 90430)	9:11	10:10	1:1	0.20	.68
(Gy 57u × LJ 90430)	11:9	10:10	1:1	0.20	.68
Pooled	52:48	50:50	1:1	0.16	.71

* Gall index: resistant ≤35% of roots galled, susceptible >35% of roots galled.

tible (129 expected of each; $\chi^2 = 0.04$, $P = .86$).

We propose that the single recessive gene for resistance to *M. javanica* be designated *mj*. This is the first gene identified in cucumber that confers resistance to a nematode (Pierce and Wehner 1990; Wehner 1993). The genotype for resistance in LJ 90430 is therefore *mj/mj*. The simple nature of inheritance of resistance to *M. javanica* indicates that it could be incorporated easily into elite inbreds using backcross methods. Cucumber cultivars with *M. javanica* resistance would benefit growers in the southeastern United States, as well as in Texas, Arizona, and California, because this nematode species is widely distributed in those areas (Walters and Barker 1994).

From the Department of Horticultural Science (Walters and Wehner) and the Department of Plant Pathology (Barker), North Carolina State University, Raleigh, NC

27695. The research reported in this article was funded in part by the North Carolina Agricultural Research Service and Pickle Packers International. The use of trade names in this article does not imply endorsement by the NCARS of the products named, nor criticism of similar ones not mentioned. This article was part of a thesis submitted by the senior author in partial fulfillment of the requirements for a Ph.D. degree.

The Journal of Heredity 1997:88(1)

References

- Amritphale D, Dixit S, and Singh B, 1993. Effect of acetone on the induction and breakage of secondary dormancy in seeds of cucumber. *J Exp Bot* 44:1621-1625.
- Barker KR, Townshend JL, Bird GW, Thomason JJ, and Dickson DW, 1986. Determining nematode population responses to control agents. In: *Methods for developing pesticides for control of plant pathogens* (Hickey KD, ed). St. Paul, Minn.: APS Press; 283-287.
- Byrd DW Jr, Ferris H, and Nusbaum CJ, 1972. A method for estimating numbers of eggs of *Meloidogyne* spp. in soil. *J Nematol* 4:266-269.
- Hadisoeganda WW and Sasser JN, 1982. Resistance of tomato, bean, southern pea, and garden pea cultivars

to root-knot nematodes based on host suitability. *Plant Dis* 66:145-150.

Hartman KM and Sasser JN, 1985. Identification of *Meloidogyne* species on the basis of differential host test and perineal-pattern morphology. In: *Advanced treatise on Meloidogyne*, vol. II. Methodology (Barker KR, Carter CC, and Sasser JN, eds). Raleigh, N.C.: North Carolina State University Graphics; 69-77.

Jewell DL, 1987. *Agricultural statistics*. Washington, D.C.: U.S. Government Printing Office; 157.

Netscher C and Sikora RA, 1990. Nematode parasites of vegetables. In: *Plant parasitic nematodes in subtropical and tropical agriculture* (Luc M, Sikora RA, and Bridge J, eds). Wallingford, U.K.: CAB International; 237-283.

Pierce LK and Wehner TC, 1990. Review of genes and linkage groups in cucumber. *HortScience* 25:605-615.

St. Amand PC and Wehner TC, 1991. Crop loss to 14 diseases in cucumber in North Carolina for 1983 to 1988. *Cucurbit Genet Coop Rpt* 14:15-17.

USDA (United States Department of Agriculture), 1993. *Agricultural statistics*. Washington, D.C.: U.S. Government Printing Office.

Walters SA and Barker KR, 1994. Update on the distribution of five major *Meloidogyne* species in the United States. *Plant Dis* 78:772-774.

Walters SA, Wehner TC, and Barker KR, 1993. Root-knot nematode resistance in cucumber and horned cucumber. *HortScience* 28:151-154.

Wehner TC, 1993. Gene list update for cucumber. *Cucurbit Genet Coop Rpt* 16:92-97.

Weston LA, Geneve RL, and Staub JE, 1992. Seed dormancy in *Cucumis sativus* var. *hardwickii* (Royle) Alef. *Sci Hort* 50:35-46.

Whitaker TW and Davis GN, 1962. *Cucurbits*. London: Leonard Hill.

Received July 21, 1995

Accepted May 24, 1996

Corresponding Editor: James L. Hamrick

Table 4. Segregation of resistance to *Meloidogyne javanica* in F₂ families developed from self-pollination of resistant and susceptible F₁ plants from five crosses

Family	F ₂ parent reaction	F ₁ observed			Fitted ratio (R:Sg:S)	χ^2	P
		R ^a	Sg ^b	S ^c			
Addis × LJ 90430	R ^a	5	0	0	All R	—	—
	S ^c	0	7	3	0 R:2 Sg:1 S	0.05	.84
Sumter × LJ 90430	R	5	0	0	All R	—	—
	S	0	7	3	0 R:2 Sg:1 S	0.05	.84
Gy 14 × LJ 90430	R	5	0	0	All R	—	—
	S	0	8	2	0 R:2 Sg:1 S	0.80	.40
Poinsett 87 × LJ 90430	R	5	0	0	All R	—	—
	S	0	7	3	0 R:2 Sg:1 S	0.05	.84
Gy 57u × LJ 90430	R	5	0	0	All R	—	—
	S	0	7	3	0 R:2 Sg:1 S	0.05	.84
Pooled	R	25	0	0	All R	—	—
	S	0	36	14	0 R:2 Sg:1 S	0.64	.45

^a All plants resistant.

^b Some plants resistant, some susceptible.

^c All plants susceptible.

^a F₂ families developed from resistant F₁ plants (F_{2,1}). Expect 5:0:0 (25:0:0 pooled).

^b F₂ families developed from susceptible F₁ plants (F_{2,2}). Expect 0:7:3 (0:33:17 pooled).