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Evaluation of NemX, a New Cultivar of Cotton with High Resistance to *Meloidogyne incognita*[1]

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Abstract: The level of resistance to root-knot nematode, *Meloidogyne incognita,* in NemX, a new cultivar of the Acala-type upland cotton, was evaluated in relation to four resistant breeding lines (N6072, N8577, N901, and N903) and four susceptible cultivars (Maxxa, SJ2, Royale, and Prema). In growth pouch tests, an average of only 4 nematode egg masses was produced on roots of NemX or the resistant lines, compared to a significantly higher average of 21 on the susceptible cultivars. In pot tests, the nematode reproduction factor (RF = Pf/Pi) in NemX and the resistant lines averaged 0.7, compared to a significantly higher average of 10 on the susceptible cultivars. Root galling in NemX or other resistant cotton averaged 15%, compared to 74% on the susceptible cultivars, in either pot or field tests. In plots with low levels of nematode infestation (Pi ≤ 150 second-stage juveniles [J2]/500 g soil), lint yield of NemX averaged 1,370 kg/ha and was less than the yield of susceptible Maxxa (1,450 kg/ha). However, in plots with medium or high levels of nematode infestation (Pi = 151–300 or >300 J2/500 g soil, respectively), yields of NemX decreased only slightly and averaged 1,300 or 1,050 kg/ha, respectively, whereas yields of Maxxa were severely reduced to 590 or 503 kg/ha, respectively. Fusarium wilt symptoms were observed on both NemX and Maxxa, and percent occurrence increased with increasing preplant nematode density. In plots with the highest nematode densities, 22% of NemX and 65% of Maxxa plants were wilted. NemX was highly effective against five *M. incognita* isolates and moderately effective against a sixth isolate that had been exposed to resistant cotton over several seasons. These results showed that NemX is as resistant to *M. incognita* as the four breeding lines, and much more resistant than the tested susceptible cultivars of cotton.

Key words: cotton, Fusarium wilt disease complex, *Gossypium hirsutum, Meloidogyne incognita,* nematode, resistance, root-knot, growth pouch.

The southern root-knot nematode, *Meloidogyne incognita,* is a major pathogen of cotton (*Gossypium hirsutum*) and many other crops worldwide (Goodell and Montez, 1994; Mai, 1985). Second-stage juveniles (J2) of the nematode infect roots, causing galling and general stunting of plants. Commonly, *M. incognita* interacts with the cotton wilt fungus *Fusarium oxysporum* f. sp. *vasinfectum* to cause the root-knot-Fusarium wilt disease complex that is more damaging to plants than the individual pathogens separately (Jeffers and Roberts, 1993; Roberts et al., 1985; Starr et al., 1989). In the San Joaquin Valley, California, where cotton is grown intensively under irrigation, *M. incognita* and *F. oxysporum* f. sp. *vasinfectum* occur on at least 20% of cotton plantations and cause about $10 million in losses in lint yield annually (Anonymous, 1996; Goodell et al., 1992). Until the 1980s, fumigant nematicides such as ethylene dibromide and 1,3-dichloropropene had provided effective control of the problem (Jorgenson, 1979), but their usage has since been banned or restricted due to environmental and health concerns.

Although several breeding lines of cotton with resistance to *M. incognita* and *F. oxysporum* f. sp. *vasinfectum* have been available for more than 30 years, high levels of resistance to both pathogens rarely have been incorporated into acceptable commercial cotton cultivars (Hyer et al., 1979; Kirkpatrick et al., 1995; Roberts, 1992). Previous attempts to develop cotton cultivars with resistance to the wilt fungus alone were disappointing because the resistance was not effective in the presence of nematodes (Shepherd, 1986). Therefore, resistance to *M. incognita* is regarded as the key factor for controlling the root-knot-Fusarium wilt disease complex.

In March 1995, the California Planting Cotton Seed Distributors (CPCSD), a grower-owned organization for the develop-

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ment of improved cultivars of cotton, released cultivar NemX, an Acala-type upland cotton developed for high resistance to root-knot nematode populations in the San Joaquin Valley, California (Garber et al., 1996; Oakley, 1995). NemX was derived from several lines, including the Acala types collected by A. H. Hyer from Mexico, and maintained at the U.S. Department of Agriculture Cotton Research Station, Shafter, California (Basset and Kerby, 1996). These cotton lines and the cultivars derived from them are commonly called Acala-type, in reference to the Acala region in Mexico where original seeds were collected. Acala-type cotton is characterized by high-quality lint. California growers plant predominantly Acala-type cotton, and current commercial cultivars include Maxxa (about 70% of total acreage), Prema, Royale, SJ2, and a few others (Basset and Kerby, 1996). Although most of these cultivars are moderately tolerant to root-knot nematode damage, they can support substantial levels of nematode reproduction (Goodell and Montez, 1994; Goodell et al., 1995). The objective of this study was to determine the level of resistance to *M. incognita* in the new cotton cultivar NemX, and to compare it with four resistant breeding lines and four cultivars commonly grown in California.

Materials and Methods

Cotton genotypes: Nine genotypes of Acala-type upland cotton were obtained from Steven Oakley of the California Planting Cotton Seed Distributors (CPCSD). The genotypes were NemX, the new cultivar developed for resistance to root-knot nematodes; resistant breeding lines N6072, N8577, N901, and N903; and susceptible cultivars Maxxa, SJ2, Royale, and Prema.

Nematode inoculum: The cotton root-knot nematode, *Meloidogyne incognita* race 3, was used in most of the laboratory and greenhouse experiments. This isolate, designated Project 77, was extracted originally as a single egg mass from cotton in the San Joaquin Valley, California. Species and race identifications were done with isoenzyme typing and differential host tests (Eisenback and Triantaphyllou, 1991). The nematode inoculum was increased on susceptible tomato cv. Tropic in a greenhouse. Galled roots were macerated in a solution of 0.5% NaOCl for 30 seconds to extract nematode eggs, and the extract was washed immediately through nested screens of 85-, 45-, and 25 µm-pore sizes (Eisenback and Triantaphyllou, 1991). Nematode eggs were retained on the 25-µm screen, from which they were washed into beakers. Egg concentrations were determined from measured aliquots with a stereoscopic microscope. The required inoculum concentration was prepared by serial dilution. Inoculum of infective second-stage juveniles was collected daily for 5 days from eggs placed in a bowl with clean water at room temperature.

Growth pouch experiment: Cotton seedlings were grown inside a controlled environment growth chamber, using a growth pouch technique developed by Omwega et al. (1988). The pouches consisted of plastic bags (17 cm × 17 cm) with paper wick inserts. A single germinating seed was placed between folds of the wick at the top portion of the pouch. The pouches were placed inside manila file folders and positioned side by side in a growth chamber. The chamber was maintained at 28 °C and 16-hour photoperiod. The seedlings were irrigated as needed with half-strength Hoagland's nutrient solution (Omwega et al., 1988). Two weeks after planting, when the root systems had secondary and tertiary branches, *M. incognita* race 3 was dispensed into the root zone at 500 J2/pouch. The treatment was applied to nine cotton genotypes (NemX, N6072, N8577, N901, N903, Maxxa, SJ2, Royale, and Prema), with 10 replications per genotype. The pouches were placed inside the growth chamber in a completely randomized design. Thirty days after inoculation, the pouches were individually flooded with erioglaucine dye, which selectively stained the gelatinous matrix of nematode egg masses blue. Egg masses were counted with the aid of a simple magnifying glass. The growth pouch technique was much

more rapid and required little space in comparison to pot or field evaluations. However, preliminary tests showed that only 60% of initial cotton seedlings developed good root systems suitable for inoculation and evaluation. In order to ensure availability of the minimum number of plants needed for evaluation, the final trials were started with twice the number of seedlings actually needed.

Greenhouse pot experiments: Rates of nematode reproduction in different cotton genotypes were determined in two pot experiments under greenhouse conditions. In the first experiment, all nine cotton genotypes (NemX, N6072, N8577, N901, N903, Maxxa, SJ2, Royale, and Prema) were evaluated against the laboratory-maintained *M. incognita* race 3 (Project 77). Ten plants of each genotype were grown singly in pots (15-cm diam. and 20 cm high) filled with steam-sterilized sandy soil (93% sand, 4% silt, 3% clay). They were maintained in a greenhouse at 28 °C. Each plant was fertilized with 15 g of N-P-K (17-6-10). Thirty days after planting, 30,000 eggs of *M. incognita* race 3 were dispensed into the root zone of each plant. The plants were arranged on greenhouse tables in a randomized complete-block design. After 60 days, the root systems were washed clean of soil and evaluated for root galling and nematode reproduction. Root galling was scored on a scale of 0 to 5, where 0 = no galls, 1 = 1 to 20%, 2 = 21 to 40%, 3 = 41 to 60%, 4 = 61 to 80%, and 5 = 81 to 100% of roots galled. Nematode eggs were extracted from a 20-g subsample of each root system, as described earlier. Egg counts were converted to eggs per root system (Pf), which was divided by the initial inoculum rate (Pi) to obtain a nematode reproduction factor (RF = Pf/Pi).

In a second pot experiment, six isolates of *M. incognita* from different sites in the San Joaquin Valley, California, were compared for their relative ability to reproduce on NemX, resistant line N8577, and susceptible cultivar Maxxa. The nematode isolates, designated by source location, were Hernstedt, Wegis East, Wegis West, Shafter South West, Shafter Station, and the laboratory-maintained Project 77. The isolates were increased separately on tomato cv. Tropic, and the inoculum processed as described earlier. Sixty plants from each cotton genotype were grown in steam-sterilized soil, and 10 individuals of each genotype were inoculated with one of the six isolates at 30,000 eggs per plant. Maintenance and evaluation of plants were as described earlier.

Field plot experiments: In one experiment, resistant NemX and susceptible Maxxa were grown in adjacent paired sub-plots within commercial cotton plantations at nine different sites in the San Joaquin Valley, California. Criteria for site selection were the historical incidence of either low, moderate, or high occurrences of root-knot and wilt disease problems, and the determined preplant nematode population density (Pi). From a 15-m × 100-m area within a preselected site, 10 soil cores, 3 cm wide and 20 cm deep, were taken randomly and composited. A 500-g subsample was processed with a sieving-flotation method (Eisenback and Triantaphyllou, 1991) to extract nematodes. The second-stage juveniles (J2) of *M. incognita* were counted under a stereoscopic microscope. Three sites from each of the following nematode infestation levels were selected for the experiment: i) low (Pi $\leq$ 150 J2/500 g of soil), ii) medium (Pi > 150 and $\leq$300 J2/500 g of soil), or iii) high (Pi > 300 J2/500 g of soil). Each plot was divided into two equal subplots, and each consisting of 100-m-long rows, 1 m apart, with 10 plants/m. NemX and Maxxa were planted next to each other in six replications. Planting was done in mid-April 1995. The crop was furrow-irrigated and received standard cultural practices, including insect pest control measures that were applied to the commercial crops surrounding the test plots. Sampling for Fusarium wilt symptoms, based on defoliation and browning of xylem tissues, was conducted in July. The number of wilted plants in four middle rows of each sub-plot was recorded as a percentage of the sample size. In November, each sub-plot was separately machine-harvested, ginned, and the yields recorded in kilograms per hectare of lint cotton.

In a second experiment, all nine cotton genotypes (NemX, N6072, N8577, N901, N903, Maxxa, SJ2, Royale, Prema) were compared for their relative ability to suppress the increase of *M. incognita* for a subsequent susceptible crop of lima bean (*Phaseolus lunatus* cv. Henderson). The experiment was done on a plot at Kearney Agricultural Center, Parlier, California. Each genotype was planted in 6-m × 10-m subplots replicated six times in a randomized complete-block design. Ten soil cores were taken randomly from each plot and processed as described above to determine root-knot nematode Pi. The plots were planted with the different cotton genotypes in April 1995, and managed as described above. The Pf in soil and percent root galling were determined in November. The next year, in April 1996, all the plots were prepared and planted with lima bean cv. Henderson, while maintaining a record of the cotton genotype previously planted. Plot size, seeding rate, and most cultural practices were similar to those used on the previous cotton crop. Nematode Pi in soil and percent root galling at harvest were determined as described above. Lima bean plants were stunted severely and bore no pods in several plots; thus, the yields from all plots were determined on the basis of fresh weight of tops from four middle rows (40 m^2) of each subplot.

Statistical analysis: Analysis of variance with SAS software (SAS Institute, Cary, NC) was carried out on actual values of data from growth pouch and pot experiments, and on $\log_{10}$ (x + 1)-transformed data from field plot experiments. The treatment means were compared with Schefe's Protected Least Significant Difference at the 5% level of probability.

Results

Growth pouch experiment: M. incognita race 3 reproduced poorly on NemX and on the resistant lines N6072, N8577, N901, and N903 but better on cultivars Prema, Royale, SJ2, and Maxxa (Table 1). An average of 4 egg masses was produced on NemX, which was not different ($P \leq 0.05$) from the average count in the resistant lines. A few galls were observed on roots of the resistant genotypes, but they did not contain mature nematodes. Egg mass counts on susceptible Prema, Royale, SJ2, and Maxxa averaged 15, 18, 20, and 32, respectively, and were higher than on resistant genotypes.

Greenhouse pot experiments: In the experiment comparing reproduction factors (RF) of *M. incognita* race 3 on nine cotton genotypes, RF on NemX averaged 0.8 and was not different from the average value on the resistant lines (Table 1). The RF values on susceptible Prema, Royale, SJ2, and Maxxa

Table 1. Reproduction of and root galling caused by *Meloidogyne incognita* race 3 on nine cotton genotypes in growth pouch and pot tests.

Cotton genotype	Expected reaction[a]	Pouch test	Pot test	
		Egg mass count[b]	Nematode RF[c]	Percent root galling
NemX	R	4 a	0.8 a	15 a
N6072	R	3 a	1.0 a	10 a
N8577	R	2 a	0.8 a	13 a
N901	R	2 a	0.5 a	18 a
N903	R	3 a	0.5 a	20 a
Prema	S	15 b	5.0 a	63 b
Royale	S	18 b	7.8 b	75 b
SJ2	S	20 b	7.5 b	78 b
Maxxa	S	32 c	18.0 c	80 b

Values are means of 10 replicates, and those within a column followed by the same letter are not significantly different ($P \leq 0.05$).

[a] R: resistant, S: susceptible.

[b] Initial inoculum was 500 infective juveniles (J2) per pouch.

[c] Reproduction factor (final nematode egg counts ÷ initial 30,000 eggs per plant).

averaged 5.0, 7.8, 7.5, and 18.0, respectively, and, except for Prema, were significantly higher than values on resistant genotypes. Percent root galling in each of the nine cotton genotypes closely approximated RF. The proportion of galling on NemX (15%) was not different from the average galling in the resistant lines but was lower than the galling on susceptible genotypes (63% to 80%).

In the experiment comparing virulence of six *M. incognita* isolates, RFs of five isolates (Hernstedt, Wegis East, Wegis West, Shafter South West, and Project 77) on resistant NemX or N8577 cotton ranged from 1 to 4 and did not differ among the isolates (Table 2). However, RFs of the Shafter Station isolate were significantly higher and averaged 8 on NemX and 18 on N8577. RFs of all *M. incognita* isolates on susceptible Maxxa cotton were higher and more variable, ranging from 12 (Project 77) to 25 (Shafter Station). Percent galling on NemX was lower for the Hernstedt (8) and Wegis East (8) isolates, and highest for the Shafter Station isolate (20). Percent root galling on N8577 did not differ among the nematode isolates. Maxxa supported relatively higher percent galling than NemX or N8577, ranging from 60 (Hernstedt or Project 77) to 75 (Shafter Station). We concluded that the *M. incognita* isolate from Shafter Station, which had been exposed to resistant cotton over several seasons, was more virulent than the other five isolates on both resistant and susceptible cotton genotypes.

Field plot experiments: Lint yields of NemX on plots with low or medium preplant levels of *M. incognita* were similar ($P \leq 0.05$) and averaged 1,335 kg/ha, whereas the yield on highly infested plots (1,050 kg/ha) was lower (Table 3). On the other hand, lint yield of Maxxa on plots with a low level of nematode infestation was 1,450 kg/ha, which was higher than corresponding yield of NemX by 6%. However, Maxxa's yields on plots with medium and high levels of nematode infestation were similar and severely suppressed to an average of 547 kg/ha. Fusarium wilt symptoms increased with increasing Pi on both NemX and Maxxa cotton, but wilt expression was consistently lower in NemX than in Maxxa (Table 3). In plots with low or high levels of nematode infestation, 1% or 22% of NemX plants were wilted, whereas 2% or 65% of Maxxa plants were wilted, respectively.

In the experiment comparing protective value of nine cotton genotypes on *M. incognita*-susceptible lima bean, resistant genotypes generally suppressed the nematode population, leading to a subsequent reduction of galling on bean plants, whereas susceptible genotypes had opposite effects (Table 4). On cotton plots, average J2 Pf values were lowest (132 J2/500 g soil) after resistant NemX and highest (930 J2/500 g soil) after susceptible SJ2. Top weight yield of lima beans was lowest (40 g/40 m^2) following susceptible Maxxa and highest (93 g/40 m^2) following resistant N903.

Discussion

The new cotton cultivar NemX had the highest level of resistance to *M. incognita*

TABLE 2. Reproduction of and root galling caused by six isolates of *Meloidogyne incognita* on resistant NemX and N8577, and susceptible Maxxa cotton genotypes in a pot test.

	NemX		N8577		Maxxa	
M. incognita isolate	RF[a]	Percent root galling	RF[a]	Percent root galling	RF[a]	Percent root galling
Hernstedt	3 a	8 a	1 a	12 a	18 ab	60 a
Wegis E.	3 a	8 a	4 a	15 a	17 ab	65 ab
Wegis W.	2 a	10 ab	3 a	15 a	13 a	70 ab
Project 77	2 a	15 ab	1 a	10 a	12 a	60 a
Shafter S. W.	2 a	15 ab	3 a	10 a	14 a	65 ab
Shafter Station	8 b	20 b	18 b	16 a	25 b	75 b

Values are means of 10 replicates, and those within a column followed by the same letter are not significantly different ($P \leq 0.05$).
[a] Reproduction factor (final nematode egg counts ÷ initial 30,000 eggs per plant).

TABLE 3. Lint yield and percent wilted plants of resistant NemX and susceptible Maxxa in field plots with low, medium, and high preplant population density of *Meloidogyne incognita.*

Preplant J2 per 500 g soil	Lint yield (kg/ha)		Percent wilted plants	
	NemX	Maxxa	NemX	Maxxa
0–150 (low)	1,370 b	1,450 b	1 a	2 a
151–300 (medium)	1,300 b	590 a	6 a	52 b
301 or more (high)	1,050 a	503 a	22 b	65 b

Values are means from replicated plots at three field sites, and were transformed by $\log_{10}$ (x + 1) for analysis of variance. Values within a column followed by the same letter are not significantly different ($P \leq 0.05$).

among genotypes of Acala-type cotton currently grown in California. NemX suppressed nematode population increase in growth pouch, pot, and field tests consistently. The level of resistance was about equal to that of four resistant breeding lines (N6072, N8577, N901, and N903). In addition, NemX had good resistance to the *M. incognita*-Fusarium wilt disease complex in the field. However, resistance of NemX to the wilt fungus, *Fusarium oxysporum* f. sp. *vasinfectum,* was not determined. Although NemX is not immune to infection by *M. incognita,* it has the potential to lower nematode inoculum substantially for succeeding crops. In growth pouch tests, some galls on roots of resistant plants did not appear to contain nematodes when they were examined. Apparently, after penetration of root tissue and initiation of the galls, induced defense mechanisms in the plants killed the nematodes.

Results from the growth pouch technique were comparable to pot or field evaluations. This technique was rapid, with each trial lasting only 60 days, and required little space. However, not all cotton seedlings grew well in the pouches. Up to 40% of the seedlings did not develop sufficient root systems or died prematurely, and in order to ensure adequate number of test plants, final trials were started with double the required seedlings. Other plants, such as common bean, grow well in pouches (Omwega et al., 1988). Although susceptible cultivars Maxxa, SJ2, Royale, and Prema allowed substantial reproduction of *M. incognita,* Goodell and Montez (1994) reported them to be moderately tolerant to the nematode. The breeding lines N6072, N8577, N901, and N903 demonstrated high levels of nematode resistance and are good candidates for the development of new resistant cultivars.

Five of six isolates of *M. incognita* from different sites in the San Joaquin Valley, California, reproduced poorly on NemX and resistant line N8577 but better on susceptible cultivar Maxxa. However, the sixth isolate (Shafter Station) reproduced better on all three cotton genotypes. This isolate originated from a site where resistant cotton lines had been maintained for several seasons, and hence might have been selected

TABLE 4. Percent root galling and top weight yield of lima bean cv. Henderson grown after different cotton genotypes in field plots.

Cotton in 1995				Lima bean in 1996	
Cotton genotype	Expected reaction[a]	Postplant J2 per 500g soil	Percent root galling	Percent root galling	Top weight (g/40 m^2)
NemX	R	132 a	17 a	70 a	75 b
N6072	R	340 b	20 a	74 ab	62 b
N8577	R	400 b	16 a	73 a	64 b
N901	R	430 b	20 a	72 a	80 b
N903	R	440 bc	18 a	67 a	93 c
Prema	S	480 cd	51 b	79 b	60 b
Royale	S	580 d	52 b	83 b	57 b
Maxxa	S	800 e	56 b	80 b	40 a
SJ2	S	930 f	55 b	80 b	42 a

Values are means of 10 replicates. Nematode counts and lima bean top weights were transformed by $\log_{10}$ (x + 1) for analysis of variance. Values within a column followed by the same letter are not significantly different ($P \leq 0.05$).

[a] R: resistant, S: susceptible.

for virulence. Virulence selection in nematode populations has been reported, especially against the *Mi* resistance gene in tomato (Roberts, 1992, 1995). Therefore, it is important that durability of resistance in NemX be safeguarded by use of crop rotation and other strategies that minimize selection of virulent nematode populations.

Although cotton genotypes with high resistance to *M. incognita* have been available for more than 30 years, resistance has been incorporated only rarely into acceptable commercial cotton cultivars (Hyer et al., 1979; Kirkpatrick et al., 1995; Roberts, 1992). As such, the development and release of the highly resistant cotton cv. NemX are important advances in the control of nematodes by host resistance. Since *Meloidogyne incognita* is the only common root-knot species that reproduces well in cotton (Goodell et al., 1992), utilization of NemX will enhance the rotational value of the cotton crop in integrated root-knot management cropping systems.

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