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New Sources of Resistance to Root-Knot Nematodes among Primitive Cottons[1]

Raymond L. Shepherd[2]

ABSTRACT

Presently, Auburn 634 RNR and related cottons are the only germplasm in upland cotton (*Gossypium hirsutum* L.) with extremely high resistance to root-knot nematodes (RKN) [*Meloidogyne incognita* (Kofoid & White) Chitwood]. Genetic vulnerability could result from using only this one source for breeding resistant cultivars. The purpose of this study was to evaluate primitive race stocks of *G. hirsutum* to identify new, genetically diverse sources of RKN resistance, and to compare the new sources with those now in upland cottons. Of 471 primitive race stocks evaluated, 18 (3.8%) were resistant. None were as resistant as Auburn 634 RNR, although several approached it. Number of eggs produced on the eight race stocks having the highest resistance ranged from 2.8 to 5.5 times fewer than were used initially to inoculate them. Resistance was widely distributed among stocks of different races (originating from the same and different geographic areas), indicating the potential diversity of the resistant germplasm. Evidence is presented that RKN resistance should be relatively stable over time and should control RKN across many geographic regions of the world.

Additional index words: **Genetic vulnerability, Cotton nematodes, Upland cotton, *Gossypium hirsutum* L., *Meloidogyne incognita* (Kofoid & White) Chitwood, Cotton breeding.**

ROOT-KNOT nematodes (RKN) [*Meloidogyne incognita* (Kofoid & White) Chitwood] are a major pest of cotton (*Gossypium hirsutum* L.). They not only are destructive alone (Brodie et al., 1960; Minton and Minton, 1966), but they also increase the incidence and severity of the cotton fusarium wilt disease [*Fusarium oxysporum* Schlecht. f. *vasinfectum* (Atk.) Snyd. & Hans.] (Smith, 1948; Martin et al., 1956; Minton and Minton, 1966) and of many cotton seedling diseases (Brodie and Cooper, 1964; Cauquil and Shepherd, 1970).

Because of the advantages for controlling RKN by means of resistant cultivars, several investigators made extensive searches for sources of high resistance to the nematode among upland cultivars and strains, but with little success (Smith, 1941; Jones et al., 1958). After many years of evaluating cottons, Smith (1953) concluded that " . . . high resistance to root knot is not present in any upland varieties."

The search for high resistance was also conducted among world cultivated and wild cotton species (Smith, 1953; Regional Research Project S-1, Technical Committee, undated). The most resistant *G. hirsutum* germplasm found in these searches was Clevewilt-6, an older fusarium wilt-resistant strain of upland cotton, and Mexico Wild, a wild *G. hirsutum* (Jones et al., 1958; Minton, 1962); but these cottons lacked sufficient resistance to prevent significant RKN reproduction and damage. Crosses between the two cottons led to the development of the breeding stock Auburn 623 RNR which has the highest resistance known in *Gossypium* (Shepherd, 1974). Germplasm of this stock and its progenies is presently the only source of high RKN resistance available in upland cotton. It has been suggested (National Research Council, Committee on Genetic Vulnerability of Major Crops; 1972) that using only one source of resistance to breed widely grown cultivars greatly increases their genetic vulnerability.

Because of the need for multiple sources of high RKN resistance in upland cotton, studies reported herein were initiated in 1975 to find new sources. The probability of success appeared to be greater in evaluating the large collection of primitive cottons (*G. hirsutum*) collected over many years in Mexico and Central America (the center of origin for upland cotton) and being maintained in Texas (Regional Research Project S-77, Technical Committee; 1974). This supposition is based on the premise that great genetic diversity in a species exists in its center of origin and, therefore, that pest-resistant germplasm is likely to exist there. Hutchinson (1951) proposed a system for classifying the primitive *G. hirsutum* cottons into the geographic races: *latifolium, punctatum, richmondi, morrilli, marie-galante, palmeri,* and *yucatanense*. Although "race" is not a formal taxonomic classification (Fryxell, 1976), this system simplifies the description of a primitive *G. hirsutum* by identifying it with a group having similar characteristics.

The purpose of this study was to identify new, genetically diverse sources of RKN resistance by evaluating the above primitive cottons, and to compare the new sources with those now in upland cottons.

MATERIALS AND METHODS

Number of stocks of each race evaluated were 177 *latifolium*, 17 *punctatum*, 5 *richmondi*, 28 *morrilli*, 8 *mari-galante*, 4 *palmeri*, and 232 unknown as to race. Seed of the race stocks to be evaluated were obtained from a Regional collection (Regional Research Project S-77, Technical Committee; 1974) and from J. N. Jenkins, Mississippi State, Miss. Because many race stocks are photoperiodic, seed were increased in Mexico. The race stocks were not evaluated in any particular sequence, but as adequate numbers of seed became available. Because several races were hardseeded, all seed were hot-water treated at 85°C for 60 s prior to use. The same four checks were included in each test. The checks, representing a wide range in resistance, were: Auburn 634 RNR (A634), an upland cotton breeding stock derived from a cross between Auburn 623 RNR and 'Auburn 56' [A634 has the same high RKN resistance as Auburn 623 RNR (Shepherd, 1982b)]; Clevewilt-6 (CW), an older upland cotton strain with moderate resistance; Auburn 56 (A56), an obsolete cultivar intermediate between CW and M-8 in resistance; and M-8 a highly susceptible doubled haploid derived from 'Deltapine 14' by J. R. Meyer, Stoneville, Miss. Procedures used to inoculate plants with RKN were as previously reported (Shepherd, 1979). Briefly, 7.6 × 7.0 cm pots on greenhouse benches were filled with methyl bromide-fumigated soil which was then wetted. About 8000 RKN eggs were deposited into a hole 2 cm

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[2] Research agronomist, USDA-ARS, Auburn Univ., AL 36849.

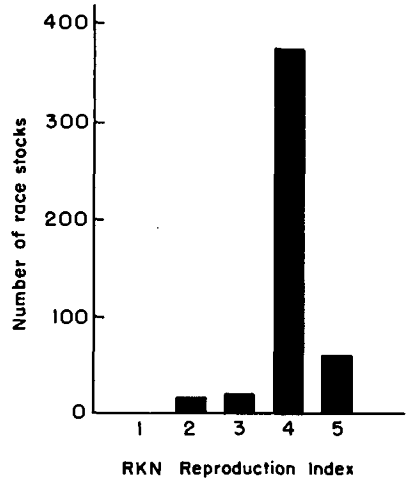

Fig. 1. Distribution of 471 primitive race stocks of cotton among RKN reproduction indices in tests 1 through 10. (Indices based on numbers of egg masses were: 1, < or = those on A634; 2, > on A634, but < on CW; 3, = or > on CW, but < on A56; 4, = or > on A56, but < on M-8; 5, = or > on M-8.)

deep in the center of each pot. Eggs were covered with dry soil which was wetted immediately. Seven to 10 days later, a newly emerged cotton seedling was transplanted into each pot. About 40 days after transplanting, soil was washed from roots; and RKN egg masses on roots were counted when those numbers were less than those on CW. When numbers obviously equaled or exceeded those on CW, they were estimated in comparison with numbers on CW, A56, and M-8. Actual counts and estimated numbers of egg masses were converted to a RKN-reproduction index of 1 to 5 as follows: 1 = numbers less than or equal to those on A634; 2 = more than on A634; but less than on CW; 3 = equal or more than on CW, but less than on A56; 4 = equal or more than on A56, but less than on M-8; and 5 = equal or more than on M-8.

Beginning in 1975, each of the 471 primitive race stocks were first evaluated in a series of 10 tests (tests 1 through 10) with six plants of an entry constituting an experimental unit. Each test contained one experimental unit of each check. A race stock exhibiting reproduction indices of 1 or 2 in its first evaluation was reevaluated as was done initially, along with other race stocks being evaluated for the first time. Reevaluations of a race stock were continued through several tests to confirm its resistance.

In 1976, after initial evaluations of the first 150 race stocks and after at least four reevaluations of each race stock with reproduction indices of 1 or 2 (as described above), those race stocks that had consistently exhibited RKN reproduction indices of 2 or lower (and random samples of race stocks that had indices of 3, 4, and 5) were tested together (test 11) for resistance in a randomized, complete block experimental design with four replications. A replication consisted of six plants of an entry. Plants in this test were evaluated for RKN resistance as described above, except that numbers of eggs produced on roots about 40 days after inoculation were used as the criterion of resistance. Procedures used for removing RKN eggs from roots and counting were as previously described (Shepherd, 1979).

Beginning in 1979, after all 471 primitive race stocks had been evaluated at least once and after each of those race stocks with reproduction indices of 1 or 2 had been reevaluated (as described above), race stocks exhibiting high resistance in all previous tests were retested together in a series of five tests (tests 12 through 16) over the next 2 years. Procedures used for conducting these tests were the same as those used in the 1976 test.

Table 1. Mean number of RKN eggs from roots of 25 race stocks and four upland cotton checks with different RKN reproduction indices in test 11.†

Race stock (T-) or check	Reproduction index‡	Eggs/plant (thousands)§
		no.
A634 (check)	1	0.38 a*
78	2	0.97 b
188	2	3.04 c
487	2	3.07 cd
495	2	3.43 cd
177	2	7.63 de
155	3	10.10 ef
497	3	12.00 ef
122	2	12.24 ef
278	3	13.60 efg
CW (check)	3	13.70 efg
283	3	14.40 efg
149	3	19.53 efgh
277	3	20.00 efghi
190	4	22.70 efghij
466	4	27.30 fghijk
272	3	31.00 fghijk
159	3	41.85 ghijk
A56 (check)	4	44.99 hijk
182	4	45.00 hijk
169	5	46.00 hijkl
134	4	47.93 hijkl
266	4	51.00 ijkl
281	5	58.00 ijklm
294	5	65.00 jklm
275	5	73.00 klm
470	5	84.00 klm
186	5	132.96 lm
M-8 (check)	5	160.28 m

* Means not followed by the same letter were significantly different at the 0.05 level of probability, according to Duncan's Multiple Range Test.
† Eggs were counted 40 days after inoculating seedlings with 8000 eggs/plant.
‡ Basis of previously assigned indices in tests 1 through 10 given in title of Fig. 1.
§ Each value is the mean of four replications, six plants/replication.

RESULTS AND DISCUSSION

Distribution of the 471 primitive race stocks of cotton among RKN reproduction indices in the preliminary tests of each race stock (tests 1 through 10) is given in Fig. 1. None were as resistant as A634, although several approached its resistance and were much more resistant than CW. Eighteen race stocks exhibited a reproduction index of 2, i.e., were more resistant than CW. The largest number (374) of the stocks had an index of 4. Very few were completely susceptible, even fewer were highly resistant.

A wide range in levels of resistance was found among race stocks in test 11, with numbers of RKN eggs/plant ranging from 380 on A634 to 160 280 on M-8 (Table 1). Significantly fewer RKN eggs (at the 0.05 probability level) were produced on roots of the four most resistant race stocks in this test than the 8000/plant initially used to inoculate them! Although none of the race stocks were as resistant as A634 in this test, race stock T-78 exhibited exceptionally high resistance. The high level of resistance of this particular stock has been previously reported (Jenkins et al., 1979). Race stocks T-188, T-487, and T-495 were also significantly more resistant than CW. These results and those for other races generally were

Table 2. Mean number of RKN eggs from roots, race classification, and origin of four upland cotton checks and 18 race stocks selected for RKN resistance from among 471 primitive race stocks in tests 12 through 16.†

Race stock (T-) or check	Eggs/plant (thousands)‡	Race§	Origin§
	no.		
A634 (check)¶	0.32 a*		
78¶	1.46 b	*latifolium*	Guatemala
75	1.78 b	*latifolium*	Guatemala
188¶	2.06 b	*latifolium*	Guatemala
27	2.08 b	*punctatum*	Chiapas, Mex.
22	2.56 bc	*latifolium*	Chiapas, Mex.
25	2.74 bc	*punctatum*	Chiapas, Mex.
487¶	2.89 bcd	*punctatum*	Yucatan, Mex.
28	2.90 bcd	*punctatum*	Chiapas, Mex.
247	2.98 cde	*latifolium*	Guatemala
26	3.67 cde	*punctatum*	Chiapas, Mex.
177¶	3.97 cde	*latifolium*	Guatemala
29	4.38 cde	*punctatum*	Chiapas, Mex.
70	4.49 cde	*latifolium*	Guatemala
176	4.50 de	*latifolium*	Guatemala
19	4.66 de	*richmondi*	Chiapas, Mex.
495¶	4.91 de	*punctatum*	Unknown
122¶	5.16 e	*latifolium*	Guatemala
44	7.55 e	*punctatum*	Chiapas, Mex.
CW (check)¶	11.70 f		
A56 (check)¶	34.91 g		
M-8 (check)¶	88.00 h		

* Means not followed by the same letter were significantly different at the 0.05 level of probability, according to Duncan's Multiple Range Test.
† Eggs were counted 40 days after inoculating seedlings with 8000 eggs/plant.
‡ Averaged over five tests: four replications/test, 6 plants/replication.
§ (Regional Research Project S-77, Technical Committee, 1974).
¶ Entries also in test 11 reported in Table 1.

consistent with those from the preliminary tests of these races.

Results of the series of five tests (tests 12 through 16) which included the most resistant race stocks in all previous tests are given in Table 2. As in previous tests, no race stock in these tests was as resistant to RKN reproduction as A634; however, 18 or (3.8%) of the 471 race stocks tested were significantly more resistant than CW. The eight most resistant race stocks ranged from 1460 to 2900 eggs/plant, significantly less than 8000 eggs/plant initially used to inoculate them These races were thus inoculated with 2.8 to 5.5 times more eggs/plant than were produced on their roots. The RKN should not be able to maintain their numbers on cultivars with the same levels of resistance. Therefore, upland cottons with these levels of resistance should control RKN; the RKN-resistant race stocks identified are important new sources of resistance.

Resistance was found in three different *G. hirsutum* races: *latifolium, punctatum,* and *richmondi* originating from the same geographic area (Chiapas, Mexico) and from three different areas (Chiapas and Yucatan, Mexico, and Guatemala) (Table 2). This wide distribution of resistance suggests potential diversity of the resistant germplasm. The more diverse the sources, the more effective they should be for reducing genetic vulnerability when transferred into upland cotton. Because upland cultivars susceptible to RKN are resistant to all RKN species except the cotton RKN, *M. incognita,* cottons resistant to this species would be resistant to all RKN species. Therefore, resistant cultivars should be valuable not only for reducing RKN-related losses in cotton, but also for rotating with susceptible crops thus depressing all RKN species (Shepherd, 1982a).

The potential for evolution of the pest into resistance-breaking biotypes or virulent races is an important consideration in breeding for resistance. Because resistance to RKN in cotton is multigenic (Shepherd, 1974), it is more likely to remain stable than if it were monogenic. Potential stability was further indicated by the wide distribution of resistance among race stocks, as mentioned above. Wide distribution suggests that it 1) was adaptationally advantageous, 2) probably evolved over a long time, and 3) could have evolved independently at one or more locations. If resistance could have been easily broken, the nematode would likely already have evolved resistance-breaking biotypes because cotton's perennial nature in the tropics would have kept plants in continuous contact with RKN for years in the many areas where they probably coexisted.

Another strong indication of the stability of RKN resistance in cotton is the relative uniformity of host response regardless of geographic origin of the RKN population (T. Kirkpatrick, personal communication, North Carolina State Univ., Raleigh). According to Kirkpatrick, of 12 RKN populations from nine regions of the world, several reproduced profusely on 'Deltapine 16'; but more importantly, A634 and its parent stock, Auburn 623 RNR, exhibited high resistance to all populations. Therefore, considerable evidence exists that resistance to this nematode would be relatively stable and would probably control RKN across many geographic regions of the world.

Although variation in RKN resistance was observed among race stocks, relationships of genes for resistance within and between race stocks and between race stocks and A634 are not yet known. However, the differences in phenotypes themselves imply genetic differences. Thus, it may be possible to select within crosses of race stock × race stock or of race stock × A634 for even higher RKN resistance than was exhibited in this study. Because of the potential benefits of RKN-resistant cultivars, further research should be done to determine whether the high resistance identified can be transferred into agronomically acceptable upland cultivars and to measure the effects of that resistance on the performance of such cultivars.

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