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# Transgressive Segregation for Root-Knot Nematode Resistance in Cotton[1]

R. L. Shepherd[2]

**ABSTRACT**

**$F_6$ families of *Gossypium hirsutum* L. × 'Mexico Wild' (a root-knot tolerant primitive *G. hirsutum* from Mexico) were advanced through $F_{10}$, with selection in greenhouse tests for resistance to root-knot nematodes [*Meloidogyne incognita acrita* (Kofoid & White, 1919) Chitwood, 1949]. A disinfected larval screening technique effectively classified 85% of the $F_6$ families as intermediate to highly susceptible, even though they had been selected in $F_2$ through $F_4$ in the field for root-knot resistance in highly infested soil.**

**Two $F_{10}$ lines, designated A623 and A61, having the highest resistance known to root-knot in *G. hirsutum*, were developed. These lines were transgrassive segregates for resistance. Root-knot resistance in the $F_1$ generation from resistant A623 × susceptible 'Stoneville 213,' 'Coker 201,' and 'Dixie King II' was incompletely dominant.**

**Fusarium wilt [*Fusarium oxysporum* Schlecht. f. *vasinfectum* (Atk.) Snyd. & Hans.] resistance in the above material was highly associated with root-knot resistance. Therefore, it may be possible to breed for fusarium wilt resistance by breeding solely for high root-knot resistance.**

***Additional index words:* Gossypium hirsutum L., *Meloidogyne incognita acrita*, Cotton breeding, Root-knot resistance screening, Host plant resistance, *Fusarium oxysporum* Schlecht, f. *vasinfectum*, Correlated traits, Cotton pests, Pedigree selection.**

ROOT-KNOT nematodes [*Meloidogyne incognita acrita* (Kofoid & White, 1919) Chitwood, 1949] are destructive pests of cotton (*Gossypium* sp.). Besides seriously disrupting normal root functions, they are major factors in many cotton disease complexes (4, 5, 16, 19), the root-knot-fusarium wilt complex being the most important. Cotton wilt caused by *Fusarium oxysporum* Schlecht. f. *vasinfectum* (Atk.) Snyd. & Hans. is greatly reduced when nematodes are controlled (7, 10, 13).

There is a long history of breeding for resistance to the root-knot-fusarium wilt complex by agricultural experiment stations, the USDA, and private companies. In 1892 at the Alabama Agricultural Experiment Station, Atkinson (2) published the first description of root-knot and fusarium wilt and recognized the association of these two organisms in a disease complex. Later, he concluded that *Fusarium* could not enter roots unaided (3). Ware (18) traced the history of breeding cotton for resistance to the nematode-wilt complex from 1895 to 1936, and Smith (15) through 1953. Ware noted that this complex became so prevalent and destructive at the turn of the century that it threatened the cotton industry in the Southeast. According to him, the first tolerant variety was released in 1905, a result of the first systematic breeding for disease resistance in a crop planted in the United States.

In 1940 and again in 1953, Smith (12, 16) compared field reaction to root-knot and fusarium wilt of current major varieties. He noted a positive correlation between root-knot and fusarium wilt resistance, with the latter being the dependent variable. After studying the problem many years, Smith (14) concluded that breeding for wilt resistance was primarily a problem of breeding for nematode resistance.

'Auburn 56,' released in 1952, represented the culmination of more than 50 years of breeding for root-knot and fusarium wilt resistance. Although only moderately tolerant (8), it still represents the highest level of resistance available in a commercial variety. Thus, higher resistance to these organisms should significantly increase yields of cotton cultivars.

Information regarding inheritance of root-knot resistance in cotton is limited. Jones, Wright, and Newsom (6) crossed a root-knot-tolerant Clevewilt-6 line × susceptible 'Deltapine 15' and speculated from $F_3$ data that resistance was a quantitative character, influenced greatly by environment. On the other hand, in crosses of root-knot-resistance × susceptible *Gossypium barbadense* L. breeding stocks, Turcotte et al. (17) found that the $F_1$ was similar in resistance to the susceptible parent and that in $F_2$, two different homozygous recessive genes conditioned resistance. Also, Wiles (20) reported that $F_1$ of crosses between *G. hirsutum* and resistant *G. barbadense* var. *darwinii* were susceptible.

In a search in the early 1950's for better sources of root-knot resistance, researchers screened large numbers of varieties, species, and subspecies (1). Two accessions, a wild *G. barbadense* and 'Mexico Wild,' a primitive, short-day *Gossypium hirsutum* L., were reported as highly promising (6, 9). Because the *G. barbadense* was most resistant, it was believed to promise more, but results with it were disappointing (11).

The purpose of this paper is to report successful use of the Mexico Wild source of resistance for devel-

[1] Contribution from the Crops Science Research Unit, Southern Region, ARS, USDA, in cooperation with the Department of Agronomy and Soils, Alabama Agricultural Experiment Station, Auburn University, Auburn, AL 36830. Received April 25, 1974.

[2] Research Agronomist, USDA, Auburn, AL 36830.

oping breeding stocks of *G. hirsutum* highly resistant to root-knot nematodes and fusarium wilt.

## MATERIALS AND METHODS

*Base Population.* Mexico Wild was crossed, by A. L. Smith, with Auburn 56, 'Coker 100A,' strain Hybrid 257, and strain Clevewilt 6-8. In $F_2$ and $F_4$ generations after these crosses, Smith selected for root-knot resistance in fields heavily infested with root-knot nematodes and fusarium wilt at Tallassee, Ala.

The $F_3$ and $F_5$ generations were grown without selection for root-knot resistance. In 1966 and 1967, $F_6$ families from these crosses were among materials I evaluated in greenhouse tests to determine best sources of root-knot resistant germplasm.

*Pedigree Selection for Resistance.* In a greenhouse maintained at about 27 C, all $F_6$ plants and later generations were screened for resistance by use of the disinfected root-knot larval screening technique (11). A mixture of *M. incognita acrita* strains obtained from several locations, including sites where field tests were later conducted, was used for inoculum.

More than 12,000 plants from 210 $F_6$ families, 35 advanced breeding lines, 5 parent lines, and resistant and susceptible check lines were screened for resistance in a series of 7 tests during 2 years. Each test was designed as a randomized complete block, replicated 4 times, with 10 plants/entry in each replicate. Plants were scored for resistance, based on a relative scale of 1 to 5: 1 = no galls or an occasional gall, 2 = light galling, 3 = moderate galling, 4 = heavy galling, and 5 = very heavy galling. Plants scoring 1 and 2 were selected from those families with an average galling score of 2.4 or lower, transplanted, and grown to maturity during each cycle of pedigree selection for resistance.

In the fourth selection cycle, 87 $F_8$ selections were grown in plant-progeny rows in field nurseries, rated for agronomic potential, and tested for resistance as $F_9$ lines in Test No. 6. Included as checks in Test No. 6 were 1) three of the most resistant $BC_3F_7$ lines developed, as previously reported (11), by crossing *G. hirsutum* × *G. barbadense* RNR and backcrossing to *G. hirsutum*; 2) parents of the original crosses; 3) line A10, selected for high root-knot susceptibility; 4) line A48 selected for intermediate susceptibility; and 5) M-8, a highly susceptible doubled haploid. The best $F_9$ lines were advanced to $F_{10}$ and tested for resistance in Test No. 7 to make final selections (fifth selection cycle). Except for progeny of the *G. barbadense* cross, the above checks also were included in Test No. 7.

## RESULTS AND DISCUSSION

Of the 210 $F_6$ families that had been field-selected for root-knot resistance in $F_2$ and $F_4$, only 179 (85% of families screened) had root-knot index means of 2.5 and higher and were classified as intermediate to highly susceptible (based on one cycle of selection) by use of the disinfected root-knot larval greenhouse screening technique. This indicates that field selection for resistance in $F_2$ and $F_4$ had been ineffective and that the greenhouse selection technique was highly effective.

Progeny tests showed that a few $F_7$ selections from two of the most resistant $F_6$ families were highly root-knot resistant. However, because of slight differences observed among sib lines within these families, selection was continued in the $F_8$ generation solely for root-knot resistance and in the $F_9$ for root-knot resistance plus agronomic potential in field nurseries. To maintain a wide gene base, selection was continued within 21 families through $F_9$.

Progress from selection for root-knot resistance is summarized graphically in Fig. 1. Genetic variation for root-knot resistance existed among and within $F_6$ families. Five cycles of selection were effective in shifting the population mean from 3.3 (the mean of 210 $F_6$ families) to 1.1 (the mean of 2 highly resistant $F_{10}$ lines). Of 210 $F_6$ families, mean root-knot indices of 31 were graded resistant to moderately tolerant (1.6 to 2.4) and, of these, 7 $F_6$ families (10-1, 88-2, 30-1, 33-1, 24-2, 20-2, and 15-2) produced 7 highly resistant $F_9$ selections in the fourth selection cycle. Root-knot resistance data for $F_6$ families (from first selection cycle) and $F_9$ progeny lines (from fourth selection cycle) are given in Table 1. Several of the most resistant $F_9$ lines developed from the Mexico Wild crosses were much more resistant than the most

**Table 1. Mean root-knot indices of $F_6$ families and $F_9$ lines of *G. hirsutum* × Mexico Wild; of $BC_3F_7$ lines of *G. hirsutum* × (*G. hirsutum*$^3$ × *G. barbadense* RNR); and of parents and susceptible checks.**

| Entry | Tests 1 to 5† | | Test 6‡ | |
|---|---|---|---|---|
| Group I. Families and lines from G. hirsutum × Mexico Wild: | | | | |
| Cross | $F_6$ family | Mean root-knot index | Selected $F_9$ line | Mean root-knot index |
| Clevewilt × Mex. Wild | 10-1 | 2.0 | A623 | 1.0a* |
| Hybrid 257 × Mex. Wild | 88-2 | 2.4 | A61 | 1.1a |
| Coker 100A × Mex. Wild | 30-1 | 1.6 | A45 | 1.2a-b |
| Coker 100A × Mex. Wild | 15-2 | 2.2 | A28 | 1.4a-c |
| | | | A47 | 1.4a-c |
| Coker 100A × Mex. Wild | 33-1 | 2.2 | A48 | 3.5h-i |
| | | | A36 | 1.5a-d |
| Coker 100A × Mex. Wild | 24-2 | 2.2 | A35 | 2.1c-g |
| Coker 100A × Mex. Wild | 20-2 | 2.2 | A29 | 1.7a-e |
| Hybrid 257 × Mex. Wild | 99-1 | 5.0 | A10 | 4.5j-k |
| Group II. Lines from G. hirsutum × G. barbadense RNR: | | | | |
| Cross | | | Selected $BC_3F_7$ line no. | Mean root-knot index |
| Auburn 56 × (G. hirsutum$^3$ × G. barbadense RNR) | | | S3 | 2.5c-g |
| | | | S28 | 2.6f-g |
| | | | S22 | 2.8g-h |
| Group III. Parental stocks and check: | | | | |
| Parental stocks | | | | Mean root-knot index |
| G. barbadense RNR | | | | 1.1a* |
| Mexico Wild | | | | 2.2c-g |
| Clevewilt 6-3 | | | | 3.5h-i |
| Auburn 56 | | | | 3.8i-j |
| Hybrid 257 | | | | 4.3i-k |
| Coker 100A | | | | 4.4j-k |
| Susceptible check | | | | |
| M-8 | | | | 5.0k |

* Means with letters in common within tests do not differ at the 0.05 level. † Mean root-knot indices were obtained from tests 1 through 5 in first selection cycle. ‡ Mean root-knot indices were obtained from Test 6 in fourth selection cycle.

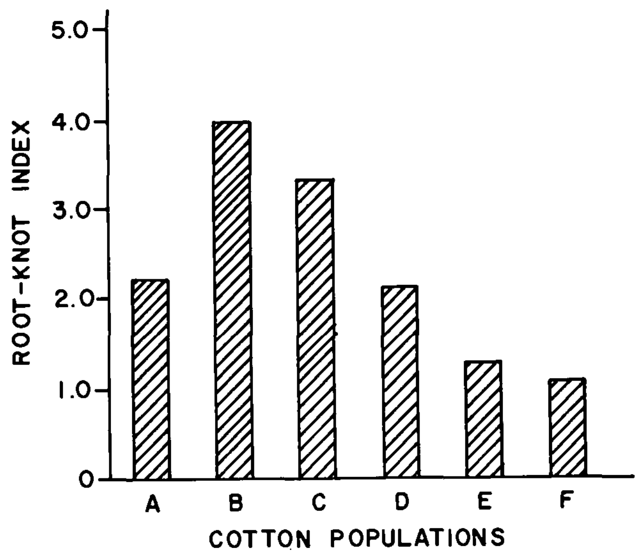


Fig. 1. Average root-knot indices of cotton populations: (A) Mexico Wild parent, (B) 4 upland parents (indices ranged from 3.6 to 4.5), (C) 210 $F_6$ families derived from the four upland parents × Mexico Wild (indices ranged from 1.6 to 5.0), (D) 31 $F_6$ families (after first selection cycle, indices ranged from 1.6 to 2.4), (E) 7 $F_9$ lines (after fourth selection cycle, indices ranged from 1.0 to 1.7), (F) 2 $F_{10}$ lines (after fifth selection cycle, indices ranged from 1.0 to 1.1).

resistant lines (S3, S22, and S28) derived from *G. barbadense* RNR crosses, as previously described (11). Yet, *G. barbadense* RNR was much more root-knot resistant than Mexico Wild. Two lines, A623 and A61 selected from families 10-1 and 88-2, respectively, showed resistance equal to or higher than that of *G. barbadense* RNR, which was the highest root-knot resistant germplasm previously available. These lines were not significantly more resistant than lines A28, A29, A36, A45, and A47 (Table 1), but they showed slightly smaller galls and fewer egg masses and had lower average scores than the latter lines. Lines A48 and A10 were selected to demonstrate results of selection for both intermediate and high levels of susceptibility, respectively, and for use as checks.

Three $F_9$ lines—A623 from Clevewilt 6-8 × Mexico Wild, A61 from Hybrid 257 × Mexico Wild, and A45 from Coker 100A × Mexico Wild—were transgressive segregants for root-knot resistance (Table 1). Each was more resistant than its parents. Thus, each parent may have contributed effective genes for resistance to make the new genetic combinations with higher resistance. A623 and A61 had the highest resistance in a progeny test of $F_{10}$ lines (Test 7) and in other experiments (unpublished data). Of the resistant lines developed, A623 showed the greatest agronomic potential.

Results of inoculating A623 and susceptible M-8 with equal numbers of root-knot larvae are shown in Fig. 2. A few small galls appeared on inoculated roots of A623, and rarely a root-knot female favorably located in a root reached maturity and produced abnormally small masses of eggs. Egg masses on M-8 contained about eight times more eggs per egg mass than those on A623.

Resistant A623 was crossed with several susceptible cultivars. The resulting $F_1$'s were intermediate, with a tendency toward partial dominance for resistance. The $F_2$ segregated widely. All progeny of each $F_2$ plant have not yet been tested but according to present information, resistance in these crosses is incompletely dominant and may be controlled by multiple genes.

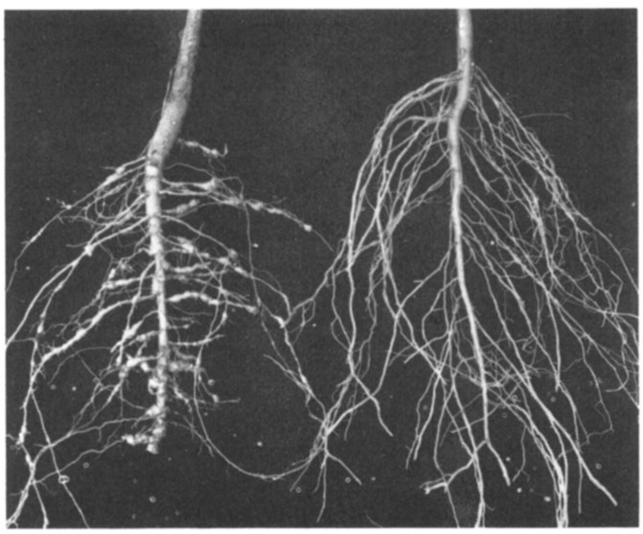

Fig. 2. Typical heavily galled cotton roots of susceptible M-8 (left) and relatively gall-free roots of resistant line A623 (right). Each plant was exposed to about 2,500 larvae.

**Table 2. Root-knot indices of cotton roots and number of root-knot larvae recovered from field soil at Tallassee, Ala.**

| Line or variety | Mean root-knot index | Mean no. root-knot larvae recovered/500 cc soil |
|---|---|---|
| A623 | 1.0a* | 44a* |
| Auburn 56 | 3.9b | 1560b |
| Deltapine 16 | 4.8c | 3145c |

* Means within a column followed by letters in common do not differ P = 0.01.

**Table 3. Mean percentage of plants with fusarium wilt grown on heavily root-knot- and fusarium wilt-infested soil at Tallassee, Ala.**

| Line or variety | Percentage wilted† |
|---|---|
| A623 | 2a* |
| A61 | 5a |
| Clevewilt 6-8 | 12b |
| Auburn 56 | 16b |
| Mexico Wild | 23c |
| M-8 | 90d |
| Rowden | 94d |

* Means followed by letters in common do not differ at P = 0.05. † Each value is mean of 4 replicates in each of 3 years, except Mexico Wild is mean of 4 replicates in 2 years.

**Table 4. Mean root-knot indices of three cotton lines tested in a greenhouse against root-knot nematode isolates from four states.†**

| Line | Cotton root-knot nematode strain from: Ala. | Ariz. | La. | Mo. |
|---|---|---|---|---|
| A61 | 1.1a* | 1.0a | 1.1a | 1.0a |
| A623 | 1.1a | 1.0a | 1.2a | 1.1a |
| M-8 | 4.9b | 4.6b | 5.0b | 4.8b |

* Means within an isolate followed by letters in common do not differ at P = 0.01. † Each value is mean of 4 replicates, 5 plants/replicate.

This mode of inheritance appears different from that reported for a *G. barbadense* cross (17), in which resistance was determined by two recessive genes. Inheritance of A623 resistance is still being studied.

Line A623 also showed high mature-plant resistance and suppressed root-knot populations in field plots (Table 2). Root-knot larvae were extracted from 500-cc soil samples from A623 plots in a four replicate, randomized complete block experiment and counted. Roots from these test plots were rated for resistance on the 1 to 5 scale. The reduction by A623 of root-knot nematode populations suggests that varieties with similar levels of resistance may be used to reduce root-knot populations in soils and to increase yields. Further studies are in progress to determine the root-knot population-suppressing value of this line.

High root-knot resistance in the crosses reported herein appears to provide high resistance to fusarium wilt. Studies have shown the wilt fungus to be highly dependent on parasitic nematodes, primarily root-knot, for invasion of cotton roots (7, 14). Until the 1950's, most cotton varieties with significant levels of fusarium wilt resistance showed positively correlated levels of slight root-knot tolerance, although the major breeding emphasis during the development of these varieties was on fusarium wilt resistance. The present work clearly shows a similar positive correlation between high levels of root-knot and fusarium wilt resistance. All root-knot-resistant lines developed in this program have shown higher resistance in at least one field test than have the most wilt-tolerant varieties presently available. Lines A623 and A61 were compared with the most resistant cultivars available in field tests during each of 3 years in highly wilt-infested soil at Tallassee, Ala. (Table 3). Lines A623 and A61 were transgressive segregants for fusarium wilt resistance, because they had a higher level of resistance than did either parent.

These results add to previous observations about the relationship of root-knot nematode and fusarium wilt resistance (3, 5, 7, 12, 14, 16). They show that it may not be necessary to breed for resistance to both root-knot and fusarium wilt, if the high root-knot resistance of A623 and A61 can be transferred to cotton varieties.

The most significant result of the present work was the development of lines A623 and A61 with the highest levels of root-knot and fusarium wilt resistance now known in *G. hirsutum*. These two lines have shown high resistance to *M. incognita acrita* strains from several cotton-growing states (Table 4). Also, high root-knot resistance of A623 in fields in California and Georgia has been reported (personal communication with A. H. Hyer and S. H. Baker, respectively) and as has high fusarium wilt resistance of this line in fields in Georgia and Louisiana (personal communication with S. H. Baker and J. E. Jones, respectively). Thus, present information indicates that resistance of A623 and A61 would be valuable over large areas of the Cotton Belt.

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