Resistance to the Root Knot Disease of Tobacco[1]

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Introduction

Root knot is a major disease throughout the flue-cured tobacco producing area, beginning in Virginia and extending into Florida. The need for root knot resistant varieties of tobacco, and other important southern crops, has long been recognized. The first report on such crop resistance was by Webber and Orton (1902). The general situation with respect to the occurrence of root knot resistance was summarized by McClintock (1922). He listed as highly resistant Iron and Brabham cowpeas, and all varieties of velvet beans and peanuts. A somewhat less resistant group included certain varieties of soybeans, the cereals, and the grasses. A study of the use of resistant crops in rotations designed to reduce root knot damage in tobacco was initiated in 1928. It was found that peanuts, oats, and native weeds were very helpful in such rotations (Clayton, Gaines, Smith, Shaw, and Graham, 1944), but rotation alone could not be depended on to give adequate control. More recently extensive use has been made of soil fumigation to control root knot. Such treatments are expensive and, perhaps more important, they may result in absorption by tobacco roots of undesirable chemical residues that accumulate in the leaves. Thus the development of root-knot-resistant tobacco is highly desirable.

This work was initiated in 1935 at which time the root-knot nematode was considered to be a single species. Recently the identification of this parasite has undergone change. Christie and Albin (1944) showed that the root knot species included a number of physiologic races that differed in pathogenicity. Chitwood (1949) described some of these races as separate species. To clarify the situation with respect to tobacco, the writers, beginning in 1950, made numerous tobacco root collections throughout the flue-cured area. All root samples contained root knot nematodes which were identified as either *Meloidogyne incognita* Chitwood or *M. incognita* var. *acrita*. As a further check, the records of the Section of Nematology, Crops Research Division, U. S. D. A. were consulted, through the courtesy of A. L. Taylor. These records showed numerous identifications of *M. incognita* and *M. incognita* var. *acrita*, and one identification of *M. arenaria* Chitwood from Georgia. This latter species is known to be general in the peanut growing area of eastern Alabama and western Georgia but its occurrence in the flue-cured tobacco area is rare. Consequently it is concluded that tobacco root knot, in the United States, is caused primarily by *Meloidogyne incognita*, and its varieties. The present study is concerned with this species.

A few words are necessary regarding root knot disease development, and the manner in which resistance functions. The root knot nematode larvae enter young roots, and, as they feed, the cortical cells multiply to form galls or knots. At first the galls are firm, with the cortex and epidermis unbroken. Steiner (1942) refers to these as "healthy galls." During this phase of disease development, plant growth may not be visibly affected. Soon, however, the female larvae mature and egg masses are produced. The

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extrusion of egg masses ruptures both cortex and epidermis, and provides entry for decay organisms that destroy the galls and roots (figure 1). Wilting and retarded growth are closely associated with the root decay phase of the disease. With susceptible tobacco, the nematodes multiply and root damage becomes more and more severe. Root knot resistant tobacco plants are freely invaded by the nematodes. Small swellings are commonly observed, and occasionally galls of some size are produced. The critical difference is that in resistant roots the larvae fail to mature and produce eggs; and galls, if formed, remain smooth and firm.

Figure 1. Root knot disease of tobacco: These galls are fully developed and decay has already begun. In a short time the entire root system would decay and the plant would then die.

Evaluation of Resistance to Root Knot

Both field and greenhouse tests were used for resistance evaluation. Field plot work was usually complicated by the uneven distribution of nematode populations, which tended to vary greatly from one year to the next. These problems were met by repeating tests at different locations for more than one year. This was readily possible with stable lines that were being evaluated for type, yield, and quality. Field testing, however, was less satisfactory with segregating lines where the problem was to measure resistance levels promptly and to pick out occasional highly resistant individuals. For such work greenhouse tests were superior, and most of the data here reported were secured in the greenhouse. Very uniform inoculation was obtained by composting and storing diseased roots, diluting with sand, adding fertilizer, and mixing thoroughly, before filling the pots. Testing was limited to the spring, summer, and fall months, since results obtained in mid-winter were unreliable. Plants were generally grown to seed maturity before making root examinations, to allow time for egg production and root decay. As a further precaution, the efficiency of each experiment was measured by plantings of susceptible tobacco. It was necessary to discard the results from experiments in which the check plants were not uniformly and severely affected. Direct comparisons between greenhouse and field tests showed that the former were more critical and consistent. However, greenhouse results were constantly field checked, since there are important facts that can only be learned by field test. Starting in 1937, and continuing throughout the investigation, data were taken in terms of "Disease Index," which was a visual estimate of the amount of disease. A class value of 0 indicated no disease symptoms and a value of 4 indicated all roots were diseased or dead. In preparing this report it has been found desirable in most cases to reduce class values to three groups: (a) 0-½ highly resistant, (b) 1-1½ moderately resistant, (c) 2-4 susceptible. In cases where a disease index was desired, class values of 0 to 4 were transformed to index values on a scale of 0-100.

Root Knot Resistance in Nicotiana tabacum

Among the varieties and strains of tobacco grown in the United States several were found that showed some root knot tolerance. The most promising was a variety of flue-cured type called Faucett Special. This was crossed with other varieties having better yield and quality, and the progeny were selected for increased resistance, plus yield and quality. The results were not good and the material was ultimately discarded. Search for root knot resistance was continued with collections of tobacco from Mexico, Central America, and South America. During the years 1935, 1936, and 1937, some 970 collections were tested and all showing evidence of resistance were retested several times. Numerous collections had some degree of tolerance, thus corroborating the statement by Colla (1943) that certain native South American varieties of tobacco were less injured by root knot than such imported varieties as Burley, Maryland, and Virginia. However, in addition to the many collections with some tolerance, a total of 42 collections—mostly from Central America—had definite resistance to root knot. This group was subjected to an intensive program of selfing and selection through four generations with the result that of the original 42 all but four—TI 419, 422, 517, and 706*—were finally eliminated. In repeated tests, the selections from these four were usually equally and highly resistant. All had small leaves and produced numerous suckers. These four collections were crossed with flue-cured varieties and the progeny were tested for resistance. Resistant lines were recovered from some crosses with TI 706, but not from similar crosses with TI 419, 422, and 517. Consequently from the total of 970 collections tested, only one, TI 706, was finally selected as the source of root knot resistance within the cultivated species, *N. tabacum*.

Breeding Work with Root Knot Resistance from N. tabacum var. TI 706

TI 706 was subjected to critical field testing in North Carolina, South Carolina, and Georgia. In all these tests it appeared highly resistant with only occasional development of small hard galls, whereas plantings of susceptible flue-cured varieties were severely galled. However, similar work was conducted in the greenhouse, and there 706 did not show uniform high resistance in all tests.

*"Tobacco introduction, T.I. 706 or (P.I. 114,080). This selection was obtained from Honduras in 1936 by Dr. W. A. Archer, Plant Introduction, Section, ARS.

Table 1.—The resistance to root knot of TI 706

TI 706 Test No.	Disease Class* 0—½ No. of Plants	1—1½ No. of Plants	2—4 No. of Plants
1	35	0	0
2	15	0	0
3	27	0	36
4	0	28	0
5	20	1	3
6	28	0	0
7	0	14	26
Total	125	43	65
Flue-cured Check Test No.			
1	0	0	29
2	0	0	24
3	0	0	24
4	0	0	24
5	0	0	22
6	0	0	40
7	0	0	28
Total	0	0	191

*0 = no disease
4 = all roots diseased or dead

Some of these data are given in table 1.

Table 1—The TI 706 seed used in the tests reported in table 1 had been selected a number of generations for uniformly high resistance, and the seed that gave apparent segregation in tests 3 and 7 was the same as that which appeared uniformly resistant in tests 1, 2, and 6 The difference between tests can be attributed to environmental effects, but the seven check lots were uniformly and severely diseased in all tests. It was apparent, consequently, despite uniformly favorable field results, that TI 706 resistance was basically unstable.

These studies were continued in the F1 and F2 generations with crosses between 706 and flue-cured tobacco, (table 2).

Table 2—The reaction of F1 populations was not entirely consistent. As the data in table 2 indicates, in some cases the F1 plants showed intermediate root knot resistance; in other cases they appeared to be completely susceptible. Erratic behavior was observed also in the F2 plantings. In three progenies, an appreciable number of plants were classed disease index 0-½. In the other seven populations no plants fell in the 0-½ class. Different flue-cured lines and varieties were used in these crosses, so it was not certain how much of the variability resulted from slight genetic differences between the susceptible flue-cured parents, and how much resulted from gene-environment interaction. Over 100 F2 selections were tested for resistance in the F3. Most of them proved to be moderately to highly susceptible and from the entire group a single selection N 57 was finally picked to initiate the backcross program.

Root Knot Resistance in the First, Second, and Third Backcrosses to Flue-cured Tobacco

The best resistant selection from the original cross had the parentage 706 X Flue-cured var. White Stem Orinoco. This selection, N 57, was then backcrossed to the same flue-cured variety. The results obtained in 1942 with a group of F4 lines from this first backcross are of interest. More than 60 lines were included in the experiment but the 10 lots listed in table 3 are an adequate sample.

Table 3.—There were indications that the original N 57 selection had higher root knot resistance than the 706 parent. N 122 H in table 3 was distinctly more resistant to root knot than 706. This line was repeatedly tested in the greenhouse and field over a period of five years and it showed high resistance. Thus it was definitely possible, not only to maintain the original 706 resistance during backcrossing, but, by critical selection, to increase resistance. The performance of certain F4 lines listed in table 3 was followed through the F2 generation of the second backcross (BC^2) and some of these data are given in table 4.

Table 4.—The results in table 4 are further evidence of the fact that lines with apparently equal root knot resistance may give quite different results when used as parents. The

Table 3.—Resistance of F4 Lines from BC^1

F4 lines	Disease Class 1—1½ No.	1—1½ No.	2—4 No.
N 116 G	0	0	30
N 120 A	6	21	4
N 120 B	19	7	0
N 120 E	0	0	28
N 122 C	0	9	18
N 122 F	0	0	27
N 122 H	12	13	0
N 123 B	3	10	9
Flue-cured Ck.	0	0	30
TI 706	0	21	39

data in table 3 indicated that N 120 B and N 122 H were equally and highly resistant. However, when these lines were crossed with flue-cured, and the F2 population tested, N 122 H gave six times as many highly resistant plants as did N 120 B. Thus at this stage the best procedure for continuing high level root knot resistance was first to eliminate by critical testing all but the most resistant lines and plants; then to cross a number of these with susceptible flue-cured, and finally to continue only with the occasional F2 that showed a large number of resistant plants.

From BC^2 resistance was recovered more readily and a number of F3 and F4 lines were obtained that were uniformly and highly root knot resistant. The variation in the number of resistant plants recovered in F2 populations was now much less. Consequently, it seemed best to check carefully the ratio of resistant to susceptible plants that might be obtained under certain different conditions.

The first comparison was made with 21 F2 lines of BC^3, first grown in a warm greenhouse (temperature 70° F. or above) during the winter, and then seed from the same lots was sowed in the spring and the plants grown in the greenhouse during the summer. The results are given in table 5.

The second comparison used a similar group of F2 lines, first indexed in the field and second indexed in the greenhouse during the summer (table 6).

Tables 5 and 6.—The data in tables 5 and 6 show how four carefully conducted tests with segregating F2 populations gave a range of 3.5 to 27.2 percent resisting plants. This matter was studied further by planting seed from 55 of the 110 apparently resistant winter selections (table 5). This planting was grown in the greenhouse during the summer and of the 55 only 7 were highly resistant. The 26 selections made originally during the summer were retested and all proved highly resistant. In the same manner a large sample of the field selections (table 6) were tested in the F3 and only about 20 percent of these showed high resistance. On the basis of these and other similar data it was concluded that after the original cross and two backcrosses there were, on the average, 4 to 5 percent of highly resistant plants in segregating F2 populations. However, under some

Table 2.—Root knot resistance of F1 and F2 progenies from TI 706 X Flue-cured tobacco

706 X Flue-cured—F1 Test No.	Disease Class 0—½ No.	1—1½ No.	2—4 No.
1	0	12	4
2	0	6	14
3	0	0	16
4	0	0	18
5	0	11	5
6	0	0	0
Total	0	29	57
706 X Flue-cured—F2 Test No.			
1	23	0	44
2	28	0	34
3	0	35	4
4	0	35	8
5	0	11	52
6	0	0	62
7	23	0	44
8	0	28	34
9	0	35	4
10	0	35	8
Total	74	179	294

Table 4.—Resistance of F2 populations from BC^2

F2 lines	Disease Class 0—½ No.	1—1½ No.	2—4 No.
N 120 A X Flue-cured	2	6	52
N 120 B X Flue-cured	5	11	37
N 122 C X Flue-cured	2	0	53
N 122 F X Flue-cured	0	8	44
N 122 H X Flue-cured	31	15	31
N 123 B X Flue-cured	3	15	42
Flue-cured Ck.	0	0	40

Table 5.—Winter vs. summer indexing for root knot resistance

Time of Testing	Total Plants No.	Highly Resistant (DI 0 - ½) No.	%
Winter	404	110	27.2
Summer	738	26	3.5

conditions a far larger number of plants may appear to be highly resistant.

The Commercial Value of the Root-Knot-Resistant Tobacco at the End of BC^2

TI 706 had very undesirable growth characters and at every stage, during the progress of the backcross program, intensive efforts were made to obtain a plant type comparable to the better flue-cured varieties, such as 402. As early as BC^2 lines were obtained that were satisfactory as to cured leaf quality. The tendency of 706 to branch and flower was eliminated completely. In fact, the selection program for type was successful in every respect except one; all root knot resistant lines had small leaves and associated with this was reduced yield. The leaves of the better lines, such as RK 42, were much larger than those of 706, but they were definitely smaller than flue-cured var. 402 and other similar varieties. A fourth backcross was made with no gain so that by 1952 the 706-flue-cured-backcross program was at dead end. Fortunately at this time a different approach to this undesirable situation began to show definite results.

Table 6.—Greenhouse vs. field indexing of F2 lines

Location	Total Plants	Highly Resistant (DI 0 - ½)	
	No.	No.	%
Field	2279	530	23.
Greenhouse	470	26	5.5

Table 7.—The performance of root knot resistance after the cross with alloploid *N. sylvestris X N. tomentosiformis*

Genotype	Disease Class		
	0—½ No.	1—1½ No.	2—4 No.
(RK 42 X S-T) XF-c — F3	91	0	0
Same X Flue-cured var. 402 — F1	37	0	0
Same X Flue-cured var. Va. Gold — F1	34	0	0
Same X Flue-cured var. Va. Gold — F2	51	0	20
Flue-cured Check	0	0	60
Alloploid *N. sylvestris* X *N. tomentosiformis*	0	0	30
RK 42	24	11	5

Table 8.—Leaf size of root knot resistant plants after the cross with alloploid *N. sylvestris X N. tomentosiformis*

Resistant F3 lines	Plants Measured	Average Maximum	
	No.	Width In.	Length In.
635	8	14.7	27.1
700	11	13.4	26.8
727	7	13.2	27.0
728	9	16.0	30.3
760	7	14.4	28.8
761	12	14.2	27.6
762	8	14.6	26.4
765	10	13.2	26.7
766	10	14.2	27.3
Mean	..	14.2	27.5
RK 42	20	10.8	19.8
Flue-cured var. 402	20	13.2	25.1

The Cross Between Root-Knot-Resistant N. tabacum and Alloploid* N. sylvestris X N. tomentosiformis

It was evident that the small leaf character associated with TI 706 root knot resistance was due to pleiotropism or to block inheritance and linkage. If pleiotropism was the problem, then the situation was hopeless. If the problem was linkage, then it was apparent that repeated backcrosses had not provided a solution. In searching for a new means to break such a linkage, it was decided to cross root-knot-resistant tobacco with the alloploid *N. sylvestris* X *N. tomentosiformis*.

In 1950, an advanced root knot resistant line, RK 42, was crossed with the alloploid. F1 plants with the largest leaves and highest root knot resistance were backcrossed to a flue-cured breeding line. A BC^1 generation of some 50 plants was grown to seed maturity. Some of the plants possessed a completely healthy root system, and also large leaves. A rigid selection program was continued in the F2 generation and 15 plants were selected for F3 tests. Twelve of these F3 lines were homozygous for root knot resistance and three were segregating. A striking feature of the results, after the alloploid cross, was the ease with which high level resistance was recovered in either F1, F2, or F3 generations, and in all instances in combination with apparently normal leaf size. The new pattern of segregation is illustrated by the following data.

Table 7.—The data given in table 7 are very different from those obtained previously (tables 1 to 6). Then many plants were classed as intermediate in resistance, (Disease Index 1 to 1½). Now, regardless of whether it was a parent F3 line or an F1 or F2, all plants fell into two classes — resistant (Disease Index 0—½) and susceptible (Disease In-

*A plant with sets of chromosomes from two or more different species.

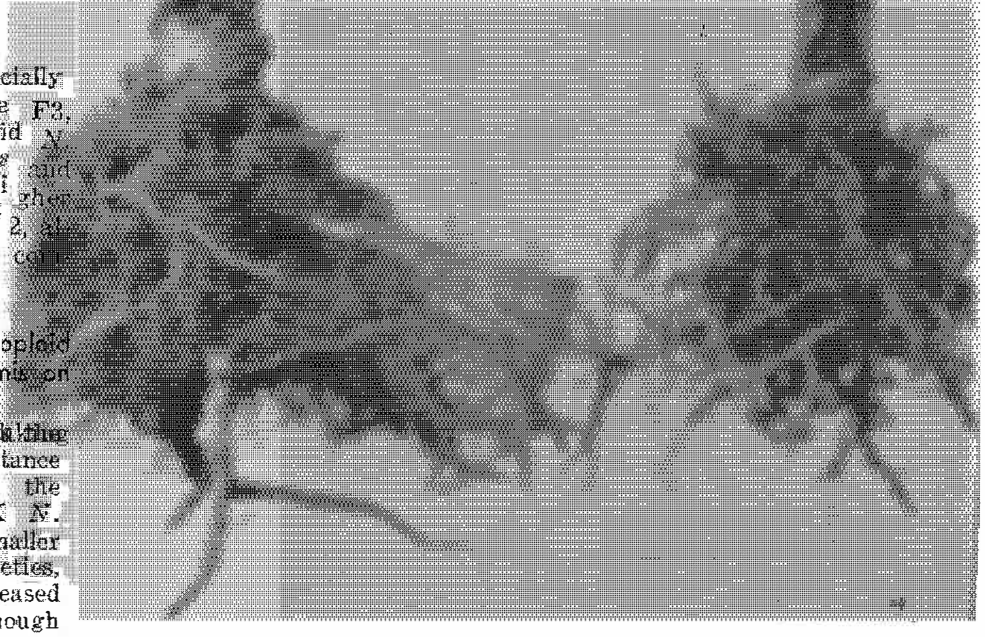

Figure 2. Left, a root knot resistant plant and, right, a check plant of the same were grown in nematode infested soil.

...ex 2—4) (figure 2). It is especially interesting to observe that the F3, ...ived from RK 42 X Alloploid *N. sylvestris* X *N. tomentosiformis* and ...backcrossed to flue-cured, had hi... root knot resistance than RK 4... though the alloploid parent was ...lely susceptible.

The Effect of the Cross with Allo... *N. sylvestris* X *N. tomentosifor...* Leaf Size

The primary reason ... the alloploid cross was to break the linkage between root knot resistance and small leaf size. Since the leaves of *N. sylvestris* X *N. tomentosiformis* are much smaller than those of flue-cured varieties, there was no expectation of increased leaf size in the progeny, although transgressive segregation is not uncommon. As has been indicated, at each step the continuation was always with plants having the largest leaves. The results of leaf measurements were consistently encouraging but conclusions were not drawn until the root knot resistance was definitely stabilized and all growth characters were typically *N. tabacum*. This stage was reached by 1954, at which time the parentage was Flue-cured X ((RK 42 X *N. sylvestris* X *N. tomentosiformis*) X Flue-cured)). Twelve F3 lines were selected for the test. The seedlings were set in 2-inch pots filled with nematode infested sand. Plants were grown to maturity under ordinary field conditions. The check plantings of RK 42 and flue-cured var. 402 were grown in healthy soil and transplanted into the same field which was free of root knot. Check plots of 402, inoculated in the same manner as the resistant lines, were also planted to measure the effectiveness of the inoculation procedure. About half of these plants were dead by mid-summer and all roots of the remainder were severely galled. Nine of the F3 lines planted were homozygous for root knot resistance and these were used for leaf measurements. The procedure followed was to measure the largest leaf of each plant when the plant was in full bloom. The roots of all plants were examined when the seed was mature. The measurements in table 8 are from the nine homozygous resistant lines.

Table 8. — The F3 plants, measurements of which are given in table 8, were root knot inoculated, while RK 42 and flue-cured var. 402 plants were not inoculated, so the latter had every opportunity to produce large leaves. The measurements of RK 42 and 402 show to what degree the previous breeding program of selection and backcrossing had failed. Thus RK 42 leaves averaged 2.4 in. narrower and 5.3 in. shorter than 402 leaves. In contrast the leaves of the resistant F3 plants averaged 1 in. wider and 2.4 in. longer than the leaves of 402. Thus the data provide concrete evidence that the cross with alloploid *N. sylvestris* X *N. tomentosiformis* had eliminated the linkage between resistance and small leaf size, and actually had increased leaf size beyond that found in flue-cured var. 402.

Table 9.—Segregation for root knot resistance in the second, third, and fourth backcrosses, following the alloploid cross

Genotype	Resistant		Susceptible		Ratio
	No.	%	No.	%	R to S
		BC² — F2			
Lot 1	48	84	9	16	5.3–1
2	42	81	11	19	3.8–1
3	45	85	8	15	5.6–1
4	46	81	11	19	4.2–1
5	90	82	20	18	4.5–1
6	82	75	27	25	3–1
7	117	71	48	29	2.4–1
		BC³ — F2			
Lot 1	40	38	64	62	0.62–1
2	57	38	92	62	0.62–1
3	118	44	148	56	0.79–1
4	103	54	86	46	1.2–1
5	111	67	54	33	2.0–1
		BC⁴ — F2			
Lot 1	60	75	20	25	3–1
2	118	66	60	34	1.9–1
3	105	72	41	28	2.56–1
Mean	...	67.5	...	32.5	2.76–1

The Behavior of Root Knot Resistance Following the Cross with Alloploid N. sylvestris X N. tomentosiformis

Further tests confirmed the fact that resistance was now dominant, figure 3, and that segregation was in two classes. Both findings indicated simple monogenic inheritance. However, the segregation in subsequent F2 populations proved so variable that it seemed desirable to continue the tests and to consider the effect of repeated backcrosses to susceptible flue-cured tobacco. The following are the results from an extended series of such tests. (table 9).

Table 9. — The second backcross (BC^2) of RK 42 X *N. sylvestris* X *N. tomentosiformis* with susceptible flue-cured tobacco was represented, table 9, by seven F2 lots. In general there was a large excess of resistant plants, many more than would be expected with simple monogenic inheritance. BC^3 was represented by five F2 population and here the situation was completely reversed. The fit for a 3 to 1 ratio was poor because of a great deficiency of resistant plants. BC^4 gave mixed results. Lot 2 had a deficiency of resistant plants, while Lots 1 and 3 gave good 3 to 1 segregation.

All these data were from tests conducted with the utmost care. There appears to be no doubt that resistance was due to a single gene pair. Likewise it was apparent that the expression of this resistance was subject to the effect of modifier genes. The wide range of results in table 9 suggests that the modifier gene situation varied in different crosses between resistant and susceptible lines and varieties. It seems quite apparent that in work with root knot resistance much variation will be encountered, affecting the degree of dominance, the level of resistance, and resistant-susceptible ratios. Critical testing will be required to obtain best results.

Resistance of Nicotiana Species to Root Knot

Search within the genus for resistance to root knot was initiated early and species showing any promise were subjected to repeated tests. The summarized data from these experiments follow: (see table 10.)

Table 10. — The average disease index figure for all checks of susceptible tobacco was 94, which indicates the uniformity and severity of the tests. Under such conditions, index values below 35 indicate distinct resistance. Nine of the species listed fell into this resistant group, and most of the other species were less susceptible than *N. tabacum*. The object of this work, however, was not primarily to make an exhaustive survey of the genus, but rather to find resistant species and then to attempt to use this resistance in the breeding program. It was early established that *N. repanda*, *N. megalosiphon*, and *N. longiflora* had high resistance (Clayton and Foster, 1940). These species, along with *N. plumbaginifolia*, were also tested in the field in North Carolina, South Carolina, and Georgia. All these species except *N. longiflora* appeared to be completely immune. *N. longiflora* showed no evidence of galls in most tests, but occasionally became severely galled, figure 4. Every effort was made to cross each of these species with *N. tabacum* and to transfer the resistance into the tobacco genome. As a first step, a careful check was made of the progeny from the same *N. tabacum* X *N. longiflora* cross that had yielded wildfire immunity (Clayton, 1947). Root knot resistance was not found in any of this material.

Repeated attempts were made to cross *N. repanda* with *N. tabacum*, but with no success. Kincaid (1949) reported a cross with *N. repanda* which appears to be the only record of success with this species.

Several crosses were obtained between *N. plumbaginifolia* and *N. tabacum*, and numerous progeny were tested for root knot resistance. It was readily possible to obtain lines with a moderate level of resistance, but these were not outstanding. A number of these lines were crossed with genotypes having TI 706 resistance and the behavior of

Table 10.—Resistance of Nicotiana species to root knot

Nicotiana species	No. of Tests	Disease Index*
alata Link & Otto	9	30
acuminata (Grah.) Hook.	10	49
arentsii Goodsp.	1	25
attenuata Torr.	2	100
bigelovii (Torr.) Wats.	5	100
benthamiana Domin	2	100
benavidesii Goodsp.	1	75
bonariensis Lehm.	1	100
debneyi Domin	5	57
exigua Wheeler	5	66
glauca Grah.	7	24
glutinosa L.	3	58
gossei Domin	3	36
goodspeedii Wheeler	4	87
langsdorffii Weinm.	3	75
longiflora Cav.	7	26
maritima Wheeler	3	78
megalosiphon Heurck & Muell.	8	16
nesophila Johnst.	3	50
noctiflora Hooker	4	29
nudicaulis Wats	3	30
otophora Griseb.	3	67
paniculata L.	3	55
plumbaginifolia Viv.	7	22
raimondii Macbr.	5	52
repanda Willd.	8	9
rotundifolia Lindl.	4	40
rustica L.	5	86
stocktoni Brandeg.	3	92
suaveolens Lehmann	4	76
sylvestris Speg. & Comes	4	75
tabacum L.	12	94
tomentosa R. & P.	4	60
tomentosiformis Goodsp.	2	54
trigonophylla Dun.	4	52
undulata R. & P.	2	75
wigandioides Koch & Fint.	7	66

*0 = no galls 100 = all roots diseased or dead

the progeny indicated that the major genes controlling resistance in *N. plumbaginifolia* and in TI 706 were similar or identical.

At one time *N. megalosiphon* was regarded as a very promising source of root knot resistance (Clayton, 1953). Several successful crosses were obtained between *N. megalosiphon* and *N. tabacum*, and the best one was received from Dr. Q. L. Holdermau of the South Carolina Experiment Station. This species was of particular interest not only because it appeared immune in field tests but also because it had been shown by Graham (1952) to be immune to four different species of Meloidogyne. Tests with this material were carried on actively, during the period 1950 to 1953, since species resistance did not appear to be linked with a leaf size factor. *N. tabacum* X *N. megalosiphon* and backcrossed to flue-cured tobacco, produced selections that had good root knot resistance and a large degree of self fertility. However, the resistance was no better, and the plant types were not as good, as those obtained from the TI 706 material after the cross with alloploid *N. sylvestris* X *N. tomentosiformis*. All the evidence to date indicates that this species, like *N. plumbaginifolia*, has resistance controlled by genes that are similar or identical with those obtained from 706, and that the apparent immunity of *N. megalosiphon*, probably results from modifiers present in the *N. megalosiphon* genome.

Thus depending on the background genome, it appears that TI 706 genes for root knot resistance may provide anything from moderate resistance to immunity. In this connection the results reported by Knight (1953) are most interesting. Working with resistance to the black arm disease of cotton, he isolated a gene which alone had no effect, but in combination with other genes it gave resistance closely approaching immunity.

Discussion

Root knot resistant lines of tobacco are now available which are well suited to the production of flue-cured varieties of desirable type. However, this has only been accomplished after a study that began in 1935 and continued without interruption up to the present time. Many problems have been encountered and possibilities for future progress are very evident. The following discussion touches briefly on some of the problems and possibilities.

A source of root knot resistance was located by 1937 in *Nicotiana tabacum*—var. TI 706, obtained from Central America. TI 706 resistance proved non-dominant and segregation in F2 generations led to the conclusion that resistance was inherited on the multiple factor basis. A backcross program was initiated, with the flue-cured type as the recurrent parent, and early results were encouraging. In BC^1 it was possible to recover stable lines that were more resistant than the original 706 parent. By careful selection such undesirable 706 characters as early flowering and profuse branching were completely eliminated. TI 706 had small leaves and from the start every effort was made to secure root knot resistance in combination with normal leaf size. This work was carried on intensively during the period 1940 to 1950. A total of four backcrosses were completed and resistant lines with larger leaves than 706 were obtained, but the best of these had leaves much smaller than the flue-cured tobacco parents. Assuming that resistance was polygenic, efforts were made to recover a part of the root knot resistance in combination with large leaves. This likewise failed, and all large leaf selections ultimately proved to be root knot susceptible. Thus, at the end of 15 years work, it appeared that root knot resistance had no practical application.

It seemed that this situation could only be due either to pleiotropism or to block inheritance and tight linkage. Work was continued on the assumption that it was the latter.

As early as 1928, Goodspeed and Clausen had shown that cultivated tobacco originated from a cross between *N. sylvestris* and a member of the *tomentosa* group. They found that, during the long period that followed, the chromosones of tobacco and the present day forms of the progenitor species, had become greatly differentiated both genically and structurally. Despite these changes, however, pairing still took place between all tobacco and all species chromosones. It seemed that the disrupting effect of such pairing might be an effective means of breaking linkage. On the basis of this reasoning, in 1950, a root knot resistant line (RK 42) was crossed with alloploid *N. sylvestris* X *N. tomentosiformis*. The alloploid cross was successful beyond expectations. After the cross, and two backcrosses to susceptible flue-cured tobacco, the leaves of a group of F3 resistant lines were shown to be broader and longer than the leaves of 402, a large leafed, flue-cured variety. Furthermore, root knot resistance behaved as a simple dominant, with a deficiency of resistant plants, evidently due to modifier effect. The average for 15 F2 populations was 67.5% resistant plants, with clear segregation into only two classes—resistant and susceptible. Furthermore the degree of resistance obtained was distinctly increased. It might appear possible that a dominant gene pair for high level resistance had been obtained from either *N. sylvestris* or *N. tomentosiformis*. However, *N. sylvestris* is susceptible to root knot and *N. tomentosiformis* possesses only moderate resistance. In addition the alloploid *N. sylvestris* X *N.*

Figure 3. Left, an F1 plant—root knot resistant x root knot susceptible. In this instance resistance was completely dominant. On the right is a root knot susceptible plant grown under the same conditions.

tomentosiformis is susceptible to root knot. Therefore a logical conclusion appears to be that the alloploid cross, followed by critical selection in the F1 and F2 generations, effectively eliminated both resistance and leaf size modifiers which were present in the RK 42 genome and possibly added desirable genes from the two species. With this alteration in genetic background, the original root knot resistance obtained in 1937 from TI 706 emerged as a monogenic dominant.

These findings adequately explain why, in earlier years it was not possible to obtain lines having high or moderate root knot resistance and large leaves. All such resistant lines had the major gene pair from 706 with which small leaf size was linked, and lower levels of resistance were due to the presence of resistance modifiers.

These conclusions, with respect to root knot resistance, raise a question as to whether there may not be other instances of supposedly complex, multiple-factor resistance, that are actually simply inherited resistance, with the degree of expression modified by the genome in which the resistance genes happen to be located. Results with the black shank breeding work are pertinent. About 1931, Florida 301 was crossed with flue-cured varieties and, after several backcrosses, it was possible to obtain Oxford 1 (Bullock and Moss, 1943), a flue-cured variety having much less resistance than the 301 parent. About this time W. A. Jenkins, of the Virginia Agricultural Experiment Station at Chatham, Virginia, crossed a moderately resistant flue-cured line with Burley tobacco, a type that was highly susceptible to black shank. Out of this material Burley 11A and 11B were selected at the Tobacco Station, Greeneville, Tenn. (Heggestad and Neas, 1957). These varieties were much more highly resistant to black shank than the flue-cured parent, as was clearly shown by comparisons in the field both at Chatham, Va., and Greeneville, Tenn. It would appear that the basic genes for black shank resistance are the same in Oxford 1 and Burley 11A and 11B, and that the cross to Burley provided a background genome that increased the expression of resistance. Reasoning along the same line, it may be that highly resistant Florida 301 and moderately resistant Oxford 1 have the same major genes for resistance, but the presence of modifier genes in the flue-cured genome reduces the expression of this resistance. With these concepts, a somewhat different approach can be made to the problem of obtaining higher resistance.

Are any of the Nicotiana species or tobacco genotypes actually immune to root knot? Barrons (1939) and Steiner (1942) pointed out that root-knot-immune plants were freely invaded by the nematode larvae, but the larvae died without reproducing. In this way the immune plant served as an effective trap crop. In seeking to decide whether a species or variety is immune, some have taken the position that successful parasitism in the greenhouse, or by special inoculation procedures, proves non-immunity. Graham (1952) concluded that *N. megalosiphon* was immune to root knot. Christie (1946) and Chapman (1957) regarded this species as highly resistant, since they obtained limited egg production. However, these tests were conducted in the greenhouse, and greenhouse tests cannot be accepted as final. A species might even be susceptible as a seedling in the greenhouse, and yet immune in the field. Thus some years ago soybeans and cowpeas were considered to be susceptible to bacterial wilt (*Pseudomonas solanacearum* E. F. Sm.), and their culture on wilt-infested land was discouraged. Smith (1939) showed that both were susceptible as seedlings in the greenhouse, but completely immune in the field. He also proved that both could be used with perfect safety in tobacco rotations on wilt-infested land. The problem of high resistance vs immunity can be academic, but the question of crop effect on soil parasite populations is of vital importance. The writers accept as the final test for root knot immunity, complete inability of the nematode larvae to mature and produce eggs under field conditions. On this basis both *N. repanda* and *N. megalosiphon* appear to be immune. Definite information is not yet available with respect to root-knot-resistance-*N. tabacum* genotypes, but field observations have indicated a marked reduction in the amount of root knot following a crop of resistant tobacco.

This work has been concerned with resistance to *M. incognita*, the common southern root knot nematode. The relation of this resistance to (1) resistance to other species of *Meloidogyne*, (2) resistance to nematode root rot (*Pratylenchus* spp.) and (3) resistance to certain other important tobacco diseases, needs consideration.

First resistance to other species of *Meloidogyne:* Drolsom *et al* (1957) reported that some root knot resistant lines of tobacco were susceptible to attack by *M. arenaria*. While *M. arenaria* presents no serious threat to tobacco at present, nevertheless, it is interesting to consider the possibility that *M. incognita* resistance may be extended to include other closely related species of *Meloidogyne*. There is first the fact that the immunity of *N. megalosiphon* to four species of *Meloidogyne* appears to be due to 706 type resistance plus modifiers in the *N. megalosiphon* genome. Further the expression of 706 resistance in tobacco has been shown to be greatly affected by modifier genes. Recent results with the very similar tomato root knot resistance are most suggestive. Taylor and

Figure 4. Left, N. plumbaginifolia and right N. longiflora; both grown in nematode infested soil. In this test, N. longiflora developed large galls. However, few nematodes matured and the galls remained firm.

Chitwood (1951) found that *Lycopersicon peruvianum* was resistant to *M. incognita*, and moderately to highly susceptible to *M. incognita* var. *acrita*, *M. hapla* and *M. arenaria*. Winsted and Barham (1957) tested tomato lines, with resistance derived from *L. peruvianum*, and found them resistant to *M. incognita*, *M. incognita* var. *acrita*, *M. javanica*, *M. arenaria*, and susceptible only to *M. hapla*. Also Thomason and Smith (1957) started with resistance to *M. incognita* var. *acrita* and, after backcrossing to a susceptible tomato, they isolated a line resistant to *M. incognita* var. *acrita* and *M. javanica*. Presumably all lines had the original gene pair from *L. peruvianum*, and it seems evident that this could be modified by genes present in susceptible tomatoes to provide resistance to as many as four different species of *Meloidogyne*. This situation may closely parallel the one in tobacco where the major gene pair was derived from TI 706.

Nematode root rot (Pratylenchus spp.) is second only to root knot in economic importance in the southern tobacco growing area. During the course of these investigations, root knot resistant lines have repeatedly been observed that had moderate resistance to root rot. No resistance to root rot has been observed in root knot susceptible lines and repeated backcrossing to susceptible flue-cured tobacco did not prevent the reappearance of lines with evident root rot resistance. These observations suggest that there are in root knot susceptible tobacco, not only genes that modify the expression of 706 resistance to root knot, but also genes which, in combination with the 706 pair, give a degree of resistance to nematode root rot. Still another indication in this same direction is the report by Moore *et al.* (1956) who found that certain old flue-cured varieties, such as Hicks, were distinctly tolerant to attack by *Pratylenchus*. The effect of adding 706 root knot resistance to the Hicks type genome will be a matter of much interest.

Combining root knot resistance with resistance to other diseases is in progress and this presents additional possibilities. Root knot has long been recognized as a factor predisposing plants to attack by other root invading parasites. Sasser *et al* (1955) found that root knot infection made plants more susceptible to black shank (*Phytophthora parasitica* Dast. var. *nicotianae* (B. de Hann) Tucker. Lucas *et al.* (1955) showed that the wounding, incident to invasion by root-knot-nematode larvae, facilitated rapid development of bacterial wilt. Still more recently Morgan (1957) has reported a definite association between root knot infection and the development of fusarium wilt (*Fusarium oxysporum* (Schlect.) Wr. var. *nicotianae* J. Johnson). It may be that combining root knot resistance with resistance to bacterial wilt, black shank, and fusarium wilt will improve resistance to those diseases. However, this cannot be assumed in advance since the root knot nematode larvae freely invade the roots of resistant plants and hence cause wounding.

In any event it is quite apparent that the development of usable root knot resistance presents many possibilities with respect to the entire tobacco root disease situation in the South. The present paper is merely a progress report. Some of the problems and interrelationships have been indicated, and further progress will require extensive research.

Summary

This study of resistance to the root-knot-nematode disease of tobacco (*Meloidogyne incognita*) was begun in 1935. Collections of *Nicotiana tabacum* were obtained from Mexico, Central and South America and tested for resistance. TI 706 was selected as the best source of root knot resistance within the cultivated species.

Resistance was carried through the original TI 706 X flue-cured cross and four backcrosses to susceptible flue-cured tobacco. The 706 resistance proved to be intermediate with respect to dominance. F2 populations from crosses between resistant lines and susceptible flue-cured varieties had from 3 to 5% of highly resistant individuals. TI 706 plants branched freely, flowered early, and had small leaves. During the backcross program all undesirable growth characters were eliminated except small leaf size. The best resistant selections had much larger leaves than 706, but in no case were leaves equal in size to the flue-cured parents.

In an attempt to break the apparent linkage between small leaf size and resistance, a breeding line, RK 42, was crossed with alloploid *N. sylvestris* X *N. tomentosiformis*. Following this cross, very high root knot resistance was recovered in the F1 and F2 generations, and simple monogenic, dominant segregation was indicated. In subsequent F2 generations, from backcrosses to susceptible flue-cured tobacco, there was a deficiency of resistant plants, indicating modifier gene action. The leaf size problem was completely eliminated. Measurements of resistant F3 plants showed that these had broader and larger leaves than flue-cured variety 402.

The cross with alloploid *N. sylvestris* X *N. tomentosiformis* changed the expression of TI 706 root knot resistance from an intermediate to a dominant and the yield of resistant plants in the F2 from 5 per cent or less to over 60 per cent, and eliminated all linkage between root knot resistance and small leaf size.

During the progress of this investigation the level of recoverable root knot resistance has been raised twice. First, out of TI 706 X flue-cured and backcrossed to flue-cured, stable lines were isolated that were more highly resistant than the 706 parent. Second, when a resistant line was crossed with alloploid *N. sylvestris* X *N. tomentosiformis* and backcrossed to flue-cured, genotypes with still higher resistance were recovered.

Investigation of the root knot resistance of species of *Nicotiana* indicated that *N. repanda* and *N. megalosiphon* were immune. However, resistance transferred from *N. megalosiphon* into the tobacco genome appeared to be similar to that already obtained from TI 706. Other crosses with *N. plumbaginifolia* also failed to yield superior root knot resistance.

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