Observations and preliminary studies on the occurrence of resistance breaking biotypes of *Meloidogyne* spp. on tomato

Caspar NETSCHER
Laboratoire de Nématologie,
ORSTOM, B.P. 1386, Dakar, Sénégal.

ABSTRACT

A series of laboratory tests complemented by field observations has shown that great differences exist between natural populations of Meloidogyne *from Senegal, the Gambia, and Mauritania in the capacity to form biotypes capable of breaking resistance of resistant tomatoes.*
The practical implications of this phenomenon are discussed and recommendations to prevent the development of these biotypes are given.

RÉSUMÉ

Une série d'expériences au laboratoire complétées par des observations aux champs a montré qu'il existe de grandes différences entre des populations naturelles de Meloidogyne *originaires du Sénégal, de la Gambie et de la Mauritanie en ce qui concerne leur capacité de former des biotypes capables de briser la résistance de tomates résistantes.*
Les conséquences pratiques de ce phénomène sont discutées et des recommandations proposées pour éviter le développement de ces biotypes aux champs sont données.

INTRODUCTION

Root-knot nematodes, *Meloidogyne* spp., cause significant losses to many crops in the tropics and several different methods have been developed to control these parasites. Among these methods, the use of resistant varieties of normally susceptible crops constitutes an inexpensive and effective way to cope with root-knot.

On the basis of research by Smith (1944), Gilbert and Mc Guire (1952, 1956), Barham and Sasser (1956), Laterrot (1973) and others, resistant varieties of market and canning tomatoes have been developed. The efficiency of such varieties is illustrated by the result of Southards (1973) who compared yields of 11 resistant varieties growing in root-knot infested soil with that of the susceptible Manapal. He found that variety Big Seven yielded about 50 % more than Manapal. In a field trial in Senegal the resistant variety Rossol grown in a moderately infested field yielded as well as the comparable variety Roma grown in fumigated soil (Netscher & Mauboussin, 1973).

In spite of good results obtained with resistant tomato varieties, resistance breaking biotypes (or "B-races" as they are often called), either developed by repeatedly growing resistant varieties in *Meloidogyne* infested soil, or occurring spontaneously without previous selection, represent a threat to tomato production. Riggs & Winstead (1959) were able to develop B-races of *Meloidogyne arenaria* and *M. incognita*, on the resistant tomato variety Hawaii 5229 from populations that attacked this variety to only a very limited extent. Triantaphyllou and Sasser (1960) were able to repeat this with single egg-mass cultures of *M. incognita.* A B-race of *M. incognita* and one of *M. javanica* had been reported from Senegal (Netscher, 1970). In this study, observations are presented on the potential capacity of natural *Meloidogyne* populations collected from Senegal, the Gambia and Mauritania to develop B-races complemented by a field experiment and field observations.

OBSERVATIONS ON NATURAL POPULATIONS

Populations of *Meloidogyne* collected from different areas in Senegal, were maintained in the greenhouse on susceptible tomato or cowpea (*Vigna sinensis*). Egg-masses of each of these populations were incubated in water and 4-week-old seedlings of the resistant variety Ronita, growing in 10 cm pots in sterile soil, were inoculated with juveniles that hatched. Inoculum level did not exceeed 2000 juveniles per plant; thus the number of plants inoculated depended on the availability of juveniles.

Four to six weeks after inoculation, plants were uprooted, and the roots washed free of soil and examined for the presence of galls in a glass beaker filled with water. The cylindrical shape of the beaker acted as a magnifying-glass making galls and adhering egg-masses easily detected. The length of time between inoculation and examination of the roots permitted the development of egg-masses of the first generation only. Egg-masses were kept in a 0.3 mol NaCl solution for periods ranging from two to four weeks and subsequently transferred to water. Juveniles collected were added to plants of Ronita as described above and if nematode reproduction occurred, the process was repeated. If reproduction of the population allowed, the process was repeated for 6 generations.

Table I summarizes the data obtained from 18 different populations and one subculture (3280 c) obtained by inoculating a susceptible tomato with one egg-mass of population 3280. Only four populations reproduced during 6 generations; the other 14 failed to reproduce during the first or second generation. In the populations that were able to reproduce on resistant tomato a marked increase in the number of females (as egg-masses recovered) occurred in the sixth generation as compared to the first generation, suggesting either a selection during the experiment or an adaptation to the resistant variety by the populations concerned. This is further illustrated by the increase in the figures expressing the number of egg-masses as the percentage of juveniles inoculated. The same tendency was observed with the single egg-mass culture 3280 c.

In another series of tests, the susceptible tomato variety Roma and the resistant Rossol, an improved variety derived from Ronita, were inoculated with 1 000 to 2 000 juveniles of 35 populations from Senegal, the Gambia, and Mauritania. After six weeks, roots of the resistant plants were examined for the presence of galls.

TABLE I

Number of egg-masses recovered from tomato, variety Ronita, inoculated with juveniles from 18 *populations and one sub-culture of* Meloidogyne *from Senegal. Data were collected after* 1, 2, *and* 6 *generations.*

									Number of egg-masses expressed as percentage of juveniles inoculated		
			Generation						Generation		
			I		II		VI		I	II	VI
Sample number	Location	Species[1]	J[2]	E.m.[3]	J	E.m.	J	E.m.			
3 239	K. Mamadou N'déné	*incognita*	1 850	3	1 500	0			0,16	0	0
3 244	Kaolack	*javanica*	11 000	0					0	0	0
3 249	Bambey	*jananica*	9 500	0					0	0	0
3 257	Dakar	*javanica*	2 800	11	8 150	350	3 300	285	0,39	4,29	8,64
3 262	Koumoune	*javanica*	19 000	0					0	0	0
3 264	Koumoune	*incognita*	1 800	10	4 400	0			0,56	0	0
3 265	Koumoune	*incognita*	3 300	0					0	0	0
3 273	Kaniak	*incognita*	5 000	1	103	1	4 600	147	0,02	0,97	3,20
3 277	Tampe	*javanica*	2 000	1	790	0			0,05	0	0
3 280	Tilène	*javanica*	7 000	4	2 000	35	4 400	310	0,06	1,75	7,05
3 407	M'Boro	*inc + java*	4 800	0					0	0	0
3 418	M'Boro	*javanica*	8 000	0					0	0	0
3 414	M'Boro	*javanica*	4 500	0					0	0	0
3 424	Lampoul	*javanica*	4 700	0					0	0	0
3 433	K. Koura	*arenaria*	3 700	3	81	3	6 200	37	0,08	3,70	0,60
3 438	St. Louis	*incognita*	7 000	0					0	0	0
3 448	Dak. Bango	*incognita*	1 800	0					0	0	0
3 488	Bambey	*incognita*	3 700	0					0	0	0
3 280 C	Laboratory	*javanica*	7 000	7	12 700	> 40	2 000	192	0,24	n.c.[4]	9,6

1 Identified on the basis of perineal patterns.
2 J = Number of juveniles inoculated.
3 E.M. = Number of egg-masses recovered.
4 n.c. = Percentage not calculated.

Among the populations tested, one each of *M. incognita* (11304) and of *M. javanica* (11310) produced considerable galling on Rossol. Cultures established from juveniles of these two populations recovered from Roma have been maintained on this variety since early 1973 without losing their aggressivity towards Rossol.

FIELD TRIALS AND OBSERVATIONS

In order to verify under field conditions whether formation of B races occurred after a number of consecutive crops of resistant tomatoes grown in a field infested by *Meloidogyne,* an experiment was made at the Centre National de Recherches Agronomiques at Bambey, Senegal. In this trial the influence of different sequences of resistant and susceptible tomatoes grown in a field infested by *M. javanica* was studied. The different sequences of susceptible and resistant tomatoes are depicted in table II. Seedlings were grown in nurseries sterilized with Vapam and transplanted 40 days after sowing. Treatments were randomized within each of the 10 replications of the trial. Each plot consisted of 9 plants growing in 3 rows of 3 plants each surrounded by a border row of Roma; spacing was 50 by 50 cm. At the end of each crop, plants were uprooted and examined for the presence of root-knot using a galling index from 0 (roots free of symptoms) to 5 (presence of many large decomposing galls).

Throughout the experiment, examination of the roots of Rossol in the field failed to reveal root-knot symptoms visible to the naked eye. However, plants were often slightly infested since juveniles of *Meloidogyne* frequently were extracted from the roots in a Seinhorst mistifier. Roots of Roma always were heavily galled.

At the Centre pour le Développement de l'Horticulture, Cambérène, Sénégal a number of varieties, reputed to be resistant to *Meloidogyne,* was tested in a field infested with a mixed population of *M. arenaria, M. incognita, M. javanica,* and intermediate forms. Varieties were grown during two consecutive cultures in the same field. In one series, 6 varieties were grown in rows of 10 plants each. Distance between plants in each row was 50 cm; rows were spaced 1 m apart. In another series 17 varieties growing in rows of 5 plants were compared. The first experiment consisted of four replicates whereas the limited space available did not allow the second series to be replicated.

TABLE II

Crop sequences using resistant and susceptible varieties of tomato at Bambey, Senegal.

Sequence N°	Sequence of tomato varieties*			
	1st Crop	2nd Crop	3rd Crop	4th Crop
I	R	R	R	R
II	S	R	R	R
III	S	S	R	R
IV	S	S	S	R
V	S	R	S	R

* S = Susceptible variety Roma; R = Resistant variety Rossol.

Results of the first test expressed as degree of galling are given in table III. Results for the second test are presented in table IV. Data from these tables do not suggest that an increase in pathogenicity took place after one crop of resistant tomatoes. Varietal differences certainly exist as shown by the different reactions of Hope I and Canton B. (table III). Differences also exist between the varieties listed in table IV. In this series a slight increase in pathogenicity was observed as illustrated by the increase of the mean gall index from 1.7 to 2.2.

TABLE III

Average gall indices of six tomato varieties grown in the same field for two consecutive cultures.

Variety	Gall indices	
	1st Crop (data 10-3-75)	2nd Crop (data 27-6-75)
Canton B	5	4.3
UHN 52	2	2.4
UHN 11	2	1.8
Hope 1	1	0.7
Hope 2	1	1.8
Rossol	1.5	1.7
Average gall index	2.1	2.1

Prior to these tests Taylor (1975) had compared the reactions of *Meloidogyne* towards Roma and Rossol by growing these two varieties in the same field from which data of table III and IV were collected. He reported that adult females had been occasionally observed on Rossol and that from egg-masses produced by these females several populations had been increased on Rossol.

Table V summarizes the results of his 14 single egg-mass inoculations on Rossol grown in a constant temperature chamber at 28 °C. It is interesting to note that from the 14 populations studied, those belonging to both *M. incognita* and *M. javanica* did not reproduce or only to a limited extent and that those populations

TABLE IV

Gall indices of 17 tomato varieties grown in the same field for two subsequent cultures.

Variety	Gall indices 1st Crop (10-3-75)	Gall indices 2nd Crop (27-6-75)
Fengshan Manalu	5	4.3
Hybrid C.Q. 6911	2	0.5
Piersol	2	0.3
Resa plan NVF	2	1
Catala NVF	1	2
Master N 52 H	1	2.8
U H N 64 H	1	2.5
U H N 65 H	1	2.4
Master N 53 H	2	2.6
Tavasque F 1	1	2.2
Marsol	1	1
Montfavet 68-18 F1	1	4
V 1 Davis	2	2.4
Atkinson	3	3
Bonus VNF	1	3.2
VNF Bush	1	2
VF 18	2	1.8
Average gall index	1.7	2.2

TABLE V

[redacted]
egg-masses collected from Rossol.

Egg-mass	number	Species*	Number of egg-masses recovered
[redacted]			
"	3	"	5
"	4	"	2
"	5	"	0
"	8	"	27
"	13	"	0
"	14	"	0
"	12	*incognita*	0
"	1	interm. jav. aren.	0
"	6	" *jav. aren.*	> 40
"	11	" *jav. aren*	> 40
"	7	" *inc. aren.*	> 40
"	9	" *inc. aren.*	> 40
"	10	" *inc. aren.*	> 40

* Species determined after perineal pattern of the female that produced the egg-mass.

considered intermediate between two species practically all produced many egg-masses.

Recently a heavy *M. javanica* attack was observed in Senegal on the resistant variety Piersol growing in a newly cleared field that as far as is known had never been cultivated (Population 12367). To exclude the possibility that the seed lot was incorrectly labeled or of mixed nature, juveniles collected from tomato plants from this field were inoculated in the laboratory to resistant varieties Piersol and Rossol of known genetic purity. Six weeks after inoculation severe galling was observed on both resistant varieties as well as on the susceptible variety Roma, confirming that in the field concerned a B-race not only existed but became predominant during the first growing season.

DISCUSSION

Results of this study show the differences in the ability of the different *Meloidogyne* populations to establish B-races on resistant tomatoes. The differences observed are not necessarily due only to the different natures of the populations tested, because environmental factors (e.g. temperature) may render resistant varieties susceptible (Dropkin, 1969). Also varietal differences may exist, as it has been shown that different resistant varieties may derive their resistance from different genes (Sidhu & Webster, 1973). These facets of the problem must be taken into account when these results are interpreted.

The data of table I were all obtained in the Nema-
[redacted]
situated in the tropical forest belt of West Africa. Air temperatures seldom exceed 32 °C and average air temperature is about 28 °C. The experiments were all [redacted] experiments, it seems unlikely that soil temperatures in the pots exceeded 28 °C, a temperature at which, according to Dropkin (1969), resistance of the cultivars considered is not broken. As all tests took place simultaneously and the same variety was used throughout the experiment, it seems logical to assume that the different reactions observed were due to genetic differences between the *Meloidogyne* populations.

Since the species of *Meloidogyne* investigated are all characterized by a mitotic parthenogenetic reproduction, all individuals descending from one individual form a clone. Therefore, it should be expected that the resistant variety will serve as a screen, selecting individual mutants possessing the ability to parasitize these cultivars and that the off-spring of these mutants all would be able to attack the resistant tomatoes. However, the gradual increase in aggressivity of the populations that developed B-races seems to indicate that apart from selection, an adaptation of selected individuals under the pressure of the continuous presence of a resistant cultivar may be responsible for the establishment of B-races. This possibility is suggested by the difference in number of egg-masses produced per number of juveniles inoculated, during the first and sixth generations of the single egg-mass culture 3280 c. It should be noted that the number of egg-masses counted during the first and second generations in reasonably accurate because only a few galls each containing one female were formed. During the 6th generation galls tended to be large, and many egg-masses deposited inside these galls were not counted. Therefore, the degree of adaptation probably is larger than shown by the figures.

Sauer and Giles (1959) observed a progressive increase in aggressivity of *M. javanica* to resistant tomatoes grown continuously for five successive cultures. Unfortunately, in this case the population contained a small proportion of *M. hapla,* too small to be observed at the beginning of the experiment, making it difficult to distinguish between the action of this species, normally aggressive towards resistant tomato, and the appearance of B-races of *M. javanica.* In the trial at Bambey, comparable to that of Sauer and Giles, the culture of successive crops of Rossol did not result in the establishment of a B-race of *M. javanica,* while a slight increase in aggressivity occurred during two consecutive crops of resistant tomato in Cambérène, and an attack of Piersol at M'Bour was instantaneous.

The fact that off-spring of females possessing characters intermediate between two species seem to be more aggressive than those from females whose identity may be more easily determined (table V), agrees with observations of Riggs and Winstead (1959). These workers developed B-races of *M. incognita, M. incognita acrita,* and *M. arenaria.* They observed that, "Perineal patterns of each of the 3 new strains differed from those of the parent populations. The differences were not sufficient to exclude any of the new strains from the parental type. The differences fell within the range of previously observed natural variation within each subspecies, but were more consistent. The patterns found in each of the new 3 strains were more similar to each other than to the parental types". It would be interesting to determine whether a correlation exists between morphology of the nematodes and their aggressivity towards resistant varieties.

The field observations reported here suggest that differences in the nature of the populations rather than environmental conditions are responsible for the attack of *Meloidogyne* on resistant tomatoes. Actually preliminary studies carried out at 33 °C and 35 °C have given indications that contrary to the findings of Dropkin (1969), resistance is not broken at these temperatures working with *Meloidogyne* populations from Senegal (Netscher, unpublished data).

Differences in degree of resistance between varieties, such as observed between resistant varieties in Cambérène, may be due to different sources of resistance. Sidhu and Webster (1973) showed that at least three major genes, $LMiR_1$, $LMiR_2$, and $LMir_3$ are involved in the genetic control of resistance. Preliminary studies with populations of *Meloidogyne* from Senegal suggest that different biotypes of the nematodes exist, characterized by their ability to overcome the resistance of varieties possessing genes $LMiR_1$ and $LMiR_2$ (Netscher, unpublished data).

The breeding of resistant varieties is expensive and demands several years work of geneticists and breeders. Though the dissemination of B-races of root-knot nematodes will be slow compared with airborne diseases, such as rusts, one should avoid selection and increase of these biotypes as much as possible in order to preserve the performances of resistant varieties.

In order to limit the creation of B-races, soils intended to be cropped with resistant varieties should be tested for their presence. Fields can be tested easily by growing resistant and susceptible varieties in pots containing composite soil samples from the fields in question. Examination of the roots of the susceptible variety will indicate the presence and the size of the nematode population by the degree of galling. Galling of the resistant varieties will indicate the probable presence of a B-race in the field examined. If few galls are present on the roots of resistant plants one should be prepared for the possible development of B-races. Subsequent crops of resistant cultivars should be avoided and in case of large populations of *Meloidogyne,* the population level should be decreased by other control methods such as soil fumigation.

In general, heavily infested soils should not be cropped with resistant varieties as it is probable that the number of nematodes belonging to a B-race is proportional to the number of individuals present. Apart from the danger of B-races, it should not be recommended to grow resistant tomatoes in heavily infested soil so as to avoid damage caused by root necrosis as a result of the penetration of juveniles of *Meloidogyne.* In fact such a hypersensitivity has been observed on tomato by several authors (Riggs and Winstead, 1959, Dropkin, 1969, Taylor, 1975), and in groundnuts, death of young seedlings has been caused by the penetration of *Meloidogyne* juveniles in great numbers provoking root necrosis (Netscher, 1975).

Therefore, the best way to avoid the creation of B-races is to incorporate the use of resistant plants in a general system of integrated control. As a preventive control method the sole use of resistant varieties in soils free of root-knot nematodes should be recommended to avoid the build-up of a population from unobserved small infection sites.

The author wishes to express his thanks to Dr. D.P. Taylor who allowed the use of the data presented in table V; to Dr. E. Collingwood who furnished the varieties used in the trial at Cambérène; and the Director of the Centre National de Recherches Agronomiques who made it possible to carry out the field trial at Bambey.

Manuscrit reçu au S.C.D. de l'ORSTOM le 30 *juillet* 1976.

LITERATURE CITED

BARHAM (W.S.), SASSER (J.N.), 1956. — Root-knot nematode resistance in tomatoes. *Proc. Ass. Sth. agric. Wkrs, 53rd Ann. Conv.* : 150-151. (Abstract).

DROPKIN (V.H.), 1969. — The necrotic reaction of tomatoes and other hosts resistant to *Meloidogyne*: reversal by temperature. *Phytopathology,* 59 : 1632-1637.

GILBERT (J.C.), MC GUIRE (D.C.), 1952. — Root-knot resistance in commercial type tomatoes in Hawaii. *Proc. Am. Soc. hort. Sci.,* 60 : 401-411.

GILBERT (J.C.), MC GUIRE (D.C.), 1956. — Inheritance of resistance to severe root-knot from *Meloidogyne incognita* in commercial type tomatoes. *Proc. Am. Soc. hort. Sci.,* 68 : 437-442.

LATERROT (H.), 1973. — Sélection de variétés de tomate résistantes aux *Meloidogyne. OEPP/EPPO Bull.,* 3 : 89-92.

NETSCHER (C.), 1970. — Les nématodes parasites des cultures maraîchères au Sénégal. *Cah. ORSTOM, Sér. Biol.,* n° 11 : 209-229.

NETSCHER (C.), 1975. — Studies on the resistance of groundnut to *Meloidogyne* sp. in Senegal. *Cah. ORSTOM, Sér. Biol.,* vol. X, n° 3 : 227-232.

NETSCHER (C.), MAUBOUSSIN (J.C.), 1973. — Résultats d'un essai concernant l'efficacité comparée d'une variété de tomate résistante et de certains nématicides contre *Meloidogyne javanica. Cah. ORSTOM, Sér. Biol.,* n° 21 : 97-102.

RIGGS (R.D.), WINSTEAD (N.N.), 1959. — Studies on resistance in tomato to root-knot nematodes and on the occurence of pathogenic biotypes. *Phytopathology,* 49 : 716-724.

SAUER (M.R.), GILES (J.E.), 1959. — A field trial with a root-knot resistant tomato variety. C.S.R.I.O. *Austr. Irrig. Res. Sta., Tech. Pap.,* 3 : 1-10.

SIDHU (G.), WEBSTER (J.M.), 1973. — Genetic control of resistance in tomato. I. Identification of genes for host resistance to *Meloidogyne incognita. Nematologica,* 19 : 546-550.

SMITH (P.G.), 1944. — Embryo culture of a tomato species hybrid. *Proc. Am. Soc. hort. Sci.,* 44 : 413-416.

SOUTHARDS (C.J.), 1973. — A field evaluation of nematode-resistant tomato varieties for vine-ripe tomato production. *Tennessee Farm and Home Science,* 85 : 18-20.

TAYLOR (D.P.), 1975. — Observations on a resistant and a susceptible variety of tomato in a field heavily infested with *Meloidogyne* in Senegal. *Cah. ORSTOM, Sér. Biol.,* vol. X, n° 3 : 239-245.

TRIANTAPHYLLOU (A.C.), SASSER (J.N.), 1960. — Variation in perineal patterns and host specificity of *Meloidogyne incognita. Phytopathology,* 50 : 724-735.

WATTS (V.M.), 1947. — The use of *Lycopersicon peruvianum* as a source of nematode resistance in tomatoes. *Proc. Am. Soc. hort. Sci.,* 49 : 213-234.