A naturally occurring resistance breaking biotype of *Meloidogyne arenaria* on tomato. Reproduction and pathogenicity on tomato cultivars Roma and Rossol

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Summary

A naturally occurring resistant breaking biotype of *Meloidogyne arenaria* was collected in Senegal on resistant tomato cultivar Rossol. At a constant temperature of 25° this population reproduced on five resistant tomato cultivars (Anahu, Healani, Nemavite, Pannevis 42166 and Rossol) as well as on the susceptible cultivar Roma. This population also reproduced on the resistant cultivars Caesar, Royal Chico and Slumac.

The reproduction and the pathogenicity, of this « B race » and of a *M. javanica* population unable to break the resistance of the tomato cultivar Rossol at low temperature, were compared on tomato cv. Roma and Rossol at different temperature regimes. Whatever was the temperature regime, the « B race » of *M. arenaria* reproduced on the resistant tomato cultivar as well as on the susceptible. When the average maximum temperature exceeded 33°, the *M. javanica* population was able to break the resistance of the cultivar Rossol but its reproduction on this cultivar was still significantly lower than that observed on the susceptible cultivar Roma. The *M. arenaria* « B race » was more pathogenic than the *M. javanica* strain in reducing top linear growth, top weight, number of new leaves and number of plants killed when the initial inoculum reached 10 000 juveniles.

Résumé

Observation d'une population de Meloidogyne arenaria *spontanément capable de briser la résistance variétale des tomates. Sa reproduction et sa pathogénie sur les cultivars Roma et Rossol*

Une population de *Meloidogyne arenaria* spontanément capable de briser la résistance variétale des tomates était récoltée sur tomate (cv. Rossol) dans une parcelle expérimentale de l'Institut Sénégalais de Recherches Agricoles située dans le nord du Sénégal. A une température constante de 25° cette population se reproduisait aussi bien sur cinq cultivars résistants de tomate (Anahu, Healani, Nemavite, Pannevis 42166 et Rossol) que sur le cultivar sensible Roma. Cette population était aussi capable de briser la résistance des cultivars Caesar, Royal Chico et Slumac.

La reproduction et la pathogénie de cette « race B » étaient comparées, sur les cultivars Roma et Rossol et à différents régimes de température, à celles d'une population de *M. javanica* incapable de briser la résistance du cultivar Rossol à basse température. A toutes les températures utilisées la « race B » de *M. arenaria* se reproduisait aussi bien sur le cultivar résistant que sur le cultivar sensible. Lorsque le maximum moyen de température dépassait 33°, la souche de *M. javanica* devenait capable de briser la résistance du cultivar Rossol mais sa capacité de reproduction sur celui-ci restait significativement inférieure à celle observée sur le cultivar sensible. La « race B » de *M. arenaria* était un parasite plus efficace que la souche de *M. javanica* pour réduire la croissance, le poids des parties aériennes, le nombre de nouvelles feuilles et pour tuer les plantes quand l'inoculum atteignait 10 000 juvéniles.

Allen (1952) reported that two populations of *Meloidogyne* spp., collected from cotton plants, were able to reproduce lightly on *Lycopersicon peruvianum* (L.) Mill., the species from which the resistance to *Meloidogyne* is derived in tomato *(Lycopersicon esculentum* L.*)*.

Since the selection of resistant cultivars is usually the result of limited testing the new cultivars to a few *Meloidogyne* populations only, it is not surprising that reports of slight reproduction on resistant cultivars are numerous (Khan *et al.*, 1975 ; Ogunfowora, 1976 ; 1977 ; Philis & Vakis, 1977 ; Viglierchio, 1978 ; Contreras Lopez, 1979 ; Hadisoeganda & Sasser, 1982). These observations may be explained by the physiological variability of *Meloidogyne* species (Netscher, 1978) and the occurrence of observations in tropics where soil temperature at 10 to 15 cm may exceed 35°, inducing a possible loss of resistance which may occur when the temperature reach 33° (Dropkin, 1969 ; Paulson, 1976 ; Araujo, Dickson & Augustine, 1979).

Riggs and Winstead (1959) observed that a limited number of females of *Meloidogyne incognita*, *Meloidogyne incognita acrita* and *Meloidogyne arenaria* produced eggs on a resistant tomato cultivar ; from these juveniles, they obtained, by successive transfers on the same tomato cultivar, populations capable of breaking the resistance of this cultivar which they termed « B races ». This phenomenon was also observed by Giles and Hutton (1958), Triantaphyllou and Sasser (1960), Netscher (1970, 1977), Taylor (1975) and Caveness (1976). Recently Netscher (1977) reported a severe attack of *Meloidogyne* on a resistant tomato cultivar in a field that had never been cultivated previously to tomato.

This paper reports the observation of a naturally occurring « B race » of *Meloidogyne arenaria* which has severely attacked the resistant cultivar Rossol in the field and was able to break the resistance of all the cultivars tested. The reproduction and the pathogenicity on tomato cultivars Roma and Rossol were compared at different temperature regimes between this « B race » of *M. arenaria* and a *M. javanica* population unable to break the resistance of the cultivar Rossol at low temperature regimes.

Field observations

In February 1981, *M. arenaria* (Strain 14568) was found severely attacking the resistant cultivar Rossol in a research field at the experimental station of the Institut Sénégalais de Recherches Agricoles (ISRA) of N'Diole. Many plants were dead and the surviving plants were stunted, had discoloured leaves and their roots were completely galled.

This field was cleared of native vegetation in June 1975 and put into production in 1977. Since then the crop rotation was practiced as follows :

— rainy season 77 : millet (cv. Souna 3)
— dry season 77-78 : tomato (cv. Rossol)
— rainy season 78 : millet (cv. Souna 3)
— dry season 78-79 : french bean (cv. Vadenel) and potato (cv. Bentch)
— rainy season 79 : ground-nut
— dry season 79-80 : egg-plant
— rainy season 80 : ground-nut
— dry season 80-81 : tomato (cv. Rossol).

Of these crops grown in Sénégal, ground-nut is immune ; millet is a poor host ; french bean, potato and egg-plant are susceptible.

Greenhouse experiments

Materials and methods

All the experiments were conducted using autoclaved (120° for 30 mn) sandy soil. Nurseries were prepared in plastic pots (2 400 cm³ capacity) by planting ten seeds in each. Nurseries and plants after transplantation were irrigated with water as needed and with half-strength Hoagland's nutrient solution added once a week.

In the first experiment, seedlings of one susceptible tomato cultivar (Roma) and of five resistant cultivars (Anahu, Healani, Nemavite, Pannevis 42166 and Rossol) were transplanted singly four weeks after germination in a glass pot containing 200 cm³ of soil. At the same time, 100 second-stage juveniles of *M. arenaria* (Strain, 14568) were inoculated on each plant. Juveniles were extracted from Rossol tomato galled roots taken from the infested field using a mist chamber (Seinhorst, 1950) ; only individuals collected within 48 h were used. The pots were placed in a water tank at a constant temperature of 25°. Each treatment was replicated ten times.

In the second experiment, seedlings of four resistant cultivars of tomato (Caesar, Slumac, Rossol and Royal Chico) were transplanted singly four weeks after germination in plastic pots (2 400 cm³ in capacity). At the same time 1 000 or 5 000 second-stage juveniles of *M. arenaria* (14568) obtained as in the first experiment were inoculated. Pots were placed in a sand bed at a constant temperature of 30°. Each treatment was replicated seven times.

In both experiments plants were grown for four weeks after inoculation. Then, individual root systems were washed and incubated for two weeks in a mist chamber (Seinhorst, 1950) to collect juveniles for analysis.

A third experiment was realized in order to compare the pathogenicity and the reproduction of a *M. javanica* population and the "B race" of *M. arenaria* on tomato cv. Roma and cv. Rossol at five temperature regimes. In this experiment juveniles of *M. javanica* derived from a clone established from a single egg mass and maintained on kenaf *(Hibiscus cannabinus* L.) in the greenhouse were compared with juveniles of *M. arenaria* (14568) derived from the population originally isolated from tomato cv. Rossol in N'Diole (Sénégal) and increased on that host in the greenhouse. Juveniles were extracted from galled roots using a mist chamber (Seinhorst, 1950). Only individuals collected within 24 h were used in this experiment.

Four week old tomatoes (cv. Roma and Rossol) were transplanted singly in plastic pots (450 cm³ capacity) or in glass pots (250 cm³ capacity). Plastic pots were placed on benches in the greenhouse and glass pots in water tanks at various temperatures. The temperatures of benches and tanks was not controlled. A temperature sensor was placed at the center of a check pot and the soil temperature

Table 1

Experimental conditions of the third experiment comparing the pathogenicity and the reproduction of *M. javanica* population and « B race » of *M. arenaria* on tomato cv. Roma and Rossol at five different temperature regimes

Temperature regimes N°	*Average minimum and maximum temperature (°)*	*Inoculum/plant*	*No of replicates*	*Time of growth after inoculation (weeks)*	*Volume of soil/pot (cm^3)*
1	22-38.5	0 100 1 000 10 000	10	4	400
2	18.5-34	0 1 000 10 000 15 000	10	4	400
3	17.5-32.5	0 1 000 10 000 15 000	10	6	400
4	20-24	0 1 000 10 000	6	4	200
5	17.5-23	0 1 000 10 000	6	6	200

recorded permanently. The different temperature regimes were differenciated by their average minimum and maximum temperature. Immediately after transplantation, plants were inoculated by pipetting the appropriate number of water-suspended juveniles in four holes around the plant roots. Non inoculated plants were included as checks for comparison. Height and number of true leaves of each plant were recorded at transplantation. Table 1 indicates the experimental conditions (replicates, temperature regimes, time of growth and volume of soil/pot).

Plants were harvested four or six weeks after inoculation. The tops were cut off and the roots washed free of soil. The tops and roots were weighed. Height of top and the number of true leaves of each plant were recorded. Roots were incubated for two weeks in a mist chamber (Seinhorst, 1950) to collect juveniles for analysis.

Results and discussion

Experiment 1

Table 2 indicates the numbers of juveniles and males recovered from roots of one susceptible tomato cultivar (Roma) and five resistant cultivars four weeks after inoculation with 100 juveniles per plant. No significant difference was observed. The population of *M. arenaria* (14568) reproduced on the five resistant tomato cultivars as well as on the susceptible.

Table 2

Numbers of juveniles and males recovered from roots of one susceptible tomato cultivar (Roma) and five resistant cultivars inoculated with 100 juveniles of *M. arenaria* (14568) after four weeks growth at 25°

Cultivar	*Numbers of juveniles* *	*Numbers of males* *
Roma	13 900	15
Anahu	10 900	25
Healani	6 900	20
Nemavite	12 000	20
Pannevis 42166	10 500	30
Rossol	11 400	30

* Means of ten replications.

Experiment 2

This population was also able to break the resistance of the cultivars Caesar, Slumac and Royal Chico grown at 30° (Tab. 3).

Table 3

Numbers of juveniles recovered from roots of four resistant tomato cultivars inoculated with 1 000 or 5 000 juveniles of *M. arenaria* (14568) after four weeks growth at 30°. Numbers of juveniles * recovered four weeks after inoculation

Cultivar	*Inoculum* *1 000*	*5 000*
Rossol	58 000	160 000
Caesar	131 000	315 000
Slumac	58 000	283 000
Royal Chico	73 000	170 000

* Means of seven replications.

Experiment 3

Reproduction. At the low temperature regimes, the population of *M. javanica* was unable to break the resistance of the tomato cv. Rossol as shown in Table 4. On the other hand, when the average maximum temperature exceeded 33°, this population was capable of reproduction on the resistant cultivar but generally the reproduction was significantly less than reproduction observed on the susceptible cv. Roma.

The "B race" of *M. arenaria* reproduced equally well on the resistant tomato cultivar as on the susceptible cultivar at all the temperature regimes (Tab. 4). Males were always produced in this population of *M. arenaria;* and their numbers increased at low temperature regimes or high levels of inoculum.

Pathogenicity. The tomato cultivars Roma and Rossol did not grow well at the lowest temperature regime (17.5-23°) and the results obtained at this temperature regime were not considered in the final analysis.

Table 4

Reproduction of *M. javanica* and *M. arenaria* 14568 on a susceptible tomato cultivar (Roma) and a resistant tomato cultivar (Rossol) at different temperature regimes. Average numbers of juveniles recovered from roots four or six weeks after inoculation

Temperature regimes N°	*Inoculum level*	M. javanica *Mean * No. juveniles/plant* *cv. Roma*	*cv. Rossol*	M. arenaria *14568* *Mean * No. juveniles/plant* *cv. Roma*	*cv. Rossol*
	0	0	0	0	0
1	100	10 580 a	2 930 b	9 420 a	11 770 a
	1 000	53 830 a	12 290 b	48 450 a	49 880 a
22-38.5°	10 000	70 920 a	25 260 b	62 750 a	38 960 a
	0	0	0	0	0
	1 000	290 a	40 b	4 230 c	1 990 c
2	10 000	40 a	70 a	670 c	1 180 c
18.5-34°	15 000	10 a	10 a	590 c	170 c
	0	0	0	0	0
3	1 000	116 720 a	—	128 980 a	94 250 a
	10 000	34 260 a	90 b	82 340 a	19 890 a
17.5-32.5°	15 000	60 930 a	80 b	78 730 a	61 500 a
	0	0	0	0	0
4	1 000	1 150 a	0 b	6 210 c	17 970 c
20-24°	10 000	280 a	0 b	1 820 c	5 440 c
	0	0	0	0	0
5	1 000	46 380 a	20 b	67 120 c	42 770 c
17.5-23°	10 000	5 070 a	50 b	plants dead	plants dead

Line means followed by the same letter are not significantly different (P = 0.01) according to Mann-Whitney's test.

* Means of ten replicates for the three first experiments and six replicates for the two last.

Table 5

Effect of 1 000 juveniles of *M. javanica* or *M. arenaria* (14568) on the top linear growth of tomato plants (cv. Rossol and Roma) after four or six weeks growth at different temperature regimes. (Means of ten replications for the three first experiments and six replications for the last).

Growth period (weeks)	*Average minimum and maximum temperature* (°)	*Inoculum*	*Top linear growth (cm)*	
			Rossol	*Roma*
		0	28.5	29.2
4	22-38.5	1 000 *M. javanica*	28.9	26.8
		1 000 *M. arenaria*	20.2 *	24.1 *
		0	26.6	20.9
4	18.5-34	1 000 *M. javanica*	27.7	18.6
		1 000 *M. arenaria*	18.2 *	17.2 *
		0	22	17
6	17.5-32.5	1 000 *M. javanica*	—	15
		1 000 *M. arenaria*	6.9 *	13.5 *
		0	16	12.8
4	20-24	1 000 *M. javanica*	13.7	12
		1 000 *M. arenaria*	10.8 *	9.8 *

* Significant difference (P = 0.01) between inoculated and check plants after a Mann-Whitney's test.

At all temperatures 1 000 juveniles of *M. arenaria* 14568 significantly reduced the top linear growth of the two tomato cultivars (Tab. 5). On the other hand, with an inoculum of 1 000 juveniles the strain of *M. javanica* was unable to reduce significantly the top linear growth. At the two high temperature regimes, with 1 000 juveniles of *M. arenaria* the top linear growth of the cultivar Rossol was reduced by 30% at 22-38.5° and by 32% at 18.5-34° ; while that of the cultivar Roma, at the same temperature regimes was reduced by 18% and 13% respectively. The resistant variety Rossol appeared more vulnerable to the "B race" of *M. arenaria* than the susceptible cultivar. With 10 000 juveniles of *M. arenaria* 14568 the top linear growth was severely reduced and 80% of the plants were killed with 15 000 juveniles. The "B race" of *M. arenaria* was more efficient in reducing the linear growth and in killing the plants than the strain of *M. javanica*, this as well on the susceptible cultivar as on the resistant cultivar.

At the two high temperature regimes with an inoculum of 10 000 or 15 000 juveniles, both populations reduced the number of new leaves and the fresh top weight of the two cultivars ; the *M. arenaria* "B race" was always more aggressive on the cultivar Rossol than the *M. javanica* strain.

Conclusion

The *M. javanica* population was unable to break the resistance of the cultivar Rossol at low temperature regimes but was able to reproduce on this cultivar when the average maximum temperature exceeded 33°. At high temperature regimes the resistance of the cultivar Rossol was broken but the reproduction of the *M. javanica* strain was still significantly lower than that observed on the susceptible cultivar Roma.

On the other hand, the population 14568 of *M. arenaria* which was isolated from a severely affected resistant tomato Rossol, was able to break the resistance of eight resistant tomato cultivars tested. At a constant temperature of 25° this population reproduced on five resistant tomato cultivars (Anahu, Healani, Nemavite, Pannevis 42166 and Rossol) as well as on the susceptible cultivar Roma. It may be concluded that this population of *M. arenaria* is a "B race". As in the field, from which this population was collected, the resistant cultivar Rossol was grown only twice in three years and these two crops alternating with susceptible crops, the development of the "B race" can not be explained by a gradual adaptation to this resistant cultivar. Therefore, this *M. are-*

naria population appears to be a naturally occurring "B race" capable of severely attacking resistant tomato cultivars and to reproduce as well on the resistant cultivar Rossol as on the susceptible cultivar Roma whatever the temperature regime is. Moreover this *M. arenaria* "B race" appeared to be a more efficient parasite than the *M. javanica* strain.

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