

Reproduction of Virulent Isolates of *Meloidogyne incognita* on Susceptible and *Mi*-resistant Tomato

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Abstract: The reproductive potential of natural and laboratory-selected *Meloidogyne incognita* isolates virulent against the tomato *Mi* resistance gene, all derived from a single egg-mass, were compared when the nematodes were inoculated on susceptible and resistant tomato. Fewer second-stage juveniles ($P = 0.01$) of the two virulent populations selected under laboratory conditions matured to females on the resistant tomato compared to the susceptible cultivar. In contrast, no differences were found between the number of egg masses produced on the resistant versus the susceptible tomato by the two natural virulent isolates. No clear general trends concerning the fecundity of the females could be inferred from the comparative analysis of the numbers of eggs per egg mass \times tomato cultivar combination. These observations suggested that the genetic changes induced under environmentally controlled nematode growth might be different from those occurring in natural *Mi*-resistance breaking biotypes grown without environmental control.

Key words: fecundity, *Lycopersicon esculentum*, *Meloidogyne incognita*, *Mi* gene, nematode, root-knot nematode, tomato, virulence.

Resistance to root-knot nematodes in tomato is generally thought to be conferred by a single dominant gene, designated *Mi* (8), which controls maturation of *Meloidogyne incognita*, *M. arenaria*, and *M. javanica*. All the currently available resistant cultivars are derived from breeding lines carrying the *Mi* gene (11), although recent works suggest the occurrence of new resistance factor(s), some of them not allelic to *Mi* (1,4,16).

Although reproduction of *M. incognita* isolates sometimes occurs, this nematode generally fails to reproduce significantly on resistant tomato genotypes grown under greenhouse or field conditions (17,18). However, the ability of some *Meloidogyne* populations to overcome the *Mi* gene (i.e., to develop and produce fertile females at a rate incompatible with plant resistance) is also well documented. Virulent field isolates have been reported in many parts of the world (2,13,14,19). Moreover, under laboratory conditions, it is possible to select virulent lineages from nonvirulent *M. incognita* isolates by repetitive inoculations onto resistant tomato genotypes (3,9,15). No conclusive opinion emerges from those

studies concerning the fecundity of the *Mi*-virulent females. Bost and Triantaphyllou (3) found no significant differences in egg mass contents when a selected virulent *M. incognita* population was reared on the susceptible Rutgers or the resistant Small Fry tomato cultivar, whereas Jarquin et al. (9) observed a greater fecundity when virulent females developed on the susceptible host, whether these females came from a laboratory-selected or from a wild virulent *M. incognita* isolate. Prot (14) reported that a naturally occurring virulent *M. arenaria* population reproduced as well on five different resistant tomato cultivars as on the susceptible control.

The objective of the present study was to compare the reproduction of virulent laboratory-selected and wild *M. incognita* isolates on both susceptible and *Mi*-resistant tomato. For that purpose, we analyzed the ability of the juveniles to mature and produce eggs.

MATERIALS AND METHODS

Biological material: Susceptible tomato (*Lycopersicon esculentum*) cv. Saint Pierre and the near-isogenic resistant cv. Piersol (10) carrying the *Mi* gene were used in this study.

Six nematode populations, each obtained from a single egg mass, were selected for the experiments. They were

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identified as *M. incognita* and were indistinguishable by their perineal patterns and isoesterase electrophoretograms (7). Their name, geographical origin, and avirulence (virulence) against the *Mi* gene are reported in Table 1. The virulent lines from Calissane and Adiopodoumé were laboratory selected and reared on Piersol for more than 25 generations according to the procedure of Jarquin-Barberena et al. (9). The avirulent isolates and the virulent field isolates were routinely cultured on Saint Pierre and Piersol, respectively.

Experimental procedures and evaluation: All experiments were conducted in a climate controlled room at a mean temperature of 23 C. Tomato seeds were germinated in steam-sterilized sandy soil in flats, and 2-week-old seedlings were transplanted singly into 50-ml plastic tubes containing the same substrate and allowed to establish for 2 weeks before inoculation.

Before the experiment started, avirulent nematode lines were increased on Saint Pierre and virulent ones on Piersol, respectively. Second-stage juveniles (J2) were collected each day from infected roots held in a mist chamber and stored at 4 C before use within 5 days. Seedlings of tomato were inoculated at a rate of 25 J2 per plant. The miniaturized tube test conditions have been used previously and gave reproducible results (5). The inoculum in water suspension was pipetted onto the soil surface around the stem base and lightly watered. Plants were arranged in a randomized

complete block design with 10 replicates for each nematode \times plant combination tested, and the experiment was repeated.

Eight weeks after inoculation, the washed root systems were placed in cold eosin yellow (0.1 g/liter water) and stirred for 30 minutes to stain egg masses. Numbers of egg masses per root system were counted and a reproductive index (Ri) was calculated according to the following ratio: $Ri = \text{no. egg masses}/\text{no. juveniles inoculated}$. The reproductive index theoretically ranged from 0 (no reproduction at all) to 1 (each juvenile inoculated gave one egg mass).

Three randomly selected plants of each combination were assayed for the average number of eggs per egg mass. For each plant, five egg masses were removed at random from the root system. They were individually dissociated in 0.9% NaOCl, mounted between a glass slide and a coverslip, and the number of eggs per egg mass counted under a stereoscopic microscope.

Analysis of variance (ANOVA) was performed for the two sets of experiments (after angular transformation of the data for the Ri values), and Duncan's multiple-range test was used to separate the different mean values.

RESULTS

Because no significant differences existed between the repeated treatments in the experiments, results were pooled together for the statistical analysis.

Development of J2: All the *M. incognita* populations tested reproduced well on the susceptible Saint Pierre, with Ri values ranging from 0.85 to 0.99 (Table 2). Except for the virulent field population from N'Gorom, which produced slightly fewer egg masses, there were no differences ($P = 0.01$) between their respective Ri values.

As expected, both avirulent populations from Calissane and Adiopodoumé were unable to reproduce on the resistant Piersol (Ri values of 0.03 and 0.01, respectively). However, all the virulent populations exhibited a high reproductive rate on

TABLE 1. *Meloidogyne incognita* isolates used and their (a)virulence against the resistance gene *Mi* in tomato.

Origin	Description
Calissane, France	Avirulent, field isolate
Calissane, France	Virulent, laboratory-selected†
Adiopodoumé, Ivory Coast	Avirulent, field isolate
Adiopodoumé, Ivory Coast	Virulent, laboratory-selected†
Valbonne, France	Virulent, field isolate
N'Gorom, Senegal	Virulent, field isolate

† Virulent isolate selected from the avirulent one of the same origin.

TABLE 2. Reproduction and fecundity of *Meloidogyne incognita* isolates on susceptible (S) 'Saint Pierre' and resistant (R) 'Piersol' tomato cultivars.

Population	Tomato cultivar	Ri†	Eggs/egg-mass
Calissane (avirulent)	Saint Pierre (S)	0.93 abc‡	700 de
	Piersol (R)	0.03 e	—§
Calissane (selected virulent)	Saint Pierre	0.98 a	485 ef
	Piersol	0.62 d	476 ef
Adiopodoumé (avirulent)	Saint Pierre	0.99 a	1,101 bc
	Piersol	0.01 e	—
Adiopodoumé (selected virulent)	Saint Pierre	0.94 ab	845 cd
	Piersol	0.60 d	365 f
Valbonne (natural virulent)	Saint Pierre	0.91 abc	1,222 b
	Piersol	0.93 abc	1,345 b
N'Gorom (natural virulent)	Saint Pierre	0.85 c	1,623 a
	Piersol	0.87 bc	1,095 bc

† Values for Ri and eggs/egg-mass are the mean of 20 and 15 replicates, respectively.

‡ Values within a column followed by the same letter are not significantly different ($P = 0.01$) according to Duncan's multiple-range test.

§ Not enough egg masses were produced to give a reliable result.

the resistant tomato, even if differences ($P = 0.01$) were found between the laboratory-selected and the virulent field isolates (Table 2). The two laboratory-selected virulent populations from Calissane and Adiopodoumé produced fewer ($P = 0.01$) egg masses on the resistant Piersol compared to the number of egg masses produced on the susceptible Saint Pierre (Ri values of 0.62 and 0.60, respectively). No differences could be found between the number of egg masses produced on the resistant versus the susceptible tomato by the two natural virulent isolates from Valbonne and N'Gorom (Table 2).

Fecundity of females: Very few avirulent J2 were able to develop into mature females on the resistant tomato cultivar, with only 4 and 17 egg masses produced on the 20 replicate plants for the isolates of Adiopodoumé and Calissane, respectively. Moreover, these egg masses contained very few eggs (data not shown). Since the number of eggs per egg mass could not be determined for these two populations, they were not taken into account in the statistical analysis of the data.

No clear trends are apparent from comparative analysis of the numbers of eggs per egg mass produced in each nematode × tomato cultivar combination (Table 2). Females of the virulent field isolates always

produced high egg numbers (from 1,095 to 1,623 per egg mass) on resistant and susceptible plants. The Valbonne isolate showed no differences ($P = 0.01$) in the number of eggs per egg mass developed on the susceptible or the resistant tomato. On the contrary, the fecundity of the N'Gorom isolate was higher ($P = 0.01$) on the susceptible compared to the resistant cultivar. A similar difference was observed between the two isolates laboratory-selected for virulence. The fecundity of the virulent Calissane isolate was not affected by the tomato cultivar on which it had been cultured, whereas females of the virulent Adiopodoumé isolate produced fewer eggs ($P = 0.01$) when propagated on the resistant Piersol.

DISCUSSION

The ability of some *Meloidogyne* isolates to overcome the tomato *Mi* resistance gene has been reported under both natural (13, 14, 19) and laboratory conditions (3, 9), and the genetic determinism of this character has been demonstrated for *M. incognita* (6). But no definitive information was provided on the reproductive potential of these virulent isolates. This study indicates that the ability of J2 to develop and produce egg masses on resistant, compared to

susceptible, tomato is less for laboratory-selected virulent *M. incognita* isolates, but is not affected for virulent field isolates. Conversely, there is no relationship of female fecundity with either the origin of their virulence or the cultivar on which they are grown.

Assuming that virulence on resistant cultivars is expressed as the ability of J2 to develop and produce egg masses, the virulent field isolates and the laboratory-selected ones exhibited differences. The number of egg masses produced on the resistant tomato was lower for both selected isolates compared to the number produced on the susceptible cultivar, while no such differences occurred for the two virulent field isolates. These observations may be correlated with the way these populations acquired their virulence, suggesting that the genetic changes induced under managed pressure in climate-controlled room conditions are different from those occurring in natural *Mi*-resistance breaking biotypes. Therefore, in *M. incognita*, two different genetic mechanisms may be involved in the acquisition of virulence against the tomato *Mi* gene. Virulent field and laboratory-selected isolates also differ in the stability of their virulence when the selection pressure of the resistance gene is removed (5).

The fecundity of virulent isolates of the potato cyst nematode on susceptible and resistant cultivars varies, depending on the species. The fecundity of *Globodera pallida* females on *Solanum vernei*-derived resistant clones was not different from those produced on susceptible hosts (20). Conversely, the number of viable eggs and juveniles was significantly less in *G. rostochiensis* cysts produced on resistant cultivars containing the single dominant *H1* resistance gene compared with those from susceptible potato (12). These data on potato cyst nematodes, and ours, suggest that there is no general assumption that can be made to estimate the fecundity of virulent nematodes on various plant cultivars, and also that no strict relation exists

between *M. incognita* virulence and fecundity.

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