REPRODUCTION OF NATURAL AND SELECTED RESISTANCE-BREAKING *MELOIDOGYNE* POPULATIONS ON NEAR-ISOGENIC TOMATO LINES

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Summary. The near-isogenic tomato cultivars Motelle (bearing the *Mi*-gene for resistance to *Meloidogyne* spp.) and Moneymaker (susceptible) were inoculated with 16 *Meloidogyne* populations. An average reproduction index for each population was obtained from a series of inoculations carried out over a period of three years. Most of the populations were able to break the resistance and to reproduce successfully on the cv. Motelle. Resistance-breaking populations came directly from fields in different countries (naturally virulent populations) or were selected under greenhouse conditions by repeated inoculations on resistant tomatoes. Naturally virulent populations from North Africa showed the greatest degree of reproduction on resistant tomato, whilst other tropical and Mediterranean populations had lower rates of reproduction, but still comparable with that of the populations generated by selection. Generally, a given population reproduced better on the susceptible than on the resistant isoline. The resistance-breaking ability of *Meloidogyne* populations has been found to be durable on resistant tomato over many years; moreover, populations retained their virulence even if reproduced for several generations on susceptible plants.

Tomato (*Lycopersicon lycopersicum* (L.) Karsten *et* Farw.) is the most important vegetable crop in Italy with about 7,5 millions tonnes produced in 1999 (ISTAT report), and the region of Apulia is the largest producer of tomatoes for processing (2 million tonnes). The sedentary endoparasitic nematodes *Meloidogyne* spp., known as root-knot nematodes (RKN), are widely distributed and cause severe damage to this crop, both outdoors and in glasshouses (Lamberti, 1981). From an economical point of view, two species are the most important, *M. incognita* (Kofoid *et* White) Chitw. and *M. javanica* (Treub) Chitw., whilst *M. arenaria* (Neal) Chitw. is less important. Chemical nematicides have been used extensively to control such pests, although increasing environmental concerns are leading governments to ban or restrict their application. In the case of tomato, genetic host-plant resistance (HPR) against RKN has been available for a long time in many processing and fresh-market cultivars. The resistance factor that has been used so far is the dominant *Mi*-gene, which confers resistance to *M. incognita*, *M. javanica* and *M. arenaria*, but several novel genes from wild species have been described (Liharska and Williamson, 1997). Although *Mi*-bearing tomato cultivars have been successfully and frequently cropped, *Mi*-mediated resistance has some serious limitations. It does not work with *M. hapla* Chitw. or at temperatures above 28 °C. Moreover, several resistance-breaking *Meloidogyne* populations have already been isolated from the field in different countries (Prot, 1984; Kaloshian *et al.,* 1996; Ornat *et al.,* 2001). Normally, such "natural virulence" characterizes populations that have not been subjected to selection by repeated exposure to *Mi* (Roberts *et al.,* 1990). Virulent populations can also be selected from avirulent wild-type isolates by repeated inoculation on resistant plants grown under controlled conditions in the greenhouse (Castagnone-Sereno *et al.,* 1994). Identification of virulence factors has been attempted by using molecular methods to compare selected virulent and avirulent isolates (Castagnone-Sereno, 1994; Molinari, 2000). In this paper, the degree of reproduction of natural and selected virulent and avirulent *Meloidogyne* populations has been assayed on near-isogenic tomato cultivars that differ only in their resistance to RKN. A difference of reproductive behaviour of these two types of virulent RKN populations is reported and some features of their virulence are discussed.

MATERIALS AND METHODS

The tomato cultivars Roma VF and VFN8, respectively susceptible and resistant to RKN, were used for rearing nematode populations, and two near-isogenic tomato lines differing only in their RKN resistance (Moneymaker, susceptible and Motelle, resistant) were used to detect the reproduction index of 16 different nematode populations. The dominant resistance gene *Mi* is homozygous in both VFN8 and Motelle.

Tomato seeds were sown in steam-sterilized loamy sand for germination. At about one month old, seedlings were transplanted singly into 12-cm diameter pots filled with the sterilized loamy sand and put in a glasshouse at 25-27 °C. Inocula were prepared by a modification of the method of Hussey and Barker (1973). A suspension of 3,000 fresh eggs in a minimum volume of 3-5 ml was pipetted into the root zone via two holes made in the soil near the plant in each pot. During each experiment, the pots were arranged in a randomised complete block design and replicated at

least six times on benches with temperature-regulated bases that maintained the soil in pots at 24-27 °C. About 40 days after inoculation, plants were removed from the pots and roots attacked by the same nematode population were collected, washed free of soil debris, gently dried, chopped into pieces of *c.* 1 cm length and weighed. The index of reproduction (IR) of nematode populations was expressed as the number of eggs per gram of fresh root (Roberts and Thomason, 1986). To calculate an average IR, six experiments were carried out over a period of three years; data are given as means plus standard deviation.

The nematode populations used in this paper came from Italy or other countries as egg masses on roots of various crops attacked in the field or glasshouse. Eggs were recovered and inoculated onto either susceptible or resistant tomato (cvs Roma VF and VFN8, respectively). Most, but not all, of the populations coming from subtropical and tropical areas were able to reproduce on resistant tomato in the glasshouse at a soil temperature of 24-27 °C. Extracts of females from most populations were used for species determination by esterase isozyme electrophoresis patterns according to Molinari (2001). The virulent nematode populations described in this paper can be divided into two groups: i) "naturally" virulent populations taken directly from the field or virulent isolates generated from single egg masses of field populations; ii) isolates selected for virulence by repeated inoculation on cv. VFN8, starting from avirulent populations (Tab. I). Virulent isolates were generally produced from those field populations that had previously shown a modest reproduction index on resistant tomatoes. Single egg masses from the field population reared on susceptible tomato were then used to produce genetically homogeneous lines. When eggs of these different isolates were inoculated onto resistant tomato seedlings, the roots were observed to suffer various degrees of infestation at the end of the cycle, with a significant number of isolates not reproducing at all, suggesting that the original population consisted of a mixture of virulent and avirulent individuals. The isolates that showed the greatest reproduction (cM-Cu0, cM-Ven0, cM-AP1, cMi-1, cMi-13), together with the naturally highly virulent field populations from Africa (Mj-T, Mj-E) and other virulent field populations from Mediterranean areas (Mi-Esp2, M-It1, M-It4), were chosen to be reared on resistant tomato and used for IR experiments.

A third class of virulent isolates was raised by selection with repeated inoculations on resistant tomato VFN8. Selection started from three Italian *M. incognita* populations that had previously shown negligible reproduction on resistant roots. Large-scale inoculation of tens of resistant seedlings with many nematodes did result in the production of a significant number of egg masses (Tab. II). Two of the populations used (isolates *z, k*) produced progenies that did not survive beyond a

Table I. The RKN populations used to inoculate near-isogenic tomatoes.

Acronym	Species	Origin	Virulence	Status
Mj-T	*M. javanica*	Tunisia	+	field population
Mj-E	*M. javanica*	Egypt	+	field population
M-It4	undetected	Italy	+	field population
M-It1	undetected	Italy	+	field population
Mi-Esp2	*M. incognita*	Spain	+	field population
M-It3	undetected	Italy	-	field population
Ma-Esp1	*M. arenaria*	Spain	-	field population
cM-Cu0	undetermined	Cuba	+	isolate from field population
cM-Ven0	undetermined	Venezuela	+	isolate from field population
cM-AP1	undetected	Italy	+	isolate from field population
cMi-1	*M. incognita*	Italy	+	isolate from field population
cMi-13	*M. incognita*	Italy	+	isolate from field population
cMi-10	*M. incognita*	Italy	-	isolate from field population
cMi-*selj1*	*M. incognita*	Italy	+	selected isolate
cMi-*selj3*	*M. incognita*	Italy	+	selected isolate
cMi-*selavr*	*M. incognita*	Italy	-	selected isolate

Table II. Egg masses produced during the selection for virulence of three Italian populations of *M. incognita* on the resistant tomato cv. VFN8.

Generation	Isolate *z*	Isolate *k*	Isolate *j*
mass-inoculation	13	38	4
first	0	32	32
second		6	full reproduction
third		0	full reproduction

third generation on VFN8; on the other hand, the third one (isolate *j*) reproduced fully on the resistant tomato by the second generation. Progenies from two single egg masses of strain *j* (cMi*selj1* and cMi*selj3*) have been reared on resistant tomato for five years and used for experiments described in the present paper. Egg masses from isolates that did not produce virulent lineages were reproduced on susceptible tomatoes and considered as avirulent selected populations (cM*selavr1*). Additionally, some avirulent field populations (M-It3, Ma-Esp1) and one isolate raised from an avirulent field population (cM-10) were included in the experiments (Tab. I).

RESULTS AND DISCUSSION

There was no significant difference among IRs of avirulent wild-type populations and avirulent selected isolates on the susceptible cv. Moneymaker, as expected (Fig. 1A). Only the virulent field populations of *M. javanica* from North Africa had indices of reproduction on the resistant cv. Motelle as high as the wild-type populations on susceptible plants; naturally virulent populations from other areas showed a lower reproduction, comparable with that of populations selected for virulence (Figs 1B, 2). Genetically homogeneous isolates produced from those field populations that had previously been found moderately virulent (6-8000 eggs/gram fresh root weight), when reproduced, did not show a significantly higher IR with respect to the parent populations (not shown). Thus, some of the "tropical" populations (from Cuba and Venezuela) assayed, as well as the many Italian ones, did not reproduce at levels equivalent to isolates on susceptible controls, suggesting that the issue is more complicated than reported in the literature (Roberts, 1995).

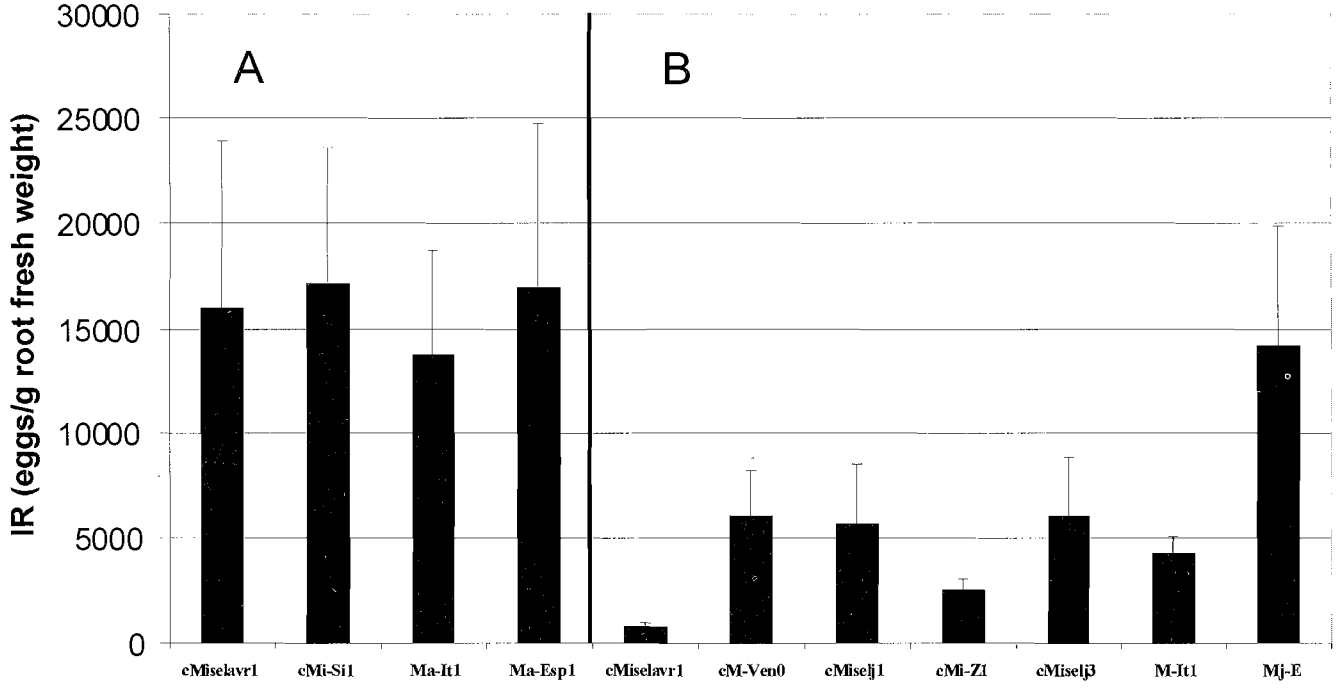

Fig. 1. Index of Reproduction (IR) of (A) *Meloidogyne* wild-type avirulent populations (M-It3, Ma-Esp1) and avirulent isolates (cMi-*selavr*, cMi-10) on the susceptible cv. Moneymaker; (B) selected populations (cMi-*selj1*), virulent field populations (M-It1, Mj-E), and isolates from field populations (cM-Ven0, cMi-AP1) on the resistant tomato isoline Motelle; the selected avirulent population cMi-*selavr* is shown as a control. Data are expressed as means of at least six experiments plus standard deviation.

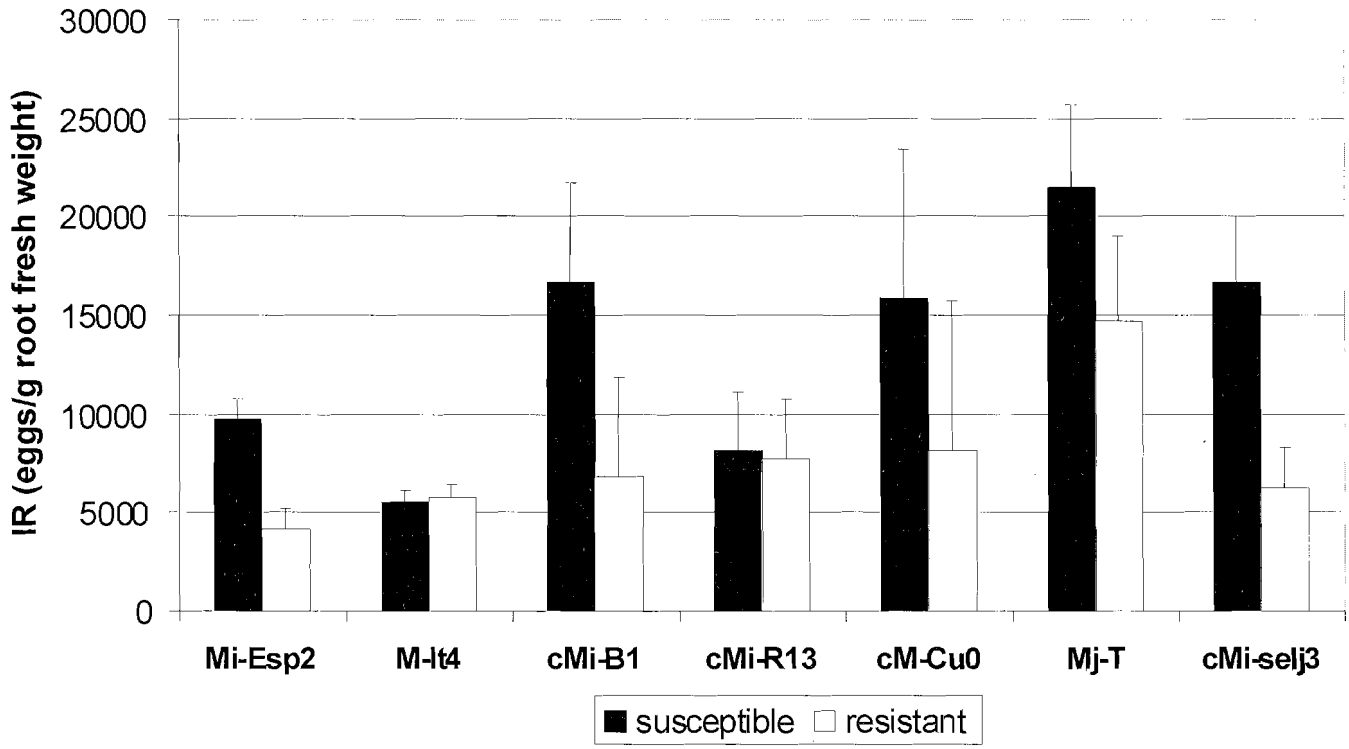

Fig. 2. Index of Reproduction (IR) of selected *Meloidogyne* populations (cMi-*selj3*), virulent field populations (Mi-Esp2, M-It4, Mj-T), and isolates from field populations (cMi-1, cMi-13, cM-Cu0) on susceptible (black bars) and resistant (white bars) near-isogenic tomato lines. Data are expressed as means of at least six experiments plus standard deviation.

When virulent populations were used to inoculate both tomato isolines, they generally showed a much higher IR on susceptible than on resistant plants, and this was particularly evident with the selected population cMi-*selj3* (Fig. 2). Only two populations (M-It4 and cM-13) had equally low IRs on both isolines. This finding is in agreement with other data that show that laboratory-selected virulent isolates do not reproduce as well on plants carrying *Mi* as they do on susceptible controls (Castagnone-Sereno *et al.,* 1994). Furthermore, data presented here suggest that this behaviour also occurs in naturally virulent populations, although it is more evident in selected isolates.

The structure of a field population that is able to reproduce on resistant tomatoes in experimental tests has been found to consist of avirulent individuals mixed with others with different degrees of virulence. The virulence is inherited and the same pattern is seen in the progeny if reproduced on a susceptible plant. Selected resistance-breaking isolates retained for a long time (i.e. several generations on resistant hosts) the ability of the parent populations to infest susceptible tomatoes heavily; they also retained the ability to reproduce on *Mi*-bearing plants after many generations on susceptible tomato (Molinari, unpublished). Hence, these findings confirm that RKN virulence towards the *Mi*-gene is genetically stable (Roberts, 1995).

Finally, data in the present paper suggest that virulence towards the tomato *Mi*-gene appears as an additional character of wild-type *Meloidogyne* populations that does not impair their ability to develop on susceptible plants of the same species; although, reproduction on resistant plant is constantly lower than that on susceptible counterparts. It is likely that the appearance of such a character is independent of *Mi* selection pressure; conversely, the selection of resistance-breaking populations in fields cropped with *Mi*-bearing tomatoes is likely to be slow and uncertain, considering the many attempts using characterised populations under the controlled conditions of our greenhouses that failed.

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