Stable Virulence Against the Tomato Resistance *Mi* Gene in the Parthenogenetic Root-Knot Nematode *Meloidogyne incognita*

Philippe Castagnone-Sereno, Michel Bongiovanni, and Antoine Dalmasso

INRA Laboratoire de Biologie des Invertébrés, 123 bd Francis Meilland, BP 2078, 06606 Antibes Cédex, France.
This research was supported in part by a grant from the Institut National de la Recherche Agronomique AIP 92/9502.
We thank E. Wajnberg for statistical advice.
Accepted for publication 18 April 1993.

ABSTRACT

Castagnone-Sereno, P., Bongiovanni, M., and Dalmasso, A. 1993. Stable virulence against the tomato resistance *Mi* gene in the parthenogenetic root-knot nematode *Meloidogyne incognita*. Phytopathology 83:803-805.

Natural and laboratory-selected *Meloidogyne incognita* lineages virulent against the *Mi* resistance gene of tomato were propagated over 18 successive generations on a resistant and on a susceptible tomato cultivar. After every three generations, their behavior on *Mi*-resistant tomatoes was assessed using miniaturized culture and inoculation conditions in order to determine whether the nematode was able to revert back to an avirulent phenotype in the absence of the selection pressure. Virulence appeared to be a stable character in *M. incognita*. Even if slight differences in the evolution of nematode reproduction were observed between the wild and selected virulent populations over successive generations, none of the populations lost the ability to overcome the *Mi* resistance gene.

Meloidogyne incognita (Kofoid & White) Chitwood is found worldwide in tropical and temperate climates. This root-knot nematode causes extensive damage to a wide range of crops, including many vegetables (16). *Meloidogyne incognita* females are obligate mitotic parthenogenetic organisms (19), which theoretically should sustain strong genetic stability.

The use of resistant cultivars is the most economical and environmentally sound method of controlling root-knot nematodes. Resistance in tomato was first identified in the wild species *Lycopersicon peruvianum* (L.) Mill. (5) and was later transferred into *L. esculentum* (18,24). All currently available cultivars with root-knot resistance are derived from this source (9). Resistance is generally thought to be controlled by a single dominant gene designated "*Mi*" (6), although the exact number of responsible genes is unknown (14,17). The phenotypic expression of incompatibility in tomato roots to *M. incognita* consists of a hypersensitive reaction that involves localized host-cell necrosis, cellular disorganization, and restricted nematode development at the infection site (8).

Despite their mitotic parthenogenetic mode of reproduction, natural populations of *M. incognita* (1,10,11) or populations developed by selection (2,7,13,21) can overcome the *Mi* gene. The latter virulent lineages were selected from avirulent populations by repeated propagation on resistant tomatoes. The existence of such a virulence phenotype suggests that an avirulent apomictic organism (*M. incognita*) may be able to modify its genotype in response to the selection pressure of a resistance gene (12). It would be of interest to determine whether the alternation of susceptible and resistant cultivars may prevent or at least delay the development of such virulent isolates.

In the context of this research, "virulence" was defined as the ability of a second-stage juvenile (J2) to develop into a female and to produce an egg mass on a tomato plant carrying the *Mi* gene. The objectives of the present work were to determine whether virulent *M. incognita* populations revert back to a predominantly avirulent phenotype (i.e., lose their ability to reproduce on resistant tomatoes) when selection pressure of the *Mi* resistance gene is removed and whether natural and laboratory-selected virulent *M. incognita* populations differ in virulence when *Mi* resistance selection pressure is removed.

MATERIALS AND METHODS

Plant materials and nematode populations. Two cultivars of *L. esculentum* were used in this study: Saint Pierre, a susceptible standard, and the near-isogenic Piersol, which possesses the *Mi* gene for root-knot resistance. Three *M. incognita* populations were selected from the collection of the Institut National de la Recherche Agronomique, based on their avirulence or virulence against the *Mi* resistance gene of tomato. Before the start of the experiment, the populations were specifically identified according to their isoesterase electrophoregrams (3). Valbonne is a naturally virulent population collected from a tomato field in the south of France. A natural population from Adiopodoumé, Ivory Coast, was chosen as the avirulent standard for this study. From this population, a virulent line was selected on Piersol for more than 20 generations according to the procedure of Jarquin-Barberena et al (7). Before the experiments started, both virulent nematode populations were routinely reared on Piersol. The avirulent population, used as a negative control, was propagated on Saint Pierre.

Experimental procedures and evaluations. Experiments were conducted from May 1989 to May 1992 in a climate-controlled room at a mean temperature of 23 C. The successive generations for each nematode population were allowed to develop in tomato pot cultures. The avirulent population was increased on Saint Pierre, and the natural and laboratory-selected isolates were reared on both Saint Pierre and Piersol. From G1 to G18, three plants were used as independent sources of inoculum for every nematode × plant combination tested. In each case, 2-mo-old tomato plants, grown individually in 1-L plastic pots containing 1.0–1.2 g of steam-sterilized sandy soil, were inoculated with 5,000 J2 from the previous generation. Inoculum collected from infected roots in a mist chamber was pipetted onto the soil surface around the stem base and lightly watered. Generation time was estimated from regular observations of the tomato root systems, the emergence of white egg masses outside the root tissues, and the hatching of the eggs, which indicated that the biological cycle was completed.

After every three generations, evaluation of nematode virulence was performed in miniaturized tube tests. For each tomato plant that was used as a nematode population source, eight 2-wk-old Piersol seedlings were transplanted singly in 50-ml plastic tubes containing the substrate described above. After 2 wk, each plant was inoculated with 25 J2 of the corresponding inoculum source as described for the pot cultures. Based on preliminary experiments, this inoculum concentration gave reproducible results.

Plants were arranged in a randomized complete block design, each block including eight Piersol plantlets for each of the five nematode × plant combinations tested. Because there were three replicates (three independent inoculum sources) for each original nematode isolate, 24 Piersol plants were assessed for each combination. Eight weeks after inoculation, resistant tomato root systems were washed, placed in cold eosin yellow (0.01%), and stirred for 30 min to stain the egg masses. The number of egg masses per root system was then determined. This procedure was repeated over 18 successive generations.

Because no significant ($P = 0.05$) differences were determined between the three repetitive units during the whole experiment, the results at each virulence test generation were pooled for each nematode population. An analysis of variance was performed for each nematode population over the course of the experiment and for the different populations after every three generations, and Duncan's multiple range test was used to separate the mean egg-mass numbers. All statistical analyses were conducted using SAS procedures (15).

RESULTS

Under our experimental conditions, it generally took from 53 to 57 days for the nematodes to complete their life cycle (data not shown). Based on this information, an average generation time of 8 wk was chosen for the entire experiment. The results are summarized in Table 1. As expected, the wild avirulent *M. incognita* population reared on the susceptible cultivar Saint Pierre and used as a negative control exhibited little or no reproduction on the resistant tomato. Conversely, the reproduction of the virulent lines on the resistant tomato cultivar Piersol remained high, indicating that the nematodes were still able to overcome the *Mi* gene. The highest reproduction rate on the resistant tomato was observed for the Valbonne wild virulent population reared on Piersol. Of these J2, 63–88% were able to produce egg masses. When routinely propagated on the susceptible tomato cultivar, this population exhibited a significantly ($P = 0.01$) lower ability to multiply on Piersol, even if 35–67% of the J2 were still able to produce egg masses. A very similar behavior was observed for the selected virulent line reared on Saint Pierre. From 40 to 50% of the J2 of the laboratory-selected virulent line were able to multiply on Piersol, thus showing the ability to reproduce on the resistant genotype. Regardless of the tomato cultivar used for multiplication, variation in rating of replicate plants was usually small for both virulent isolates.

The virulence of the selected population continually propagated on the resistant tomato was very stable. No variation ($P = 0.01$) in reproduction was observed over the 18 generations analyzed. Even if an overall decrease of its reproductive ability on Piersol seemed to occur over the 18 generations of the experiment, no significant variability ($P = 0.01$) of the egg-mass production of the homologous population reared on the susceptible cultivar was observed from G6 to G18. Moreover, except for G2, numbers of egg masses produced on Piersol at each generation were identical whether the population was maintained on the resistant or on the susceptible cultivar (Table 1).

Regardless of the tomato cultivar on which it was propagated, the naturally virulent *M. incognita* population from Valbonne showed the same evolution in its ability to reproduce on Piersol over the 18 generations. Egg-mass production appeared to be stable ($P = 0.01$), except for two lower values at G6 and G12. This reduction, though indicative of a biological phenomenon, cannot be interpreted as evidence for a modification in nematode virulence, because Piersol roots still experienced strong attacks compared with roots inoculated with the avirulent population. The discrepancy between the egg-mass numbers for the Valbonne isolate reared on Saint Pierre or on Piersol indicated a significantly ($P = 0.01$) higher reproduction rate when the nematode was continually reared on the resistant tomato cultivar (Table 1).

DISCUSSION

Nematode population data over 18 generations of constant propagation on susceptible tomato plants demonstrated that revert-selection for avirulence was not possible in *M. incognita,* thus indicating that virulence against the *Mi* resistance gene of tomato is a stable character for this nematode, at least at the phenotypic level. In earlier experiments, Riggs and Winstead (13) reported that maintenance of laboratory-selected virulent *M. incognita* lines on susceptible tomato for three generations did not affect their virulence to the *Mi* gene of the tomato breeding line Hawaii 5229. More recently, Turner (22) obtained similar results in assessing the stability of *Globodera pallida* virulence once the selection pressure of resistant *Solanum vernei* hybrids was removed; after four generations on a susceptible potato cultivar, the nematode populations remained virulent. The present data are in agreement with these previous investigations and constitute the first report of the stability of the virulence of a plant-parasitic nematode in the absence of the plant resistance gene selection pressure over a long-term experiment.

Quite different behavior was nevertheless observed between artificially-selected and natural virulent populations. The population from Valbonne displayed a significant decrease in its ability to reproduce on resistant tomato plants when it was propagated on the susceptible cultivar, while the selected virulent population produced similar egg-mass numbers when multiplied on Saint Pierre or on Piersol. According to that result, one should therefore surmise that the laboratory-selected virulent line showed the same ability to overcome *Mi* at both the qualitative and quantitative levels regardless of the cultivar on which it was reared. On the contrary, the naturally virulent Valbonne population showed a higher rate of multiplication when maintained on the resistant rather than on the susceptible tomato cultivar. This relative loss of reproductive ability might be related to an adverse genetic background associated to virulence, which decreases the nematode fitness on nonresistant hosts. This process, commonly known as "stabilizing selection," has already been proposed in gene-for-gene interactions between plants and pathogenic fungi (23). Whatever the nature of the difference may be between Valbonne and the laboratory-selected virulent line, it should be correlated with the way these two populations acquired their abilities to overcome the plant resistance. That one lineage was selected for

TABLE 1. Egg-mass production on Piersol of *Meloidogyne incognita* lines reared on susceptible and resistant tomato cultivars over 18 successive generations

Nematode line	Tomato cultivar used for multiplication	Generations[x,y]					
		G2	G6	G9	G12	G15	G18
Avirulent[z]	Saint Pierre (susceptible)	0.2	0.1	0.2	0.1	0.3	0.1
Selected for virulence	Saint Pierre	16.5 aB	13.0 abAB	10.8 bcC	8.8 cB	10.8 bcB	9.8 bcC
	Piersol (*Mi*-resistant)	10.1 aC	12.0 aAB	12.5 aBC	10.5 aB	10.9 aB	11.1 aC
Wild virulent	Saint Pierre	16.6 aB	10.1 bB	16.7 aB	9.4 bB	14.3 abB	16.7 aB
	Piersol	21.8 aA	15.7 cA	20.9 abA	18.2 bcA	22.0 aA	22.1 aA

[x]Values are the mean of 24 replicates. Each plant was inoculated with 25 second-stage juveniles.

[y]Values within a line followed by the same lower case letter are not significantly different ($P = 0.01$) according to Duncan's multiple range test. Values within a column followed by the same upper case letter are not significantly different ($P = 0.01$) according to Duncan's multiple range test.

[z]Serves as the negative control.

virulence under managed pressure in greenhouse and climate-controlled room conditions may have induced genetic changes different from those occurring in a natural *Mi*-resistance breaking *M. incognita* biotype.

Until now, no obvious correlation has been found between virulence and any modification of the nematode at the biochemical level. One protein was found to be different between the virulent and avirulent *M. incognita* isogenic lineages when compared by two-dimensional electrophoresis (4), but no information on the nature or function of this protein is currently available. This lack of a strong protein variability is to be related to the law rate of biochemical polymorphism previously reported for this nematode species (3) in relation to the parthenogenetic mode of reproduction of *M. incognita,* which should account for clonal genotypes within this species.

In a recent review, Triantaphyllou (20) suggested that an unusually high number of mutations occurring on a set of few genes with major effects may be involved in the gradual increase of parasitism in *M. incognita* on resistant tomato cultivars. Although selection for avirulence was not possible, it is unlikely that increased virulence was due only to the selection of virulence alleles already present in the avirulent nematodes. In that case, revert-selection should have occurred, unless all the virulent genotypes had become homozygous for that character. For the same reason, the hypothesis of random mutational events seems unconvincing. Based on current knowledge, no definite explanation can be put forward. The fact that *M. incognita* reproduces by obligate parthenogenesis prevents (or at least strongly limits) any crossing event and thus limits experiments of classical genetics. Nevertheless, the ability to select, under a controlled environment, near-isogenic virulent and avirulent lineages from a single juvenile should be considered an advantage, and the use of that specific material may provide useful information. Work is in progress to determine the genetic nature and inheritance of (a)virulence in this nematode.

LITERATURE CITED

1. Berthou, F., Ba-Diallo, A., De Maeyer, L., and De Guiran, G. 1989. Caractérisation chez les nématodes *Meloidogyne* Goeldi (*Tylenchida*) de types virulents vis-à-vis du gène Mi de la tomate dans deux zones maraîchères au Sénégal. Agronomie 9:877-884.
2. Bost, S. C., and Triantaphyllou, A. C. 1982. Genetic basis of the epidemiologic effects of resistance to *Meloidogyne incognita* in the tomato cultivar Small Fry. J. Nematol. 14:540-544.
3. Dalmasso, A., and Bergé, J. B. 1978. Molecular polymorphism and phylogenetic relationship in some *Meloidogyne* spp.: Application to the taxonomy of *Meloidogyne*. J. Nematol. 10:323-332.
4. Dalmasso, A., Castagnone-Sereno, P., Bongiovanni, M., and De Jong, A. 1991. Acquired virulence in the plant parasitic nematode *Meloidogyne incognita*. II. Two-dimensional analysis of isogenic isolates. Rev. Nematol. 14:305-308.
5. Ellis, D. E. 1943. Root-knot resistance in *Lycopersicon peruvianum*. Plant Dis. Rep. 27:402-404.
6. Gilbert, J. C., and McGuire, D. C. 1956. Inheritance of resistance to severe root-knot from *Meloidogyne incognita* in commercial type tomatoes. Proc. Am. Soc. Hortic. Sci. 63:437-442.
7. Jarquin-Barberena, H., Dalmasso, A., De Guiran, G., and Cardin, M. C. 1991. Acquired virulence in the plant parasitic nematode *Meloidogyne incognita*. I. Biological analysis of the phenomenon. Rev. Nematol. 14:299-303.
8. Kaplan, D. T., and Keen, N. T. 1980. Mechanisms conferring plant incompatibility to nematodes. Rev. Nematol. 3:123-134.
9. Medina-Filho, H. P., and Tanksley, S. D. 1983. Breeding for nematode resistance. Pages 904-923 in: Handbook of Plant Cell Culture. Vol. 1. D. A. Evans, W. R. Sharp, P. V. Ammirato, and Y. Yamada, eds. Macmillan, New York.
10. Netscher, C. 1977. Observations and preliminary studies on the occurrence of resistance breaking biotypes of *Meloidogyne* spp. on tomato. Cah. O.R.S.T.O.M., Ser. Biol. 11:173-178.
11. Prot, J. C. 1984. A naturally occurring resistance breaking biotype of *Meloidogyne incognita* on tomato. Reproduction and pathogenicity on tomato cultivars Roma and Rossol. Rev. Nematol. 7:3-28.
12. Riddle, D. L., and Georgi, L. L. 1990. Advances in research on *Caenorhabditis elegans*. Applications to plant parasitic nematodes. Annu. Rev. Phytopathol. 28:247-269.
13. Riggs, R. D., and Winstead, N. N. 1959. Studies on resistance in tomato to root-knot nematodes and on the occurrence of pathogenic biotypes. Phytopathology 49:716-724.
14. Roberts, P. A., Dalmasso, A., Cap, G., and Castagnone-Sereno, P. 1990. Resistance in *Lycopersicon peruvianum* to isolates of *Mi* gene-compatible *Meloidogyne* populations. J. Nematol. 22:585-589.
15. SAS Institute. 1988. SAS User's Guide: Statistics. Release 6.03 ed. SAS Institute, Cary, NC.
16. Sasser, J. N. 1979. Pathogenicity, host range and variability in *Meloidogyne* species. Pages 257-267 in: Root-Knot Nematodes (*Meloidogyne* species). Systematics, Biology and Control. F. Lamberti and C. E. Taylor, eds. Academic Press, London.
17. Sidhu, G. S., and Webster, J. M. 1975. Linkage and allelic relationships among genes for resistance in tomato (*Lycopersicon esculentum*) against *Meloidogyne incognita*. Can. J. Genet. Cytol. 17:323-328.
18. Smith, P. G. 1944. Embryo culture of a tomato species hybrid. Proc. Amer. Soc. Hortic. Sci. 44:413-416.
19. Triantaphyllou, A. C. 1971. Genetics and cytology. Pages 1-32 in: Plant Parasitic Nematodes. B. M. Zuckerman, W. F. Mai, and R. A. Rohde, eds. Vol. 2. Academic Press, New York.
20. Triantaphyllou, A. C. 1987. Genetics of nematode parasitism on plants. Pages 354-363 in: Vistas on Nematology. J. A. Veech and D. W. Dickson, eds. E.O. Painter, DeLeon Springs, FL.
21. Triantaphyllou, A. C., and Sasser, J. N. 1960. Variation in perineal patterns and host specificity of *Meloidogyne incognita*. Phytopathology 50:724-735.
22. Turner, S. J. 1990. The identification and fitness of virulent potato cyst-nematode populations (*Globodera pallida*) selected on resistant *Solanum vernei* hybrids for up to eleven generations. Ann. Appl. Biol. 117:385-397.
23. Van Der Plank, J. E. 1982. Host pathogen interactions in plant disease. Academic Press, New York.
24. Watts, V. M. 1947. The use of *Lycopersicon peruvianum* as a source of nematode resistance in tomatoes. Proc. Amer. Soc. Hortic. Sci. 49:233-234.