Acquired virulence in the plant parasitic nematode *Meloidogyne incognita*. 1. Biological analysis of the phenomenon[1]

Horacio JARQUIN-BARBERENA, Antoine DALMASSO, Georges de GUIRAN and Marie-Claire CARDIN

INRA, Station de Recherches de Nématologie et de Génétique Moléculaire des Invertébrés, B.P. 2078, 06606 Antibes Cedex, France.

SUMMARY

Four isolates of the root-knot nematode *Meloidogyne incognita* were analyzed for their virulence against the dominant Mi gene which controls resistance in commercially grown tomatoes. One isolate was found to be virulent under natural conditions and served as a standard for comparison of virulence. Two others were selected from an avirulent population by propagating them for 12 and 21 generations on the resistant tomato cv. Piersol. For comparison, populations derived from a single juvenile were maintened on susceptible cv. St Pierre. Comparisons of penetration of juveniles into the roots, the numbers of egg-masses produced from a standardized inoculum, the number of eggs per egg-mass produced in each generation on the susceptible and the resistant cultivars, showed a progressive increase in the proportion of virulent nematodes in spite of the reproduction being by obligatory mitotic parthenogenesis. Selection for virulence was accompanied by a general increase in fecundity and in the case of the acquired virulence generation after each generation. On the other hand, virulence remained stable after nine generations of propagation on susceptible tomato in the naturally virulent and the old acquired virulent isolates, while virulence seemed to continue to increase in the recently virulent one. Possible genetic mechanisms involved in these phenomena are discussed.

RÉSUMÉ

Acquisition d'une virulence chez le nématode phytoparasite Meloidogyne incognita. *1. Analyse biologique du phénomène*

Quatre isolats de l'espèce *Meloidogyne incognita* ont été analysés pour leur virulence vis-à-vis du gène dominant Mi qui est à l'origine de la résistance chez les tomates cultivées. Un isolat trouvé naturellement virulent dans une serre a servi de témoin de référence pour un fort degré de virulence. Deux autres isolats ont été sélectionnés à partir d'une population avirulente et multipliés respectivement pendant 12 et 21 générations sur le cultivar résistant Piersol. On a comparé la pénétration des juvéniles dans les racines, les nombres de pontes produites à partir d'un inoculum déterminé, ainsi que le nombre d'œufs par ponte; ceci pour chaque génération sur les cultivars Saint-Pierre (hôte) et Piersol (résistant). Lorsqu'une pression de sélection est exercée par la variété résistante, on observe une augmentation très progressive de la virulence, en dépit de la reproduction par parthénogenèse mitotique de cette espèce. Des différences dans la fécondité ont été remarquées entre les isolats et dans le cas de la virulence acquise au fil des générations successives. De plus, la virulence est restée stable après neuf générations sur la tomate hôte pour deux isolats virulents étudiés : celui à virulence naturelle et celui à virulence anciennement acquise. Ce caractère semble même s'accroître chez l'isolat à virulence récemment acquise. Les mécanismes génétiques pouvant expliquer ces observations sont discutés.

The root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood, is polyphagous. The number of egg-masses, the number of eggs in the egg-masses, and the length of the life cycle are useful criteria for evaluating the status of host of plants. Some sources of resistance to root-knot nematodes have been observed in different botanical families, particularly in the solanaceae. Resistance of *Lycopersicon peruvianum* (L.) Mill. thought to be controlled by the dominant Mi gene, has been transferred into several tomato cultivars. This resistance operates against the three most important *Meloidogyne* species : *M. incognita, M. javanica* (Treub) Chitwood, and *M. arenaria* (Neal) Chitwood. Tomato cultivars which prevent the development and multiplication of root-knot nematodes are considered to be resistant. However, in avirulent populations a few individuals may produce small egg-masses and wild, naturally virulent populations have been recovered from some cultivated fields and natural areas.

As the three species of *Meloidogyne* mentioned above reproduce by obligate mitotic parthenogenetic process (Triantaphyllou, 1971, 1981) the progeny of a single female should be homozygous. Consequently isogenic lines may be obtained from a single juvenile (J2) and the

(1) Research supported in part by EEC Program TS 2A 0054 F.

selection of virulent lines from an avirulent enables comparisons at several different levels (for instance for their proteins see Dalmasso *et al.* (1991).

Several workers have reported the existence of virulence (Riggs & Winstead, 1959; Netscher, 1976; Prot, 1984; Roberts & Thomason, 1986). However, few investigations have demonstrated that selection of virulent strains from avirulent by continous breeding on resistant cultivars. Netscher (1976) first reported differences in number of egg-masses produced during six generations on a resistant tomato cultivar by inoculating a constant number of juveniles of a single egg-mass population. He suggested an adaptation of selected individuals under the pressure of the resistant cultivar. It was however reported by Netscher and Taylor (1979) that not all populations have the capacity to adapt on resistant tomatoes. During twelve generations Bost (1982) observed an increase in the compatibility between *M. incognita* and resistant cultivar Small Fry. After selection the number and the content of the egg-masses on this cultivar were lower than on the susceptible cultivar Rutgers. Bost and Triantaphyllou (1982) observed that parasitism of *Meloidogyne incognita* on resistant tomatoes increases stepwise and Triantaphyllou (1982) suggested that each step may represent a mutation of a gene that controls parasitism.

The first objective of this study was to investigate the possibility of obtaining virulent lines of *M. incognita* from other isogenic, largely avirulent lines using population from different origins and to explain the source of genetic variation that allows such an adaptation to occur, in spite of the mitotic parthenogenetic reproduction of this species.

A second objective was to analyse increase in virulence over successive generations in order to determine if the increase is progressive or gradual. Moreover, we attempted to compare lines selected for increased virulence with a naturally virulent population with regard to fecundity and stability of their respective degree of virulence when the selection pressure imposed by resistant cultivars is removed during several generations.

Materials and methods

The susceptible tomato cultivar St Pierre was used to multiply and maintain the avirulent line. The resistant cultivar Piersol was used to select virulent *Meloidogyne* isolates. On it, population of *M. incognita* collected from tomato in the Ivory Coast appeared to be largely avirulent, whereas another from the South of France (Valbonne) was found naturally virulent. The identifications were established according to the perineal patterns and the isoesterase proteinograms (Dalmasso & Bergé, 1983).

The unselected avirulent line (IC avir.) originated from a single juvenile approximatively thirty generations before and was continuously reared on St Pierre to maintain its avirulence. From this avirulent population, two virulent isolates were selected by inoculating the resistant cultivar Piersol : one designated " old virulent " (IC old), was selected before the experiment, for twelve generations on Piersol, the other " recent virulent " (IC rec) was selected only three generations from the start of the experiment. In this way it was obtained virulent isolates, whose genotypes should differ from the avirulent isolate only for the gene(s) involved in the plant nematode interaction.

All the experiments were carried out in a controlled environment at 20-25 °C. Seeds were germinated in sterilized soil in plastic tubes (50 cm^3) or 1 liter plastic pots and allowed to become established for two weeks (plastic tubes) or one month (pots) before inoculation.

Seedlings growing in pots were inoculated by pipetting 1 000 J2 of *M. incognita* in a water suspension directly onto the soil, followed by light watering as needed. For seedlings growing in plastic tubes 20 juveniles were added.

In one experiment the two lines which were being selected for virulence and the naturally virulent isolate (Valbonne) were reared for nine more generations on the resistant Piersol. Concurrently, egg-masses of the same isolates were inoculated to the susceptible St Pierre to provide unselected lines for comparison to determine the rate of selection for virulence. A second experiment was made to determine the stability of virulence. The selected virulent lines were reared on susceptible tomato St Pierre for a further nine generations; at intervals of three generations the reproduction on the susceptible cultivar was compared with the resistant cultivar inoculated with the same number of juveniles (1 000 J2). Growing conditions were the same as in the first experiment.

The fitness of each isolate on both cultivars was evaluated every generation (1st experiment) or every three generations (2nd experiment) by counting the number of juveniles which penetrated into roots according to the technique described by de Guiran (1966), the number of galls, egg-masses, and eggs in each egg-mass. Five days were enough to evaluate proportions of the juveniles which entered into the roots. Experiments were made in ten replicates (juvenile penetration) or four replicates; i.e. 4 × 5 egg-mass contents dissociated in sodium hypochlorite 0.5 % (eggs production).

Results

Root invasion by juveniles and development

Examination of Fig. 1 shows the following trends :

Penetration into the resistant cultivar of IC old (selected for twelve generations) increased progressively at each generation but remained stable at high levels for Valbonne and was negligible in the case of Ivory Coast

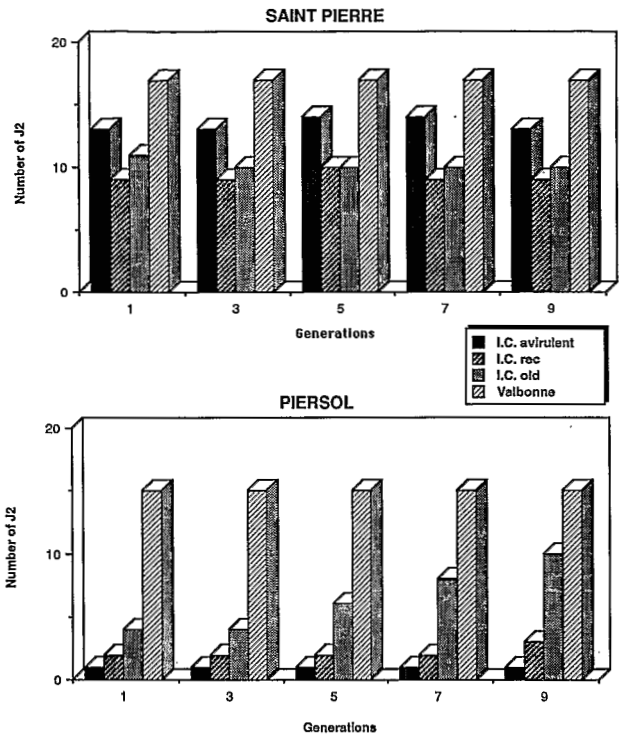

Fig. 1. Numbers of juveniles of *Meloidogyne incognita* having penetrated into the roots of the cvs Saint-Pierre (susceptible) and Piersol (resistant), 5 days after inoculation with 20 freshly hatched juveniles in 50 cm^3 tubes. IC : avirulent (standard); Valbonne : wild virulent (Mean of 10 replications).

unselected line (IC avir). There was no significant difference between the number of larvae which entered the roots of cv. St Pierre, and cv. Piersol for the wild virulent Valbonne isolate. After nine generations the penetration value of IC old (21th generation) on resistant cultivar tends to reach the stable value of IC avir on the susceptible one. Juvenile penetration into cv. St Pierre was lower for both the induced virulent isolates than for Ivory Coast unselected line (Fig. 1).

Table 1 gives the number of egg-masses which were produced on both cultivars inoculated with the four isolates. On the susceptible St Pierre, Valbonne produced more egg-masses than the unselected line from Ivory Coast, and similar numbers were produced at each generation. On resistant Piersol, the Valbonne line produced as many egg-masses as on the susceptible, but the unselected Ivory Coast line produced less than one egg-mass per plant. Selection progressively increased the number of egg-masses produced on the resistant cultivar, and to a less degree on the susceptible (Table 1).

Table 1

Changes, along the generations, in the number of egg-masses collected on a susceptible (Saint-Pierre) and a resistant (Piersol) cv. inoculated with four lines of *Meloidogyne incognita*. IC rec. : virulent line recently selected, IC old : line maintained for 12 generations on resistant tomato before experiment, both coming from the IC avirulent. One liter pots were inoculated with 1 000 juveniles. Means of four replications. The means followed by the same letter are not significantly different at P = 0.05 according to the Newman & Keuls test.

Lines	Generations	Saint-Pierre (S)	Piersol (R)
IC AVIR.	1	515 a	+
(avirulent	3	530 *a*	+
standard)	5	540 *a*	+
	7	522 *a*	+
	9	530 *a*	+
IC RECENT	4	360 *a*	30 *a*
	5	340 *a*	40 *b*
	6	360 *a*	30 *a*
	7	390 *b*	90 *c*
	8	390 *b*	120 *d*
	9	410 *b*	170 *e*
	10	410 *b*	150 *f*
	11	440 *c*	180 *g*
	12	410 *b*	180 *g*
IC OLD	13	350 *a*	310 *a*
	14	350 *a*	310 *a*
	15	400 *b*	320 *a*
	16	350 *a*	300 *a*
	17	410 *b*	390 *b*
	18	630 *d*	400 *b*
	19	650 *d*	460 *d*
	20	530 *c*	440 *c*
	21	590 *c*	451 *cd*
VALBONNE	1	720 *a*	590 *a*
(wild virulent)	3	750 *a*	623 *a*
	5	720 *a*	626 *a*
	7	750 *a*	603 *a*
	9	730 *a*	599 *a*

+ The very low number of egg masses (< 1) did not allow to give reliable values.

Table 2 evaluates the fecundity of the four isolates : while the number of egg-masses was increasing progressively during the adaptation of Ivory Coast recently selected (IC rec) and Ivory Coast selected for twelve generations (IC old) on the resistant cultivar, there was a reduction of fecundity on both cultivars for these two isolates if compared with the egg-mass contents of the unselected avirulent isolate on St Pierre, i.e. IC avir.

Table 2

Changes in the number of eggs/egg-mass under the same conditions as Fig. 2. Means of 20 egg masses. The means followed by the same letter are not significantly different at P = 0.05 according to the Newman and Keuls test.

Lines	Generations	Saint-Pierre (S)	Piersol (R)
IC AVIR.	1	1 765 *a*	+
(avirulent	3	1 789 *a*	+
standard)	5	1 741 *a*	+
	7	1 759 *a*	+
	9	1 751 *a*	+
IC RECENT	4	425 *a*	402 *a*
	6	496 *b*	427 *a*
	8	498 *b*	477 *ab*
	10	538 *b*	533 *b*
	12	536 *b*	527 *b*
IC OLD	13	1 418 *a*	733 *a*
	15	1 450 *a*	816 *b*
	17	1 516 *ab*	872 *b*
	19	1 564 *b*	893 *b*
	21	1 578 *b*	895 *b*
VALBONNE	1	2 471 *b*	1 893 *a*
(wild virulent)	3	2 550 *b*	1 938 *a*
	5	2 385 *ab*	2 018 *ab*
	7	2 317 *a*	2 030 *b*
	9	2 429 *b*	1 917 *a*

+ The very low number of egg-masses did not allow to give reliable values.

The egg-masses of IC old contained about twice as many eggs as those of IC rec on Piersol and three time more on St Pierre. It also appears that the initial fecundity of the IC avir on St Pierre was almost regained by IC old. Somewhat aberrant significant differences between successive generations which were sometimes observed can be considered as minor accidents and do not alter really the general tendency of egg-masses and egg production on several generations.

The adaptation of the isolates IC rec and IC old was progressive. At the end of the first generation a mean of 3 egg-masses/1 000 juveniles inoculated and 300 eggs/egg-masses were produced on the resistant cultivar and 451 egg-masses which each contained at least 1 500 eggs by the 21th generation. The fecundity of the Valbonne isolate was higher, even on the resistant tomato than that of the avirulent Ivory Coast on the susceptible one. Tyler (1938) confirmed by de Guiran (1980) reported a fecundity of 3 000 eggs/egg-mass. Such high values are observed when growing conditions are good, in absence of intra specific competition and counting empty shells.

STABILITY OF VIRULENCE

The objective of the second experiment was to check the stability of the natural and induced virulence in the absence of the selection pressure involving the Mi gene. Every three generations, juveniles collected from the susceptible cultivar were reinoculated on the resistant one and virulence checked by counting egg-masses and galls two months later. Fig. 2 shows that no variation occured for IC old and Valbonne while there was an increase in virulence of IC rec. An unexpected result was the increase in number of galls and egg-masses for this isolate in spite of having been reared for several generations on the susceptible host (see below).

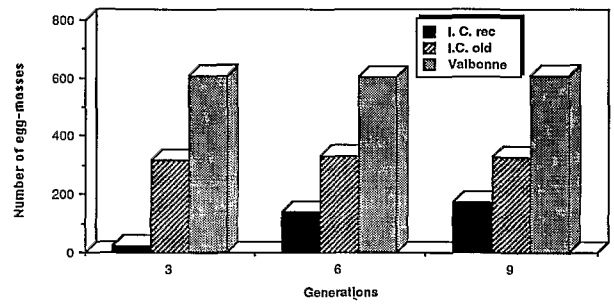

Fig. 2. Evaluation of the stability of the acquired and wild virulence on resistant tomato. Number of egg-masses of the virulent isolates recovered from cv. Piersol (resistant) after rearing the isolates several generations on susceptible tomato (Means of four replications; plants were grown in 1 liter pots and inoculated with 1 000 juveniles).

Discussion

Our results show that isolates of *M. incognita* originating from single juveniles may be progressively selected for virulence against the Mi gene which controls the resistance to several *Meloidogyne* spp. in tomato. The isolates overcame the resistance of the Mi gene by increasing the proportion of juveniles which invaded, the proportion of females which produced egg-masses and their fecundity (number of eggs/egg-mass).

Although *Meloidogyne* reproduce by mitotic parthenogenesis this did not prevent the selection of virulent lines from the avirulent single juvenile line from the Ivory Coast population. Moreover other virulent isolates were selected in our laboratory from *M. incognita* from different origin (Dalmasso *et al.*, 1991).

The percentage of virulent nematodes continued to increase up to the 21th generation when the observations ceased but the rate of evolution appeared to be slowing. The number of eggs per egg-mass progressively increased in the selected lines, but selection initially reduced their number, regardless of the cultivar. Fecundity was generally lower on cv. Piersol, but the naturally virulent

isolate from Valbonne always produced large egg-masses which contained numerous eggs in both cultivars, by contrast to the unselected Ivory Coast isolate. We can conclude that the fecundity, which may vary in *M. incognita* independantly of the environmental conditions, is dependent on several, yet undetermined, genetic factors. The increases in several components (penetration, development, ovogenesis) also indicates that several mechanisms or one rather general mechanism are involved in selection for virulence. Further studies at a molecular level are needed to understand the kinds of genetic changes induced by the host plant.

Significantly, the old acquired or natural virulences appeared genetically stable, even after nine generations on the compatible cultivar. The further increase in virulence of the recently selected Ivory Coast line when maintained on susceptible cultivar (IC rec) is the most surprising result and, until now no genetic explanation can be proposed. One may suppose that the phenomenon is autoamplyfing after its induction. We are trying to confirm this on other populations and species.

Triantaphyllou (1987) advanced the hypothesis that a series of mutations might explain a step by step progression of the parasitism. The present observations may also be explained by a gene amplification system that could be caused by the selection pressure resulting in a very progressive increase of virulence in a similar manner as it has been demonstrated by Mouchès *et al.* (1986) for insecticide resistance in *Culex*. In fact there is a closer analogy with what occurs in apomictic clones of *Myzus persicae* (Bunting & Van Emden, 1980) which have been selected for resistance to organo-phosphorous insecticides. Recently Field, Devonshire and Ford (1988) showed that this type of resistance is based on a gene amplification as in the mosquito.

The obligatory parthenogenetic mode of reproduction of several root-knot nematodes, which prevents any interbreeding between the selected lines, prevents determination of the number of genes involved and their characterisation (recessivity or, less likely, dominance). Even so, the genus *Meloidogyne* offers a unique model for studying the molecular changes induced by plants on phytoparasitic nematodes.

References

Bost, S. C. & Triantaphyllou, A. C. (1982). Genetic basis of epidemiologic effects of resistance to *Meloidogyne incognita* in tomato cultivar Small Fry. *J. Nématol.*, 14 : 540-544.

Bunting, S. & Van Emden, H. F. (1980). Rapid response to selection of increased esterase activity on small populations of an apomictic clone of *Myzus persicae*. *Nature*, 285 : 502-503.

Dalmasso, A. & Bergé, J. B. (1983). Enzyme polymorphism and the concept of parthenogenetic species exemplified by *Meloidogyne*. In : Stone, A. R., Platt, S. H. & Khalil, L. F. (Eds). *Concepts in nematode systematics*, New York & London, Academic Press : 186-196.

Dalmasso, A. Castagnone Sereno, P., Bongiovanni, M. & de Jonk, A. (1991). Acquired virulence in the phytophagous nematode *Meloidogyne incognita*. 2. Bidimensional analysis of isogenic isolates. *Revue Nématol.*, 14 : 305-308.

Field, L. M., Devonshire, A. L. & Ford, B. G. (1988). Molecular evidence that insecticide resistance in peach-potato aphids (*Myzus persicae* Sulz.) results from amplification of an esterase gene. *Biochem. J.*, 251 : 309-312.

de Guiran, G. (1966). Coloration des nématodes dans les tissus végétaux par le bleu coton à froid. *Nematologica*, 12 : 646.

de Guiran, G. (1980). Facteurs induisant chez *Meloidogyne incognita* un blocage du développement des œufs considéré comme une diapause. *Revue Nématol.*, 3 : 61-69.

Mouchès, C., Pasteur, N., Bergé, J. B., Hyrien, O., Raymond, M., Robert de Saint-Vincent, B., de Silvestri, M. & Georghiou, G. (1986). Amplification of an esterase gene is responsible for insecticide resistance in a California *Culex* mosquito. *Science*, 233 : 778-780.

Netscher, C. (1976). Observation and preliminary studies on the occurence of resistance breaking biotypes of *Meloidogyne* spp. on tomato. *Cah. ORSTOM, Sér. Biol.*, 11 : 173-178.

Netscher, C. & Taylor, D. P. (1979). Physiologic variation within the genus *Meloidogyne* and its implications on integrated control. In : Lamberti, F. & Taylor, C. E. (Eds). *Root-knot nematodes. Systematics, biology and control.* New York & London, Academic Press : 269-294.

Prot, J. C. (1984). A naturally occuring resistance breaking biotype of *Meloidogyne arenaria* on tomato : Reproduction and pathogenicity on tomato cultivars Roma et Rossol. *Revue Nématol.*, 7 : 23-28.

Riggs, R. D. & Winstead, N. N. (1959). Studies on resistance in tomato to root-knot nematodes and the occurrence of the pathogenic biotypes. *Phytopathology*, 49 : 716-724.

Roberts, P. A. & Thomason, I. J. (1986). Variability in reproduction of isolates of *Meloidogyne incognita* and *M. javanica* on resistant tomato genotypes. *Plants Dis.*, 70 : 547-551.

Triantaphyllou, A. C. (1971). In : Zuckerman, B. M., Mai, W. F. & Rhode, R. A. (Eds). *Genetics and cytology in plant parasitic nematodes. Vol. II*, New York & London, Academic Press : 1-32.

Triantaphyllou, A. C. (1981). Oogenesis and the chromosomes of the parthenogenic root-knot nematode *Meloidogyne incognita*. *J. Nematol.*, 13 : 95-105.

Triantaphyllou, A. C. (1987). Genetics of nematode parasitism on plants. In : Veech, J. A. & Dickson, D. (Eds). *Vistas on Nematology.* Hyattsville MD, USA, Soc. Nematologists. Inc. : 354-363.

Tyler, J. (1938). Egg output of the root-knot nematode. *Proc. helminth. Soc. Wash.*, 5 : 49-54.

Accepté pour publication le 9 mai 1990.