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The reproductive potential of the root-knot nematode *Meloidogyne incognita* is affected by selection for virulence against major resistance genes from tomato and pepper

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Abstract The emergence of virulent root-knot nematode populations, able to overcome the resistance conferred by some of the resistance genes (R-genes) in Solanaceous crops, i.e., *Mi*(s) in tomato, *Me*(s) in pepper, may constitute a severe limitation to their use in the field. Research has been conducted to evaluate the durability of these R-genes, by comparing the reproduction of several laboratory-selected and wild virulent *Meloidogyne incognita* isolates, on both susceptible and resistant tomatoes and peppers. We first show that the *Me1* R-gene in pepper behaves as a robust R-gene controlling avirulent and virulent *Me3, Me7* or *Mi-1* isolates. Although the reproductive potential of the virulent isolates was highly variable on susceptible and resistant plants, we also confirm that virulence is highly specific to a determined R-gene on which selection has occurred. Another significant experimental result is the observation that a reproductive fitness cost is associated with nematode virulence against *Mi-1* in tomato and *Me3* and *Me7* in pepper. The adaptative significance of trade-offs between selected characters and fitness-related traits, suggests that, although the resistance can be broken, it may be preserved in some conditions if the virulent nematodes are counter-selected in susceptible plants. All these results have important consequences for the management of plant resistance in the field.

Key words *Meloidogyne* spp. · *Solanaceae* · *Me(s)* and *Mi-1* resistance genes · Virulence specificity · Fitness cost of virulence

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Introduction

Root-knot nematodes (RKNs), *Meloidogyne* spp., are extremely polyphagous plant parasites, responsible for estimated losses of more than 80 billion Euros/year (Blok et al. 2008). These yield losses are a problem throughout the EU and are exacerbated by the sophisticated and intensive character of EU agriculture. In addition, new regulations have withdrawn the use of most chemical nematicides since 2006, and nematode problems have been increasing in horticultural crops consequently. In this context,

cultivation of grafted vegetables is expanding in Europe, and has been adopted as a non-chemical alternative to methyl bromide in several countries (Methyl Bromide Technical Options Committee 2006). Because of this situation, breeding programs for the selection of resistant cultivars and/or rootstocks have recently become a major challenge for breeders. However, the diversity of *Meloidogyne* species and isolates infecting vegetables in most major production areas worldwide, is a threat to the use of single major resistance genes (R-genes) (Castagnone-Sereno 2002). Since resistance sources against these nematodes are rare, management of the R-genes currently available is of crucial importance to preserve their durability.

In Solanaceous species (e.g., tomato, pepper), several R-genes have been identified, that control the three main RKN species, *M. arenaria*, *M. incognita* and *M. javanica* (Williamson and Kumar 2006), and are currently used in breeding programs. However, the diversity and availability of such R-genes is limited. In tomato, all the modern fresh-market and processing resistant cultivars carry the single dominant gene *Mi-1*, introgressed from a single interspecific hybrid (Williamson and Roberts 2009). For more than 60 years, this gene has been the only source of resistance to RKNs in all the available tomato cultivars worldwide, and may be considered as a very stable R-gene in terms of durability. However, although this gene has been an excellent example of the use of host resistance to effectively reduce the need for pesticide application (Sorribas et al. 2005), the lack of diversity in the resistance sources exploited explains in part the emergence and spread of *Mi-1*-virulent nematode populations able to overcome it (Castagnone-Sereno 2002). Indeed, natural virulent isolates have been reported in many areas of the world where tomato is a major crop, and this trend has been increasing recently (e.g., Tzortzakakis et al. 2005, 2008; Verdejo-Lucas et al. 2009; Devran and Söğüt 2010), which could significantly reduce the duration of the commercial exploitation of the *Mi-1* gene in tomato. Moreover, the artificial selection of virulent lineages from progenies of avirulent *Meloidogyne* isolates, was demonstrated by repeated inoculations onto *Mi-1*-resistant tomato genotypes under laboratory and greenhouses conditions (Jarquin-Barberena et al. 1991; Castagnone-Sereno et al. 1994b; Meher et al. 2009).

In pepper, resistance to RKNs is controlled by several linked dominant genes, i.e., the *Me* genes. Three of them (*Me1, Me3, Me7*), in inbred lines originating from three genetically distant accessions, are effective against *M. incognita*, *M. arenaria* and *M. javanica* (Hendy et al. 1985; Djian-Caporalino et al. 1999; 2001; 2007). However, in laboratory experiments, the selection of virulent variants toward the *Me3* gene was achieved by using drastic selection pressure of avirulent *M. incognita* populations, whereas the resistance conferred by the *Me1* gene could not be overcome (Castagnone-Sereno et al. 1996; 2001). Although *Mi*-1 and *Me*- virulent populations were obtained in experimental assays, the results suggested that these R-genes may differ in their durability if repeatedly used under field conditions.

Few data are available in the literature about the reproductive potential of RKNs virulent against R-genes from Solanaceous vegetables, and reports showed differences that might be revealed at different developmental stages during the nematode-host interaction. Experimental evaluation of the fecundity (i.e., production of egg-masses by mature females) of *Meloidogyne* field populations virulent against the tomato *Mi-1* gene did not show any significant differences when the nematodes were inoculated onto susceptible versus resistant plants (Castagnone-Sereno et al. 1994a; Tzortzakakis et al. 1998). Another study indicated that *Mi-1*-virulent nematodes produced fewer eggs per gram of root on resistant tomatoes compared to susceptible ones (Huang et al. 2004). More recently, a lower reproduction of *M. incognita* was found associated to virulence against *Mi-1* when inoculated onto susceptible tomatoes, using a combination of female fecundity, fertility and egg hatching as an overall measure of reproduction (Castagnone-Sereno et al. 2007). In addition, as far as we know, no such information is currently available concerning RKN populations exhibiting virulence against the pepper *Me* R-genes.

The first objective of the present study was to compare the reproduction of several laboratory-selected and wild *Mi-1*- and *Me*-virulent isolates of *M. incognita* on both susceptible and resistant tomatoes and peppers. For that purpose, we evaluated the ability of the juveniles to mature into adult females and produce eggs. A further objective was to determine whether reproductive fitness costs may

be associated with the ability of the nematodes to overcome the tomato and pepper R-genes, by comparing reproductive parameters of *M. incognita* isogenic lines that differ only for their virulence on a set of resistant and susceptible host genotypes. Specificity of virulence and fitness costs of virulence could have important consequences for the management of plant resistance in the field.

Materials and methods

Plant material

Tomato (*Solanum lycopersicon*) cultivars Saint Pierre and Marmande, susceptible to *M. arenaria*, *M. incognita* and *M. javanica*, and cultivars Piersol and VFN8, homozygous for the *Mi-1* gene and resistant to the same three *Meloidogyne* species, were used in the experiments. Saint Pierre and Piersol are near-isogenic lines (Laterrot 1975).

Pepper (*Capsicum annuum*) genotypes used in this work were inbred lines with differential resistances to the three RKN species. Doux Long des Landes is a susceptible cultivar; Yolo Wonder exhibits a partial (i.e., a low level of quantitative) resistance to some RKN populations; the two resistant haplo-diploid lines, HD149 and HD330, produced through in vitro androgenesis (Dumas de Vaulx et al. 1981) are homozygous for the *Me3* and *Me1* genes, respectively (Hendy et al. 1985); the inbred pepper line Criollo de Morelos 334 (CM334) was selfed in insect-proof cages to eliminate outcrossing and was assumed to be homozygous for the major dominant resistance gene previously named *Me7* (Djian-Caporalino et al. 2007).

Nematode isolates

Twelve *M. incognita* isolates were used. Their geographical origin and (a)virulence status against the tomato *Mi-1* or pepper *Me3* and *Me7* R-genes are reported in Table 1. All the nematode isolates originated from single egg mass cultures, except the greenhouse naturally-selected isolates CREAT, pop A, pop B and pop C that consisted of mixed cultures. Prior to multiplication, each isolate was specifically identified according to its isoesterase electrophoretic pattern (Dalmasso and Bergé 1978) and/or by SCAR-PCR (Zijlstra et al. 2000). All nematode isolates have been maintained for at least 2 years (i.e., 10 successive generations) in the greenhouse on susceptible or resistant cultivars for the avirulent or virulent isolates, respectively. Hatched second-stage juveniles (J2s) were obtained in a mist chamber from previously inoculated roots. Nematodes were collected in water every 48 h and used immediately for inoculating the plants.

Table 1 *Meloidogyne incognita* isolates used in this study

Code	Geographic origin	Features	(a)virulence
avir F	Morelos, Mexico	Reference isolate from the INRA collection	Avirulent[b]
avir Mifield1	Italy	Field isolate	Avirulent[b]
avir MifieldV	Venezuela	Field isolate	Avirulent[b]
vir *Mi-1*	France[a]	Laboratory-selected on *Mi-1* from avir F	Virulent *Mi-1*
vir SM1	Italy	Laboratory-selected on *Mi-1* from avir Mifield1	Virulent *Mi-1*
vir SM2V	Italy	Laboratory-selected on *Mi-1* from avir MifieldV	Virulent *Mi-1*
vir *Me3*	France[a]	Laboratory-selected on *Me3* from avir F	Virulent *Me3*
vir *Me7*	France[a]	Laboratory-selected on *Me7* from avir F	Virulent *Me7*
vir CREAT	La Baronne, France	Greenhouse isolate naturally-selected on *Me3*	Virulent *Me3*
vir pop A	Hungary	Greenhouse isolate naturally-selected on *Me3*	Virulent *Me3*
vir pop B	Hungary	Greenhouse isolate naturally-selected on *Me3*	Virulent *Me3*
vir pop C	Hungary	Greenhouse isolate naturally-selected on *Me3*	Virulent *Me3*

[a] the geographic origin indicates the country on which selection over the R-gene was conducted

[b] for both the *Mi-1*-gene in tomato and *Me*-genes in pepper

The *Mi-1* and *Me*-avirulent nematode isolates used in this study were collected originally from heavily infested fields or greenhouses, in areas where resistant tomatoes and peppers have not been cultivated. They are avirulent for both the *Mi-1*-gene in tomato and *Me*-genes in pepper.

From the Morelos *M. incognita* avirulent isolate, virulent lines were laboratory-selected and reared by successive re-inoculation on the *Mi-1*-tomato cultivar Piersol, or the *Me3*-pepper HD149 line, or the *Me7*-pepper CM334 line for more than 25 generations, starting from the progeny of one single female, according to the procedure of Jarquin-Barberena et al. (1991). The *Mi-1* virulent SM1 and SM2V isolates have been laboratory-selected under controlled greenhouse conditions from the avirulent wild-type isolates avir Mifield1 and avir MifieldV, respectively, by repeated inoculations on *Mi-1*-resistant tomato for about 40 generations. The *Me3*-virulent isolates CREAT from La Baronne, France, and pop A, pop B, and pop C from Hungary have been naturally selected on resistant peppers carrying *Me3* in experimental field (greenhouses). Vir CREAT was issued from a single year of selection in field (approximately 3 generations). Pop A, pop B, pop C from Hungary were well-established virulent populations arising from several years (not precisely determined) of selection in field.

Experimental procedures and infestation parameters

Tomato and pepper seedlings of cultivars susceptible and resistant to RKNs, were grown individually in 100 ml pots containing steam-sterilized sandy soil covered by a 1 cm layer of loam. Six to twenty replicates were performed for each cultivar and each nematode isolate tested. Experiments were conducted in climatic chambers maintained at 24°C (±2°C) with a 12-h light cycle and a relative humidity of 60–70%. For each nematode isolate, six to seven-week-old plants (4–6 true leaves) were inoculated with a water suspension of 300 to 500 J2s.

Six to seven weeks after inoculation (i.e., a duration that allowed completion of the nematode life cycle), plants were harvested, carefully washed individually with tap-water, and stained for 10 min in a cold aqueous solution of eosin yellow (0.1 g/l water), to specifically stain egg masses (EMs) in red (Roberts et al. 1990).

The roots were then rinsed and examined under a magnifying glass and the number of EMs counted for each plant.

For each plant, up to 10 EMs were removed at random from the root system and the eggs were separated from the gelatinous matrix in 0.9% NaOCl, mounted in water between a glass side and a cover slip and counted under a stereomicroscope. Three repeats per plant were assessed.

Since two different tomato cvs (for both nematode susceptible and resistant), a variable inoculum rate (300–500 J2s) and a variable number of replicates (6–20) were described, three parameters of infection were calculated :

- Infection Frequency (IF) was calculated according to the following ratio: IF=number of EMs/number of J2s inoculated. IF theoretically ranged from 0 (no reproduction at all) to 1 (each juvenile inoculated could develop into a gravid female and generate one EM);
- Female Fertility (FF) was evaluated as the average number of eggs per EM (i.e., the number of eggs produced by each female);
- Reproduction Potential (RP) of the nematode population defined as the number of eggs/number of J2s inoculated, following formula: RP=IF x FF.

The virulence costs (C_h) of the laboratory-selected virulent lines on the susceptible host were estimated as follows: C_h (%)=1—RP(virulent line)/RP(avirulent line) (Castagnone-Sereno et al. 2007).

Statistical analysis

In order to investigate a possible cost of virulence, the RP on the susceptible tomato or pepper of a restricted set of nematode genotypes (avir F, avir Mifield1, avir MifieldV, vir SM1, vir SM2V, vir *Me3*, and vir *Me7*) were compared. A Kruskal-Wallis test was first carried out. Wilcoxon-Mann-Whithney unilateral tests were then used for post hoc comparisons in order to check if, for a similar nematode genetic background, avirulent strains have higher RP than virulent ones. This led us to perform four pairwise comparisons (avir F versus vir *Me3* or vir *Me7*; avir Mifield1 versus vir SM1; avir MifieldV versus vir SM2V). Bonferroni correction was consequently applied (significance

level at $\varepsilon = 0.05/4 = 0.0125$ instead of $\alpha = 0.05$). Analyses were performed using the free software R (http://www.r-project.org/).

Non-parametric tests were also applied to compare the RP of the nematode isolates on each plant genotype except "R *Me1*-pepper" (see Fig. 2 and associated Results). After a significant Kruskal-Wallis test, Wilcoxon-Mann-Whithney bilateral tests were carried-using Bonferonni correction (significance level at $\varepsilon = 0.05/28 = 0.0018$). These post hoc comparisons should nevertheless be interpreted with caution since they are not only sensitive to differences of RP between populations but also to differences in sample sizes.

Results

All the *M. incognita* isolates tested were able to infect both the susceptible tomatoes and peppers, with IF ranging from 0.10 to 0.87 (IF not determined for avir *Mifield1* and *vir Mi-1*) (Table 2). The *Mi-1* and *Me*-avirulent isolates avir MifieldV and avir F produced fewer egg masses on the partially resistant pepper cultivar Yolo Wonder compared to the susceptible pepper Doux Long des Landes and tomato St Pierre or Marmande plants. As expected, i) avirulent isolates (avir MifieldV and avir F) were unable to infect the resistant tomatoes or peppers (IF values ranging from 0 to 0.002); ii) selected isolates virulent on *Mi-1*-tomato exhibited a high infection frequency on resistant tomatoes ($0.35 \leq IF \leq 0.82$); and iii) both selected and natural isolates virulent on *Me3*-pepper and selected isolates virulent on *Me7*-pepper exhibited a high infection frequency on the *Me3*-pepper ($0.23 \leq IF \leq 0.81$). Nevertheless, none of the *Mi-1, Me3* and *Me7*-virulent isolates (overcoming either tomato or pepper R genes) were able to infect *Me1*-pepper, and both selected and natural isolates virulent on resistant peppers were unable to reproduce on *Mi-1*-tomato. One or two egg-masses were obtained on a few *Me1*-peppers, but these egg masses contained very few eggs (i.e., < 65 eggs; data not shown) compared to those on *Me3*-peppers (i.e., > 800 eggs; data not shown) and the nematodes obtained from these eggs did not survive to a successive inoculation, so it was not possible to obtain a *Me1*-virulent isolate.

Considering the RP of the nematodes, we first observed that the RP of avirulent isolates was higher

Table 2 Infection frequency (IF) of greenhouse and laboratory-selected *Meloidogyne incognita* isolates on susceptible vs. resistant tomato and pepper genotypes. Values are the mean (±standard error) of 6–20 replicates

Host genotypes[a]	Avirulent populations			Laboratory selection on *Mi-1*-tomato[b]			Laboratory selection on *Me3*- pepper[b]	Laboratory selection on *Me7*- pepper[b]	Greenhouse selection on *Me3*-pepper	Greenhouse selection on *Me3*-peppers		
	avir F	avir Mifield1	avir MifieldV	vir *Mi-1*	vir SM1	vir SM2V	vir *Me3*	vir *Me7*	Vir CREAT	vir pop A	vir pop B	vir pop C
S tomato	0.41±0.03	0.73±0.08	0.49±0.06	0.46±0.12	0.35±0.03	0.33±0.04	0.31±0.03	0.35±0.03	0.32±0.02	0.60±0.03	0.42±0.03	0.52±0.06
R tomato *Mi-1*	0.00±0.00	nd	0.002±0.66	0.82±0.14	0.43±0.04	0.35±0.06	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
S pepper	0.24±0.02	nd	0.66±0.05	nd	0.44±0.02	0.38±0.04	0.17±0.01	0.26±0.02	0.27±0.02	0.71±0.03	0.87±0.03	0.67±0.04
PR pepper	0.10±0.01	nd	0.27±0.03	0.00±0.00	0.51±0.02	0.41±0.05	0.18±0.02	0.12±0.02	0.16±0.02	0.71±0.03	0.66±0.08	0.43±0.03
R pepper *Me3*	0.002±0.001	nd	0.00±0.00	0.00±0.00	nd	0.00±0.00	0.38±0.02	0.25±0.03	0.23±0.02	0.61±0.08	0.81±0.06	0.43±0.02
R pepper *Me1*	0.00±0.00	nd	0.00±0.00	0.00±0.00	nd	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

[a] *S* susceptible, *R* resistant, *PR* partially resistant

[b] The virulent isolates were selected from the avirulent ones by successive rearing on resistant tomatoes and peppers, respectively

nd data not determined

on susceptible tomato than on susceptible pepper (496.78±46.30 and 371.21±71.77 for the avir F isolate on tomato and pepper, respectively; and 238.78±35.13 and 157.73±17.93 for the avir MifieldV isolate on tomato and pepper, respectively) (data not shown). When comparing the RP of a restricted set of the avirulent and virulent nematode populations on susceptible plants, we firstly demonstrated significant differences between nematode populations (χ^2_{6df}=40.8, $p<10^{-3}$) (Fig. 1). More interestingly, the hypothesis of a virulence cost was strongly supported in three of the four pairwise comparisons: avir F versus vir *Me3* (W=200; $p<10^{-3}$); avir F versus vir *Me7* (W=279; $p<10^{-3}$); avir MifieldV versus vir SM2V (W=34; p=0.004). In these three cases, the estimated fitness cost ranged from 54 to 71%. In the last comparison (avir Mifield1 versus vir SM1), the same tendency was observed but the difference was not significant (W=23; p=0.24); it is noteworthy that the sample size was nevertheless low (6 replicates).

Except for the resistant *Me1*-pepper, significant differences of RP were found between virulent nematode lines on a same host plant (Fig. 2). The number of eggs produced ranged from 106 to 496 times the number of J2s inoculated on susceptible cultivars (RP=106±9 to 496±19) and from 0 to 436 times the number of J2s inoculated on resistant cultivars (RP=0 to 436±32). The *Me3*-virulent isolates A, B and C from Hungary reproduced much more on all susceptible and *Me3*-resistant pepper genotypes (RP ranging from 200±3 to 496±19) than the *Me3*-virulent isolate arising from a single year (approximately 3 generations) of selection in field (vir CREAT) (RP ranging 117±11 from to 209±21), and than the laboratory selected isolates (vir *Me3* and vir *Me7*) (RP ranging from 63±10 to 322±18).

Discussion

Selection for RKN resistance in crops, including tomato and pepper, is a major challenge for plant breeders. Therefore, because the introgression of R-genes into cultivars of agronomic interest by successive back-crosses generally takes several years, a long-term goal of breeders is to select the genotypes (robust R-genes, their combination, and associated genetic backgrounds) that are expected to exhibit the best durability under field conditions (late emergence and low fitness of nematode virulent populations). In pepper, the *Me1* R-gene appears to be a good candidate for such a purpose. Previous studies had shown that none of the 22 RKN avirulent isolates

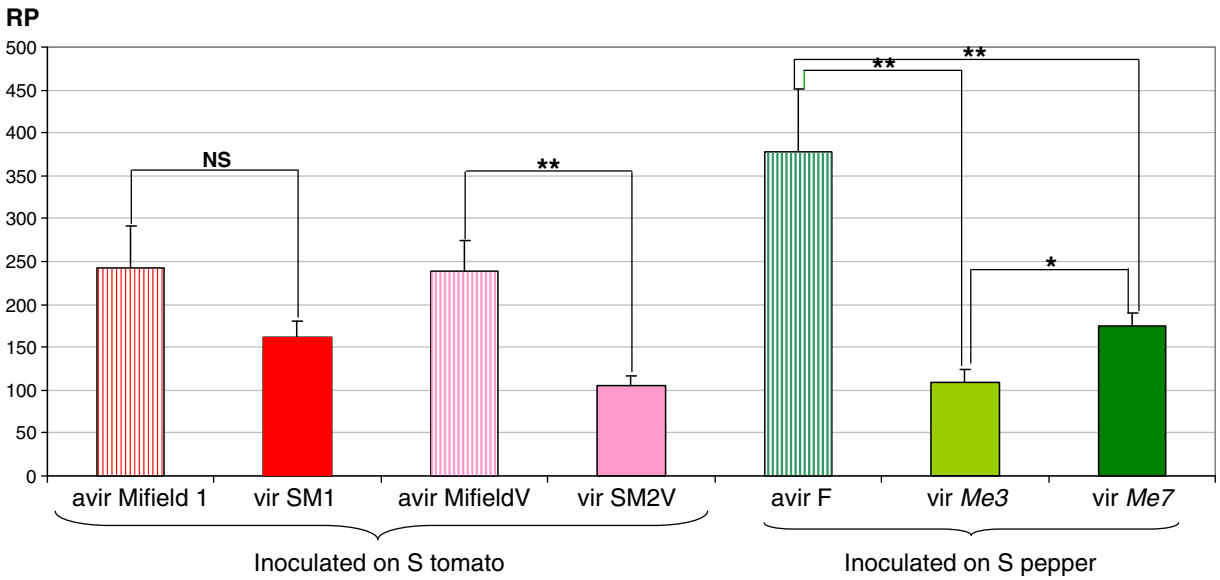

Fig. 1 Comparison of the reproduction potential (mean RP of 6 to 20 replicates±standard error) of avirulent and virulent laboratory-selected *M. incognita* isolates on tomato and pepper susceptible genotypes. NS=no significant difference; *=significant difference; **=highly significant difference (significance level at ε=0.05/4=0.0125 instead of α=0.05)

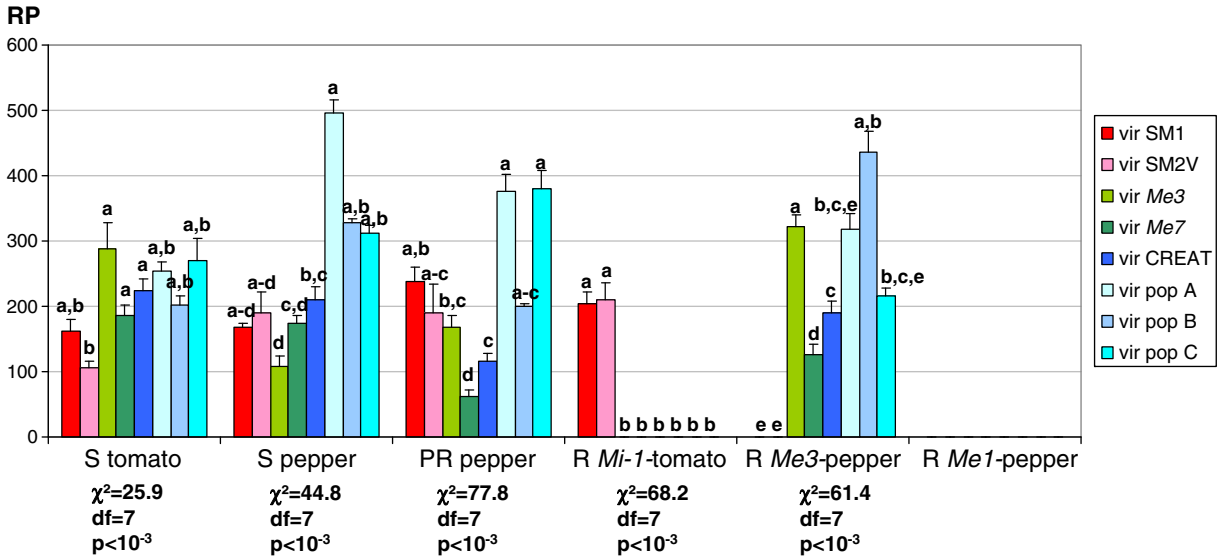

Fig. 2 Comparison of the reproduction potential (mean RP of 6 to 20 replicates±standard error) of laboratory-selected and natural *M. incognita* virulent isolates on both susceptible and resistant tomatoes and peppers. The combinations missing a datum bar (for tests on R *Mi-1*-tomato, R *Me3*-pepper and R *Me1*-pepper) have values of 0. The results of Kruskal-Wallis tests are given under each line

tested, belonging to the three major species (*M. incognita, M. arenaria* and *M. javanica*), were able to overcome this R-gene under laboratory artificial conditions (Castagnone-Sereno et al. 1996; 2001). In the present study, we further showed that neither natural nor selected virulent RKN isolates (overcoming either *Mi-1* from tomato or *Me3* or *Me7* from pepper) were able to reproduce on *Me1*-peppers. Such a result is in good agreement with previous data indicating that these R-genes are not equally overcome by RKNs, and that *Me1* behaves as a robust R-gene. Conversely to *Mi-1, Me3* and *Me7*, *Me1* induces a late hypersensitive reaction in the vascular cylinder of infected roots, thus inhibiting the development of egg-laying females (Bleve-Zacheo et al. 1998; Pegard et al. 2005). Our observations here support previous data suggesting that late expression of the hypersensitive reaction by *Me1* prevents (or at least strongly reduces) the frequency of emergence of *Me1*-virulent nematode genotypes.

Additionally, this study demonstrates that both natural and selected RKN isolates virulent on *Me3* or *Me7* resistant peppers are unable to reproduce on *Mi-1* tomato, and confirmed that selected isolates virulent on *Mi-1* tomato are unable to reproduce on *Me3* and *Me1* resistant peppers. The virulent isolate overcoming *Me7* was able to reproduce on *Me3*-pepper, which may indicate that *Me3* and *Me7* may be one single allele at the same gene. Indeed, two independent allelism tests performed with 385 test-cross lines [F1(CM334xHD149) x DLL] strongly suggested that *Me3* and *Me7* are allelic, since no susceptible plant was obtained on these 385 lines inoculated with 500 avirulent *M. incognita* juveniles (Djian-Caporalino & Castagnone-Sereno, unpublished). Moreover, the fact that RKN selected for virulence over *Me7* also broke down *Me3* (and reciprocally, data not shown) indicates these are a single allele. So, RKN isolates virulent on one resistant crop are definitively not virulent on a different resistant crop. These findings indicate that virulence is highly specific to the R-gene on which selection has occurred. As a result of such strict specificity of virulence, once virulent isolates are selected on a determined R-gene, it is very likely that alternation in the rotation with a different gene will reduce the number of nematodes in the soil under their damage threshold, except if multiple virulence can be progressively selected, which remains to be demonstrated.

The reproduction potential of *Mi-1*-, *Me3*- or *Me7*-virulent laboratory-selected and wild *M. incognita* isolates exhibited significant high levels of variability on a same host, either susceptible or resistant. As nematode reproduction on resistant genotypes is

generally explained by the interaction between the plant genotype and nematode isolate, but not by either factor alone (Ornat et al. 2001; Jacquet et al. 2005; Lopez-Perez et al. 2006; Cortada et al. 2008), we can hypothesize that the variability observed here in the infection and reproduction of virulent lines on a same host can be attributed to the nematode genetic background, because they did not originate from the same nematode population and they were not selected on the same resistant plants in laboratory and in fields, even if the R-gene in these plants was the same. The natural *Me3*-virulent isolates selected on resistant peppers seemed to reproduce better on susceptible and resistant *Me3*-peppers than laboratory-selected isolates. This observation may be correlated to the way these isolates acquired their virulence, and suggests that the genetic changes induced under managed pressure in artificial conditions are different from those occurring in natural *Me3* resistance-breaking biotypes, as previously reported for laboratory-selected or natural isolates virulent against the tomato *Mi-1* R-gene (Castagnone-Sereno et al. 1994a; Roberts 1995). It may also result from the fact that laboratory-selected *Me3*-virulent isolates are very recent and have not yet been submitted to alternative hosts, conversely to the case with natural virulent isolates that may be older and whose genetic background may have co-evolved with the virulence, thus restoring the nematode fitness.

Comparing the RP of the avirulent and laboratory-selected virulent nematodes revealed that both *Me3*- (or *Me7-)* and *Mi-1*-virulent isolates showed significantly lower RPs on susceptible plants compared to the avirulent isolates, mainly due to a decrease in female fertility. This result indicates that some fitness cost reduced the nematode reproduction on the susceptible plants because of unnecessary virulence. This fitness cost may be associated to the virulence trait (pleiotropic effect), but may also result in part from the reduction of genetic variability operated by the selection pressure of the R-genes. Experimental evidence of such virulence fitness costs penalizing the aggressiveness of virulent pathogens on susceptible plants has been frequently reported for bacteria, fungi and viruses (Vera Cruz et al. 2000; Parlevliet 2002; Desbiez et al. 2003; Ayme et al. 2007; Janzac et al. 2009). However, similar data on RKNs remain scarce (Petrillo and Roberts 2005; Castagnone-Sereno et al. 2007). The present study reinforces these previous observations and suggests that such a fitness cost of virulent lines on susceptible crops is a general trend in plant pathogens, including nematodes. In our study, the virulence costs were estimated at 33 to 56% on susceptible tomatoes and at 54 to 71% on susceptible peppers. Thus, *Me3* (or *Me7)* seemed to exert a higher selection pressure than *Mi-1* on RKN populations, resulting in an increased fitness cost of virulence. Moreover, RP of avirulent isolates was higher on susceptible tomatoes than on susceptible peppers, suggesting that the so-called 'susceptible' peppers had some undetermined and partial resistance factors, conferring a quantitative resistance to some *Me3*- (or *Me7-)* virulent RKN isolates that are not present in susceptible tomatoes. In that respect, it should be noted that resistance to some isolates of the RKN species *M. chitwoodi* was previously observed in Doux Long des Landes (Berthou et al. 2003; Djian-Caporalino et al. 2007). Further investigations are currently underway in the laboratory to confirm this hypothesis, which may have major practical implications in terms of durability of the resistance (Palloix et al. 2009).

Host resistance is considered as an important component of integrated management of RKNs. Because few R-genes acting against these pests are currently available, it is an urgent need to protect them and promote their durability. Since resistance deployment is influenced by the variation in (a) virulence and host range, two primary attributes of host resistance for nematode resistance breeding and management are relevant: i) the value of resistance in crop self-protection, based on the level of resistance to injury caused by initial infection, and ii) the rotational value of resistance in cropping systems for protecting subsequent crops, based on the ability to decrease the nematode population densities in soil by restricting nematode reproduction (Roberts 1995). In pepper, the *Me1* R-gene confers a high level of resistance without affecting the plant growth, is still active at high temperature conversely to the tomato *Mi-1* resistance gene, and is not overcome by both selected and natural virulent isolates overcoming *Mi-1* in tomato nor *Me3* nor *Me7* in pepper. This R-gene therefore appears to be suitable for either combination with *Me3* in pepper cultivars ('pyramiding') or alternating with other R-genes (*Me3* or *Me7, Mi-1*) in crop rotations to strengthen and increase the durability of resistance. Experiments are now necessary to validate the obtained results under field agronomic conditions

comparing: i) the alternation of single R-genes in rotation; ii) the mixture of genotypes bearing single R-genes sown in the same plot; and iii) the pyramiding of two R-genes in one genotype. Results will allow the identification of conditions lowering the emergence of virulent biotypes of RKN in the field, and the time required for the reduction of parasites under their damage threshold using the R-plants as RKN 'traps'. This approach will help to promote the durability of resistance against RKN, and making breeders and farmers (resistance users) sensitive to the 'directions for use' necessary to maintain their durability.

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References

Ayme, V., Petit-Pierre, J., Souche, S., Palloix, A., & Moury, B. (2007). Molecular dissection of the potato virus Y VPg virulence factor reveals complex adaptations to the pvr2 resistance allelic series in pepper. *Journal of General Virology, 88*, 1594–1601.

Berthou, F., Palloix, A., & Mugniery, D. (2003). Characterisation of virulence in populations of *Meloidogyne chitwoodi* and evidence for a resistance gene in pepper *Capsicum annuum* L. line PM217. *Nematology, 5*, 383–390.

Bleve-Zacheo, T., Bongiovanni, M., Melillo, M. T., & Castagnone-Sereno, P. (1998). The pepper resistance genes *Me1* and *Me3* induce differential penetration rates and temporal sequences of root cell ultrastructural changes upon nematode infection. *Plant Science, 133*, 79–90.

Blok, V. C., Jones, J. T., Phillips, M. S., & Trudgill, D. L. (2008). Parasitism genes and host range disparities in biotrophic nematodes: the conundrum of polyphagy versus specialisation. *BioEssays, 30*, 249–259.

Castagnone-Sereno, P. (2002). Genetic variability of nematodes: a threat to the durability of plant resistance genes? *Euphytica, 124*, 193–199.

Castagnone-Sereno, P., Bongiovanni, M., & Dalmasso, A. (1994a). Reproduction of virulent isolates of *Meloidogyne incognita* on susceptible and *Mi*-resistant tomato. *Journal of Nematology, 26*, 324–328.

Castagnone-Sereno, P., Wajnberg, E., Bongiovanni, M., Leroy, F., & Dalmasso, A. (1994b). Genetic variation in *Meloidogyne incognita* virulence against the tomato *Mi* resistance gene: Evidence from isofemale line selection studies. *Theoretical and Applied Genetics, 88*, 749–753.

Castagnone-Sereno, P., Bongiovanni, M., Palloix, A., & Dalmasso, A. (1996). Selection for *Meloidogyne incognita* virulence against resistance genes from tomato and pepper and specificity of the virulence/resistance determinants. *European Journal of Plant Pathology, 102*, 585–590.

Castagnone-Sereno, P., Bongiovanni, M., & Djian-Caporalino, C. (2001). New data on the specificity of the root-knot nematode resistance genes *Me1* and *Me3* in pepper. *Plant Breeding, 120*, 429–433.

Castagnone-Sereno, P., Bongiovanni, M., & Wajnberg, E. (2007). Selection and parasite evolution: a reproductive fitness cost associated with virulence in the parthenogenetic nematode *Meloidogyne incognita*. *Evolutionary Ecology, 21*, 259–270.

Cortada, L., Sorribas, F. J., Ornat, C., Kaloshian, I., & Verdejo-Lucas, S. (2008). Variability in infection and reproduction of *Meloidogyne javanica* on tomato rootstocks with the *Mi* resistance gene. *Plant Pathology, 57*, 1125–1135.

Dalmasso, A., & Bergé, J. B. (1978). Molecular polymorphism and phylogenetic relationship in some *Meloidogyne* spp.: Application to the taxonomy of *Meloidogyne*. *Journal of Nematology, 10*, 323–332.

Desbiez, C., Gal-On, A., Girard, M., Wipf-Scheibel, C., & Lecoq, H. (2003). Increase in Zucchini yellow mosaic virus symptom severity in tolerant zucchini cultivars is related to a point mutation in P3 protein and is associated with a loss of relative fitness on susceptible plants. *Phytopathology, 93*, 1478–1484.

Devran, Z., & Söğüt, M. A. (2010). Occurrence of virulent root-knot nematode isolates on tomatoes bearing the *Mi* gene in protected vegetable-growing areas of Turkey. *Phytopathology Mediterranea, 38*, 245–251.

Djian-Caporalino, C., Pijarowski, L., Januel, A., Lefebvre, V., Daubeze, A., Palloix, A., et al. (1999). Spectrum of resistance to root-knot nematodes and inheritance of heat-stable resistance in pepper (*Capsicum annuum* L.). *Theoretical and Applied Genetics, 99*, 496–502.

Djian-Caporalino, C., Pijarowski, L., Fazari, A., Samson, M., Gaveau, L., O'Byrne, C. V., et al. (2001). High-resolution genetic mapping of the pepper (*Capsicum annuum* L.) resistance loci *Me3* and *Me4* conferring heat-stable resistance to root-knot nematodes (*Meloidogyne* spp.). *Theoretical and Applied Genetics, 103*, 592–600.

Djian-Caporalino, C., Fazari, A., Arguel, M. J., Vernie, T., VandeCasteele, C., Faure, I., et al. (2007). Root-knot nematode (*Meloidogyne* spp.) *Me* resistance genes in pepper (*Capsicum annuum* L.) are clustered on the P9 chromosome. *Theoretical and Applied Genetics, 114*, 473–486.

Dumas de Vaulx, R., Chambonnet, D., & Pochard, E. (1981). Culture in vitro d'anthères de piment (Capsicum annuum): amélioration des taux d'obtention de plantes chez différents génotypes par des traitements à +35°C. *Agronomie, 1*(10), 859–864.

Hendy, H., Pochard, E., & Dalmasso, A. (1985). Transmission héréditaire de la résistance aux nématodes *Meloidogyne* Chitwood (Tylenchida) portée par deux lignées de *Capsicum annuum* L.: étude de descendances homozygotes issues d'androgenèse. *Agronomie, 5*, 93–100.

Huang, X., McGiffen, M., & Kaloshian, I. (2004). Reproduction of *Mi*-virulent *Meloidogyne incognita* isolates on *Lycopersicon* spp. *Journal of Nematology, 36*, 69–75.

Jacquet, M., Bongiovanni, M., Martinez, M., Verschave, P., Wajnberg, E., & Castagnone-Sereno, P. (2005). Variation in resistance to the root-knot nematode *Meloidogyne incognita* in tomato genotypes bearing the *Mi* gene. *Plant Pathology, 54*, 93–99.

Janzac, B., Fabre, F., Palloix, A., & Moury, B. (2009). Constraints on evolution of virus avirulence factors predict the durability of corresponding plant resistances. *Molecular Plant Pathology, 10*, 599–610.

Jarquin-Barberena, H., Dalmasso, A., de Guiran, G., & Cardin, M. C. (1991). Acquired virulence in the plant parasitic nematode *Meloidogyne incognita*. I. Biological analysis of the phenomenon. *Revue de Nématologie, 14*(2), 299–303.

Laterrot, H. (1975). Séries de lignées isogéniques de tomate ne différant que par certains gènes de résistance aux maladies. *Phytopathologia Mediterranea, 14*, 129–130.

Lopez-Perez, J. A., Le Strange, M., Kaloshian, I., & Ploeg, A. T. (2006). Differential response of *Mi* gene-resistant tomato rootstocks to root-knot nematodes (*Meloidogyne incognita*). *Crop Protection, 25*, 382–388.

Meher, H. C., Gajbhiye, V. T., Chawla, G., & Singh, G. (2009). Virulence development and genetic polymorphism in *Meloidogyne incognita* (Kofoid & White) Chitwood after prolonged exposure to sublethal concentrations of nematicides and continuous growing of resistant tomato cultivars. *Pest Management Science, 65*, 1201–1207.

Methyl Bromide Technical Options Committee (2006). *Report of the Methyl Bromide Technical Options Committee. Non-chemical Alternatives Adopted as Replacements to Methyl Bromide on a Large Scale*. Nairobi, Kenya: United Nation Environmental Programme, UNON Publishing Section Services, 39–73.

Ornat, C., Verdejo-Lucas, S., & Sorribas, F. J. (2001). A population of *Meloidogyne javanica* in Spain virulent to the *Mi* resistance gene in tomato. *Plant Disease, 85*, 271–276.

Palloix, A., Ayme, V., & Moury, B. (2009). Durability of plant major resistance genes to pathogens depends on the genetic background, experimental evidence and consequences for breeding strategies. *New Phytologist, 183*, 190–199.

Parlevliet, J. E. (2002). Durability of resistance against fungal, bacterial and viral pathogens; present situation. *Euphytica, 124*, 147–156.

Pegard, A., Brizzard, G., Fazari, A., Soucaze, O., Abad, P., & Djian-Caporalino, C. (2005). Histological characterization of resistance to different root-knot nematode species related to phenolics accumulation in *Capsicum annuum* L. *Phytopathology, 95*, 158–165.

Petrillo, M. D., & Roberts, P. A. (2005). Fitness of virulent *Meloidogyne incognita* isolates on susceptible and resistant cowpea. *Journal of Nematology, 37*, 457–466.

Roberts, P. A. (1995). Conceptual and practical aspects of variability in root-knot nematodes related to host plant resistance. *Annual Review of Phytopathology, 33*, 199–221.

Roberts, P. A., Dalmasso, A., Cap, G. B., & Castagnone-Sereno, P. (1990). Resistance in *Lycopersicon peruvianum* to isolates of *Mi* gene-compatible *Meloidogyne* populations. *Journal of Nematology, 22*, 585–589.

Sorribas, F. J., Ornat, C., Verdejo-Lucas, S., Galeano, M., & Valero, J. (2005). Effectiveness and profitability of the *Mi*-resistant tomatoes to control root-knot nematodes. *European Journal of Plant Pathology, 111*, 29–38.

Tzortzakakis, E. A., Trudgill, D. L., & Phillips, M. S. (1998). Evidence for a dosage effect of the *Mi* gene on partially virulent isolates of *Meloidogyne javanica*. *Journal of Nematology, 30*, 76–80.

Tzortzakakis, E. A., Adam, M. A. M., Blok, V. C., Paraskevopoulos, C., & Bourtzis, K. (2005). Occurrence of resistance-breaking isolates of root-knot nematodes on tomato in Greece. *European Journal of Plant Pathology, 113*, 101–105.

Tzortzakakis, E. A., da Conceicao, I. L. P. M., dos Santos, M. C. V., & de O Abrantes, I. M. (2008). Selection of virulent *Meloidogyne* individuals within mixed isolates by continuous cultivation on a *Mi* gene resistant tomato genotype. *Journal of Plant Diseases and Protection, 115*(5), 234–237.

Vera Cruz, C. M., Bai, J., Oña, I., Leung, H., Nelson, R. J., Mew, T. W., et al. (2000). Predicting durability of a disease resistance gene based on an assessment of the fitness loss and epidemiological consequences of avirulence gene mutation. *Proceedings of the National Academy of Sciences of the USA, 97*, 13500–13505.

Verdejo-Lucas, S., Cortada, L., Sorribas, F. J., & Ornat, C. (2009). Selection of virulent isolates of *Meloidogyne javanica* by repeated cultivation of *Mi* resistance gene tomato rootstocks under field conditions. *Plant Pathology, 58*, 990–998.

Williamson, V. M., & Kumar, A. (2006). Nematode resistance in plants: the battle underground. *Trends in Genetics, 22*, 396–403.

Williamson, V. M., & Roberts, P. A. (2009). Mechanisms and genetics of resistance. In R. N. Perry, M. Moens, & J. L. Starr (Eds.), *Root-knot Nematodes* (pp. 301–325). Wallingford, UK: CAB International.

Zijlstra, C., Donkers-Venne, T. H. M., & Fargette, M. (2000). Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterised amplified region (SCAR) based PCR assays. *Nematology, 2*, 847–853.