Genetic Basis of the Epidemiologic Effects of Resistance to *Meloidogyne incognita* in the Tomato Cultivar Small Fry[1]

S. C. Bost and A. C. Triantaphyllou[2]

Abstract: The genetic nature of resistance and its epidemiologic effects on two *Meloidogyne incognita* populations were assessed in the F_1 hybrid tomato cv. Small Fry. The progeny of a Small Fry × Small Fry cross segregated in a 3:1 resistant:susceptible ratio, indicating the presence of a single, completely dominant resistance gene ($LMiR_2$) in Small Fry. In a subsequent experiment, infection frequency and the rate of development of primary infection on resistant Small Fry × Small Fry segregates were compared to those on susceptible segregates and the susceptible cultivar Rutgers. Suppression in both infection frequency and rate of development of primary infection was entirely attributable to gene $LMiR_2$. A single egg-mass population of *M. incognita* propagated for 12 generations on Small Fry showed an increased ability over the wild type population to parasitize plants containing the $LMiR_2$ gene but failed to completely overcome resistance. The relationship of this phenomenon to the genetics of the *Lycopersicon esculentum-M. incognita* interaction is discussed. *Key words:* infection frequency, primary infection, selection, rate of development.

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Resistance to *Meloidogyne incognita* (Kofoid and White) Chitwood in the hybrid tomato (*Lycopersicon esculentum* Mill.) cv. Small Fry is conferred by a single, dominant gene in a heterozygous state (J. C. Watterson, plant breeder, Petoseed Co., Inc., personal communication). This gene was considered to be the Mi gene that confers resistance to many other resistant tomato cultivars including Nematex. However, genetic studies by Sidhu and Webster (10,11) have indicated that the resistance gene in Small Fry is different from the Mi gene of Nematex, and that the two genes are closely linked. These investigators proposed the designation $LMiR_2$ for the resistance gene of Small Fry and redesignated the Mi gene of Nematex as $LMiR_1$.

Nematode variants capable of parasitizing resistant tomato cultivars occasionally occur (2,7,9,13). Continued culture of resistant cultivars may result in increased compatibility by *M. incognita* populations. A single egg-mass population of *M. incognita* propagated for 12 generations on Small Fry showed increased compatibility with this cultivar (2). However, infection frequency (the proportion of juveniles that establish infection) and egg production were significantly lower during primary infection on Small Fry than on cv. Rutgers. It was unknown whether the suppression of both of these parameters was attributable to the $LMiR_2$ gene, or whether horizontal resistance was involved.

In the present study, the reactions of resistant and susceptible Small Fray × Small Fry (SF × SF) progeny inoculated with either a wild type or a resistance-

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[2]Respectively, Graduate Research Assistant, Department of Plant Pathology, and Professor, Department of Genetics, North Carolina State University, Raleigh, NC 27650.

overcoming population of *M. incognita* were analyzed. The objectives were (i) to identify the effects of the $LMiR_2$ gene in Small Fry on *M. incognita* infection and rate of development, (ii) to assess the relative level of horizontal resistance to *M. incognita* in this cultivar, and (iii) to determine whether a *M. incognita* population propagated for 12 generations on Small Fry has adaped to components of the Small Fry genome other than the $LMiR_2$ gene.

MATERIALS AND METHODS

A preliminary test to ascertain heterozygosity at the $LMiR_2$ locus in Small Fry was conducted. Flowers on three Small Fry plants were emasculated upon opening, self-pollinated, and protected with a gelatin capsule. Seeds obtained from self-pollinated fruit (SF × SF) were planted in 5-cm-d clay pots containing a 2:1 mixture of steam-sterilized sandy loam and washed river sand. At the four true-leaf stage, each seedling was transferred to a 10-cm-d clay pot containing a similar soil mixture and inoculated with a suspension of 3,000 eggs of *M. incognita* (culture 108 of the International *Meloidogyne* Project). After 37 days, the roots were gently washed free of soil, immersed for 10 min in Phloxine B (0.15 gm/liter tap water), and examined for the presence of galls and egg masses.

The SF × SF progeny segregated in a 3:1 resistant:susceptible ratio as expected for a single, dominant gene in the heterozygous state. Such a segregation allowed an investigation of the epidemiologic effects of resistance in Small Fry. Additional SF × SF progeny were inoculated as described above with 400 juveniles each of either culture 108, which lacks the ability to parasitize Small Fry, or culture $108SF_{12}$, which has developed this ability during 12 generations of serial transfer on Small Fry. Culture $108SF_{12}$ originated from a single female that developed on a Small Fry plant inoculated with culture 108. Eight plants each of Small Fry and the susceptible cv. Rutgers were also included in the study. Fifty SF × SF plants were inoculated with each of the two nematode cultures to assure the inclusion of at least eight susceptible segregates in each case. The pots were arranged in a completely randomized design in a 23–28 C greenhouse. After 37 days from inoculation, roots were washed and treated with Phloxine B. The period of 37 days was chosen on the basis of 18,000 degree-h accumulation which had been determined to be sufficient for maximum egg production on Rutgers.

Eight plants of each combination of plant type (Rutgers, Small Fry, resistant segregates, or susceptible segregates) and culture (108 or $108SF_{12}$) were assayed for total number of egg masses per plant and average number of eggs per egg mass. The latter value was obtained for each plant by randomly removing 40 egg masses and the associated galls, placing them in 25 ml of 0.5% NaOCl together with a 2.8-cm magnet, and stirring for 5 min with a magnetic stirrer at 350 rpm. The suspension was then diluted to 150 ml, and the total number of eggs and the average number of eggs per egg mass were calculated based on counts made in three 1-ml aliquots. Among the SF × SF progeny inoculated with culture $108SF_{12}$, resistant and susceptible segregates were distinguishable by the presence of infection by second-generation juveniles (secondary infection) on the roots of the susceptible segregates.

The index of parasitism (IP) was used to compare the two nematode populations. The IP is the number of egg masses found on a test host expressed as a percentage of those found on the susceptible cv. Rutgers. The purpose of the IP is to compensate for any juvenile viability differences that may arise during preparation of the inocula. Any preinoculation conditions which suppress juvenile infectivity on a standard susceptible cultivar should suppress infectivity on other cultivars proportionately. Thus, the IP is not affected by such conditions.

Index of parasitism estimates were obtained only for primary infection, so that the effects of infection frequency could be distinguished from other epidemiologic effects of resistance (8). The latter include such epidemiologic parameters as latent period (the period between infection to onset of egg production), rate of egg production, and infectious period (the reproductive period) (14,15,16). The first two of

these parameters are collectively estimated in this study by a single value—the average number of eggs per egg mass following 18,000 degree-h of development.

RESULTS AND DISCUSSION

In the preliminary test, the SF × SF progeny segregated in a resistant:susceptible ratio of 85:29, closely fitting a 3:1 ratio ($X^2 = 0.0117$). Such a ratio indicates a single gene for resistance in a heterozygous state. The susceptible segregates were heavily galled and contained many egg masses, whereas none of the resistant segregates contained more than two egg masses. The lack of a difference in degree of resistance between the homozygous and heterozygous resistant segregates indicates that $LMiR_2$ is completely dominant.

In the subsequent part of the study, plants containing $LMiR_2$ (Small Fry and resistant SF × SF segregates) inoculated with culture 108 developed very few egg masses (Table 1). Plants containing $LMiR_2$ inoculated with culture $108SF_{12}$ developed fewer egg masses than did plants lacking $LMiR_2$ (Table 1). There were fewer eggs per egg mass on plants with $LMiR_2$ gene than on susceptible SF × SF segregates (Table 2). Numbers of egg masses and eggs per egg mass produced on Small Fry and resistant segregates did not differ, supporting the conclusion of complete dominance of the $LMiR_2$ allele.

It is assumed that the resistant and susceptible SF × SF segregates, considered as groups, differ only at the $LMiR_2$ locus. As an F_1 hybrid, Small Fry constitutes an essentially homogeneous group. With the exception of the resistant segregates inoculated with culture 108, the standard deviations obtained for the resistant and susceptible SF × SF segregates were no greater than those obtained for Small Fry (Tables 1 and 2). These results suggest that the two groups of SF × SF progeny are also essentially homogeneous. All genes, other than $LMiR_2$, affecting resistance or susceptibility appear to have been distributed approximately equally to all selfed Small Fry progeny. The higher coefficient of variation obtained on resistant segregates inoculated with culture 108 may be the result of segregation of a gene modifying $LMiR_2$. However, these effects were not apparent among resistant segregates inoculated with culture $108SF_{12}$. The homogeneity of the selfed Small Fry progeny enables the distinction of the effects of $LMiR_2$ on nematode development from the effects of other genes in the Small Fry genome. The suppression of both infection frequency (Table 1) and rate of development of primary infection (Table 2) are attributable solely to

Table 1. Infection frequencies for four hosts inoculated with a wild-type *Meloidogyne incognita* population (108) and with a population ($108SF_{12}$) derived from it through 12 generations of selection on tomato cv. Small Fry.

	Nematode population					
	108			$108SF_{12}$		
Host	Number of egg masses per plant*	Standard deviation (ln n+1)	IP†	Number of egg masses per plant	Standard deviation (ln n+1)	IP
Rutgers	326.9 a	0.080		211.1 a	0.208	
Small Fry	6.1 b	0.377	1.9	145.5 b	0.163	71.6
SF × SF susceptible segregates	323.0 a	0.116	99.6	234.2 a	0.067	110.9
SF × SF resistant segregates	5.9 b	1.007	1.8	145.0 b	0.069	71.5

*Mean of eight replicate plants inoculated with 400 juveniles each. Column means followed by the same letter are not different according to Waller-Duncan's Bayesian k-ratio t-test (1 = 100).

†IP (index of parasitism) = number of egg masses on test host expressed as a percentage of those on the susceptible cv. Rutgers.

Table 2. Egg production on four hosts by a wild-type *Meloidogyne incognita* population (108) and by a population ($108SF_{12}$) derived from it through 12 generations of selection on tomato cv. Small Fry.

Host	Nematode population			
	108*		$108SF_{12}$	
Rutgers	693.0 a	(0.082)†	605.0 ab	(0.090)
Small Fry	...‡		543.8 b	(0.262)
SF × SF susceptible segregates	602.6 b	(0.139)	668.9 a	(0.101)
SF × SF resistant segregates	...‡		545.1 b	(0.060)

*Number of eggs per egg mass; average from eight test plants. Column means followed by the same letter are not different according to Waller-Duncan's Bayesian k-ratio t-test.

†Numbers in parentheses are standard deviations, based on ln n+1.

‡No counts taken because of an insufficient number of egg masses recovered from these plants.

$LMiR_2$. Thus, $LMiR_2$ has the epidemiologic effect of suppressing both the number of infections and the rate at which successful infections develop.

Other investigators also have found that nematode development is slower in resistant species or cultivars than in susceptible ones, although the genetic basis for this effect has not been determined (1,3,4,5). The present study attributes the retarded development in a resistant cultivar to a single gene.

The removal of the dominant $LMiR_2$ allele from the Small Fry genome by selfing allows an analysis of residual (horizontal) resistance. The amount of infection on the susceptible segregates did not differ from that on Rutgers, for either nematode population (Table 1). Thus, without the $LMiR_2$ allele, Small Fry appears to have a level of susceptibility comparable to that of Rutgers and, therefore, does not possess any residual resistance.

There were no significant differences in the number of egg masses produced on Rutgers and on susceptible segregates, either before or after selection (Table 1). These data suggest that adaptation in the nematode was probably not directed at any components of the Small Fry genome other than $LMiR_2$. The number of eggs per egg mass produced on susceptible segregates relative to that on Rutgers increased slightly following selection, but the increase was not significant statistically (Table 2). The greater egg production by culture 108 on Rutgers may be the result of prolonged adaptation to this cultivar, on which culture 108 was maintained prior to these studies.

The reaction between culture $108SF_{12}$ and plants with $LMiR_2$ gene is instructive in an analysis of the genetics of the *Lycopersicon esculentum-M. incognita* interaction. Culture $108SF_{12}$ is derived from a single egg mass, reproduces by mitotic parthenogenesis (12), and was subjected to intense selection favoring virulence alleles for 12 generations. These factors suggest limited variation and a high degree of adaptation in this population. Nevertheless, this population has less parasitic ability on plants containing gene $LMiR_2$ than on those that do not (Tables 1, 2). As discussed earlier, this study has eliminated the possibility of a greater level of horizontal resistance in Small Fry as a cause of the lower compatibility. Gene $LMiR_2$ apparently has a residual potency that may not be subject to being overcome by the nematode, as has been suggested from the Pm4 gene for resistance in wheat to *Erysiphe graminis* f. sp. *tritici* (6). Using two near-isogenic lines of wheat, differing at the Pm4 locus, Martin and Ellingboe (6) determined that the Pm4 allele reduced the rate of development of primary infection of fungal isolates containing the corresponding virulence allele.

This study elucidates the role of the host in observed nematode population kinetics. A single, completely dominant

gene, $LMiR_2$, suppresses both infection frequency and rate of development of primary infection by *M. incognita*. The response of a nematode population to selection on resistant tomato cultivars may now be interpreted on this basis. *Meloidogyne* spp. populations characteristically progress through various intermediate degrees of adaptation during continued exposure to tomato cultivars containing resistance genes (2,7,9,13). An understanding of the cause of these intermediate degrees of virulence would be helpful to an understanding of the basis of variability in this nematode. A further genetic analysis of the ability of *M. incognita* to overcome resistance is presented elsewhere (2).

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