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The effect of heterozygosity for virulence on the development of the potato cyst nematode, *Globodera rostochiensis*

Richard JANSSEN *, Jaap BAKKER and Fred J. GOMMERS

Department of Nematology, Agricultural University Wageningen, P.O. Box 8123, Binnenhaven 10, 6700 ES Wageningen, The Netherlands.

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Summary — The genetic constitution of avirulent larvae (Aa and AA) of *Globodera rostochiensis* has no substantial influence on the development into adults on *Solanum tuberosum* ssp. *andigena* CPC 1673 containing the H_1 resistance gene. None of the AA larvae and only 0.3 % of the Aa larvae were able to develop into females. The H_1 gene was only moderately effective against males. Both AA and Aa larvae were able to develop into males. The heterozygous males (Aa) had no real advantage over homozygous males (AA).

Résumé — ***Influence de l'hétérozygotie de la virulence sur le développement du nématode à kyste de la pomme de terre Globodera rostochiensis*** — La composition génétique de larves avirulentes (Aa et AA) de *Globodera rostochiensis* n'a pas d'influence notable sur le développement au stade adulte du nématode élevé sur *Solanum tuberosum* ssp. *andigena* CPC 1673, possédant le gène de résistance H_1. Aucune des larves AA et 0,3 % seulement des larves Aa sont capables de se développer en femelles. Le gène H_1 n'est que modérément efficace en ce qui concerne les mâles. Les larves AA et Aa sont capables de se développer en mâles. Les mâles hétérozygotes (Aa) n'ont pas un avantage net sur les mâles homozygotes (AA).

Key-words : Nematodes, *Globodera*, potato, heterozygosity, virulence.

A crucial process in the development of potato cyst nematodes is the induction and maintenance of a syncytium, which regulates the transfer of nutrients towards the nematode (Gommers, 1981; Zacheo, 1986). After the induction of a feeding site the second stage larva becomes sedentary and progresses through third and fourth developmental stages to adult. Sex differentiation, which becomes visible in the third developmental stage, is epigenic and is mainly influenced by nutritional factors (Trudgill, 1967; Mugniéry & Fayet, 1981; Mugniéry, 1982, 1985; Mugniéry & Fayet, 1984; Janssen *et al.*, 1987). The imbalance of the adult sex ratio towards males in resistant plants (Turner & Stone, 1984) is thought to result from the poor nutritional potential of the syncytia. In incompatible combinations the syncytia remain small and become surrounded by necrotic tissue, which limits the transport of nutrients from plant to nematode (Kühn, 1958; Huijsman *et al.*, 1969; Rice *et al.*, 1985). In contrast with males, females cannot develop because of their greater nutritional needs.

Mendelian segregation patterns followed by the development of females on plants having the H_1 resistance gene from *Solanum tuberosum* ssp. *andigenum* CPC 1673, showed that virulence in *Globodera rostochiensis* is inherited at a single locus and is recessive to avirulence (Janssen *et al.*, 1990*c*). The resistance mechanism conferred by the H_1 gene is not absolute and homozygous dominant avirulent larvae (AA) incidentally develop into females. The underlying process is unclear and it is also unknown wether heterozygous larvae (Aa) have an advantage over homozygous dominant larvae (AA) to escape from the resistance mechanism. Also the fate of the homozygous dominant (AA) and the heterozygous larvae (Aa) has never been subjected to detailed analyses. It was hypothesized that both genotypes are able to develop into males on the resistant plant and that the heterozygous genotype has no selective advantage over the homozygous dominant genotype (Jones *et al.*, 1967).

In this study we analysed the effects of the genetic constitution of avirulent genotypes (Aa and AA) on the development of larvae into adults on plants having the H_1 resistance gene.

Material and methods

Air-dried cysts of the avirulent line Ro_1-19 and the virulent line Ro_5-22 (Janssen *et al.*, 1990*b*) were presoaked in tap water and after one week the larvae were hatched with root diffusate of the cultivar Bintje. Newly

* *Present address : Research Institute for Plant Protection (IPO-DLO), P.O. Box 9060, 6700 GW Wageningen, The Netherlands.*

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formed fresh F_1 cysts were artificially hatched (Janssen *et al.*, 1987).

Roots of sprouts of tuber segments of the susceptible cultivar Eigenheimer and cultivar Saturna carrying the H_1 gene from *S. tuberosum* ssp. *andigena* CPC 1673 were grown on 2 % water agar in 9 mm Petri dishes (Mugniéry & Person, 1976). The Petri dishes were kept in the dark at 18 °C. Freshly hatched larvae or larvae stored up to three days at 4 °C, were used. Two larvae were inoculated per root tip (Janssen *et al.*, 1990*a*). The inoculum size was 400 larvae (200 root tips) per treatment.

The F_1 of line Ro_5-22 and line Ro_1-19 (Ro_5-22 × Ro_1-19), was obtained by placing one male on the gelatinous matrix of the female. Males from Ro_1-19 were reared on cultivar Eigenheimer in clay pots (700 ml) in sandy loam soil inoculated with 50 cysts in a controlled environment with 16 h light and at 18 °C. A slow release N-P-K granulate fertilizer (Osmocote®) was added. Males were harvested after about 30 days with an elutriator (Oostenbrink, 1960). Females were reared on roots of cultivar Saturna grown on water agar (Mugniéry & Person, 1976; Janssen *et al.*, 1990*a*) by inoculating one larva per root tip per Petri dish.

Ten days after inoculation, root systems and adhering agar gel were submerged in beaker glasses filled with tap water, by piercing a rod through the tuber segment and the agar gel. Contact between the segment and water was avoided. After twenty days, the tuber segment with the root system and adhering agar was returned to the Petri dish to assess accurately the numbers of females. Males were counted from the beaker glasses.

Results

The number of larvae developing into adults on cultivar Eigenheimer ranged 228 from (Ro_1-19), 266 (Ro_5-22) to 321 (F_1), indicating that the two lines and the F_1 differ in their vitality. The effect of the hosts in compatible combinations on the growth of adults was less distinct. The numbers of adults produced by Ro_5-22 was 266 on cultivar Eigenheimer and 282 on cultivar Saturna.

In compatible combinations the male-female ratio averaged 5.5 %, whereas this figure was 143 % in the two incompatible combinations. None of the homozygous avirulent larvae (AA) and only one heterozygous avirulent larva (Aa) was able to develop into a female on cultivar Saturna. The differences between the two genotypes were more pronounced considering the development into males. On cultivar Saturna the number of heterozygous males (94) was nearly twice as high as the number of homozygous dominant males (49). These differences are also significant mathematically, when the numbers of males are expressed as a percentage of the total number of adults that developed on Eigenheimer (Table 1), accepting a maximum SD of 3.0 % for the total number of adults (Janssen *et al.*, 1990*a*).

Table 1. Number of adults of Ro_1-19 (AA), Ro_5-22 ♀ × Ro_1-19 ♂ (Aa) and Ro_5-22 (aa) that developed on susceptible cultivar Eigenheimer and resistant cultivar Saturna after inoculating 400 larvae per combination.

Pathotype (genotype)	Cultivar	Females	Males	Adults (tot.)	% males over adults (tot.) on Eigenheimer
Ro_1-19 (AA)	Eigenheimer	220	8	228	
	Saturna	0	49		21.5*a*
Ro_5-22 × Ro_1-19(Aa)	Eigenheimer	307	14	321	
	Saturna	1	94		29.3*b*
$Ro_5$22 (aa)	Eigenheimer	260	6	266	
	Saturna	253	29		10.9*c*

Figures with different letter are significant at $P < 0.05$.

Discussion

Various studies have shown that males of avirulent populations can develop on plants having the H_1 resistance gene (Jones, 1954; Den Ouden, 1958; Trudgill *et al.*, 1967; Turner & Stone, 1984; Forrest *et al.*, 1986). However, in these studies the AA and Aa genotypes were not tested separately. We demonstrated that both AA and Aa genotypes are able to develop into males. It is also apparent that the resistance conferred by the H_1 gene is to a certain extent also operating on the development of males. The total number of adults in compatible combinations, predominantly females, is much higher than the number of adults, predominantly males, in incompatible combinations. For example, the number of adults of line Ro_1-19 on cultivar Eigenheimer is 228, whereas this figure is 49 on cultivar Saturna. These data demonstrate that larvae able to develop into females on cultivar Eigenheimer do not necessarily develop into males on cultivar Saturna.

As already discussed in a previous report (Janssen *et al.*, 1990*b*), the resistance of the H_1 gene is probably not absolute. Our data indicate that heterozygous larvae have no or only a slight advantage over homozygous dominant larvae to develop into females on plants carrying the H_1 resistance gene. Only one Aa larva out of the 400 larvae inoculated developed into a female and none of the avirulent AA larvae.

The number of males of the heterozygous F_1 (94) on cultivar Saturna is much higher than the number of homozygous avirulent males of line Ro_1-19 (49). However, the absolute numbers seem not suitable for a proper evaluation of a possible selective advantage of the heterozygous larvae (Aa) over homozygous dominant larvae (AA) to develop into males. The F_1 larvae are more vital than the larvae of Ro_1-19. These differences in vitality may also explain the larger numbers of males

of the F_1 on cultivar Saturna. The increased vitality of the F_1 larvae may have resulted from heterosis or from the fact that the larvae of the F_1 were obtained by artificial hatching from young cysts, whereas the larvae of the two inbred lines were hatched from one year old air-dried cysts. The relative number of males, calculated as a percentage of the number of adult females and males on cultivar Eigenheimer, seems a more appropriate measure, because the contribution of vitality is minimized in these figures. As shown by the relative numbers in Table 1 the F_1 larvae (29.3 %) may have a slight advantage over the larvae of Ro_1-19 (21.5 %) to develop into males. However, it can not be excluded that differences in vitality of the larvae, also interfere with these relative numbers. In any case, it seems feasible to conclude, that if heterozygous larvae have a selective advantage, the selection pressure is small and has no large consequences for the population genetics of virulence. Simulation models, which assert from the assumption that AA and Aa genotypes have an equal chance to develop into males on plants having the H_1 gene, are sufficiently accurate to predict the behaviour of virulence in field populations (Jones & Perry, 1978; Jones *et al.*, 1981; Spitters & Ward, 1988).

Biochemical explanations for a gene-for-gene system are often based either on the specificity of the incompatible combination or compatible combination. In the first model, the elicitor-receptor theory, the avirulence allele produces an elicitor which triggers the hypersensitive reaction (Keen, 1981). In the alternative model of induced susceptibility, the virulence allele produces a substance, which blocks the biosynthetic pathway of the hypersensitive response (Ouchi *et al.*, 1976). Both models are also applicable to the development of females and males on plants having the H_1 resistance gene. Our data indicate that heterozygosity does not result in a less overt or delayed hypersensitive reaction. The presence of one avirulent allele in the heterozygous larvae resulting either in a elicitor (first model) or an incomplete blocking mechanism (second model), leads to a host reaction which is comparable with the reaction evoked by the double dominant larvae.

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