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Multiple selection of potato cyst nematode *Globodera pallida* virulence on a range of potato species. I. Serial selection on *Solanum*-hybrids

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Abstract

A series of selection experiments on potato cyst nematode *Globodera pallida*, pathotype Pa1, tested the virulence response of the nematode to a range of resistant potato *Solanum* genotypes. Alleles conferring virulence against all four *Solanum* sources used in the study (i.e. *Solanum vernei*, *S. multidissectum*, *S. sanctae-rosae* and *S. tuberosum* ssp. *andigena*) were detected. Selection for multiple virulence against a combination of resistant sources resulted in the originally-selected virulence genes being retained or lost. The mechanism, or basis, of potato cyst nematode resistance differs between the *Solanum* species. The appropriate use of resistance cultivars produced from a range of *Solanum*-resistant species offers a management tool for controlling potato cyst nematode levels in infested land.

Abbreviations: PCN – potato cyst nematodes; *S. andigena* – *Solanum tuberosum* ssp. *andigena*.

Introduction

It is well established that resistance in wild and cultivated potatoes to the potato cyst nematode (PCN) *Globodera pallida* is mediated by race specific major genes (Toxopeus and Huijsman, 1953; Howard et al., 1970), by unspecified 'polygenes' (Goffart and Ross, 1954; Rothaker, 1958) or by a complex of major and minor genes (Ross, 1969; Huijsman, 1974). Mapping studies are providing a clearer picture of how these different genes are distributed in *Solanum* chromosomes (van der Voort et al., 2000; Gebhart and Valkonen, 2001). It is also apparent that *G. pallida* comprises a heterogeneous range of populations that co-evolved with potato species in South America over many thousands of years (see Turner and Evans, 1998). Although the *G. pallida* populations introduced to other potato growing regions of the world during the last hundred years comprise only a part of the original gene pool in South America, great variation still exists within these populations. As such, the majority of *G. pallida* populations studied contain virulence genes with specific virulent alleles that can overcome the effects of resistance genes in potato species (Turner et al., 1983; Turner, 1990). The ability of *G. pallida* populations to develop increased virulence against the different resistance sources incorporated into potato cultivars may have important consequences for the control of PCN field populations, where resistance is an increasingly significant part of pest management strategies. Whitehead (1991) demonstrated that selection of virulent *G. pallida* populations occurs after one year cultivation on the *Solanum vernei*-based resistant cultivars Glenna or Morag. Beniers et al. (1995) found that continuous cultivation of the partially resistant cultivar Darwina led to increased *G. pallida* virulence, with the selected nematode population exhibiting enhanced virulence both against Darwina and the related resistant *S. vernei*-hybrid SVP $(Vt^n)^2$ 62.33.3.

There have also been field reports indicating that commercial potato cultivars with partial resistance to *G. pallida* derived from *S. vernei* have become less effective at controlling PCNs, apparently due to the proportion of virulent individuals in the nematode populations increasing (M. Hancock, pers. comm.). In a series of pot-based selection experiments, Turner et al. (1983) and Turner (1990) identified six field populations of *G. pallida* from Europe that contained virulence genes to resistance from the *S. vernei* hybrids 62.33.3 and 65.346/19. Repeated selection of these populations on the resistance sources resulted in PCN lines with increased virulence to *S. vernei*. These selected populations were genetically distinct from their unselected counterparts and exhibited similar levels of environmental fitness under field-type conditions. The present study examined the potential in these populations for further virulence selection on resistant potato cultivars containing *G. pallida* resistance genes from additional wild or primitive cultivated species i.e. *S. multidissectum* CPC 3246, *S. sanctae-rosae* CPC 2483 and *S. andigena* CPC 2802. A comparison of the differences in *G. pallida* resistance exhibited by these potato species would help to predict how durable resistance from alternative sources is likely to be when challenged by virulent *G. pallida* populations. This will allow better informed use of plant resistance for PCN management, leading to more effective control of the nematode pests.

Materials and methods

Starting material for the experiments comprised *G. pallida* lines derived from an original field population (pathotype Pa1) from Glarryford, Co. Antrim, in Northern Ireland. After six generations of selection on the *S. vernei*-hybrids 62.33.3 and 65.346/19, the population Glarryford had significantly enhanced reproductive ability on both clones and the two selected lines were genetically distinct from the unselected control (Turner, 1990). These populations were inoculated onto the Pa1-resistant potato cultivars P55/7 (derived from *S. multidissectum*), A27/18 (from *S. sanctae-rosae* × *S. andigena*) and D42/8 (from *S. andigena* × *S. multidissectum*). All hosts had previously been assessed as fully resistant to Pa1 populations by standard National List Test procedures (McKenzie and Turner, 1987); i.e. their reproductive ability (P_f/P_i) was less than 1.00, where P_i is the initial nematode population size and P_f the final population size. This ratio is expressed as a percentage of the ratio on a susceptible control for convenient comparison of hosts.

The method of selection was that described for *S. vernei*-hybrids by Turner et al. (1983), i.e. 30 cysts were inoculated into 15-cm pots with a sprouted potato tuber of the appropriate host in 3 : 1 loam : sand, and maintained under glasshouse conditions (temperature range 12–25 °C and RH at approx. 55%). Treatments were replicated five-fold and plants of cultivar Arran Banner (PCN susceptible) were included to provide unselected control populations. After plant senescence, cysts were extracted, counted and stored at 4 °C until the following season and inoculated as above onto the same clones on which they had been raised, and onto cultivar Arran Banner. Where new cyst production permitted, this procedure was continued for a further 11 generations, on various combinations of the clones (Table 1). The selection programmes involved initial selection on new hosts and back testing for virulence on previous hosts. Nematode reproduction for each generation was assessed as the ratio P_f/P_i. Analyses of variance among experimental means were made on $\log_{10}$ transformed data.

Table 1. Inoculation programme of *S. vernei*-virulent PCN population Glarryford (Pa1) ( ) = number of generations on host plant

Line	Original host 1	Subsequent host 2	Subsequent host 3	Subsequent host 4	Subsequent host 5
1	65.346/19 (6)	P55/7 (6)	65.346/19 (1)		
2	65.346/19 (6)	P55/7 (6)	A27/18 (3)	65.346/19 (1)	P55/7 (1)
3	65.346/19 (6)	P55/7 (6)	D42/8 (4)	65.346/19 (1)	
4	65.346/19 (6)	A27/18 (9)	65.346/19 (1)		
5	65.346/19 (6)	D42/8 (9)	65.346/19 (2)		
6	62.33.3 (6)	P55/7 (10)	62.33.3 (1)		
7	62.33.3 (6)	P55/7 (6)	A27/18 (4)	62.33.3 (1)	P55/7 (1)
8	62.33.3 (6)	P55/7 (6)	D42/8 (4)	62.33.3 (1)	
9	62.33.3 (6)	A27/18 (4)			
10	62.33.3 (6)	D42/8 (5)			

Results

Nematode reproduction for lines 1–10 are presented as Figures 1(A)–(J) respectively, with Pa1 (Glarryford) reproduction expressed as a percentage of Arran Banner control. Mean reproduction on Arran Banner (P_f/P_i, 44.13) was not significantly different between years. In all lines (initially selected for

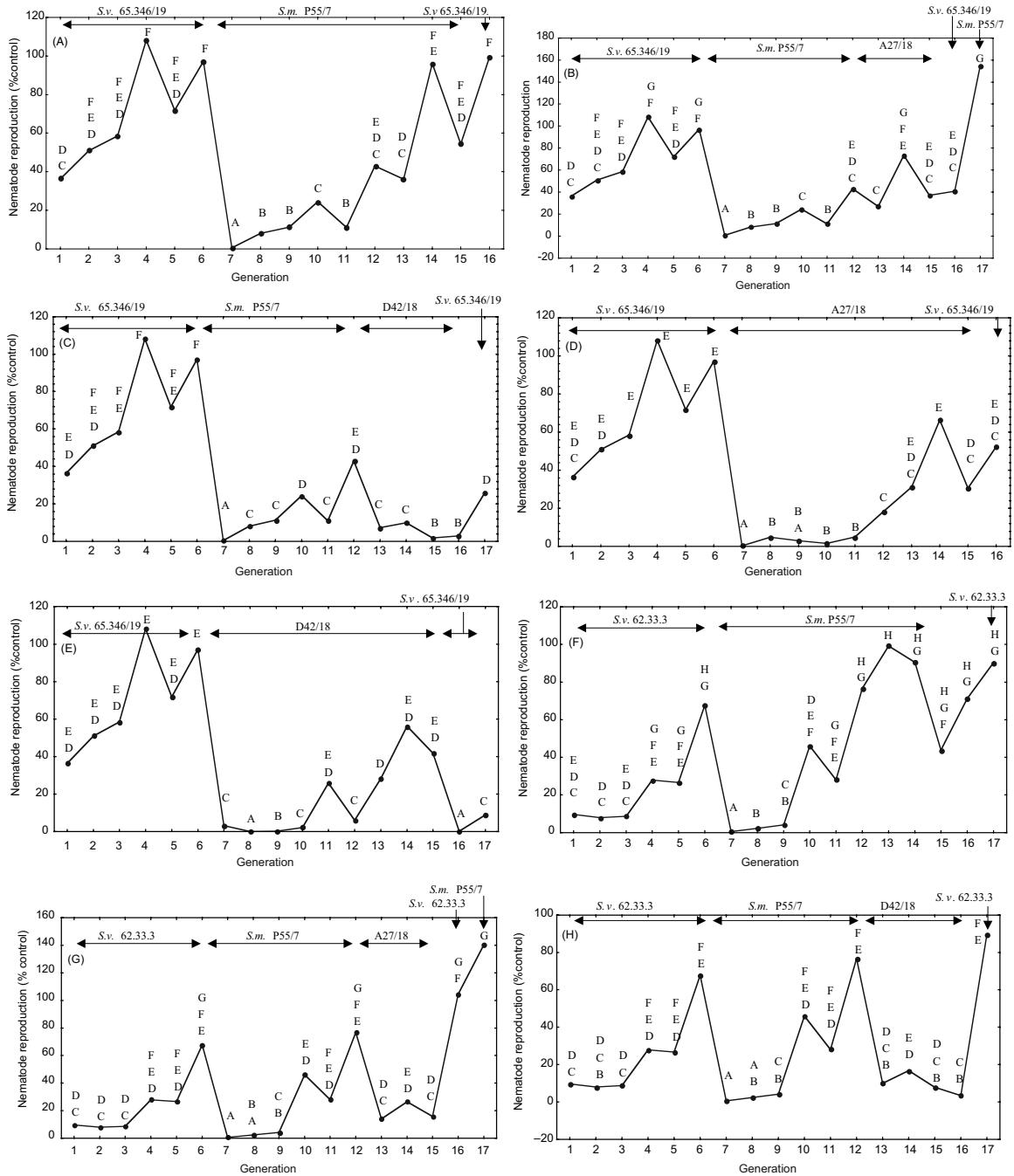

Figure 1(A)–(J).

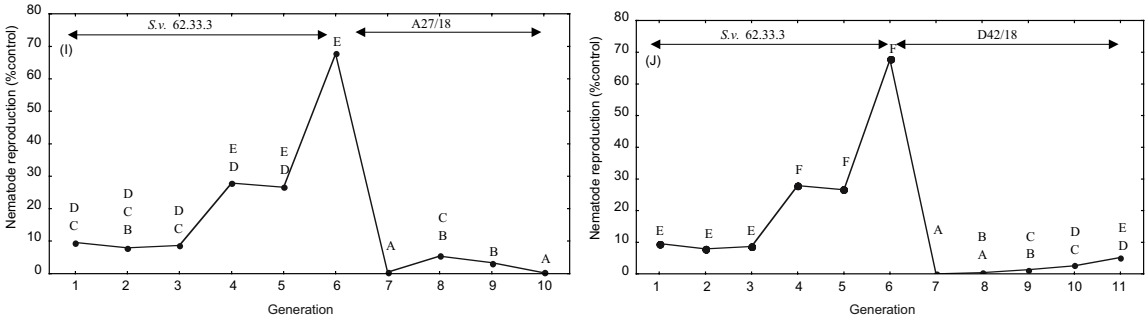

Figure 1(A)–(J). Mean *G. pallida* (population Glarryford Pa1) reproduction (as a percentage of Arran Banner control – detransformed data) during successive selection on: (A) *Solanum* clones *S. vernei (S.v.)* 65.346/19, *S. multidissectum (S.m.)* P55/7 and *S.v.* 65.346/19 (Line 1 from Table 1). (B) *Solanum* clones *S.v.* 65.346/19, *S.m.* P55/7, *S. sanctae-rosae (S.s-r.)* × *S. andigena (S.a.)*. A27/18, *S.v.* 65.346/19 and *S.m.* P55/7 (Line 2 from Table 1). (C) *Solanum* clones *S.v.* 65.346/19, *S.m.* P55/7, *S.a.* × *S.m.* D42/8, and *S.v.* 65.346/19 (Line 3 from Table 1). (D) *Solanum* clones *S.v.* 65.346/19, *S.s-r* × *S.a.* A27/18 and *S.v.* 65.346/19 (Line 4 from Table 1). (E) *Solanum* clones *S.v.* 65.346/19, *S.a.* × *S.m.* D42/8, and *S.v.* 65.346/19 (Line 5 from Table 1). (F) *Solanum* clones *S.v.* 62.33.3, *S.m.* P55/7 and *S.v.* 62.33.3 (Line 6 from Table 1). (G) *Solanum* clones *S.v.* 62.33.3, *S.m.* P55/7, *S.s-r.* × *S.a.* A27/18, *S.v.* 62.33.3 and *S.m.* P55/7 (Line 7 from Table 1). (H) *Solanum* clones *S.v.* 62.33.3, *S.m.* P55/7, *S.a.* × *S.m.* D42/8, and *S.v.* 62.33.3 (Line 8 from Table 1). (I) *Solanum* clones *S.v.* 62.33.3, and *S.s-r.* × *S.a.* A27/18 (Line 9 from Table 1). (J) *Solanum* clones *S.v.* 62.33.3 and *S.a.* × *S.m.* D42/8 (Line 10 from Table 1). Means sharing the same letter are not significantly different at $p < 0.05$ (Duncan's Multiple Range Test; Snedecor and Cochrane, 1980).

Table 2. Response of *S. vernei*-virulent PCN population Glarryford (Pa1) inoculated on to alternative hosts

Line	Original host 1		Subsequent host 2		Subsequent host 3		Subsequent host 4		Subsequent host 5	
1	65.346/19 (6)	ý	P55/7 (9)	ý	65.346/19 (1)	_				
2	65.346/19 (6)	ý	P55/7 (6)	ý	A27/18 (3)	ý	65.346/19 (1)	†	P55/7 (1)	_
3	65.346/19 (6)	ý	P55/7 (6)	ý	D42/8 (4)	ü	65.346/19 (1)	†		
4	65.346/19 (6)	ý	A27/18 (9)	ý	65.346/19 (1)	†				
5	65.346/19 (6)	ý	D42/8 (9)	ý	65.346/19 (2)	†				
6	62.33.3 (6)	ý	P55/7 (10)	ý	62.33.3 (1)	_				
7	62.33.3 (6)	ý	P55/7 (6)	ý	A27/18 (4)	ü	62.33.3 (1)	_	P55/7 (1)	_
8	62.33.3 (6)	ý	P55/7 (6)	ý	D42/8 (4)	ü	62.33.3 (1)	_		
9	62.33.3 (6)	ý	A27/18 (4)	ü						
10	62.33.3 (6)	ý	D42/8 (5)	ü						

( ) = number of generations on host plant.
ý = significant increase in reproductive rate within selection on host ($p < 0.05$).
ü = no significant increase in reproductive rate within selection on host.
_ = virulence retained with selection through alternative hosts.
† = virulence reduced with selection through alternative hosts.

S. vernei-virulence on either 65.346/19 or 62.33.3 for six generations), inoculation onto alternative resistant hosts resulted in significantly reduced reproductive rates. As selection was attempted on the subsequent host(s), the response of the lines varied (Table 2).

Lines selected on 65.346/19 (1–5) all permitted, after a varying number of generations, the selection of virulent individuals on their subsequent host 2 (P55/7, A27/18 or D42/8). Lines reproducing on 62.33.3 (6–10) permitted selection of individuals virulent to P55/7, but not to A27/18 or D42/8. Consequently, lines 9 and 10 were lost (and further testing was not possible) after four and five generations of inoculation i.e. there were, apparently, no virulent alleles within these PCN lines against the resistance genes contained within A27/18 or D42/8.

Those lines from which selection of virulence on an alternative host was achieved (lines 1–8) were inoculated either onto a third host, or returned to their original host. Of those returned to their original host (lines 1, 4 and 5 for 65.346/19, and line 6 for

62.33.3), lines 1 and 6 appeared to retain their respective *vernei*-virulence. However, lines 4 and 5 (selected on 65.346/19 and either A27/18 or D42/8) had lost their 65.346/19 virulence. When line 5 was re-inoculated for a second generation on 65.346/19 it did, however, show a significant increase in reproduction (Figure 1(E), generation 17).

Amongst those lines selected for virulence twice and inoculated onto a third resistant host (i.e. lines 2, 3, 7 and 8), a range of responses was observed. Of lines 2 and 3, which were selected on 65.346/19 and P55/7, line 2 proved to contain virulence genes/alleles against A27/18 but line 3 did not contain such genes/alleles against D42/8. The lines selected first against 62.33.3 and against P55/7 (lines 7 and 8) showed no increase of reproductive rate on either A27/18 or D42/8. These four lines (2, 3, 7 and 8) were returned to hosts against which they were previously virulent. Line 2 (selected on 65.346/19, P55/7 and A27/18) had lost its virulence to 65.346/19. Likewise, line 3 (selected on 65.346/19, P55/7 and D42/8) had lost its virulence to 65.346/19. Line 7 (virulent after selection on 62.33.3 and P55/7, but not A27/18) had retained its double virulence. Line 8 (virulent after selection on 62.33.3 and P55/7, but not D42/8) had retained its virulence to 62.33.3 but its virulence to P55/7 was not re-assessed.

Discussion

The possibility of selecting nematode populations that are virulent towards resistance sources from wild plant species is an important consideration for plant breeders. Most recent studies on the virulence selection of plant parasitic nematodes have concentrated on sedentary endo-parasites such as root-knot nematodes, *Meloidogyne* spp. (see Roberts, 1995; Janssen et al., 1998), the cyst nematodes *Heterodera* spp. (Young, 1992) and *Globodera* spp. (Turner, 1990; Schouten and Beniers, 1997). These studies have demonstrated the adaptability of such nematodes in overcoming plant resistance, and the danger of assuming, or relying on, resistance in a cultivar to offer a durable source of nematode control. The current study on the responses of one *G. pallida* population (Pa1, Glarryford) to a range of resistance sources has demonstrated the adaptability of PCNs in overcoming plant resistance and the complexity of the host–parasite relationship exhibited by the *Solanum–Globodera pallida* interaction.

The original Glarryford PCN field population contained virulence genes (and corresponding virulence alleles) to the four distinct Pa1-resistant *Solanum* genotypes studied. This was shown in tests against the *S. vernei* clones 65.346/19 and 62.33.3 (lines 1–10), *S. multidissectum* P55/7 (lines 1–3, 6–10), *S. sanctae-rosae* × *S. andigena* A27/18 (line 4) and *S. andigena* × *S. multidissectum* D42/8 (line 5), where lines developed increased reproductive ability on the clones. It is therefore reasonable to assume that this population may also contain additional virulence genes to other resistance sources that were not tested for. While most *Solanum* resistance to *G. pallida* is polygenic (which normally results in quantatative resistance), it is not known if the *G. pallida* virulence to different *Solanum* resistance sources tested in the present study is mediated through one virulence gene acting against multiple plant resistance genes (perhaps through the influence of multiple virulence alleles), or if multiple nematode virulence genes are involved.

With the exception of two lines, reproduction of the original two virulent *G. pallida* isolates recovered through successive generations after initial suppression when challenged by a new resistance source. When the Glarryford virulent lines were challenged in various combinations of these alternative sources of resistance, the range of responses found showed that the original virulence could be lost or retained. This may indicate the occurrence of genetic 'bottleneck' effects and a loss of genetic variation within the nematode lines (i.e. with only part of a nematode population able to reproduce on the resistant clone, genetic variation in individuals which could not reproduce may be lost). Due to random effects, this bottleneck may, or may not, lead to a corresponding loss of alleles of other virulence genes; e.g. virulence was retained for 65.346/19 and 62.33.3 when selected through P55/7 (lines 1 and 6), but lost when selected through A27/18 and D42/8 (lines 3 and 4). Alternatively, if nematode virulence against different *Solanum* species is mediated through a single virulence gene, then a loss of allelic variation at that gene could also account for a loss of virulence against different *Solanum* genotypes.

This study did not attempt to assess rates of virulence selection under standard cultivation practices, although other studies have shown that measurable increases in virulence can occur under these conditions (Whitehead, 1991; Beniers et al., 1995). Instead, it concentrated on qualitative assessments of the responses of *G. pallida* to successive selection pressures. However, it was

apparent that the rates of selection for virulence did vary depending on the *Solanum* species used, with those involving *S. andigena* (A27/18 and D42/8) being lower. Perhaps more significant was the observation that previous selection history can have a strong influence on the ability of a population to develop virulence to a novel resistance source. Beniers et al. (1995) demonstrated that selection of virulence on a *S. vernei*-based partial resister (Darwina) also conferred virulence to the related resistant *S. vernei* hybrid 62.33.3. The current study showed that selection on two very similar *S. vernei*-resistant genotypes could result in differences in the virulence potential of a *G. pallida* population. Specifically, initial selection on 62.33.3 resulted in an inability of the virulent line to develop virulence to A27/8 (*S. sanctae-rosae* × *S. andigena*) and D42/18 (*S. andigena* × *S. multidissectum*), while selection on 65.346/19 did not restrict the development of virulence against these clones. The effect of previous history was also seen in the reduction in virulence to 65.346/19 after a number of generations on A27/18 and D42/8 (lines 2 and 3).

Studies by Hoopes (1977) demonstrated structural differences leading to nematode feeding site malfunction between *S. multidissectum*, *S. vernei* and *S. andigena* hybrids resistant to *G. rostochiensis* (Ro1), but did not establish if this was due to genetic differences between their resistance mechanisms or due to phenotypic effects. The current work has confirmed that the development of virulence against one *Solanum* genotype does not automatically confer virulence against other *Solanum* resistance sources. This would indicate that the mechanisms of resistance between the four *Solanum* genotypes studied are different and, therefore, potentially provide a management tool for controlling PCN levels in infested potato land. With appropriate planting of potato cultivars bred from a range of *Solanum* resistance sources, and regular monitoring of the virulence characteristics of the PCN population, it should be possible to reduce the build-up of virulence. Where PCN gene-pools are well characterised, it may be possible to predict changes in nematode virulence in response to particular *Solanum* genotypes. Additional studies on a wider range of resistant *Solanum* species and the durability of their resistance will be presented in future papers.

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