Increased virulence of *Globodera pallida* during repeated rearing on different resistant potato cultivars explained by a simple model

J. E. Beniers, Y. Nöllen, H. J. van Eck and H. J. Schouten*

Wageningen Plant Research, Wageningen University & Research, PO Box 16, 6700 AA Wageningen, Netherlands

Selection for virulence of *Globodera pallida* on potato cultivars was studied for four generations under controlled conditions. The reproduction rate \( \frac{P_f}{P_i} \) of a mixed Pa2/3 population increased by a factor of 61 during rearing on the partially resistant potato cv. Darwina compared to rearing on the susceptible cv. Irene. This was a result of selection for virulence on cv. Darwina, and achieving the Hardy–Weinberg equilibrium on cv. Irene. Increased virulence also significantly raised the reproduction rate on several other *Solanum* genotypes. These changes could be explained reasonably well by the monogenic inheritance of a virulence factor breaking the \( \text{Grp}1 \) locus. The virulence changes were probably mainly evoked by this gene only, inherited from \( S. \text{vernei}^{1-3} \) or \( S. \text{vernei}^{24/20} \). The \( \text{Grp}1 \) locus has probably provided the differential \( S. \text{vernei} \) hybrid \( \left( VT^{\text{n}262-33-3} \right) \) with its resistance to the Pa2 group and not to the Pa3 group. Alternation of cultivars did not halt selection if the cultivars highly differentiated between the Pa2 and Pa3 populations. Only when alternation was with cultivars that harboured a different resistance gene against Pa3 was selection for virulence delayed. Differences in virulence levels (i.e. reproduction rates) within the nematode population determined the rate of selection, not the resistance level itself. Selection of a Pa3 population for three generations on cv. Karakter not only increased the reproduction rate on cv. Karakter itself by a factor 4.2, but also raised the reproduction on other potato genotypes. A simple monogenic model could explain these changes in virulence.

**Keywords:** *Globodera pallida*, model, potato cyst nematodes, resistance, selection, virulence

**Introduction**

Potato cyst nematodes, including *Globodera pallida*, are a serious threat for potato production, especially in short and/or narrow rotations. In naturally infected potato fields, eggs in the cysts of *G. pallida* hatch in the spring, and the emerging larvae feed on roots of potatoes. If the potato cultivar is resistant to the nematode, the larvae cannot develop a good feeding structure in the roots, required for maturing females, and so the larvae become males; this is known as epigenetic sex determination (Schouten, 1993). A susceptible potato root allows the formation of a feeding structure, leading to both female and male nematodes. After mating, the females develop into cysts, containing 200–400 eggs. In autumn/winter after the potato harvest, the cysts will remain in the soil, and the eggs become dormant. Every year in spring, without the host, about 30% of the eggs hatch spontaneously. When potatoes are grown again, the surviving eggs will hatch, leading to a new reproduction cycle (Seinhorst *et al.*, 1995; Been & Schomaker, 2000; Eves-van den Akker *et al.*, 2015).

Until now, two different pathotypes have been distinguished in the Netherlands, i.e. Pa2 and Pa3 (Hockland *et al.*, 2012). More recently, Niere *et al.* (2014) reported the presence of highly virulent *G. pallida* populations in potato fields in Emsland, Germany, possibly caused by selection for virulence.

Turner *et al.* (1983) were the first to report on selection for virulence in *G. pallida*, reared in pots over five generations on *Solanum vernei* hybrids \( \left( VT^{\text{n}} \right)^2 62-33-3 \) and MPI 65.346/19. Turner continued this experiment for up to 11 generations, and provided further proof for selection for virulence, leading to an increase in the relative susceptibility of \( \left( VT^{\text{n}} \right)^2 62-33-3 \) from 10% before selection to 89% after selection. These selected populations were genetically distinct from their unselected counterparts and exhibited similar levels of environmental fitness under field-type conditions (Turner, 1990).

Turner & Fleming (2002) used these populations for additional experiments on effects of alternation of cultivars. Inoculation of these populations onto hosts from different sources of resistance (*S. multidissectum*,...
S. sanctae-rosae and S. andigena) resulted in significantly reduced reproduction. However, in most cases these populations selected on the S. vernei progenitor also resulted in selection for virulence on their subsequent alternate host.

Whitehead (1991) also showed selection for virulence in G. pallida populations on the S. vernei-based resistant cultivars Glenna or Morag. Beniers et al. (1995) found that in field plots, 8 years of continuous cultivation with cv. Darwina increased the virulence from 8.5% to 30%.

The virulence of the G. pallida populations from the Darwina plots to (VT)² 62-33-3, one of the parents of cv. Darwina, had also increased significantly.

Phillips & Blok (2008) studied selection for virulence and effectiveness of alternation of hosts. They detected significant selection, both on S. vernei-derived potato progenitors and on clones derived from S. tuberosum subsp. andigena CPC2802. However, selection on S. vernei did not lead to significantly increased virulence to the S. andigena-derived progenitors. It is probable that the S. vernei-derived potato progenitors contained other resistance genes than S. tuberosum subsp. andigena CPC2802, and therefore other virulence alleles emerged. In this context it is interesting that Rouppe van der Voort et al. (2000) mapped a major-effect QTL conferring Pa3 resistance from S. vernei on chromosome 5, whereas Moloney et al. (2010) mapped resistance of S. tuberosum subsp. andigena on chromosome 4. However, it is still speculative whether the resistance genes that exerted the selection in the experiments of Phillips & Blok (2008) are the same genes that were mapped by Rouppe van der Voort et al. (2000) and Moloney et al. (2010).

A previous paper (Schouten & Beniers, 1997) analysed the increase in the reproduction rate of G. pallida populations that were reared for four generations on partially resistant potato cultivars. Reproduction rate is measured as the number of newly formed eggs per inoculated egg on a test host, also denoted as P/P₀. Reproduction rate was split into aggressiveness (fitness) and virulence, where aggressiveness was defined as reproduction rate (P/P₀) on the susceptible cultivar Irene, and virulence as reproduction rate on the partially resistant cultivar relative to reproduction rate on the susceptible cultivar, expressed as a percentage. Rearing G. pallida on the cultivar Elkana with a low level of resistance did not increase reproduction rate significantly, but rearing on the more resistant cultivars Darwina and Karakter did. This was caused by an increase in virulence, in turn caused by an enhanced ability of the eggs to develop into cysts, and not by an increase in the number of eggs in the new cysts. Reproduction rate on the susceptible host generally was not significantly affected (Schouten & Beniers, 1997).

Fournet et al. (2013) also observed a very significant increase in virulence after selection on resistant hosts, some with progenitor AM 78-3778 as parent, carrying the GpaVᵥᵥ QTL from S. vernei on chromosome 5, synonymous with the GpI₁ locus, rather than to Gpa5. The latter has been mapped in a diploid derived from AM 78-3704 (Rouppe van der Voort et al., 1998, 2000).

Counterintuitively, in their case, selection for virulence also led to an increase in fitness on the susceptible host, as shown by increased cyst sizes and elevated numbers of larvae per cyst (Fournet et al., 2016).

In a previous paper (Schouten & Beniers, 1997), selection experiments were described on a Pa3 population and on a mixture of a Pa2 and Pa3 population, which were very distinct regarding virulences. The observed increased virulence for the mixed population could be predicted rather well by a simple numerical model, using assumptions that Jones used in his models for selection in G. rostochiensis (Jones et al., 1981; Jones, 1985). Validation of models for selection for virulence requires the variation in virulence within a population to be known. However, for natural populations this is generally not known. For that reason, an 80% Pa2:20% Pa3 mixed population was made (Schouten & Beniers, 1997).

The present study looks at the effect of alternation of potato cultivars on the rate of selection to increased reproduction rates of the mixed Pa2/3 population and the Pa3 population. This was studied in two ways. First, after rearing a G. pallida population for four generations on one partially resistant cultivar A, the reproduction rate on another potato cultivar B was determined, and compared to the reproduction rate of the unselected population on cultivar B. This is the relative reproduction rate on cultivar B after selection on cultivar A. Secondly, two or more cultivars were alternated when rearing a population for four generations. After this selection process, the selected population was inoculated on test hosts, and relative reproduction rates were determined. Working definitions are given in Table 1.

By measuring the reproduction rates of the Pa2, Pa3 and mixed population before and after selection, a

| Parameter                  | Working definition                                                                 |
|----------------------------|------------------------------------------------------------------------------------|
| Selected population        | Globodera pallida population that was reared for several generations on a partially resistant potato cultivar |
| Unselected population      | G. pallida population that was reared for several generations on the susceptible potato cultivar |
| Reproduction rate          | The number of new eggs per inoculated egg after one reproduction cycle on a Solanum test host |
| Relative reproduction rate | The quotient of reproduction rate of a selected population and reproduction rate of the unselected population. The same starting population (either Pa3 or Pa2/3) was used for rearing the selected and the unselected population. If the relative reproduction rate deviated significantly from 1, than the selected population was different from the unselected population, e.g. because of selection for virulence. The two reproduction rates used for calculation of the quotient were determined on one test host during one growing season in one glasshouse in a fully randomized block |
| Virulence                  | The number of new eggs per inoculated egg on a test host divided by that number on the susceptible host Irene |
simple monogenic model for selection for virulence could be validated, as described earlier (Schouten & Beniers, 1997). Here, this study is extended to effects on virulence to other cultivars.

Materials and methods

Nematode populations

The *G. pallida* populations D383 (Pa2) and Coll. 1112 (Pa3) were used, as in a previous study (Schouten & Beniers, 1997). These two populations were mixed at an initial ratio of 80% Pa2:20% Pa3 by crushing approximately 8000 and 2000 cysts, respectively, and mixing the suspensions of eggs. Selection experiments were also performed using the Pa3 population alone.

Potato cultivars

Table 2 shows the sequence of alternation of the cultivars. Cultivars Elles and Darwina were chosen, as at the time when the experiments started, these cultivars were used in regions in the Netherlands for production of starch potatoes, and were known for their resistance to Pa2. Cultivar Karakter was used as it has resistance to both Pa2 and Pa3. Cultivar Irene was used as a susceptible reference cultivar, and the generally less susceptible cv. Elkana was also included. More detailed levels of resistances are shown in Table 3.

Production of successive nematode generations

The nematode populations were reared on the susceptible cv. Irene to provide the unselected populations, and on partially resistant cultivars to provide the selected populations. The populations were produced in ‘production series’. Each plastic pot was filled with 5 kg dry weight of Seinhorst soil-mixer, consisting of silver sand, hydrogravel and clay powder (12:3:2), nutrients and 100 g water per kg dry weight (Teklu et al., 2018). Suspensions of eggs were obtained by crushing approximately 1400 cysts. Four eggs per g of dry soil were inoculated into the soil, using 20 cm-long needles. After inoculation, two potato sprouts were planted per pot and placed in an air-conditioned glasshouse with a day length of 16 h and a temperature of 20 °C. Seventeen weeks after inoculation, the watering was stopped. The cysts were elutriated from the soil and stored at approximately 20 °C to obtain two generations per year.

Results

Reproduction rate of Pa2/3 mixture reared on cv. Darwina

The mixed 80% Pa2:20% Pa3 population reared for four generations on cv. Darwina resulted in a 61 times higher reproduction rate on Darwina compared to rearing for the same number of generations on the susceptible cultivar Irene (Table 3). The main reason for this very clear increase was selection for virulence by cv. Darwina, as the Pa3-nematodes had a more than 1000 times higher reproduction rate on the susceptible host, an extra step for estimating the pooled variance was applied, as described by Schouten & Beniers (1997). The statistical package GENSTAT (Payne, 2004) was used.
and 20% Pa3-nematodes (80% AA + 20% aa), but after one generation the alleles are redistributed among the new nematodes according to the Hardy–Weinberg equilibrium (64% AA, 32% Aa, 4% aa), reducing the Pa3-nematodes (aa) from 20% to 4% in one generation, i.e. a 5-fold reduction, without selection. Therefore, the 61-fold increase of the reproduction rate of the selected population compared to the unselected population can be explained by selection for virulence in the selected population, combined with the Hardy–Weinberg equilibrium in the unselected population.

**Reproduction rate of Pa2/3 mixture on other cultivars**

Table 3 shows that rearing on cv. Darwina not only raised the reproduction rate ($R_P/P_I$) on cv. Darwina itself, but also the reproduction rate on other potato genotypes.

**Selection for virulence and aggressiveness**

Rearing the Pa2/3 mixture on cvs Darwina and Karakter did not decrease the reproduction on cv. Irene (Table 3), and therefore did not reduce the aggressiveness (fitness) of these nematodes. Apparently, the selection for increased virulence did not occur at the expense of fitness on a susceptible host.

**Selection for virulence of the Pa3 population**

Selection not only occurred in the mixed Pa2/3 population, but also in the Pa3 population (Table 3), though to a far lesser extent. Continuous rearing of the Pa3 population on cv. Darwina increased the relative reproduction rate on Darwina itself, but also on cvs Elles, Karakter and several other resistant genotypes.

Rearing of Pa3 on cv. Karakter raised the relative reproduction rate on cv. Karakter itself to 4.2, and on cv. Darwina to 1.7. It also raised the relative reproduction rate on other resistant genotypes (Table 3).

**Alternation of cultivars**

Tables 4 and 5 show the effects of alternation of cultivars on relative reproduction rate. The rearing of the

---

### Table 3

| Source of resistance | RR of unselected populations | RR$^{b}$ after rearing on Darwina | RR$^{a}$ after rearing on Karakter |
|----------------------|------------------------------|-----------------------------------|----------------------------------|
| **Inoculum**         | **Pa2** | **Pa2/3** | **Pa3** | **Pa2** | **Pa3** | **Pa2/3** | **Pa3** | **Pa2** | **Pa3** | **Pa2/3** | **Pa3** |
| **Source of resistance** | **Pa2** | **Pa2/3** | **Pa3** | **Pa2** | **Pa3** | **Pa2/3** | **Pa3** | **Pa2** | **Pa3** | **Pa2/3** | **Pa3** |
| Irene                | 83     | 106     | 98     | 12     | 97     | 104     | 1.0     | 1.0     | 1.1     |            |        |
| Elkana               | 58     | 67      | 56     | 0.97   | ntc    | ntc     | ntc     | ntc     | ntc     |            |        |
| Elles                | 0.032  | 0.93    | 23     | 719    | 38     | 42      | ntc     | ntc     | ntc     | 41        | 1.8    |
| Darwina              | 0.014  | 0.36    | 14     | 1036   | 22     | 20      | 24      |        |        | 37       | 1.7    |
| Karakter             | 0.14   | 0.26    | 0.99   | 7.1    | 1.5    | 2.0     | 4.2     |        |        | 5.8      | 2.0    |
| Bintje               | 73     | 97      | 87     | 1.2    | 95     | 91      | ntc     |        |        | 0.98     | 1.0    |
| ODV 22731            | 51     | 66      | 47     | 0.92   | 69     | 69      | ntc     |        |        | 1.0      | 1.5    |
| AM 78-3778           | 0.023  | 0.018   | 0.15   | 6.5    | 0.43   | 0.16    | 0.37    |        |        | 24       | 1.1    |
| Seresta              | ntc    | ntc     | ntc    | 0.49   | ntc    | ntc     | 0.72    | 1.35    |        | ntc      | 1.5    |
| AM 78-3704           | 0.051  | 0.11    | 0.27   | 5.3    | 0.25   | 0.30    | 0.70    |        |        | 2.3      | 1.1    |
| AM 78-3787           | ntc    | ntc     | ntc    | 0.36   | ntc    | ntc     | 0.79    | 1.58    |        | ntc      | 2.2    |
| AM 78-4102           | 0.018  | 0.014   | 0.50   | 28     | 0.81   | 1.24    | 1.91    |        |        | 58       | 2.5    |
| AM 78-3848           | ntc    | ntc     | 1.09   | ntc    | 0.76   | 0.97    | 1.6     |        |        | ntc      | 0.7    |

The sources of resistance used as parents during breeding are shown (J. H. Vinke, personal communication).

$^{a}$Sources of resistance (Dellaert & Vinke, 1987): 1, Solanum tuberosum subsp. andigena CPC 1673, contributed locus Gpa2 on chromosome 12; 2, S. vernei 1-i, 3, S. vernei 24/20; 4, S. vernei LGU 8; 5, S. oplocencus EBS 1786; 6, S. spegazzinii 440; 7, S. vernei 218/16, 8, S. leptophyes EBS 1044; 9, S. sanctae-rosae EBS 1778.

$^{b}$The quotient of the RRs of the unselected Pa3 and Pa2 populations is a measure of the ability of the host to differentiate between the Pa3 and the Pa2 population. The quotient of the RR of a selected population compared to the RR of the unselected population is named relative RR (rRR). If the rRR deviated significantly from 1, the value is underlined ($P < 0.05$). The italicized data refers to rRR on a cultivar after continuous cultivation on that cultivar. Working definitions are given in Table 1. ntc, combination not tested.

$^{c}$Elkana has as additional source S. edinense. All mentioned genotypes lack this source.
Pa2/3 population for two generations on cv. Elles, one generation on cv. Darwina and one generation on cv. Irene resulted in a 29- and 39-times higher reproduction rate to Elles and Darwina, respectively, as rearing for the same number of generations on the susceptible cultivar Elles and Darwina, respectively, as rearing for the

Table 4 The relative reproduction rate (rRR) of the Pa2/3 population to different potato cultivars, after rearing for four generations on one or more cultivars.

| Cultivar   | Irene (4) | Elles (4) | Elles (4) | Darwina (4) | Elles (2), Karakter (1), Irene (1) | Elles (2), Karakter (1), Irene (1) | Elles (2), Karakter (1), Irene (1) | Karakter (2), Elles (2) | Karakter (2), Elles (2) |
|------------|-----------|-----------|-----------|-------------|------------------------------------|------------------------------------|------------------------------------|------------------------|------------------------|
| Elles      | 1.0 a     | 1.0 a     | –         | –           | –                                  | –                                  | –                                  | 0.70 a                 | 0.85 a                 |
| Darwina    | 1.0 a     | –         | 28 c      | 41 d        | 29 c                               | 14 b                               | 11 b                               | –                      | –                      |
| Karakter   | 1.0 a     | –         | –         | 61 e        | 39 d                               | 23 c                               | 22 c                               | 8.2 b                  | 5.0 b                  |

Data within a row followed by a different letter are significantly different (P < 0.05). The italicized data refers to rRR on a cultivar after continuous cultivation on that cultivar.

aContinuous cultivation of one cultivar. Number of generations in parentheses.

bAlternation of cultivars. The exact sequence of alternation is shown in Table 2.

Modelling selection for virulence

Several models for selection for virulence have been presented (Jones, 1983; Spitters & Ward, 1988; Schouten, 1993, 1994, 1996, 1997). In a previous paper (Schouten & Beniers, 1997) a simple monogenic model was described for simulation of selection for virulence. Virulence is the reproduction rate of the nematodes on a resistant cultivar relative to the reproduction rate on the susceptible reference cultivar. Table 6 shows the calculation procedure according to this model. The model predicted that after four generations of rearing the mixed Pa2/3 population on cv. Darwina, the frequency of the virulent nematodes (the Pa3-nematodes) is 0.9, and the frequency of the avirulent nematodes is 0.1. If a certain cultivar has a resistance gene effective to Pa2, but ineffective to Pa3 populations, then selection by cv. Darwina should also raise the virulence to that cultivar.

In mathematical terms: the virulence (Vir) of the Pa2/3 population would have been raised from (0.8 × VirPa2 + 0.2 × VirPa3) before selection to (0.1 × VirPa2 + 0.9 × VirPa3) after four generations of selection on cv. Darwina. Table 3 allows the estimation of VirPa2 and VirPa3, as virulence is defined as the reproduction rate on the resistant host divided by the reproduction rate on the susceptible host. From these values, the expected final virulence level could be calculated according to the model, as 0.1 × VirPa2 + 0.9 × VirPa3, and could be compared with the observed levels, shown in Table 3.

In Figure 1 these expected and observed virulence levels are plotted for a set of Solanum genotypes. Despite the simplicity of the underlying assumptions, the model predicted reasonably well. Heterosis effects that arose from mixing Pa2 and Pa3 were filtered out by using virulence data instead of reproduction rates (Schouten & Beniers, 1997).

Modelling the effect of the Hardy–Weinberg equilibrium on virulence

The effect of obtaining the Hardy–Weinberg equilibrium during rearing on the susceptible cultivar Irene was also modelled (Table 7). Although no selection occurred, according to the monogenic model the virulence would decrease from (0.8 × VirPa2 + 0.2 × VirPa3)
to (0.96 × Vir_{Pa2} + 0.04 × Vir_{Pa3}) after rearing on cv. Irene. This was validated for the same eight Solanum genotypes as shown in Figure 1. The results in Figure 2 again show a reasonable agreement between the expected outcome of the model and the observed virulence levels, despite the simplicity of the model, and the rigid assumptions underlying it.

Figures 1 and 2 indicate that the selection on Pa2/3 by cv. Darwina and obtaining the Hardy–Weinberg equilibrium can to a large extent be explained by monogenic virulence to one resistance gene.

Discussion

After four generations on cv. Darwina, the mixed Pa2/3 population had not only increased its relative reproduction rate on cv. Darwina by a factor of 61, but had also increased considerably the relative reproduction rate on other hosts such as Elles, Karakter and several AM 78–selections. For cv. Elles this can be understood from the sources of resistance, as cvs Darwina and Elles have been derived from the same three resistance sources.

Both cvs Elles and Darwina differentiated strongly between the Pa2 and Pa3 populations, by ratios of 719 and 1036, respectively. This explains the strong selection pressure towards Pa3 by cv. Darwina, and the resulting increase of relative reproduction rate on both cvs Elles and Darwina. The higher the differentiation by a host between the Pa2 population and Pa3 population, the stronger the relative reproduction rate of the Pa2/3 population to that host after selection by cv. Darwina. This is studied in more detail in the modelling paragraph.

Table 6 The calculation procedure for the frequencies of the virulent and avirulent nematodes in the Pa2/3 population during rearing on potato cv. Darwina, according to the monogenic model.

| Generation | Stage | Frequency P of genotype | Comments |
|------------|-------|-------------------------|----------|
| 0          | Eggs  | aa = 0.2, Aa = 0.0, AA = 0.8 | Mixing ratio was 20% Pa3 and 80% Pa2; Pa2 was assumed to have the genotype AA, and Pa3 aa |
|            | Males | aa = 0.2, Aa = 0.0, AA = 0.8 | Males are assumed here not to be affected by resistance |
|            | Females | aa = 1.0, Aa = 0.0 | Only virulent females (aa) are assumed to survive on cv. Darwina |
|            | Sperm frequency | —— | —— |
|            | Ova frequency | —— | —— |
| 1          | Eggs  | aa = 0.2, Aa = 0.8, AA = 0.0 | aa = a_f × a_x = 0.2 × 1.0 × 0.2; etc. |
|            | Males | aa = 0.2, Aa = 0.0, AA = 0.8 | —— |
|            | Females | aa = 1.0, Aa = 0.0 | —— |
|            | Sperm frequency | —— | —— |
|            | Ova frequency | —— | —— |
| 2          | Eggs  | aa = 0.6, Aa = 0.4, AA = 0.0 | —— |
|            | Sperm frequency | —— | —— |
|            | Ova frequency | —— | —— |
| 3          | Eggs  | aa = 0.8, Aa = 0.2, AA = 0.0 | —— |
|            | Sperm frequency | —— | —— |
|            | Ova frequency | —— | —— |
| 4          | Eggs  | aa = 0.9, Aa = 0.1, AA = 0.0 | —— |

aa = virulent; Aa or AA = avirulent; self-evidently P(aa) + P(AA) + P(AA) = 1. The assumptions are discussed by Schouten & Beniers (1997). The expected frequency of the virulent nematodes equals 0.9 after four generations.
The calculation procedure for the frequencies of the virulent and avirulent nematodes in the Pa2/3 population during rearing on the susceptible potato cv. Irene, thus obtaining the Hardy–Weinberg equilibrium.

| Generation | Stage | Frequency P of genotype* |
|------------|-------|--------------------------|
| 0          | Eggs  | 0.2                      |
| 0          | Males | 0.2                      |
| 0          | Females | 0.2                   |
| 0          | Sperm frequency | 0.2 |
| 0          | Ova frequency | 0.2 |
| 1          | Eggs  | 0.04                     |
| 1          | Males | 0.04                     |
| 1          | Females | 0.04                |
| 1          | Sperm frequency | 0.04 |
| 1          | Ova frequency | 0.04 |
| 2          | Eggs  | 0.04                     |

*aa = virulent; Aa or AA = avirulent. Monogenic resistance is assumed. Additional assumptions are discussed by Schouten & Beniers (1997). The expected frequency of the virulent nematodes equals 0.04 after obtaining the Hardy–Weinberg equilibrium.

The relationship between the expected virulence of the Pa2/3 population after rearing on cv. Irene according to the model described in Table 7 (horizontal axis), and the observed virulence (vertical axis). The arrows indicate the change in virulence from 80% Pa2 to 80% Pa2 and 4% Pa3 after obtaining the Hardy–Weinberg equilibrium, according to the model in Table 7.

The differential between the Pa2 group and the Pa3 group, as proposed by Kort et al. (1977), is S. vernei hybrid 62-33-3, also referred to as (VTn)2 62-33-3. This differential has been derived from sources 2 and 3 (Table 3). From Table 3 it can be deduced that the ability of the S. vernei hybrid (VTn)2 62-33-3 to differentiate between the Pa2 group and Pa3 group is probably also caused by the Pa2 resistance provided by Grp1.

The cultivars Darwina and Elles have been derived from sources 1, 2 and 3 (Table 3). These cultivars both have the source S. vernei 24/20, which is not present in either cv. Elkana or ODV 22731. It is probable that S. vernei 24/20 is responsible for the strong ability to differentiate between the Pa2 and Pa3 population, and for the very significant selections for virulence by cvs Elles and Darwina. These genotypes contain the resistance gene Grp1 (van Eck et al., 2017). Grp1 contributes to a major resistance to Pa2 and partial resistance to Pa3 (Roupppe van der Voort et al., 2000). Most likely, the selection exerted by cv. Darwina on the Pa2/3 population is caused by Grp1, providing resistance to Pa2. This gene probably descends from S. vernei 24/20.

The differential between the Pa2 group and the Pa3 group, as proposed by Kort et al. (1977), is S. vernei hybrid 62-33-3, also referred to as (VTn)2 62-33-3. This differential has been derived from sources 2 and 3 (Table 3). From Table 3 it can be deduced that the ability of the S. vernei hybrid (VTn)2 62-33-3 to differentiate between the Pa2 group and Pa3 group is probably also caused by the Pa2 resistance provided by Grp1.
Pa3 was also reared on cv. Karakter. Due to the poor reproduction on cv. Karakter, the population had to be multiplied for one generation on cv. Irene, leaving three generations of cv. Karakter. Whereas four generations of Pa3 on cv. Darwina increased the reproduction rate on cv. Darwina by a factor of 2, three generations on cv. Karakter increased the reproduction rate by a factor of 4.2 on cv. Karakter, suggesting that the selection pressure by cv. Karakter was stronger. Cultivar Karakter has a high level of resistance, and in addition to the three resistance sources of cv. Darwina, also has two extra sources in its pedigree, i.e. S. vernei LGU 8 and S. oploclense EBS 1786. Rouppe van der Voort et al. (2000) and van Eck et al. (2017) indicate that cv. Karakter harbours Gp1, Gpa5 and Gpa6. Rouppe van der Voort et al. (2000) showed that Gpa5 and Gpa6 together provide high levels of resistance to Pa3. This suggests that Gpa5 and Gpa6 are responsible for the stronger selection pressure. Selection on cv. Karakter also increased the reproduction rate on cv. Darwina and all other tested Solanum genotypes.

The rather constant reproduction rate of Pa3 on the susceptible cv. Irene indicates that the selection had no clear impact on aggressiveness. As shown earlier (Schouten & Beniers, 1997), the increased reproduction rate was a result of increased virulence, not of increased aggressiveness.

As a measure for selection pressure in Pa2/3 towards Pa3 at the expense of Pa2, the quotient (reproduction rate of Pa3):(reproduction rate of Pa2) may be used. This quotient appears to be predictive for the selection rate of Pa2/3 and for the efficiency of alternation of the cultivars for slowing down selection in this population.

Alternation of cvs Elles and Darwina was not effective, whilst alternating with the nonselective cv. Elkanza was more effective. This indicates that alternation of cultivars did not halt selection if the cultivars differentiated strongly between Pa2 and Pa3, in favour of Pa3. Only alternations with cultivars that did not provide a selective advantage for Pa3 were helpful in delaying selection. Unfortunately, no cultivars were available that allowed stronger reproduction of Pa2 compared to Pa3.

Globodera pallida nematode movements in the soil are very limited. As a result, populations of this nematode tend to inbreed, thus loosing heterozygosity. This inbreeding tendency favours recessive virulence (Montarry et al., 2015). This was not accounted for in the modelling here, nor were the alleles mixed randomly. The inbreeding tendency will boost the increase in virulence in the field.

Several conclusions can be drawn from this study. Rearing Pa2/3 on cv. Darwina not only raised the reproduction rate on cv. Darwina itself, but also the reproduction rate on other potato varieties. The increased reproduction rates on hosts other than Darwina were mostly predominant for the hosts that highly differentiated between the Pa2 population and the Pa3 population. Both cvs Elles and Darwina discriminated very significantly between these two populations by a ratio of 719 and 1036, respectively.

This explains the strong selection pressure towards Pa3 by cv. Darwina, and the resulting increase in reproduction rate on both cvs Elles and Darwina. These very high increases in relative reproduction rate after rearing Pa2/3 on cv. Darwina are caused not only by selection for virulence, but also by a decrease in virulence after rearing on cv. Irene, therewith achieving the Hardy–Weinberg equilibrium. These changes in virulences for Pa2/3, due to selection and the Hardy–Weinberg equilibrium, could be predicted reasonably well by a simple monogenic model. This indicates that for Pa2/3, these changes could be explained to a large extent by one major resistance gene and one complementary avirulence gene only.

Based on pedigree information, selection rate and the monogenic model, the selection exerted by cv. Darwina on the Pa2/3 population is probably caused by the resistance gene Gp1 from (VTn)2 62-33-3, the resistant parent of Darwina (Rouppe van der Voort et al., 1998; van Eck et al., 2017). This gene provided resistance to the Pa2 population but to a far lesser extent to the Pa3 population.

The differential between the Pa2 group and the Pa3 group as proposed by Kort et al. (1977), is S. vernei hybrid 62-33-3, also referred to as (VTn)2 62-33-3, the above-mentioned parent of cv. Darwina.

The genes Gp1, Gpa5 and Gpa6 are likely to be present in cv. Karakter, and the Solanum genotypes AM 78-3778 and AM 78-4102, in view of the pedigrees.

Alternating cultivars did not halt selection if the cultivars differentiated heavily between Pa2 and Pa3, in favour of Pa3. Only alternating with cultivars that did not provide a selective advantage for Pa3 were helpful in delaying selection. Selection occurred not only in the mixed Pa2/3 population, but also in the Pa3 population, though to a far lesser extent. Selection to a more virulent ‘pathotype’ took place (‘Pa4/Emstland’; Niere et al., 2014). Selection of Pa3 on cv. Karakter also increased reproduction rate to cv. Darwina and all other tested Solanum genotypes.

As appeared from selection of Pa2/3 on cvs Darwina and Karakter, it is not the resistance level of a host to a nematode population that determines the rate of selection, but the differences in virulence levels within the nematode population are the main factor. This is in agreement with the classical population genetics (Fisher, 1930). Apparently, for cv. Karakter the variance for virulence was larger within Pa3 than within Pa2/3. For cvs Elles and Darwina the opposite was true.

The simple monogenic model could also explain the virulence change of the Pa3 group to a more virulent ‘pathotype’. The differentials S. vernei hybrids AM 78-3777 and AM 78-4102 with resistance to the Pa2 and Pa3 group in particular showed selection to a more virulent ‘pathotype’ (Hockland et al., 2012).

Acknowledgements

The authors thank Ellie Meijnders, Daan Jaspers and Johnny Visser for technical assistance, and J. H. Vinke for providing information on the pedigree of genitors.
References

Been TH, Schomaker CH, 2000. Development and evaluation of sampling methods for fields with infestation foci of potato cyst nematodes (Globodera rostochiensis and G. pallida). Phytopathology 90, 647–56.

Beniers A, Mulder A, Schouten HJ, 1995. Selection for virulence of Globodera pallida by potato cultivars. Fundamentals of Applied Nematology 18, 497–500.

Dellaert LMW, Vinke JH, 1987. Testing potatoes for resistance to Globodera pallida pathotype Pa-3; resistance spectra of plant genotypes and virulence spectra of Pa-3 isolates. Revue de Nématologie 10, 445–53.

van Eck HJ, Vos PG, Valkonen JPT et al., 2017. Graphical genotyping as a method to map Nyamain and Gpa5 using a reference panel of tetraploid potato cultivars. Theoretical and Applied Genetics 130, 515–28.

Eves-van den Akker S, Lilley CJ, Reid A et al., 2015. A metagenetic approach to determine the diversity and distribution of cyst nematodes at the level of the country, the field and the individual. Molecular Ecology 24, 5842–51.

Fisher RA, 1930. The Genetical Theory of Natural Selection. Oxford, UK: Clarendon.

Fournet S, Kerlan MC, Renault L, Dantec JP, Rouaux C, Montjay J, 2013. Selection of nematodes by resistant plants has implications for local adaptation and cross-virulence. Plant Pathology 62, 184–93.

Fournet S, Esche-Bozy D, Renault L, Hamelin FM, Montjay J, 2016. Adaptation to resistant hosts increases fitness on susceptible hosts in the plant parasitic nematode Globodera pallida. Ecology and Evolution 6, 2559–68.

Hockland S, Niere B, Grenier E et al., 2012. An evaluation of the implications of virulence in non-European populations of Globodera pallida and G. rostochiensis for potato cultivation in Europe. Nematology 14, 1–13.

Jones FGW, 1985. Modelling multigenic resistance to potato cyst nematodes. EPPO Bulletin 15, 155–66.

Jones FGW, Parrott DM, Perry JN, 1981. The gene-for-gene relationship and its significance for potato cyst nematodes and their solanaceous hosts. Plant Parasitic Nematodes 3, 23–36.

Kort J, Ross H, Rumpenhorts HJ, Stone AR, 1977. An international scheme for identifying and classifying pathotypes of potato cyst nematodes Globodera rostochiensis and G. pallida. Nematologica 23, 333–9.

Moloney C, Griffin D, Jones PW et al., 2010. Development of diagnostic markers for use in breeding potatoes resistant to Globodera pallida pathotype Pa2/3 using germplasm derived from Solanum tuberosum sp. andigena CPC 2802. Theoretical and Applied Genetics 120, 679–89.

Montarry J, Jan P-L, Gracianne C et al., 2015. Heterozygote deficits in cyst plant-parasitic nematodes: possible causes and consequences. Molecular Ecology 24, 1654–77.

Niere B, Krüssel S, Osmers K, 2014. Auftreten einer außergewöhnlich virulenten Population der Kartoffelzystenmimetaten. Journal für Kulturpflanzen 66, 426–77.

Payne RW, 2004. The Guide to GenSyst Release 8: Syntax and Data Management. Oxford, UK: Lawes Agricultural Trust.

Phillips MS, Blok VC, 2008. Selection for reproductive ability in Globodera pallida populations in relation to quantitative resistance from Solanum vernei and S. tuberosum sp. andigena CPC2802. Plant Pathology 57, 573–80.

Roupee van der Voort J, Lindeman W, Folkertse R et al., 1998. A QTL for broad-spectrum resistance to cyst nematode species (Globodera spp.) maps to a resistance gene cluster in potato. Theoretical and Applied Genetics 96, 654–61.

Roupee van der Voort J, van der Vossen E, Bakker J et al., 2000. Two additive QTLs conferring broad-spectrum resistance in potato to Globodera pallida are localized on resistance gene clusters. Theoretical and Applied Genetics 101, 1122–30.

Schouten HJ, 1993. Models of incomplete selection for virulence of potato cyst nematodes caused by sex determination that depends on host-resistance. Nethedlands Journal of Plant Pathology 99, 191–200.

Schouten HJ, 1994. Preservation of avirulence genes of potato cyst nematodes through environmental sex determination. A model for incomplete resistance. Phytopathology 84, 771–3.

Schouten HJ, 1996. A model examining the effect of environmental sex determination in parasites on the breakdown of monogenic host resistance. Nematologia 42, 80–8.

Schouten HJ, Beniers JE, 1997. Durability of resistance to Globodera pallida. I. Changes in pathogenicity, virulence, and aggressiveness during reproduction on partially resistant potato cultivars for several generations. Phytopathology 87, 862–7.

Seinhorst JW, Oostrom A, Been TH, Schomaker CH, 1995. Relative susceptibilities of eleven potato cultivars and breeders’ clones to Globodera pallida Pa3, with a discussion of the interpretation of data from pot experiments. European Journal of Plant Pathology 101, 457–65.

Spitters CJT, Ward SA, 1988. Evaluation of breeding strategies for resistance to potato cyst nematodes using a population dynamic model. Euphytica 39, 87–98.

Teklu MG, Beniers JE, Been TH, Schomaker CH, Molendijk LPG, 2018. Methods for the estimation of partial resistance in the glasshouse of potato cultivars/genotypes against potato cyst nematodes & root-knot nematodes. Wageningen University & Research Report. DOI: 10.13140/RG.2.2.30798.56648.

Turner SJ, 1990. The identification and fitness of virulent potato cyst-nematode populations (Globodera pallida) selected on resistant Solanum vernei hybrids for up to eleven generations. Annals of Applied Biology 117, 385–97.

Turner SJ, Fleming CC, 2002. Multiple selection of potato cyst nematode Globodera pallida virulence on a range of potato species. I. Serial selection on Solanum-hybrids. European Journal of Plant Pathology 108, 461–7.

Turner SJ, Stone AR, Perry JN, 1983. Selection of potato cyst nematodes on resistant Solanum vernei hybrids. Euphytica 32, 911–7.

Whitehead AG, 1991. Selection for virulence in the potato cyst-nematode, Globodera pallida. Annals of Applied Biology 118, 395–402.