Some aspects of resistance to and tolerance of potato cyst nematodes in potato

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Enkele aspecten van resistentie tegen en tolerantie van aardappelcysteaaltjes in aardappel

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Stellingen

1. De door Stanton *et al.* (1990) voorgestelde selectieprocedure voor tolerantie van *Pterotylenchus cecidogenus* in *Desmodium ovalifolium* wordt onvoldoende onderbouwd door de gepresenteerde waarnemingen

   Stanton, J.M., C.A. Garcia & C. Torres, 1990. Resistance and tolerance of *Desmodium ovalifolium* to *Pterotylenchus cecidogenus*, the stem gall nematode. Nematologica 36: 424-433.

2. De toevoeging 'partiëel' bij resistentie tegen aardappelcysteaaltjes is overbodig

3. Het belang van tolerantie van aardappelcysteaaltjes wordt veelal onderschat

4. Degenen die slechts het voorwoord en curriculum vitae van een proefschrift lezen, illustreren daarmee de aantrekkingskracht van 'human interest'

5. Een goede stelling is onjuist

6. Een mens is geen aardappel

7. De groei van het aantal auto's zal pas tot stilstand komen door een gebrek aan snelheid van die auto's

8. Bij de Europese eenwording dient niet slechts het belang van de inwoners voorop te staan

9. Het is niet nodig aparte sportwedstrijden voor vegetariërs te organiseren

10. De bescherming van burgers in veel landen tegen schendingen van de mensenrechten, uitgevoerd door hun overheid, verdient meer steun

Stellingen behorende bij het proefschrift getiteld: "Some aspects of resistance to and tolerance of potato cyst nematodes in potato".
Frits K. Arntzen, 17 mei 1993.

Abstract

Potato cyst nematodes (PCN), *Globodera rostochiensis* and *G. pallida*, are major pests of the potato. In this thesis, research on some aspects of resistance to and tolerance of PCN is presented. The distinction of virulence groups with a hierarchical clustering procedure is proposed as an alternative for the currently distinguished pathotypes. Resistance in potato cultivars should be expressed in a quantitative way, tested against PCN populations representative of virulence groups. A previously unknown major gene for resistance to some *G. pallida* populations was found, thought to be derived from *S. tuberosum* ssp. *andigena* CPC 1673. Several potato cultivars appeared to carry this gene, presumably due to linkage of this gene with a trait they have been selected for. Several genotypes with *G. pallida* resistance from *S. vernei*, as currently used in potato breeding, appeared to be highly intolerant to *G. pallida*. Differences in tolerance between *G. pallida* resistant genotypes in the field were associated with differences in biomass of infested plants in the greenhouse, relatively early in growth. In progenies of crosses, tolerance and resistance appeared not to be related. Selection for tolerance in a *G. pallida* resistance breeding programme is advised, in order to avoid the development of resistant, but highly intolerant potato cultivars. Multiple regression analysis revealed that the tolerance of the tested genotypes was associated with the induced rate of hatching and the capacity of root growth after infection.

Voorwoord

De verschijning van dit boekje vormt de afsluiting van het project 'Partiële, pathotype-niet-specifieke resistentie tegen en tolerantie van aardappelcysteaaltjes'. Dit project werd gefinancierd door de Nederlandse Aardappel Associatie en uitgevoerd bij de Stichting Voor Plantenveredeling SVP, later opgegaan in het Centrum voor Plantenveredelings- en Reproductieonderzoek CPRO-DLO, in de periode van oktober 1986 tot oktober 1992.

Vele mensen hebben bijgedragen aan de totstandkoming van dit proefschrift. Om te beginnen Lidwine Dellaert. Zij nam het initiatief voor het project. Doret Wouters en nadien Johnny Visser leverden als assistent een belangrijke bijdrage aan het onderzoek. Jan Menting hielp mee aan verschillende proeven, evenals de studenten/stagiaires Arno Huijsmans, André de Kock, Martin Meinema en Marieke Förch. Henk Vinke gaf vanwege zijn lange ervaring met dit werk veel advies. Verder wil ik de samenwerking noemen met Jan Schans (Nematologie), Jaap Bakker (PD) en Rob van Haren (IPO-DLO), en met Margriet Boerma en Roland Velema (HLB Assen), die zorg droegen voor de veldproeven. De syntheseafdeling op de Broekemahoeve leverde materiaal. Harry Jonker bepaalde glycoalkaloïdengehaltes. Fred van Eeuwijk en Paul Keizer hielpen met berekeningen en gaven statistische adviezen. Van commentaar op de manuscripten werd ik ruimschoots voorzien door Coosje Hoogendoorn, J.E. Parlevliet, A.F. v. d. Wal, Mark Phillips, Marcel van Oijen en Hans Mulder. Aan Leontine Colon heb ik een leuke kamergenoot gehad. Tot slot wil ik Marianne noemen met haar eigen voor mij belangrijke inbreng.

Allen die hebben bijgedragen, hartelijk dank! Ook dank ik mijn promotoren voor de gelegenheid op dit onderwerp te promoveren.

Het proefschrift is afgemaakt onder de toeziende oogjes van Siebe.

Frits Arntzen

Contents

Note

Some chapters of this thesis are based on papers, accepted by the following journals:

Arntzen, F.K. & F.A. van Eeuwijk, 1992. Euphytica 62: 135-143 (Chapter 2).

Arntzen, F.K., J.H. Vinke & J. Hoogendoorn, 1993. Fundamental and Applied Nematology: in press (Chapter 3).

Arntzen, F.K., J.H.M. Visser & J. Hoogendoorn, 1993. Annals of Applied Biology: in press (Chapter 7).

The other chapters have been submitted to various journals.

INTRODUCTION

The two potato cyst nematode species, *Globodera rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959, and *G. pallida* Stone, 1973, are major pests of the potato crop. These species belong to the family Heteroderidae (Nematoda: Tylenchida). Potato cyst nematodes (PCN) originate, just as the potato, from South-America. PCN was first found in Europe in 1881 (Evans *et al.*, 1975), and reported for the first time in the Netherlands in 1941 (Oostenbrink, 1950). PCN is now widespread in Europe and has been found in many countries around the world (Evans & Stone, 1977). These nematodes parasitize various Solanaceous species (Stelter & Engel, 1975). The potato, *Solanum tuberosum* ssp. *tuberosum* L., is the only host species grown as an arable crop in the field in the Netherlands.

Diffusate from growing potato roots induces hatching of juveniles from eggs in the potato cysts in the soil. Juveniles subsequently penetrate these roots and induce giant cells in the roots for feeding. After three moults, adult males and females are formed. The slender males leave the roots, while the female bodies swell and burst through the epidermis, with the head remaining attached. After mating, the female body develops into a cyst, containing a large number of eggs. Mature cysts get detached from the roots, and can remain viable in the soil for several years (Oostenbrink, 1966). In temperate conditions, PCN is presumed to have one major generation a year (Evans, 1969).

When fields are infested with PCN, they form a threat to potato growing. Seed tubers are not allowed to be contaminated with PCN infested soil. Moreover, PCN causes at higher densities direct yield loss.

Control of PCN is particularly important in the Netherlands, where the potato is the second most widely cultivated crop (*Anon.*, 1992a). Control can be achieved by crop rotation, application of nematicides and growing resistant cultivars. Crop rotation is often not attractive for the arable farmer, as there are too few other crops that are economically interesting to grow. The use of nematicides is expensive. Moreover, the use of nematicides will be restricted. The Dutch Government is aiming at a 70 % reduction of the use of nematicides in arable farming in the year 2000 (*Anon.*, 1991a). In these circumstances, the growing of cultivars with resistance to PCN is the most attractive method of control and can contribute more to the control of PCN than chemical treatment (Gurr, 1992).

Table I. The pathotype scheme for potato cyst nematode populations (Kort *et al*, 1977). A multiplication ratio on a differential genotype ≤1 indicates resistance (-), a multiplication ratio >1 indicates susceptibility (+).

Differential genotypes	Species and pathotype							
	G. rostochiensis					*G. pallida*		
	Ro1	Ro2	Ro3	Ro4	Ro5	Pa1	Pa2	Pa3
S. andig. CPC 1673 hybr.	-	+	+	-	+	+	+	+
S. kurtz. hybr. 60-21-19	-	-	+	+	+	+	+	+
S. vernei hybr. 58-1642/4	-	-	-	+	+	+	+	+
S. vernei hybr. 65-346-19	-	-	-	-	-	+	+	+
S. multid. hybr. P55/7	+	+	+	+	+	-	+	+
S. vernei hybr. 62-33-3	-	-	-	-	±	-	-	+

PCN resistance has been obtained by breeding. No useful resistance was found within *S. tuberosum* ssp. *tuberosum* (Oostenbrink, 1950). Resistance was introduced by crossing with sources of resistance, such as *S. tuberosum* ssp. *andigena*, *S. vernei* and some other *Solanum* species (Ross, 1986). PCN resistance breeding has started more than forty years ago and a large number of cultivars with resistance to PCN are currently available (*Anon.*, 1992b). Nevertheless, the assortment of resistant cultivars has several important shortcomings and the use of resistant cultivars is still limited.

The resistance in many cultivars is specific to PCN populations. Kort *et al.* (1977) classified PCN populations for differences in virulence by distinguishing several pathotypes of *G. rostochiensis* and *G. pallida* (Table I). A large number of cultivars have only resistance to the prevailing pathotype of *G. rostochiensis*, pathotype Ro1 (Table II). Only a few cultivars have resistance to *G. pallida*. Moreover, nearly all cultivars with resistance to *G. pallida* are only suitable for starch production. The resistance to *G. rostochiensis* Ro1 is based on a single gene (Toxopeus & Huijsman, 1953), which could be incorporated easily in cultivars. A gene-for-gene relationship between virulence and this resistance was demonstrated recently (Janssen *et al.*, 1991). The resistance to *G. pallida* derived from *S. vernei* has a polygenic inheritance (Dale & Phillips, 1982). This type of inheritance has hampered the breeding process. The resistance to *G. pallida*

Table II. Numbers of cultivars with resistance to the various pathotypes of PCN (from *Anon.*, 1992b).

Pathotype	Number of cultivars with resistance
Ro1	252
Ro2/Ro3	45
Pa2	14
Pa3	2

pathotype Pa1 (Dunnett, 1962) is the only resistance to *G. pallida* found, based on a single gene. This pathotype has not been reported to occur in the Netherlands.

Resistance and virulence are treated as qualitative traits, as demonstrated by the pathotype scheme of Kort *et al.* (1977). However, clear differences exist between PCN multiplication rates on cultivars, which are currently classified as being susceptible (*Anon.*, 1992b; *Anon.*, 1992c). In the present situation, the information on the resistance of cultivars is incomplete.

Resistant cultivars may be intolerant. In heavily PCN infested fields, damage may also occur in resistant cultivars, as PCN juveniles are able to penetrate the roots of these cultivars. Resistant cultivars with intolerance will give control of PCN on highly infested fields, but will also suffer a relatively high yield reduction. Therefore, resistant cultivars should preferably be also tolerant to PCN (van der Wal, 1978).

Relatively little attention has been given to tolerance until now. No information on tolerance of PCN is given in the Dutch Cultivar List (*Anon.*, 1992c), although differences in tolerance between potato cultivars have been observed often. A number of characteristics is suggested to play a role in PCN tolerance (Evans & Haydock, 1990), but the relative importance of these characteristics and the mechanisms of tolerance are largely unknown. Screening for tolerance has been carried out in the field (Phillips *et al.*, 1988a), but the relationship between field and pot trials was not yet investigated. Evans & Haydock (1990) assumed that the hypersensitivity reaction in resistant genotypes confers intolerance to nematodes, suggesting that resistance and intolerance may be associated.

Some terminology should be explained. Resistance describes the effects of host genes that restrict or prevent nematode multiplication in a host species (Trudgill, 1991).

Virulence can be regarded as the ability of nematode populations to overcome host resistance, effective to other nematode populations (Shaner *et al.*, 1992). Tolerance of a potato genotype is characterized by the ability to withstand or recover from the damaging effects of nematodes and yield well (Trudgill, 1991). The tolerance of genotypes is compared at the same initial PCN density (Trudgill, 1986).

In this thesis, research is described, relevant for improvement of the breeding and use of PCN resistant cultivars, with respect to the above mentioned aspects. Initial methodological research revealed a rapid method for the determination of the number of eggs per cyst (Chapter 1), which was used in some of the succeeding research. The resistance of nine potato genotypes was tested with ten PCN populations. The results were interpreted quantitatively and an alternative way for the distinction of virulence groups and the expression of resistance was proposed (Chapter 2). The level of resistance to various *G. pallida* populations of a potato cultivar, previously regarded as susceptible, was studied (Chapter 3). The inheritance of the resistance was investigated, by testing the resistance in progenies of crosses, and its source (Chapter 3). The interaction of PCN populations with this resistance was compared with that of resistance from *S. multidissectum* (Chapter 4).

In Chapter 5, the tolerance of resistant genotypes was investigated in the field and in pots. Biomass partitioning, as affected by PCN, was studied in pots. The inheritance of tolerance and the relationship between levels of tolerance and resistance were determined in progenies of crosses (Chapter 6). Research on the possible relationship between rate of hatching and level of tolerance is described in Chapter 7. Finally, the root growth of various potato genotypes was investigated after direct inoculation of juveniles on roots (Chapter 8). Multiple regression analysis was used to identify major factors, explaining the level of tolerance of the tested genotypes (Chapter 8). The thesis concludes with a general discussion of the results, followed by a summary in English and Dutch.

CHAPTER 1

Rapid and non-destructive assessment of the number of eggs in cysts of potato cyst nematodes by weighing

Abstract

Dry cysts of potato cyst nematodes, derived from greenhouse rearing, were weighed and afterwards the number of eggs was determined by crushing the cysts and counting the eggs. A high correlation (r=0.96) was found between the number of eggs per dry cyst of potato cyst nematodes and cyst weight, measured with a supermicrobalance. The number of eggs per cyst can be assessed rapidly and in a non-destructive way by weighing.

Introduction

For the assessment of the density in soil of potato cyst nematodes, *Globodera rostochiensis* and *G. pallida*, cysts are recovered by the flotation method (Fenwick, 1943), and the number of cysts in the debris is counted (van der Wal & Vinke, 1982). In addition, the number of eggs per cyst may be assessed by crushing cysts in water and counting the number of eggs in samples of the resulting egg suspension. This method was first described by Bijloo (1954) and later modified by Seinhorst and Den Ouden (1966). The number of eggs per cyst can vary considerably (Hesling, 1959; Stelter & Gaur, 1969).

This method for determining the number of eggs per cysts is, however, destructive to the cysts and labour-intensive. A method for non-destructive assessment of the cyst content was described by Stelter and Gaur (1969). They found a good relationship between volume of cysts, as assessed by measuring their diameter, and the number of eggs in these cysts. Fenwick and Reid (1951) used the weight of cysts, but only with the aim of obtaining uniformly sized batches of cysts for experiments.

The work reported here was carried out to investigate whether weighing cysts could be a fast and accurate method for the assessment of the number of eggs in dry cysts.

Materials and methods

Samples of dry cysts of various PCN populations of differing species, pathotype designation and place of origin, were used in three experiments. The populations had been reared in different years in a greenhouse on various susceptible cultivars, with the inoculum spread freely in the soil. Per sample, the number of eggs was assessed by crushing the cysts in water (Seinhorst & den Ouden, 1966) and counting the number of eggs in the resulting egg suspension or in subsamples.

Experiment 1.1. A sample of 50 cysts of each of 17 *G. rostochiensis* and 13 *G. pallida* populations was taken. Moreover, from eight *G. rostochiensis* populations and seven *G. pallida* populations, a sample with 50 small cysts and a sample with 50 large cysts, selected using a microscope, were prepared. All samples were weighed three times on a Sartorius R160D electronic microbalance with sensitivity of 0.01 mg and an error of maximal 0.1 mg, as indicated by the manufacturer. Afterwards, the number of eggs per

sample was assessed by crushing the cysts in 50 ml water and counting the number of eggs in three subsamples of 1 ml each.

Experiment 1.2. A sample of cysts of eleven *G. rostochiensis* and nine *G. pallida* populations each was used. For each of the 20 samples, the number of cysts was randomly chosen between 10 and 250. In addition, 20 samples were prepared in the same way with small or large cysts. All samples were weighed once on a Sartorius R160D electronic microbalance and the total number of eggs was assessed by crushing the cysts in 25 to 200 ml water, depending on the size of the sample, and by counting three subsamples of 1 ml each.

Experiment 1.3. Ten individual dry cysts with different size of each of five *G. rostochiensis* and six *G. pallida* populations were chosen. The mean diameter of these cysts was measured with a microscope and the cysts were weighed twice on a Sartorius S-4 electronic supermicrobalance with sensitivity 0.1 µg and an error of maximal 1 µg (manufacturer's specification). Cyst volume was calculated, using the formula $(4/3)\pi((1/2)d)^3$, with d being the diameter. Afterwards, the number of eggs was assessed by crushing the cysts in a small volume of water and counting the total number of eggs.

Results

Experiment 1.1. The correlation between cyst weight and number of eggs per cyst in samples, selected for size, was very high ($r=0.92$; $P<0.01$). The variance between repeated weight measurements of samples accounted for less than 1% of the total variance of cyst weight. Therefore, one weight measurement appears to be sufficient. With random samples, differing less in cyst content, the correlation between cyst weight and number of eggs per cyst was still $r=0.77$ ($P<0.01$).

Experiment 1.2. The correlation between weight of cyst samples and the number of cysts was very high, both for samples of cysts, selected for size, and for random samples, not selected for size (Table 1.1). For samples of cysts, selected for size, the correlation of weight with number of eggs was considerably and significantly ($P<0.01$) higher than the correlation of weight with number of cysts (Table 1.1).

Experiment 1.3. Again, the variance between repeated weight measurements of samples accounted for less than 1% of the total variance of cyst weight. The relationship

Table 1.1. Correlations between number of cysts, total number of eggs and sample weight, with and without selection of cysts for size, in Expt 1.2.

	Number of eggs	Sample weight
No selection for size		
Sample weight	0.93 *[1])	
Number of cysts	0.79 *	0.89 *
Selection for size		
Sample weight	0.93 *	
Number of cysts	0.60 *	0.67 *

[1]) * = significant (P<0.01)

Table 1.2. Correlations between volume of cysts, as calculated from the cyst diameter, cyst weight and number of eggs per cyst, in Expt 1.3.

	Cyst weight	Number of eggs
Number of eggs	0.96 *[1])	
Cyst volume	0.89 *	0.78 *

[1]) * = significant (P<0.01)

Table 1.3. Values and confidence interval (P=0.05) of the regression coefficient b of the regression line y=b*x, with y being the number of eggs per sample and x being weight of the sample (µg), and the percentage explained variance (r^2) by regression analysis, in Expts 1.1, 1.2 and 1.3.

Experiment	b	Confidence interval	Explained variance (%)
1.1	8.39	7.90<b<8.89	74
1.2	7.99	7.47<b<8.51	86
1.3	7.99	7.73<b<8.25	91

between number of eggs per cyst and cyst weight, using a supermicrobalance, is shown in Figure 1.1. A few cysts contained less eggs than expected from their cyst weight, which may be due to the fact that the newly formed cysts at rearing may have been mixed with some largely empty cysts of the inoculum. Despite this, a very high correlation was found between the number of eggs per cyst and cyst weight (Table 1.2). The correlation between cyst volume and number of eggs per cyst was considerably and significantly ($P<0.01$) lower (Table 1.2).

Regression analysis. All samples, either selected or not selected for size, were used for linear regression analysis. In each of the experiments, the observed value of the constant did not differ significantly from zero. Therefore, a simplified regression analysis was preferred, in which the constant was omitted. Thus, a regression line $y=b*x$ was used, in which y is the number of eggs, x is sample weight (µg), and b is the regression coefficient. The value of b did not differ significantly between Expts 1.1, 1.2 and 1.3 (Table 1.3). The value of b, although slightly higher for *G. pallida* than for *G. rostochiensis*, did not differ significantly between these species.

Discussion

The high correlations between cyst weight and number of eggs show that cyst weight provides a good assessment of the average number of eggs per cyst. Weighing cysts is much less labour-intensive than crushing and counting the number of eggs. It has to be noted that the results have been obtained with clean samples of cysts, obtained from greenhouse rearing. For weighing cysts, a supermicrobalance is preferable, as it can be used for weighing of even individual cysts (Expt 1.3), and has the highest accuracy. When the cyst content of samples with ten cysts or more has to be assessed, weighing with a microbalance, which is less sensitive, may be sufficient (Expts 1.1 and 1.2). Usually, cysts will be weighed in various replicates of the treatments, and the treatment mean number of eggs thus will be assessed with increased accuracy.

The weight of cysts not only includes the weight of eggs, but also the weight of other cyst contents and the cyst walls. Despite this, the explained variances of regression of number of eggs on weight were very high (Table 1.3), and the relationship found proved to be very useful. However, the actual weight of eggs cannot be estimated in this way.

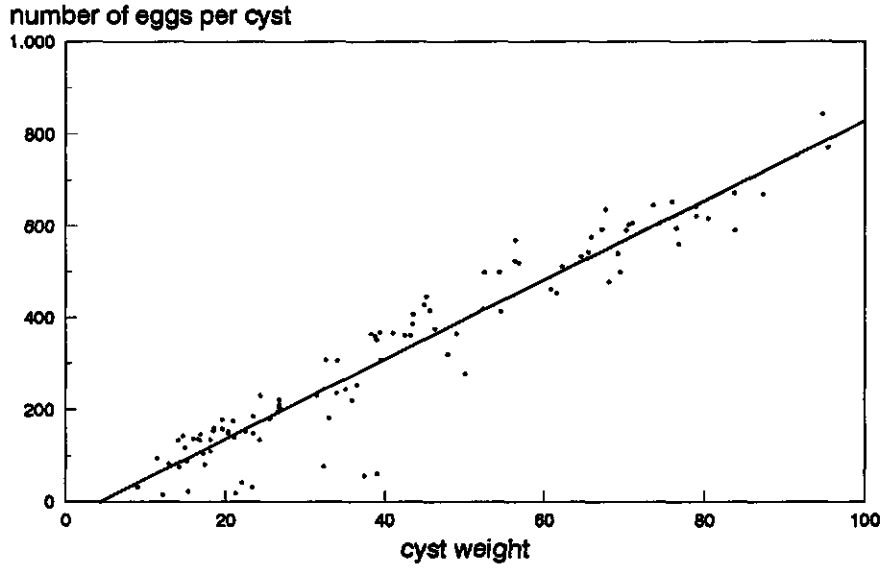


Figure 1.1. The relationship between total number of eggs per cyst and cyst weight (µg), using a supermicrobalance, in Expt 1.3.

In our experiment, a significantly lower correlation of cyst volume with number of eggs was found than the correlation of cyst weight with number of eggs. However, Stelter and Gaur (1969) found a very high correlation ($r>0.95$) between cyst volume and number of eggs. Their assessment of volume differed from ours, as they took into account both the smallest diameter and the largest diameter for calculation of the volume. Diameter measurements are, however, very labour-intensive, unless they can be done by image analysis.

The method for assessment of the number of eggs, presented here, has the advantage that it is not destructive to the cysts, as compared to fast methods for counting juveniles (Been *et al.*, 1990; Robinson *et al.*, 1992) or possibly quantitative ELISA for detection of cysts (Schots *et al.*, 1992). It can be very useful to assess final PCN densities rapidly in pot trials and afterwards, the cysts can be used for further research. Weighing may therefore be regarded as a simple and effective method of assessing the number of eggs in cysts.

CHAPTER 2

Variation in resistance level of potato genotypes and virulence level of potato cyst nematode populations

Abstract

In two experiments, using different testing methods, the number of newly formed cysts was determined on nine potato genotypes with resistance from various sources. Ten potato cyst nematode (PCN) populations were used in these experiments. Rank correlation between numbers of cysts over potato genotype-PCN population combinations for both experiments was high (r_s=0.90). Dendrograms for PCN populations and potato genotypes were constructed, based on a simultaneous hierarchical clustering procedure for potato genotype-PCN population interaction terms. Several virulence groups could be identified within *Globodera rostochiensis* as well as within *G. pallida*. Host genotypes, derived from the same sources of resistance, were clustered in different resistance groups.

Introduction

The resistance level of potato genotypes, *Solanum tuberosum* ssp. *tuberosum* L., to potato cyst nematodes (PCN), *Globodera rostochiensis* (Woll.) Skarbilovich, and *G. pallida* Stone, may differ with different PCN populations. Kort *et al.* (1977) grouped PCN populations by distinguishing five pathotypes of *G. rostochiensis* and three of *G. pallida*, based on the multiplication rate of the population being ≤ 1 or >1 on a set of differential potato genotypes. In the Netherlands, assessment of cultivar resistance is carried out using this pathotype classification. Only very high levels of resistance, with a PCN multiplication rate below 0.20, are considered satisfactory to control PCN, according to Dutch plant protection regulations. Both the pathotype of PCN populations and the resistance of potato genotypes are thus determined in a quantitative test, followed by a qualitative interpretation of the results.

The above mentioned concept of pathotypes and the classification of PCN resistance has been criticized on a number of points (Trudgill, 1985; Nijboer & Parlevliet, 1990). First, for the identification of pathotypes homogeneity of virulence is required (Parlevliet, 1985), while the pathotype concept of Kort *et al.* (1977) allows heterogeneity of virulence in PCN populations. Secondly, genotypes derived from crosses with *Solanum vernei*, show a range of resistance levels. It may therefore be more useful to measure and describe resistance in a quantitative manner. Thirdly, pathotype and resistance assessments, based on absolute PCN multiplication rates, may vary between experiments due to the large influence of environmental factors on these rates.

Several authors have described research on the quantitative expression of resistance. Phillips (1984) found little influence of PCN density on resistance level, relative to a susceptible cultivar, of *S. vernei* hybrids. Phillips & Trudgill (1985) found a good relationship between PCN resistance tests, carried out in the greenhouse and in the field, for a range of resistance levels. Seinhorst (1984) proposed to assess PCN resistance as the ratio of the PCN multiplication on the tested genotype to that on a susceptible standard cultivar. An *ad hoc* Panel of the European Plant Protection Organization EPPO suggested that, in addition to susceptible cultivars, some partially resistant genotypes should be used as reference genotypes in resistance tests (Mugniéry *et al.*, 1989).

At the EPPO workshop on Cyst Nematodes in Münster (1984), it was decided to replace the term 'pathotype' by 'virulence group', but the criteria for identification of virulence groups were not presented. The workshop concluded that only two virulence groups for

G. rostochiensis, consisting of the former pathotypes Ro1/Ro4 and Ro2/Ro3/Ro5, and two for *G. pallida*, Pa1 and Pa2/Pa3, could be clearly distinguished (Mugniéry *et al.*, 1989).

We studied the similarity of PCN populations within these virulence groups, using predominantly Dutch PCN populations, in tests with Dutch cultivars and breeding lines, which have been derived from various sources of resistance. The effects of testing method and cyst content on the observed resistance and virulence levels were investigated. Furthermore, we used a hierarchical clustering procedure for the identification of virulence groups. In this way, the relationship between resistance groups and sources of resistance was investigated also.

Materials and methods

The nine resistant potato genotypes tested are listed in Table 2.1. These cultivars and breeding lines represent the most important sources of resistance to PCN that have been used in the Netherlands. Two of the resistant genotypes, 12380 and Vantage, have been proposed as reference genotypes for determination of resistance in a quantitative manner by Mugniéry *et al.* (1989), and were included in their trials. Vantage originates from a Dutch breeding programme and 12380 is derived from a source of resistance also used in the Netherlands (Table 2.1). Désirée and Maritta were added as susceptible control genotypes. Five populations of *G. rostochiensis* and five of *G. pallida* were used in the experiments (Table 2.2). All populations originate from the Netherlands, except Harmerz, which originates from Germany. The populations represent different pathotypes (Bakker, 1987; Vinke, pers.comm.) and/or are different as shown by protein electrophoresis (Bakker, 1987). No PCN population with pathotype designation Ro2 was used, because the differential *S. kurtzianum* hybrid 60.21.19 has not been a source of resistance for Dutch potato cultivars. Therefore no distinction between the pathotypes Ro2 and Ro3 is expected when tested with the potato genotypes used here. Also no population of *G. pallida* pathotype Pa1 was used, because it has not been reported to occur in the Netherlands.

Table 2.1. Potato genotypes tested, and the sources of their resistance to potato cyst nematodes.

Potato genotype	Sources of resistance[1]			
	1	2	3	4
AM78-3778	x	x	x	x
Astarte	-	x	-	-
Elles	x	x	x	-
Mara	-	x	-	-
Pansta	x	x	x	-
Saturna	x	-	-	-
(VT")[2] 62-33-3	-	x	x	-
12380	x	-	x	-
Vantage	-	x	x	-

[1]) 1 = *S. tuberosum* ssp. *andigena* CPC 1673
2 = *S. vernei* ssp. *balssii* 2/1 (Rothacker, 1959)
3 = *S. vernei* CPC 2488-3 x *S. vernei* CPC 2487-3
4 = *S. vernei* LGU8 x *S. oplocense* (EBS, Germany)

Experiment 2.1. Tubers were planted in a pot with a volume of 325 ml, containing 500 g sandy loam soil and inoculated with cyst portions with an equal volume (Vinke *et al.*, 1992), with on average 23 cysts. With this pot size (diameter 10 cm), Phillips & Trudgill (1985) reported a good relationship between pot and field assessments of partial resistance. The mean weight of cyst portions is shown in Table 2.2. Cysts were enclosed in nylon sieve bags, with a pore diameter of 175 µ, sufficiently wide to be passed by hatched juveniles. The experiment was carried out in the greenhouse, following a split plot design with PCN populations as mainplots, and potato genotypes as subplots, in five replications. Watering was stopped after about 14 weeks and when the soil was air-dry, newly formed cysts were recovered by flotation and counted. A score of 1-5 was given for the amount of dry remains of the root system. If at least 10 cysts per pot were found, the newly formed cysts were also weighed, to a maximum of 50 cysts. Cyst weight and the mean number of eggs per cyst are highly correlated (Arntzen, 1988). In addition, a hatching test was carried out. Samples of 10 to 50 newly formed cysts were

exposed for six weeks to standard potato root diffusate, as described by Dellaert & Vinke (1987). The diffusate with hatched juveniles was diluted to 10-50 ml, depending on the number of cysts per sample. The number of hatched larvae was counted in three samples of 1 ml.

Experiment 2.2. Stem cuttings of the potato genotypes were grown in a mixture of peat, sand and perlite. After three to four weeks, the rooted cuttings were transplanted to pots with a volume of 150 ml, containing 73 g peaty soil, and inoculated using cyst portions with the same volume and of the same ten PCN populations as in Expt 2.1. A similar split plot design was used, but the experiment was carried out in nine replications. After six weeks, plants were removed from the pots and newly formed cysts visible on the rootball were counted, representing the total number of newly formed cysts (Forrest & Holliday, 1979). Furthermore, a score 1-3 was given for the size of the root system.

Statistical analysis. Data for numbers of cysts and number of hatched juveniles per cyst were transformed to the logarithm with base 10 of the numbers plus 1 to obtain approximate homogeneity of variances and normality, as required for analysis of variance. Due to the large proportion of zeroes for the numbers of cysts in Expt 2.2, transformation did not succeed in achieving homogeneity of variance and normality. Therefore no analysis of variance was done on these data, nor any analysis with the same requirements, such as the hierarchical clustering analysis described below. Various correlations were determined by calculation of the non-parametric Spearman's rank correlation coefficient (r_s).

Assessment of virulence groups among PCN populations and resistance groups among potato genotypes was done by means of an agglomerative hierarchical clustering procedure, as described by Corsten and Denis (1990). First, a two-way table of means was constructed for the log numbers of cysts of Expt 2.1. The classifying factors were potato genotypes and PCN populations. Next, the means of this table were corrected for the row and column main effect, thus leaving residuals from additivity, which represent the interaction between the row and column factor. The interaction table served as the basis for the classification of potato genotypes and PCN populations. The rationale for removing the main effects was that we were primarily interested in the relative and not in the absolute differences between genotypes with respect to the PCN populations, and, complementary, in the relative differences between PCN populations with respect to the potato genotypes (Fox & Rosielle, 1982; Phillips & McNicol, 1986).

Table 2.2. Potato cyst nematode populations used, with pathotype designation, their year of collection and origin and the mean weight of inoculum.

Populations	Pathotype designation	Year of collection	Place of origin	Weight inoculum (mg)
Mierenbos A[1]	Ro1	1954	Wageningen	0.72
C258	Ro3	1974	Odoorn	0.65
F515[1]	Ro4	1970	Emmen	0.76
G1510	Ro5	1973	Norg	0.64
Harmerz[1]	Ro5	1968	Harmerz (Germany)	0.70
P2-22[1]	Pa2	1959	Coevorden	0.56
Rookmaker[1]	Pa3	1960	Valthe	0.70
75-884-4[1]	Pa3	1975	Vriezeveen	0.67
74-768-20[1]	Pa3	1974	Sleen	0.71
(Coll.)1077[1]	Pa3	1957	Anjum	0.66

[1]) protein electrophoresis pattern is described by Bakker (1987)

The clustering algorithm consecutively merged those pairs of rows or columns that exhibited the minimum mean square for interaction between them. Except for the first step, rows or columns can be the results of mergings in earlier steps. In this way groups of genotypes and populations are formed, so that the interaction is mainly due to interaction between these groups, whereas the groups are internally quite homogeneous. Clustering takes place for rows and columns simultaneously, and stops at the moment that a certain threshold for internal heterogeneity of the groups is exceeded. We used as a stopping criterion an F-test procedure (at a conservative 0.01 level) in which variation due to within group heterogeneity was tested against an error estimate derived from the split plot analysis. The results of the clustering are presented by dendrograms for the host genotypes and the PCN populations separately. In both dendrograms, the horizontal axis represents the size of the interaction sum of squares that is explained by the interaction between the groups of host genotypes and the groups of PCN populations that have been formed until that point.

To investigate cyst content with respect to the variables mean cyst weight, reflecting number of eggs per cyst, and the number of hatched juveniles per cyst, analyses of variance were carried out. The data to be analysed were restricted to genotypes for which at least ten cysts per pot were observed for all PCN populations, thus excluding AM78-3778, 12380 and Vantage, while PCN populations were restricted to *G. pallida* only.

Results

Number of cysts. In Table 2.3 and 2.4, the mean numbers of observed cysts (untransformed) are given. Interactions between host genotypes and PCN populations can easily be observed, both within *G. rostochiensis* and *G. pallida*. Within *G. rostochiensis*, the largest differences between PCN populations were seen for Saturna, Astarte and Mara. With respect to the various *G. pallida* populations, a large variation was found for Elles and (VT^m)^2 62-33-3. For the highly resistant AM78-3778 and the rather susceptible Astarte, considerably less variation was found. The resistance level of Pansta also varied over *G. pallida* populations, but less than for Elles and (VT^m)^2 62-33-3. The number of cysts on Vantage varied over PCN populations in both

Table 2.3. Mean number of newly formed cysts of ten potato cyst nematode populations on eleven potato genotypes in Expt 2.1.

Potato genotypes	*G. rostochiensis* populations					*G. pallida* populations					
	Mier.	F515	C258	G1510	Harm.	P2-22	1077	75-884-4	Rookm.	74-768-20	Mean
AM78-3778	1	2	3	9	10	5	6	2	5	10	5
12380	1	0	2	4	3	3	8	6	19	137	18
Vantage	34	21	5	17	34	3	17	5	32	107	28
Elles	8	5	5	45	4	4	10	44	58	123	31
$(VT^m)^2$ 62-33-3	3	1	3	5	8	20	29	85	96	307	56
Pansta	8	2	2	32	19	23	105	208	199	84	68
Astarte	26	80	6	73	63	118	106	145	122	102	84
Mara	5	16	7	135	66	134	204	257	216	277	132
Saturna	1	1	123	169	387	269	357	362	385	478	253
Mean	10	14	17	54	66	64	94	124	126	181	75
Désirée	780	816	110	235	314	333	374	339	403	550	425
Maritta	669	508	428	693	396	421	261	222	427	630	466
Mean	725	662	269	464	355	377	318	281	415	590	446

Table 2.4. Mean number of newly formed cysts of ten potato cyst nematode populations on eleven potato genotypes in Expt 2.2.

Potato genotypes	*G. rostochiensis* populations					*G. pallida* populations					
	Mier.	F515	C258	G1510	Harm.	P2-22	1077	75-884-4	Rookm.	74-768-20	Mean
AM78-3778	0	1	0	0	1	0	0	0	0	0	0
12380	0	0	0	0	0	0	0	0	0	1	0
Vantage	4	3	9	10	16	1	2	2	13	13	7
Elles	1	0	1	1	1	0	2	5	6	25	4
$(VT^m)^2$ 62-33-3	0	0	1	0	3	1	3	10	9	46	7
Pansta	0	0	0	1	1	9	15	14	19	18	8
Astarte	2	21	3	55	34	35	34	44	28	53	31
Mara	0	3	3	23	65	63	91	47	42	59	40
Saturna	0	0	35	44	29	86	111	61	45	52	46
Mean	1	3	6	15	17	20	29	20	16	30	16
Désirée	158	165	110	86	157	53	92	83	59	143	111
Maritta	82	137	124	86	156	108	79	90	50	82	99
Mean	120	151	117	86	156	81	86	87	55	113	105

Table 2.5. Analysis of variance of log number of cysts, with degrees of freedom (d.f.), percentage of total sums of squares (%TSS) and the mean squares (MS), excluding the two susceptible standard cultivars Désirée and Maritta, for all ten potato cyst nematode (PCN) populations and the two species separately (Expt 2.1).

Source of variance	All PCN populations			*G. rostochiensis*			*G. pallida*		
	d.f.	%TSS	MS	d.f.	%TSS	MS	d.f.	%TSS	MS
PCN population	9	27	8.77*[1]	4	17	4.54*	4	10	3.68*
Error 1	36		0.46	16		0.43	16		0.52
Host genotype	8	26	9.55*	8	27	3.59*	8	45	8.14*
Host genotype-PCN population	72	21	0.85*	32	27	0.90*	32	10	0.46*
Error 2	320		0.21	162		0.16	162		0.25
Total	445			222			222		

[1]) *=significant (P<0.01)

experiments, that for 12380 only in Expt 2.1. The correlation between numbers of cysts in both experiments was high (r_s=0.90; P<0.01).

For both experiments, the correlation between root score and log number of cysts over all host genotype-PCN population combinations was low and not significant (r_s=-0.01 and 0.17 for Expt 2.1 and Expt 2.2 respectively). Therefore, root score did not need to be considered in further analyses.

Only the data of Expt 2.1 were considered suitable for an analysis of variance, after transformation. A significant host genotype-PCN population interaction effect was found (P<0.001), which amounted to 21% of the total sum of squares (Table 2.5). Interaction and main effects were of comparable magnitude. Within *G. rostochiensis*, the interaction effect was relatively large, when compared to *G. pallida*.

The dendrograms obtained from the clustering procedure are given for the host genotypes and PCN populations in Figure 2.1. Figure 2.1a shows that six clusters of genotypes could be distinguished. Saturna, with resistance only from *S. tuberosum* ssp. *andigena*, showed an interaction pattern which was, in accordance with expectation, quite different from the other genotypes, which were derived from other sources of resistance. Contrary to expectation, however, genotypes derived from the same sources of resistance also ended up in different groups. Resistance patterns of genotypes with shared resistance sources apparently still can differ to a considerable extent.

The dendrogram for PCN populations (Figure 2.1b) shows that six groups could be distinguished. For *G. rostochiensis*, the four groups Mierenbos/F515, C258, Harmerz, and G1510 could be distinguished, with the latter being clustered with some *G. pallida* populations. Mierenbos A and F515 appeared to be most distinct from the other populations. Within *G. pallida*, the clustering procedure indicated three groups, P2-22/1077, 75-884-4, and Rookmaker/74-768-20.

The correlation between mean log number of cysts for both variables was not significant in both experiments (r_s=-0.34 and -0.27 in Expt 2.1 and 2.2 respectively). Thus, no clear relationship was found between the mean number of cysts of PCN populations formed on the nine host genotypes with resistance and the ability of these populations to form cysts on the two susceptible host cultivars.

Cyst content. In comparison to the differences found for the number of cysts only small differences between genotypes could be observed for both mean cyst weight and the number of hatched juveniles per cyst. Analyses of variance for the restricted set of genotypes and populations showed no significant genotype main effect and

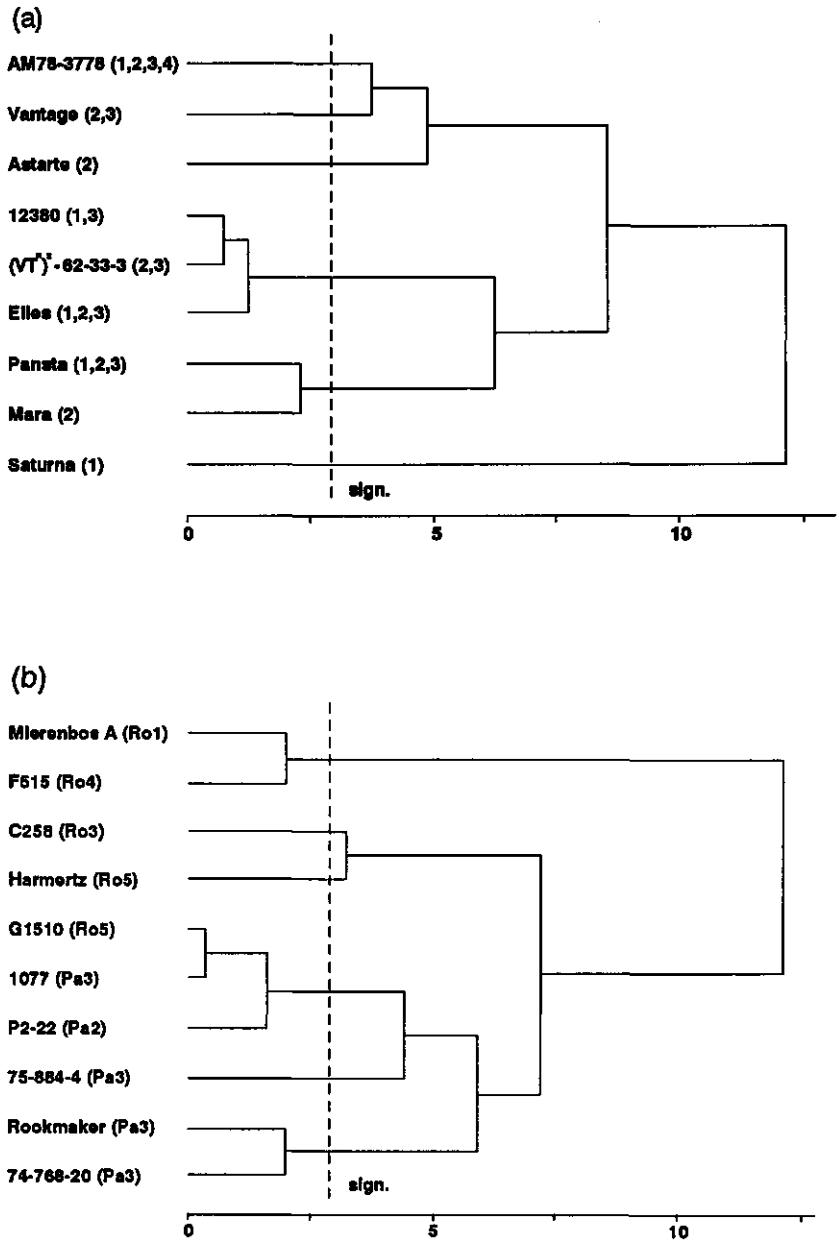


Figure 2.1. Dendrograms (a) for potato genotypes, with sources of resistance as indicated in Table 2.1, and (b) for potato cyst nematode (PCN) populations with their pathotype designation, after hierarchical clustering based on host genotype-PCN population interaction terms for log cyst numbers. The horizontal axis displays the sum of squares explained by clustering and the vertical line stands for the significance threshold (P=0.01).

Table 2.6. Mean weight per cyst (µg) and mean number of hatched juveniles per cyst, for five *G. pallida* populations, tested with eight potato genotypes, all except AM78-3778, 12380 and Vantage. Means carrying the same letters are not significantly different from each other (P<0.05).

G. pallida population	Cyst weight		Juveniles per cyst	
P2-22	21.2	a	85.6	a
Rookmaker	23.9	b	112.3	b
75-884-4	21.2	a	95.1	ab
74-768-20	20.9	a	105.1	ab
1077	22.7	ab	115.5	b

genotype-population interaction effect for both cyst weight and hatched juveniles per cyst. However, for both traits significant differences were found between some PCN populations (Table 2.6). No significant correlations were found between mean number of newly formed cysts on the one hand, and mean number of hatched juveniles per cyst and cyst weight on the other hand (r_s=-0.15 and 0.03 respectively).

Discussion

In the two experiments, variation in resistance and virulence was measured in two different ways, with tubers and cuttings, and with differences between experiments in space for plant growth, PCN density and soil type. Numbers of cysts were based on flotation recovery or rootball observation. However, differences between the two experiments were small, as indicated by the high Spearman rank correlation for the number of cysts. Results of Forrest & Holliday (1979), indicating a high correlation between flotation recovery test and rootball test, were thus confirmed.

Data on cyst weight and hatched juveniles per cyst showed that, at least for the lower levels of resistance we used, these traits were not affected by host

genotype-PCN population interaction, and that they were not correlated with numbers of newly formed cysts. Furthermore, variation in these traits was much smaller than the variation in numbers of cysts, which was found before by Phillips & Trudgill (1983). Lellbach (1983) found a high correlation between number of cysts and cyst content on *S. vernei* hybrids with relatively high levels of resistance. The results imply that although nematode multiplication rate is more accurately determined based on both number of cysts and cyst content, size and pattern of host genotype-PCN population interaction can be estimated fairly accurately through the numbers of newly formed cysts alone.

The data on numbers of cysts confirm that a range of resistance levels occurs with respect to all PCN populations, which means that the classification into resistant or susceptible potato genotypes, as presently used in the pathotype scheme, is arbitrary. Also, some major interactions between potato genotypes and PCN populations occurred within *G. rostochiensis* Ro2/Ro3/Ro5, and within *G. pallida* Pa2/Pa3, showing that these groups are heterogeneous for virulence characteristics.

The proportion of variance due to host genotype-PCN population interaction variance was larger in our experiment, compared to results of a similar type of experiment using only *G. pallida* populations, described by Phillips *et al.* (1989). They found 0.2% variance due to the host genotype-PCN population interaction effect, whereas 84% was due to the host genotype effect. We found values of 10 and 45% respectively (Table 2.5). The difference with the results of Phillips *et al.* (1989) may be explained by larger differences in virulence in the PCN populations, which we used for testing. Vantage and 12380 showed much less interaction with *G. pallida* populations in their experiment than in our experiment.

The *G. pallida* populations P2-22 and Rookmaker were collected around 1960 (Table 2.2), when no *G. pallida* resistant cultivars were available. This indicates that virulence characteristics of *G. pallida* populations varied widely, even before resistant cultivars were released.

Increased virulence of PCN populations on resistant hosts appeared not to be related to the ability to form more cysts on susceptible cultivars, as shown by the not significant correlation between these traits.

No clear relationship between sources of resistance in potato genotypes and their resistance pattern was found. Much variation in resistance pattern existed between genotypes, with resistance derived from *S. vernei*. The variation cannot

be explained by the use in potato breeding of three different seed samples of *S. vernei*, collected from different locations in South-America (Table 2.1). Also with genotypes, derived from the same sources, considerable variation occurred. This may be due to the differential loss of resistance genes from the original source in some potato genotypes during breeding, since resistance in *S. vernei* is probably polygenic (Rothacker, 1959; Dale & Phillips, 1982; Dellaert *et al.*, 1988).

With the set of genotypes used, several virulence groups of PCN populations were identified. However, the implications of the results are limited. First, the results are likely to be influenced by the sources of resistance used. Resistance from *S. spegazzini*, incorporated in a number of German cultivars (Ross, 1986), and resistance from *S. tuberosum* ssp. *andigena* CPC 2802, used for breeding in Great Britain (Phillips & Trudgill, 1983), were not included. In the Netherlands, breeding lines are currently used with amongst others resistance derived from *S. leptophyes* and *S. sanctae-rosae* (Dellaert & Vinke, 1987). Including genotypes from other sources of resistance may alter the results. Secondly, including more PCN populations in the test may increase the number of virulence groups distinguished. Thirdly, environmental variation in this experiment was quite large. More replications, or increased accuracy of the testing method may lead to the distinction of more virulence groups. Finally, when the relative frequency of PCN populations in the field and their virulence characteristics is taken into account, the number of virulence groups may be limited.

The procedure described here for the identification of virulence groups nevertheless seems promising. If the potato genotypes and the PCN populations are chosen as to fulfil the above mentioned criteria, virulence groups of PCN can be distinguished in a relevant way, and a range of resistance levels can be determined with PCN populations representing those virulence groups.

CHAPTER 3

Inheritance, level and origin of resistance to *Globodera pallida* in the potato cultivar Multa, derived from *Solanum tuberosum* ssp. *andigena* CPC 1673

Abstract

Resistance to *Globodera pallida* has been found in the potato cultivar Multa. This cultivar is derived from *Solanum tuberosum* ssp. *andigena* CPC 1673, the widely used source of the H_1 resistance gene to *G. rostochiensis*. The inheritance, level and origin of the resistance to *G. pallida*, as found in the cultivar Multa, were studied. A high level resistance to *G. pallida* population D236 was found in Multa, and appeared to be based on a single gene. A relatively low, but significant level of resistance to most other potato cyst nematode populations was also found in Multa. The monogenic resistance did not interact with the low level resistance to *G. pallida* populations P2-22 and Rookmaker. The low level resistance to these populations presumably has a separate genetic basis, with mainly recessive inheritance. Eight out of eleven cultivars with resistance to *G. rostochiensis* from *S. tuberosum* ssp. *andigena* CPC 1673 also had the high level resistance to the D236 population. The monogenic resistance to D236 therefore presumably originates from CPC 1673.

Introduction

Resistance to potato cyst nematodes (PCN), *Globodera pallida* and *G. rostochiensis*, was not found originally within *Solanum tuberosum* ssp. *tuberosum* (Oostenbrink, 1950). Breeding for resistance to PCN therefore has made extensive use of other *Solanum* species. *Solanum tuberosum* ssp. *andigena* CPC 1673 was found to have resistance to *G. rostochiensis* (Ellenby, 1952), and this resistance has been incorporated in many potato cultivars. Resistance to both PCN species was found in *Solanum vernei*, which is now the most widely used source for resistance breeding to *G. pallida* (Ross, 1986).

Resistance to *G. rostochiensis* from *S. tuberosum* ssp. *andigena* CPC 1673 is based on a single gene, the H_1 gene (Toxopeus & Huijsman, 1953), and is used to identify *G. rostochiensis* pathotype Ro1 (Kort *et al.*, 1977). In the nematode, avirulence to the H_1 gene is determined also by a single gene (Janssen *et al.*, 1991). Resistance to *G. pallida* from *S. vernei* is characterized by polygenic inheritance (Dale & Phillips, 1982; Dellaert *et al.*, 1988). For breeders, this type of inheritance is less attractive, as relatively few genotypes with a high level of resistance are found in the progeny of crosses with susceptible cultivars. Single gene based resistance to *G. pallida* has been reported in *S. multidissectum* (Dunnett, 1962). The resistance is effective to a limited number of *G. pallida* populations and was used by Kort *et al.* (1977) to identify *G. pallida* pathotype Pa1. No other monogenic resistances to *G. pallida* have been found, even although many accessions from several wild *Solanum* species have been screened (Dellaert *et al.*, 1988).

The Dutch cultivar Multa, derived from the cross Oberarnbacher Frühe x (Record x CPC 1673-1), does not carry the H_1 gene (Bakker, pers. comm.) and has been described as susceptible to PCN in Descriptive Cultivar Lists in the Netherlands. Seinhorst (1986), however, reported a reduced susceptibility of Multa to some *G. pallida* populations. The source of this resistance was unknown. No reports on *G. pallida* resistance in *Solanum tuberosum* ssp. *andigena* CPC 1673 existed. Only Parrott and Trudgill (1970) reported resistance to some *G. pallida* populations in two cultivars, which have been derived from CPC 1673. Multa has no other known source of PCN resistance in its pedigree.

In this Chapter, the results are presented of further investigations into the PCN resistance of Multa. The level of resistance of Multa to various potato cyst nematode populations was determined, and the inheritance of the resistance and its origin were investigated.

Materials and methods

Experimental procedures. Resistance tests were carried out with tubers, planted in 240 ml pots, containing 120 g peaty soil, or with rooted stem cuttings, planted in 150 ml pots, filled with 75 g peaty soil. The tubers had been presprouted and the stem cuttings had been grown for about three weeks and had a small root system before transplanting. Pots were inoculated at planting with a preset volume of cysts (Vinke *et al.*, 1992), containing on average 29 cysts, with an average total weight of 1.18 mg. Pots were placed in a greenhouse, with an average temperature of 20°C, and were watered daily. After 6 to 8 weeks, when cysts were well developed, rootballs were removed from the pots and the number of newly formed cysts, visible on the rootball, was counted. This number is representative of the total number of newly cysts formed (Forrest & Holliday, 1979). A score (1-5) was given for the size of the root system.

Numbers of cysts were transformed to the logarithm with base 10 of the numbers of cysts plus 1, to obtain homogeneity of variance, necessary for analysis of variance. Correlations between data, which did not show a normal distribution, were determined by Spearman's rank correlation coefficient r_s.

Resistance in relation to variation in the pathogen. Rooted stem cuttings of Multa and the susceptible standard cultivar Maritta were tested with twelve Dutch PCN populations, four of *G. rostochiensis* and eight of *G. pallida* (Table 3.1), with 20 replicates (Experiment 3.1). The populations varied in pathotype designation, place of origin, and protein pattern, as shown with two dimensional gel electrophoresis by Bakker (1987). Only *G. rostochiensis* populations C258 and G1510 have not been investigated by Bakker.

Inheritance of resistance. Multa was crossed with the susceptible standard cultivar Maritta, and with the diploid *S. phureja* 81-1886-524, known to have some resistance to *G. pallida* (Dellaert *et al.*, 1988) and to produce 2*n*-gametes. In addition, Multa was selfed. Tubers of 81 genotypes from the cross Maritta x Multa, 85 genotypes from the cross Multa x 81-1886-524, which were identified as hybrids by the presence of seed spots (Hermsen & Verdenius, 1973), and 90 genotypes obtained from selfing of Multa, were used in a resistance test with *G. pallida* population D236, with three replicates (Experiment 3.2).

Rooted stem cuttings of the same 81 genotypes of the cross Maritta x Multa were tested with three *G. pallida* populations, D236, P2-22 and Rookmaker, with five replicates (Experiment 3.3). Parent genotypes were also included.

Rooted stem cuttings of the same 85 genotypes from the cross Multa x 81-1886-524,

Table 3.1. Details of potato cyst nematode (PCN) populations, numbers of cysts on Multa and on the susceptible standard cultivar Maritta, and numbers of cysts on Multa relative to Maritta, when tested with these populations (Expt 3.1). Relative numbers of cysts carrying the same letter are not significantly different from each other (LSD; P<0.05).

PCN populations	Species	Pathotype designation	Origin of populations	Number of cysts on		Number of cysts on Multa relative to the number on Maritta
				Multa	Maritta	
Mierenbos A	*G. rostochiensis*	Ro1	Wageningen	132 *[1]	202	0.65 de
C258	*G. rostochiensis*	Ro3	Odoorn	140 *	233	0.60 de
F515	*G .rostochiensis*	Ro4	Emmen	115	150	0.76 e
G1510	*G. rostochiensis*	Ro5	Norg	96	140	0.69 de
D234	*G. pallida*	Pa2	Smilde	3 *	117	0.03 a
D236	*G. pallida*	Pa2	Anlo	3 *	129	0.02 a
P2-22	*G. pallida*	Pa2	Coevorden	64 *	161	0.39 c
Rookmaker	*G. pallida*	Pa3	Valthe	63 *	156	0.40 c
(Coll.) 1077	*G. pallida*	Pa3	Anjum	68 *	140	0.49 cd
74-768-20	*G. pallida*	Pa3	Sleen	73 *	221	0.33 c
75-884-4	*G. pallida*	Pa3	Vriezenveen	63 *	144	0.44 c
(Coll.) 1112	*G. pallida*	Pa3	Westerbork	29 *	121	0.24 b

[1]) an asterisk indicates significantly fewer cysts on Multa than on Maritta, tested with the same population (LSR; P<0.05)

and the parents, were tested with two *G. pallida* populations, D236 and Rookmaker, with three replicates (Experiment 3.4).

Origin of resistance. *Solanum tuberosum* ssp. *andigena* CPC 1673 was a seed sample, from which various genotypes have been used in potato breeding (Huijsman, 1957). These genotypes, from which Multa and various other cultivars have been derived, do not exist anymore. Tubers of the other ancestors of Multa, Record and Oberarnbacher Frühe, were tested for resistance, together with Multa and the susceptible standard cultivar Maritta, with *G. pallida* populations D236 and P2-22 (Experiment 3.5). Furthermore, tubers of eleven cultivars with resistance to *G. rostochiensis*, derived from *S. tuberosum* ssp. *andigena* CPC 1673, were also included in this experiment. The cultivars originate from three genotypes, CPC 1673-1, CPC 1673-11 and CPC 1673-20, backcrossed two or three times with *S. tuberosum* ssp. *tuberosum*. One of the cultivars, Amigo, has been derived from a cross between genotypes, originating from two backcrosses of different CPC 1673 genotypes with *S. tuberosum* ssp. *tuberosum*. The mentioned cultivars have been released between 1960 and 1970. Expt 3.5 was carried out with five replicates.

Results

Significant differences in resistance levels were found in all experiments. Correlations (r_s) between log transformed number of cysts and root scores were below 0.10 and not significant, except in Expt 3.3.

Resistance to a range of PCN populations. In Expt 3.1, Multa appeared to have a high level of resistance to two *G. pallida* populations, D234 and D236, as indicated by the formation of very few cysts (Table 3.1). Apart from the high level resistance to D234 and D236, the cultivar showed a lower level of resistance to most other PCN populations, characterized by the formation of significantly fewer cysts than formed on the susceptible standard cultivar Maritta. The level of this resistance varied with PCN populations (Table 3.1). The resistance level in Multa was significantly higher to most *G. pallida* populations than to the *G. rostochiensis* populations.

Inheritance of resistance. In Expt 3.2, tests with *G. pallida* population D236 showed a clear distinction between groups of highly resistant and susceptible genotypes for the Maritta x Multa progeny (Figure 3.1a). For the Multa x 81-1886-524 progeny (Figure

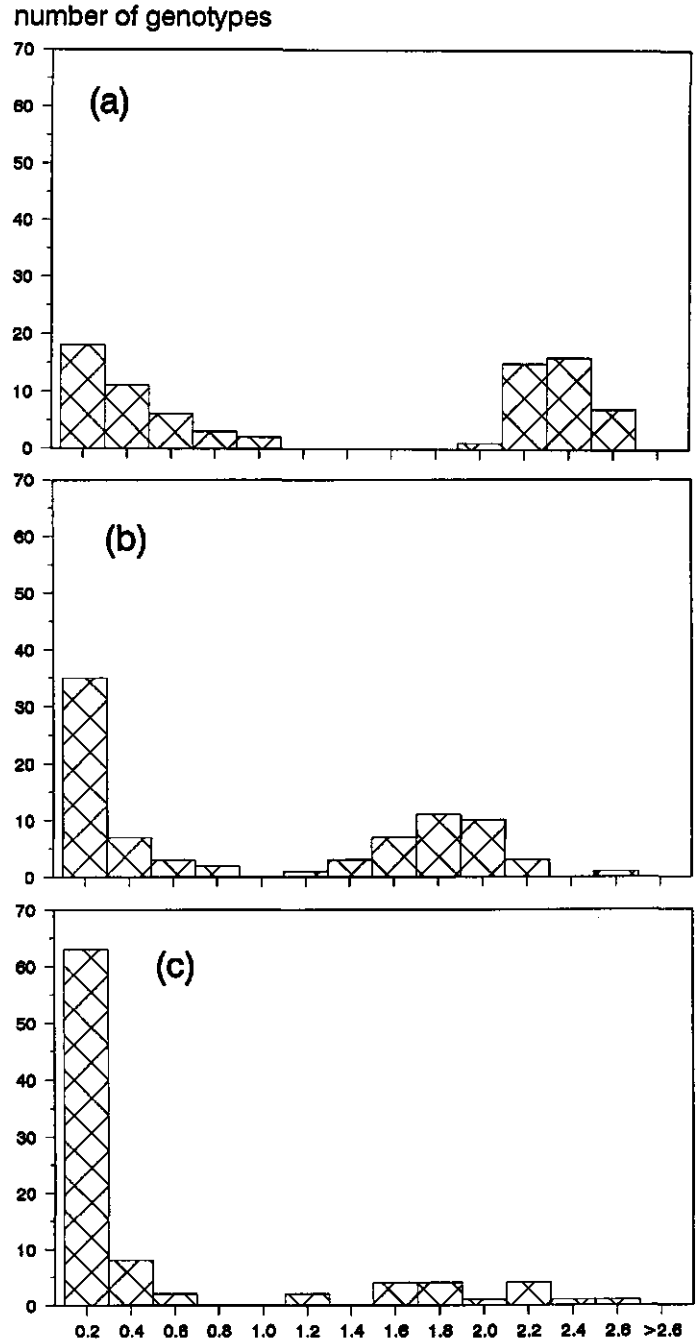


Figure 3.1. Frequency distributions of genotypes for log transformed numbers of cysts, tested with *Globodera pallida* population D236 in Expt 3.2. (a): progeny of Maritta x Multa; (b): progeny of Multa x 81-1886-524; (c): progeny of Multa selfed. Only upper limits of classes are indicated.

3.1b) and for Multa selfed (Figure 3.1c) also, although less clear, groups of highly resistant and susceptible genotypes could be distinguished. The first two progenies were retested in Expts 3.3 and 3.4. The results were very similar. The log numbers of cysts after retesting was highly correlated with the log numbers of cysts in Expt 3.2, as presented (r_s=0.94 and 0.96 respectively; P<0.01).

Based on these results, the hypothesis that the resistance to D236 in Multa is based on a single, dominant gene, was tested. Assuming that Multa is heterozygous (simplex) for the resistance, then in case of the first two progenies, a 1:1 segregation would be expected, and with Multa selfed a 3:1 segregation. The number of genotypes per group of highly resistant and susceptible genotypes was determined with a criterion of the log transformed number of cysts being > 1 or ≤ 1, based on the frequency distributions as presented in Figure 3.1a to 3.1c. Four genotypes showed a different classification after retesting. These have been listed separately in Table 3.2. The deviations from the expected segregation patterns were low and not significant (Table 3.2).

The earlier reported resistance of 81-1886-524 to *G. pallida* populations (Dellaert *et al.*, 1988) was confirmed (Table 3.3). The level of this resistance was about equal to that of Multa to P2-22 and Rookmaker in Expt 3.3 (Table 3.3), and again about equal to that of Multa to Rookmaker in Expt 3.4 (Figure 3.2c).

The distribution of genotypes within the Maritta x Multa progeny for log number of cysts numbers with P2-22 and Rookmaker (Expt 3.3) is shown in Figures 3.2a and 3.2b. Very few genotypes as resistant as Multa were found. Tested with these populations, only 9 and 7, respectively, of the 79 genotypes had a significantly lower number of cysts than the susceptible parent Maritta. Only within this progeny and with P2-22 and Rookmaker, low but significant correlations between log numbers of cysts and root score were found (r_s = 0.32 and 0.26 respectively).

Within the Multa x 81-1886-524 progeny, tested with Rookmaker in Expt 3.4, several genotypes were found with the same or fewer cysts than both parents (Figure 3.2c). Within this progeny, tested with Rookmaker, 55 of the 83 genotypes had significantly fewer cysts than Maritta. The on average higher level of resistance of this progeny, compared to that of the Maritta x Multa progeny, is likely to be due to the earlier mentioned resistance of 81-1886-524.

Genotypes with high level resistance to D236 were on average not more resistant to *G. pallida* populations P2-22 and Rookmaker than genotypes without high level resistance to D236 (Table 3.4). This indicates that there is no relationship between the presence of the monogenic resistance and the low level resistance to these populations,

Table 3.2. Segregation of Multa progenies into genotypes, highly resistant or susceptible to *G. pallida* population D236 (Expts 3.2 and 3.3). Genotypes with different classification after retesting, have been listed as unclear.

Progeny	Number of genotypes			Chi-square value with expected segregation pattern		Probability of a larger chi-square value
	Highly resistant	Susceptible	Unclear			
Maritta x Multa	40	39	2	(1:1)	0.006	>0.995
Multa x 81-1886-524	47	36	2	(1:1)	1.467	>0.100
Multa selfed	73	17		(3:1)	1.793	>0.100

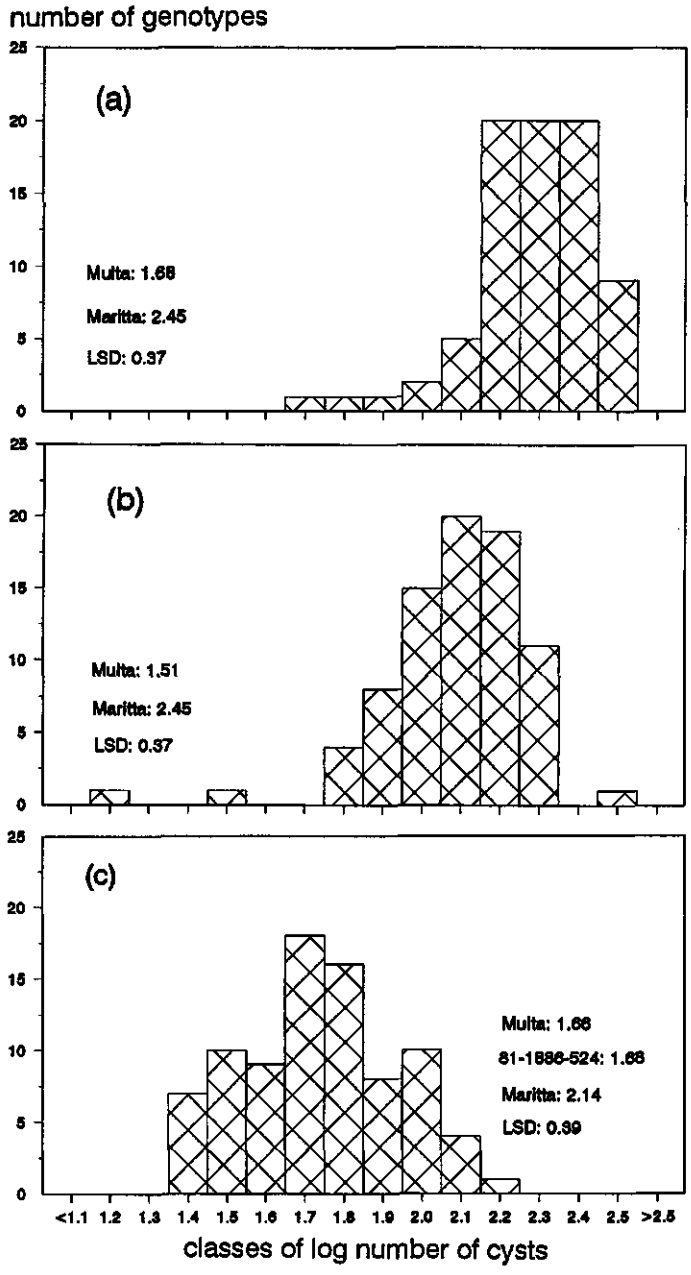


Figure 3.2. Frequency distributions of genotypes for classes of log transformed numbers of cysts. (a): progeny of Maritta x Multa, tested with *Globodera pallida* population P2-22 in Expt 3.3; (b): progeny of Maritta x Multa, tested with *G. pallida* population Rookmaker in Expt 3.3; (c): progeny of Multa x 81-1886-524, tested with Rookmaker in Expt 3.4. Only upper limits of classes are indicated. Values for parents and LSD's ($P<0.05$) are presented in the figures.

Table 3.3. Number of cysts on three genotypes, used as parents of progenies, and the number of cysts relative to the number on Maritta (Expt 3.3). Numbers of cysts, found with the same PCN population and carrying different letters are significantly different from each other (LSR; $P<0.05$).

PCN population	Genotype	Number of cysts	Number of cysts relative to Maritta
P2-22	Multa	64 a	0.22
	81-1886-524	61 a	0.21
	Maritta	297 b	
Rookmaker	Multa	49 a	0.27
	81-1886-524	48 a	0.26
	Maritta	185 b	
D236	Multa	0 a	0.00
	81-1886-524	45 b	0.26
	Maritta	175 c	

Table 3.4. Mean log number of cysts for the Maritta x Multa progeny in Expt 3.3, and the Multa x 81-1886-524 progeny in Expt 3.4, and for genotypes of these progenies, separated into two groups, either highly resistant or susceptible to D236.

Progeny	Number of genotypes	Log number of cysts with		
		D236	P2-22	Rookm.
Maritta x Multa (Expt 3.3):				
All genotypes	79	1.15	2.24	2.05
Resistant to D236	40	0.28	2.23	2.04
Susceptible to D236	39	2.05	2.25	2.06
Multa x 81-1886-524 (Expt 3.4):				
All genotypes	83	0.87		1.69
Resistant to D236	47	0.15		1.69
Susceptible to D236	36	1.81		1.69

and that these populations are highly virulent to the monogenic resistance.

Origin of resistance. The cultivars Record and Oberarnbacher Frühe, ancestors of Multa, did not show any resistance to *G. pallida* populations D236 or P2-22 (Table 3.5). However, high level resistance to D236 was found in eight other cultivars, derived from *S. tuberosum* ssp. *andigena* CPC 1673 (Table 3.5). Only Saturna, Aurora and Provita were found to be highly susceptible to D236. Resistant cultivars were derived from all three CPC 1673 genotypes, used as parent (Table 3.5). The results indicate that the resistance to D236, as found in Multa, is likely to have been derived from *S. tuberosum* ssp. *andigena* CPC 1673. The probability of the number of cultivars with resistance to D236 was calculated. Considering only the cultivars that were derived from three backcrosses with presumably susceptible *S. tuberosum* ssp. *tuberosum* cultivars, seven out of nine cultivars were found to be resistant. Even when assuming quadruplex resistance to D236 in CPC 1673, the probability of such a high number of resistant cultivars after three backcrosses without selection was only 0.051.

Three out of eleven cultivars, Ehud, Prevalent and Marijke, also showed a significantly lower number of cysts than Maritta with P2-22 (Table 3.5).

Discussion

The results presented here give evidence of an until now unknown high level, monogenic resistance to some *G. pallida* populations in the potato cultivar Multa, unnoticed since its release more than 25 years ago. After Dunnett (1962) found monogenic resistance in *S. multidissectum* to virulence group Pa1, this is only the second report of monogenic resistance to *G. pallida*. Resistance to *G. pallida* from *S. tuberosum* ssp. *andigena* CPC 2802, originally attributed to a single H_3 gene (Howard *et al.*, 1970), was later found to show polygenic inheritance (Dale & Phillips, 1982).

A clear distinction between resistance and susceptibility in the frequency distributions of the Multa progenies was observed, which formed a good basis for assessing segregation patterns. The arbitrary division of a continuous distribution for numbers of cysts into categories of resistant and susceptible genotypes may lead to wrong conclusions on numbers of genes involved (Luedders, 1989).

The high level resistance of Multa was found to be effective to two populations of *G. pallida*, D234 and D236. Two dimensional gel electrophoresis (Bakker, 1987) showed that

Table 3.5. Details of pedigree of Multa and its ancestors and eleven *G. rostochiensis* Ro1 resistant cultivars, derived from three *S. tuberosum* ssp. *andigena* CPC 1673 genotypes, the number of backcrosses with *S. tuberosum* ssp. *tuberosum*, and the number of cysts and the number of cysts relative to Maritta, with *G. pallida* populations D236 and P2-22 in Expt 3.5. Numbers of cysts for each population carrying the same letter are not significantly different from each other (LSR; $P<0.05$).

Potato cultivar	CPC 1673 genotype and number of backcrosses			Numbers of cysts with *G. pallida* populations		Number of cysts relative to Maritta with	
	CPC 1673-1	CPC 1673-11	CPC 1673-20	D236	P2-22	D236	P2-22
Multa	2			0 a	100 b	0.00	0.44
Multa ancestors:							
Oberarnb. Frühe				175 b	156 bc	0.78	0.68
Record				154 b	201 bc	0.69	0.88
Other cultivars:							
Saturna	2			193 b	173 bc	0.86	0.75
Aurora	3			280 b	161 bc	1.25	0.70
Ehud	3			4 a	86 b	0.02	0.38
Element	3			0 a	123 bc	0.00	0.54
Prevalent		3		1 a	116 b	0.00	0.51
Provita		3		174 b	155 bc	0.78	0.67
Amigo		2	2	1 a	183 bc	0.00	0.80
Alcmaria			3	1 a	185 bc	0.00	0.81
Amaryl			3	0 a	195 c	0.00	0.85
Bellona			3	0 a	149 bc	0.00	0.65
Marijke			3	0 a	25 a	0.00	0.11
Susceptible standard:							
Maritta				224 a	229 c		

these two populations have highly similar protein patterns, and that their protein patterns differ more from various other *G. pallida* populations investigated than many of those populations amongst each other. It is interesting to note that these differences in protein pattern are reflected by differences in virulence characteristics, as we found these two populations to be avirulent to the reported single resistance gene, while at least two other *G. pallida* populations, P2-22 and Rookmaker, were found to be highly virulent to this gene.

Low level resistance in Multa was found to all other *G. pallida* populations, used in our experiments, but its level varied between *G. pallida* populations. The low level resistance might be explained by these *G. pallida* populations being a mixture of nematodes, virulent and avirulent to the monogenic resistance. However, with the *G. pallida* populations P2-22 and Rookmaker, this explanation was not applicable, as no effect of the monogenic resistance on the resistance level to these populations was found within the Multa derived progenies (Table 3.4). The low level resistance to these populations thus presumably has a different genetic basis. This resistance seems to be mainly recessively inherited, as very few genotypes within the Maritta x Multa progeny were found, which were equally resistant as Multa, while many genotypes were found, which were as susceptible as Maritta. In this case, root system size may have had a small effect on numbers of cysts found, as a low but significant correlation with root score was observed.

Although the original *S. tuberosum* ssp. *andigena* CPC 1673 ancestors of Multa and related cultivars have been lost, it is clear that they have been the source of the major gene conferring resistance to *G. pallida* population D236. Other ancestors of Multa did not show this resistance while many of the other *G. rostochiensis* resistant cultivars, derived from three different CPC 1673 genotypes, were found to possess the high level resistance to D236. The high number of cultivars, derived from CPC 1673, with resistance to D236 may be explained by fairly close linkage of this resistance gene with the H_1 gene, conferring resistance to *G. rostochiensis* virulence group Ro1, or by such a linkage with some agronomical trait, for which cultivars were selected. Linkage to the H_1 gene of some unknown factor increasing *G. pallida* resistance was suggested by Dale and Phillips (1984). An explanation by assuming quadruplex resistance in the CPC 1673 sources is highly improbable, but could not be rejected, with a probability only just above 0.05. Possibly, the resistance gene found in Multa may have also been incorporated in some cultivars, derived from CPC 1673, in which Parrott and Trudgill (1970) found resistance to some *G. pallida* populations.

The source of the low level resistance in Multa remains unclear, but the mainly recessive mode of inheritance does suggest that various ancestors may have been heterozygous for this resistance. The source of the low level resistance, found in some other CPC 1673 derived cultivars, also remains unclear. Low level resistance has been reported also in other cases of major gene resistances to PCN. Dunnett (1962) found with *S. multidissectum* hybrids, that apart from the H_2 resistance gene to the PCN population Duddingston, also a low level of resistance to Duddingston was found in part of the progeny, lacking the H_2 gene. This low level resistance thus has a genetic basis, different from the H_2 gene. In cultivars with *G. rostochiensis* resistance from *S. tuberosum* ssp. *andigena* CPC 1673, apart from high level resistance to virulence group Ro1, a varying, lower level of resistance to several other *G. rostochiensis* populations has been found but it is not known whether this low level resistance is caused by the H_1 gene, or has a different genetic basis (Janssen *et al.*, 1990).

With the high level *G. pallida* resistance to populations D234 and D236, a new virulence group within *G. pallida* may be distinguished in the way, described in Chapter 2. However, the frequency of populations, avirulent for this resistance, is presumably not very high, since the resistance to D236 has not been reported before, even although many cultivars derived from CPC 1673 have been grown now for more than 20 years.

This research has led to the distinction of a single gene conferring high level resistance to *G. pallida* and low level resistance to *G. pallida* with a mainly recessive type of inheritance. The discovery of the single gene for resistance to *G. pallida* may stimulate research for single genes with resistance to a wider spectrum of *G. pallida* populations. Such resistance genes can be incorporated by breeders in new potato cultivars more easily than the polygenic resistance from *S. vernei*, as commonly used now.

CHAPTER 4

Differences in virulence between some potato cyst nematode populations to potato genotypes with monogenic resistance to *Globodera pallida* from *Solanum multidissectum* or *S. tuberosum* ssp. *andigena* CPC 1673

Abstract

The number of cysts, formed by six potato cyst nematode populations, was compared for two genotypes, with monogenic resistance to *G. pallida* derived from either *S. multidissectum* or *S. tuberosum* ssp. *andigena* CPC 1673. Differential interaction between populations and the genotypes indicated that the genes for resistance from the two sources are different.

Table 4.1. Mean weight of samples of cysts used as inoculum and the mean number of juveniles hatched *in vitro* from these samples, of the six potato cyst nematode populations used, and their species and place of origin (GB= Great Britain, NL=the Netherlands).

Populations	Species	Place of origin	Weight inoculum (mg)	Number of juveniles
Duddingston	*G. pallida*	Duddingston, Scotland, GB	1.38	4399
Glarryford	*G. pallida*	Glarryford, N. Ireland, GB	1.42	6390
D234	*G. pallida*	Smilde, NL	1.42	7490
D236	*G. pallida*	Anlo, NL	1.35	7280
Rookmaker	*G. pallida*	Valthe, NL	1.53	4910
Mierenbos A	*G. rostochiensis*	Wageningen, NL	1.49	7910

Introduction

Potato cyst nematodes (PCN), *Globodera pallida* and *G. rostochiensis*, are major pests of the potato crop. Major gene resistance to *G. pallida* was first demonstrated by Dunnett (1962) in *Solanum multidissectum* PH 1366. This gene (H_2) confers nearly complete resistance to *G. pallida* populations with pathotype designation Pa1 (Kort *et al.*, 1977). A second major gene resistance to *G. pallida* was reported in Chapter 3. No or very few cysts of *G. pallida* populations D234 and D236 are formed on genotypes with this resistance gene. The gene presumably originates from *S. tuberosum* ssp. *tuberosum* CPC 1673, which is also the source of the widely used H_1 resistance gene to *G. rostochiensis* (Ellenby, 1952).

S. multidissectum and *S. tuberosum* ssp. *andigena* are related species. They both belong to the Series Tuberosa from the subsection Potatoe of *Solanum* and originate from the Andes-region in South-America (Hawkes *et al.*, 1979). Chavez (1984) suggested that *S. tuberosum* ssp. *andigena* may have developed amongst others from *S. multidissectum*. Hosaka (1986) found considerable resemblance between the chloroplast DNA of *S. tuberosum* ssp. *andigena* and *S. multidissectum*. It is possible that a similar gene for resistance to *G. pallida* occurs in genotypes of both species. In that case, populations with pathotype designation Pa1 are expected to be avirulent to the *G. pallida* resistance gene, described in Chapter 3. The *G. pallida* populations D234 and D236 are then expected to be avirulent to the H_2 gene.

In this study, the possible similarity of the H_2 gene from *S. multidissectum* and the recently discovered gene for *G. pallida* resistance from *S. tuberosum* ssp. *andigena* CPC 1673 was investigated by comparison of the virulence level of some PCN populations to potato genotypes with monogenic resistance from these sources.

Materials and methods

Three potato genotypes were used. The genotypes were P55/7, with monogenic resistance to *G. pallida* from *S. multidissectum* PH 1366 (Dunnett, 1962), the cultivar Multa, with monogenic resistance to *G. pallida*, presumably derived from *S. tuberosum* ssp. *andigena* CPC 1673 (Chapter 3), and the susceptible standard cultivar Maritta. P55/7 has, apart from *S. multidissectum*, also *S. tuberosum* ssp. *andigena* CPC 1685 as

Table 4.2. Number of cysts on three potato genotypes (P55/7, with monogenic resistance to *G. pallida* from *S. multidissectum*; Multa, with monogenic resistance to *G. pallida* from *S. tuberosum* ssp. *andigena* CPC 1673, and the susceptible standard cultivar Maritta), with six PCN populations (Table 4.1). For each PCN population separately, means carrying different letters, are significantly different from each other (LSR; $P<0.05$).

Populations	Species	Potato genotypes		
		P55/7	Multa	Maritta
Duddingston	*G. pallida*	1 a	53 b	168 c
Glarryford	*G. pallida*	2 a	64 b	275 c
D234	*G. pallida*	31 b	0 a	208 c
D236	*G. pallida*	50 b	0 a	191 c
Rookmaker	*G. pallida*	7 a	73 b	156 c
Mierenbos A	*G. rostochiensis*	94 a	166 b	246 c

ancestor (A. Thompson, pers. comm.). *S. tuberosum* ssp. *andigena* CPC 1685 is a source of the H_1 gene (Huijsman, 1955), but P55/7 does not carry this gene (Kort *et al.*, 1977).

Stem cuttings of the genotypes were made. After three weeks, rooted cuttings were transplanted in 150 ml pots, filled with 75 g peat soil. Pots were inoculated at planting with samples of a preset volume of cysts (Vinke *et al.*, 1992) of six PCN populations (Table 4.1). Two populations, which have been designated as pathotype Pa1, originate from Great Britain. Pa1 has not been reported to occur in the Netherlands. The weight of cyst samples of the populations was determined, as this has been shown to represent the number of eggs in the samples (Chapter 1). The mean number of juveniles, hatched from four samples per PCN population, was determined *in vitro* after 6 weeks exposure to standard potato root diffusate with regular changes of the diffusate. The procedure is described in Chapter 7. Inoculated pots were placed in a greenhouse, at an average day temperature of 20°C, and were watered twice daily. After 6 weeks, when the cysts were well developed, rootballs were removed from the pots and the number of newly formed cysts, visible on the surface of the rootball, was counted. This number is representative of the total number of newly formed cysts (Forrest & Holliday, 1979). A score (1-5) was given for the size of the root system. The experiment was carried out with 15 replicates.

The observed numbers of newly formed cysts were transformed to logarithms with base 10 of the numbers of cysts plus 1, to obtain homogeneity of variance, necessary for analysis of variance. Correlations between data, which did not show a normal distribution, were calculated using Spearman's rank correlation coefficient (r_s).

Results and discussion

The mean weight of samples of cysts for inoculum varied little between PCN populations (Table 4.1). Also relatively little variation in weight between samples within populations was found, with the mean coefficient of variation for sample weight within populations being 0.08. The number of viable eggs per sample differed considerably more between PCN populations, as shown by the variation in numbers of juveniles, hatched *in vitro* (Table 4.1). These differences may partially explain the observed differences in numbers of cysts on the susceptible standard cultivar Maritta (Table 4.2). The root score was not significantly correlated (r_s=0.05) with the log transformed number of cysts.

Very few cysts were formed on P55/7 with the *G. pallida* populations Duddingston and Glarryford (Table 4.2). This is in accordance with the classification of these populations as belonging to pathotype Pa1, for which P55/7 has been chosen as the differential in the pathotype scheme of Kort *et al.* (1977). No cysts were found with *G. pallida* populations D234 and D236 on Multa. This confirms earlier observations of very few or no newly formed cysts on Multa with these populations (Chapter 3). However, a considerably higher number of cysts was found with D234 and D236 on P55/7, and with Duddingston and Glarryford on Multa (Table 4.2). These differences show clearly that the major genes for resistance from *S. multidissectum* and from *S. tuberosum* ssp. *andigena* CPC 1673 are different genes.

Multa had a significantly lower number of cysts than Maritta with Rookmaker and *G. rostochiensis* Mierenbos A, which confirms earlier results (Chapter 3). The lower number of cysts on Multa was also found with Duddingston and Glarryford, indicating that the earlier observed low level resistance of Multa to *G. pallida* populations is also found with populations, classified as Pa1. This low level resistance presumably has a separate genetic basis from the monogenic resistance to D234 and D236 (Chapter 3).

Significantly less cysts were also found on P55/7, as compared to Maritta, with *G. pallida* D234, D236 and Rookmaker and *G. rostochiensis* Mierenbos A. Kort (1974), Stone *et al.* (1979) and Ross (1986) reported already considerably less cysts on P55/7, compared to susceptible standard cultivars, with various PCN populations. The results presented here show a very low number of cysts of Rookmaker on P55/7. Rookmaker is a *G. pallida* population with a relatively high virulence level to several genotypes with resistance from *S. vernei*, the widely used source of *G. pallida* resistance in potato breeding (Chapter 2). It would be very interesting for potato breeding if the resistance to Rookmaker is conferred by the H_2 gene. However, this resistance may also have a separate genetic basis. In as well the *S. multidissectum* as the S. tuberosum ssp. *andigena* CPC 1685 ancestor of P55/7, apart from the H_2 and the H_1 gene respectively, additional resistance presumably occurred (Dunnett, 1962; Trudgill & Parrott, 1969).

The results, presented here, clearly show that the major genes conferring *G. pallida* resistance from *S. multidissectum* and *S. tuberosum* ssp. *andigena* CPC 1673 are different. A new virulence group within *G. pallida* might be distinguished (Chapter 2). At least, the results demonstrate until now unknown differences in virulence for resistance genes between *G. pallida* populations.

CHAPTER 5

Differences in tolerance in field and pot tests between potato genotypes, resistant to *Globodera pallida*

Abstract

Tolerance to *Globodera pallida* of fifteen potato genotypes with a high level of resistance to *G. pallida* was assessed by the tuber yield in a heavily *G. pallida* infested field, proportional to the tuber yield in nematicide treated strips in the same field, in each of two years. Tolerance of these genotypes was also assessed in pots in the greenhouse by the total biomass after 35 and 70 days growing in heavily *G. pallida* infested soil, proportional to the biomass in uninfested soil. Large differences in tolerance between the genotypes were observed. High correlations for tolerance assessments were found between the two field experiments and between field and pot experiments. High correlations were also found between tuber yields in untreated strips in the field and biomass of infested plants in pots. Some genotypes were considerably more intolerant to *G. pallida* than standard cultivars, but others were at least as tolerant. No relationship between tolerance and maturity score was found. Infestation with *G. pallida* in the greenhouse decreased total leaf area mainly by reducing the leaf area per leaf and much less by reducing the number of leaves. Shoot and root dry weight were about equally affected by the nematode.

Introduction

Potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) can cause considerable damage to the potato crop (Seinhorst, 1982; Brown & Sykes, 1983). The use of tolerant potato cultivars may reduce this damage. The tolerance of potato genotypes to potato cyst nematodes (PCN) is compared at the same initial PCN density (Trudgill, 1986). Resistance is important for the control of PCN, but does not prevent damage, as juveniles are able to penetrate the roots of both resistant and susceptible genotypes (Trudgill, 1986). Therefore, to maximize PCN control and tuber yield, tolerance should be combined with resistance (van der Wal, 1978; Trudgill, 1991).

Tolerance assessments in the field have been described by Dale *et al.* (1988) and Phillips *et al.* (1988a). Huijsman *et al.* (1969) and Seinhorst and den Ouden (1971) assessed tolerance by measuring plant biomass in pot trials. However, the relationship between tolerance tests in the field and in pots in the greenhouse has not been intensively investigated. Trudgill and Cotes (1983) reported a good relationship between field and greenhouse tolerance tests, but their research was carried out with few genotypes, and they did not present correlations between field and greenhouse tolerance data.

Evans and Haydock (1990) have reviewed research on plant characters, possibly related to tolerance. Differences for hatching, extent of invasion of roots, root vigour, root morphology, degree of necrosis and resistance to secondary infection have been observed and may be related to tolerance differences. However, the relative importance of these factors for the explanation of tolerance is still largely unknown.

The aim of this research was to identify differences in tolerance to *G. pallida* between resistant genotypes in the field, and to study the relationship between these differences and various plant growth characters as observed in pots in the greenhouse.

Materials and methods

Genotypes. Fifteen genotypes were used, originating from the PCN resistance breeding programme, carried out at the Centre for Plant Breeding and Reproduction Research CPRO-DLO. They have been selected for good agronomical characteristics and a high level of resistance to *G. pallida*. The resistance was mainly derived from *Solanum vernei*, but also other wild species with resistance, such as *S. oplocense*, were used in the

resistance breeding programme (Dellaert & Vinke, 1987). The genotypes had previously been tested for resistance to *G. pallida* populations P2-22 (pathotype designation Pa2) and Rookmaker (pathotype designation Pa3). The number of cysts formed on these genotypes was on average 3% and 4% respectively of the number of cysts, found on a susceptible standard cultivar, and no genotype-PCN population combination showed more than 10% of the cysts on the susceptible standard cultivar (Vinke, pers. comm.). Maturity scores (1-9) were obtained in 1988 and 1989 in field trials on PCN free clay soils, and were based on observations of leaf senescence, with a score 1 being very late and 9 being very early. The genotypes were found to vary considerably for earliness.

Three standard cultivars, Elles, Darwina and Mentor were also used in the field tests for tolerance. Elles and Darwina are resistant to *G. pallida* at varying levels (Bakker, 1988; Chapter 2), while Mentor is thought not to carry PCN resistance (*Anon.*, 1992c).

Field tests. The 15 highly resistant genotypes and 3 standard cultivars were grown in two years in fields with sandy humous soil and heavily infested with *G. pallida* (Table 5.1). For the 1989 and 1990 experiments different fields were used, both located near Smilde in the north-east of the Netherlands. Strips with a width of 3 m were treated with the nematicide Monam, with active ingredient metham-sodium. Plots with six seed tubers per genotype were laid out across treated and untreated strips of the field, resulting in three seed tubers in each strip, separated by a seed tuber of a red-skinned cultivar, and with red-skinned tubers at the borders of each plot. Planting density was 30 x 75 cm. The fields were fertilized and fungal diseases were controlled following normal agricultural practice. Harvest was carried out at full maturity. For each plot, tuber yield on the heavily infested, untreated strip, and tuber yield on the neighbouring nematicide treated strip were determined. Proportional yields and absolute yield reductions within plots were calculated and analyzed. The experiments were thus carried out in a split plot design, with four replicates per genotype.

Pot test. The 15 highly resistant genotypes were grown in clay pots, containing 3 kg soil. Sandy loam soil without organic matter was used, obtained from just reclaimed land. The soil was fertilized with 100 ml/kg soil Steiner nutrient solution and 1 g/kg soil NPK 12-18-10, and either infested with cysts, equivalent to a density of 275 eggs/g soil, or uninfested. Cysts originated from various *G. pallida* populations, which had been reared as a mixture of these populations in the greenhouse. Planting was done in April. Eye pieces of equal size and with a single sprout were used for planting and plants grew with a single stem. Pots were spaced 30 x 75 cm in the greenhouse, and watered twice

Table 5.1. Agronomic details of field experiments in 1989 and 1990.

	1989	1990
G. pallida infestation (juv./g soil)	31	20
date of nematicide treatment	30 March	29 March
planting date	4 May	24 April
harvesting date	24 October	22 October
pH	5.3	4.8
Percentage organic matter	13	29

daily when necessary. Stem length was measured at weekly intervals. Plants were harvested after 35 and 70 days. At the first harvest, the number of leaves longer than 1 cm was counted and the leaf area per leaf was measured with a Li-Cor LI3100 leaf area meter. At each harvest, shoots, including stolons, roots and tubers were dried and weighed separately. The experiment was carried out, using a split plot design, with nematodes as mainplot treatment, and genotypes as subplot treatment, with three replicates.

Statistical analysis. Correlations between data were determined by Spearman's rank correlation coefficient (r_s), which does not require normal distribution of data. Significance thresholds were, unless indicated otherwise, determined with ($P<0.05$).

Results

Considerable damage due to *G. pallida* was found in all experiments. In the field in 1989 and 1990, average tuber yield was reduced by 49% and 54% respectively on untreated strips, compared to those, treated with nematicide (Table 5.2). In 1990, the residual variance was much greater than in 1989, which may have been due to night frost in the

last week of May, killing many of the young sprouts. Data for two genotypes with extremely high variance in proportional tuber yield were considered as unreliable and excluded from further analysis. After 35 days, a mean of 76% reduction of total biomass was found due to *G. pallida* infestation in pots, and after 70 days a mean of 88% reduction was found (Table 5.2). At that date, some plants of very intolerant genotypes had died.

Large differences in tolerance in the field between genotypes were found (Table 5.2). Yields on the heavily infested strips varied from 0.16 to 1.40 kg/plant in 1989 and from 0.07 to 1.43 kg/plant in 1990. Some of the resistant genotypes tested, like Ve8542 and Ve8550, yielded very poorly on the heavily infested strips (Table 5.2). The standard cultivar Darwina yielded significantly less than Elles on these strips, but significantly more than some of the fifteen resistant genotypes tested. Other genotypes, however, gave a higher yield than Darwina on the heavily infested strips. In pots, large differences in total biomass between the fifteen tested genotypes were found, after as well 35 as 70 days (Table 5.2).

The ratio of tuber yield on infested strips and tuber yield on nematicide treated strips and the ratio of biomass in infested pots and biomass in uninfested pots were calculated. They are referred to as proportional yield and proportional biomass, respectively. Differences in proportional yield of genotypes were large in both years, and some of the resistant genotypes tested were considerably less tolerant than all standard cultivars in both years (Figure 5.1). Proportional yield was highly correlated between the two field assessments (Table 5.3). Proportional yield and proportional biomass were also highly correlated, especially for proportional biomass after 70 days (Table 5.3). Correlations between absolute tuber yield or biomass obtained with the high *G. pallida* infestation only were also high between field and pot assessments. The correlations between absolute differences in yield or biomass were much lower (Table 5.3).

Tuber yields on nematicide treated and untreated strips in the field were significantly correlated (r_s=0.66 and 0.63). In the greenhouse, the correlation between mean biomass per genotype in infested and uninfested soil was significant after 35 days (r_s=0.55) and low and not significant after 70 days (r_s=0.12). The rank correlation between root weight per genotype in infested and uninfested soil was 0.40 and 0.37 after 35 and 70 days respectively (n.s.;P>0.05).

Although the tested genotypes differed considerably in maturity score, no clear association between tolerance and maturity score was found (Figure 5.2). Correlations between proportional tuber yield and maturity score were low and not significant, with

Table 5.2. Tuber yield (kg fresh weight/plant) in heavily *G. pallida* infested strips and strips, treated with nematicide, in the same field for fifteen highly resistant potato genotypes and three standard cultivars, and biomass (g dry weight/plant) in *G. pallida* infested and uninfested pots after 35 and 70 days for the fifteen resistant genotypes.

Genotypes	Yield (1989)		Yield (1990)		Biomass 35 days		Biomass 70 days	
	Treated	Untreated	Treated	Untreated	Uninfested	Infested	Uninfested	Infested
Ve8515	1.65	1.08	1.68	1.25	3.64	1.47	13.88	1.99
Ve8530	1.09	0.33	0.74	0.22	5.04	0.63	15.79	0.53
Ve8536	1.20	0.26	0.37	0.07	3.97	0.57	13.60	0.42
Ve8538	1.54	1.09	1.68	0.94	3.60	0.79	12.21	2.96
Ve8542	1.39	0.36	1.07	0.09	2.96	0.42	13.70	0.53
Ve8550	1.04	0.16	0.56	0.07	3.19	0.30	13.99	0.37
Ve8556	1.35	0.70	-	-	3.40	1.12	14.63	1.39
Ve8557	1.47	0.73	0.65	0.34	4.48	1.14	13.65	1.71
Ve8567	1.44	0.80	1.04	0.42	3.32	0.90	12.59	1.49
Ve8571	1.36	0.87	0.78	0.70	4.07	0.95	14.40	3.19
Ve8577	1.47	0.47	1.04	0.36	4.39	0.99	15.30	1.72
Ve8590	1.59	0.99	1.03	0.50	4.65	1.75	14.21	3.37
Ve8592	1.44	1.01	-	-	3.76	0.92	12.14	2.10
Ve8598	1.34	1.08	0.88	0.60	4.42	1.15	15.57	2.66
Ve85103	1.32	0.69	0.81	0.18	3.21	0.79	12.38	1.21
Standard cvs								
Darwina	1.27	0.78	1.40	0.81	-	-	-	-
Elles	2.03	1.40	2.11	1.43	-	-	-	-
Mentor	1.57	1.13	1.81	1.16	-	-	-	-
Mean	1.38	0.71	0.95	0.44	3.87	0.93	13.87	1.71
LSD	0.43	0.27	0.57	0.40	1.74	0.44	n.s.	0.81

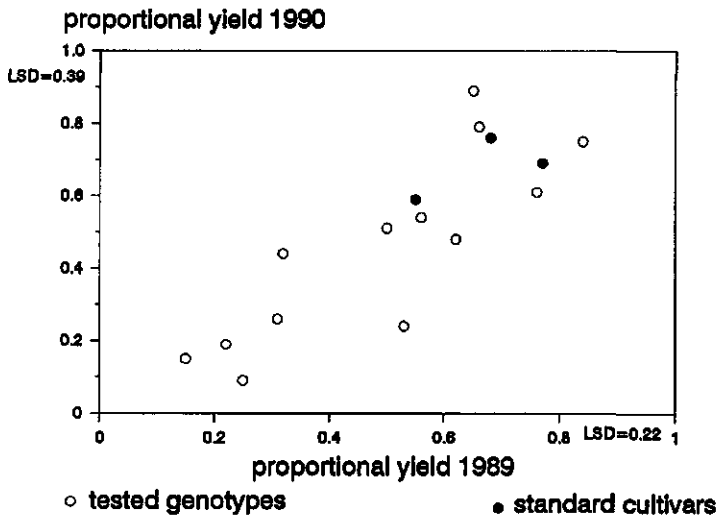


Figure 5.1. Relationship between tuber yield on heavily *G. pallida* infested, untreated strips, proportional to tuber yield in adjacent nematicide treated strips in the field, in 1989 and 1990, for thirteen *G. pallida* resistant genotypes and three standard cultivars.

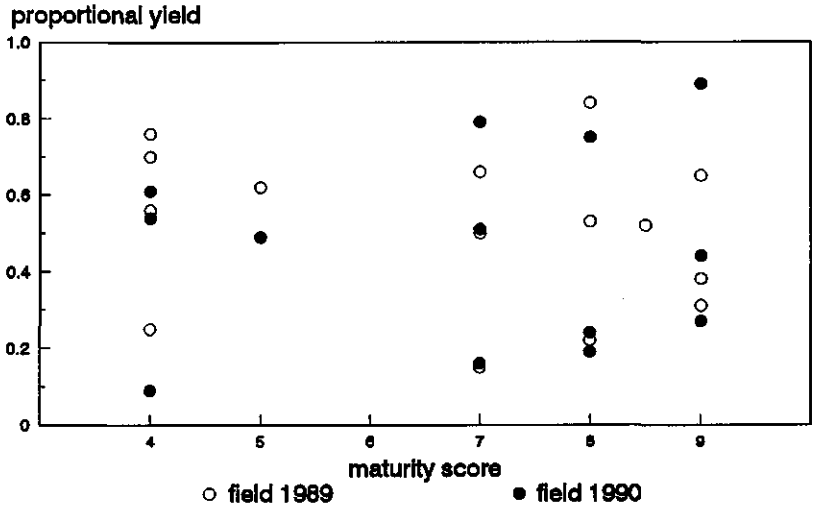


Figure 5.2. Relationship between proportional tuber yield in *G. pallida* infested fields in 1989 and 1990 (see Figure 5.1) for fifteen and thirteen resistant genotypes respectively, and maturity as scored in uninfested fields.

Table 5.3. Spearman rank correlation coefficients between field and pot assessments of tolerance, calculated as the tuber yield or biomass under heavy *G. pallida* infestation proportional to the tuber yield or biomass in control conditions, as absolute tuber yield or biomass differences between these two conditions, and absolute yield or biomass under heavy *G. pallida* infestation only.

	Proportional yield or biomass			Yield in infested strips or biomass in infested pots			Absolute yield or biomass differences		
	Field 1989	Field 1990	Pot 35 days	Field 1989	Field 1990	Pot 35 days	Field 1989	Field 1990	Pot 35 days
Field 1990	0.88**			0.92**			0.35		
Pot 35 days	0.67**	0.63*		0.74**	0.75**		0.27	-0.36	
Pot 70 days	0.88**	0.79**	0.65*	0.88**	0.87**	0.81**	0.65*	-0.18	0.58*

(*) significant (P<0.05)
(**) significant (P<0.01)

Table 5.4. Spearman rank correlation coefficients between stem length of *G. pallida* infested plants in the greenhouse at 14 to 35 days after planting and biomass in pots of *G. pallida* infested plants after 35 days, and proportional tuber yield in the field in two years.

Days after planting	Biomass infested plants 35 days	Proportional yield 1989	Proportional yield 1990
14	0.77**	0.43	0.51
21	0.64**	0.62*	0.66**
28	0.82**	0.68**	0.75**
35	0.84**	0.74**	0.79**

(*) significant ($P<0.05$)
(**) significant ($P<0.01$)

values of -0.12 and -0.07 in 1989 and 1990 respectively. In the greenhouse, correlations between biomass of infested plants and maturity score were also low and not significant, both after 35 days (r_s=0.03) and 70 days (r_s=-0.07).

Measurements of stem length showed that the length of infested intolerant plants did not increase anymore after 28 days, much earlier than for uninfested plants (Figure 5.3). Stem length of infested plants of tolerant genotypes increased for a much longer period. Stem length of infested plants in the greenhouse was not only highly correlated with biomass of infested plants after 35 days, but was also correlated with proportional tuber yield in the field (Table 5.4).

Damage due to *G. pallida* was associated with a reduction of shoot and root weight. The reductions of shoot and root weight for each genotype, after 35 days, were about equal, although with intolerant genotypes a slightly larger reduction for root weight than for shoot weight was observed (Figure 5.4). After 35 days, tuber weight was still low. For control plants, tubers accounted for on average 5% of the total biomass, for infested plants tubers accounted for 1% of the total biomass.

After 70 days, tubers accounted for 65% of the biomass of control plants and for 40% of the biomass of infested plants. The partitioning of biomass over plant parts varied much between genotypes. Early maturity was positively correlated with tuber weight and negatively correlated with shoot and root weights of control plants (Table 5.5). The same was found with infested plants, but correlations were lower. Tolerance, as assessed in

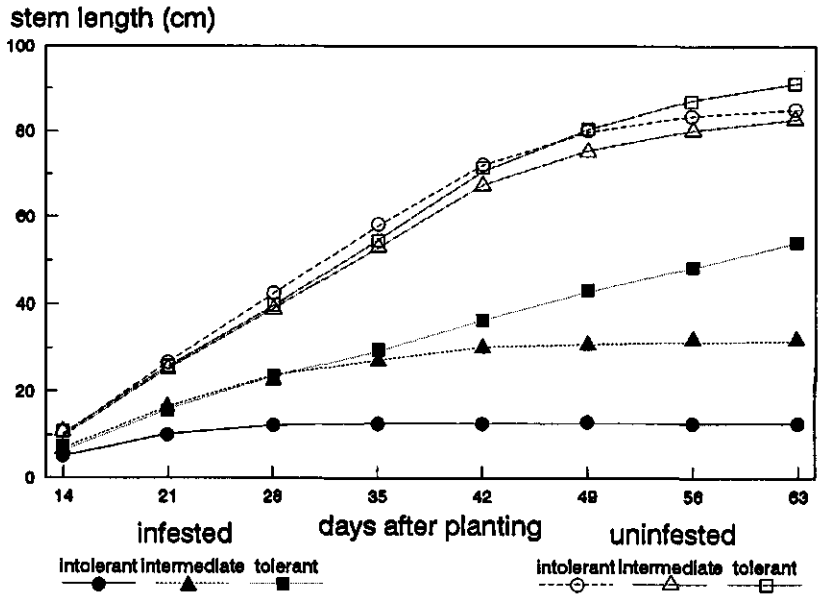


Figure 5.3. Mean stem length at weekly intervals for the five most tolerant, the five intermediate and the five most intolerant genotypes for *G. pallida* infested and uninfested plants in the greenhouse. Tolerance classifications were based on dry weights of infested plants after 70 days in the greenhouse.

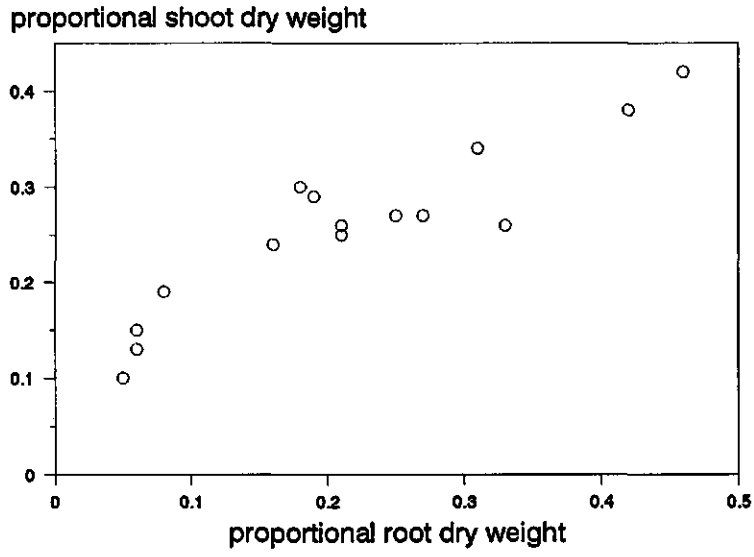


Figure 5.4. Relationship between proportional shoot dry weight and root dry weight in *G. pallida* infested soil, compared to *G. pallida* free soil, 35 days after planting in the greenhouse, for fifteen *G. pallida* resistant genotypes.

field experiments, was positively correlated with shoot and root weight of infested plants, but not with weights of plant parts of uninfested plants. The correlation between reduction of root and shoot weight after 70 days was again high (r_s=0.90; P<0.01).

Damage due to PCN was clearly associated with a reduction of total leaf area, measured in the greenhouse after 35 days. For all genotypes the number of leaves formed was considerably less reduced than the mean leaf area per leaf (Figure 5.5). The correlation between leaf area per leaf and total biomass was high (r_s=0.87; P<0.01). The correlation between number of leaves and total biomass was lower, but still significant (r_s=0.68; P<0.01).

Discussion

Clear differences in tolerance were observed between the tested *G. pallida* resistant genotypes. These differences appeared to be expressed better by the proportional yield and biomass or absolute yield and biomass with heavy *G. pallida* infestation than by absolute differences in yield and biomass between infested and control conditions, since with the latter much lower correlations between field tests, and between field and pot tests were found. The high correlations obtained with absolute yield and biomass indicate that control treatments might not be necessary for the assessment of tolerance. This confirms earlier results of Dale *et al.* (1988).

High correlations between field and pot assessments of tolerance were found, despite considerable differences in the way of measuring. In pots, total biomass of genotypes was measured in PCN-free and infested soil. In the field, tuber yield was measured on untreated and nematicide treated strips. Nematicide treatment does not completely control PCN and may also have other effects. The high correlations found indicate that assessment for tolerance may be carried out in pots with a high PCN density in the greenhouse. Assessment may even be carried out during early plant growth, by simply scoring stem length.

Some of the fifteen resistant genotypes tested in our trials were highly intolerant to *G. pallida*, when compared to the standard cultivars. The cultivar Darwina has been reported to be relatively intolerant and the cultivar Elles to be very tolerant, and considerably more tolerant than the cultivar Mentor (Velema & Boerma, 1990). The differences in yield between the standard cultivars on the untreated strips confirm these findings (Table 5.2).

Table 5.5. Spearman rank correlation coefficients between dry weights of plant parts after 70 days in pots and proportional tuber yield in the field, and maturity score.

	Uninfested plants			Infested plants		
	Shoot	Roots	Tubers	Shoot	Roots	Tubers
Proportional yield 1989	0.32	0.40	-0.18	0.86**	0.91**	0.27
Proportional yield 1990	0.18	0.27	-0.01	0.78**	0.74**	0.45
Maturity score	-0.65**	-0.51	0.89**	-0.34	-0.14	0.64**

(*) significant ($P<0.05$)
(**) significant ($P<0.01$)

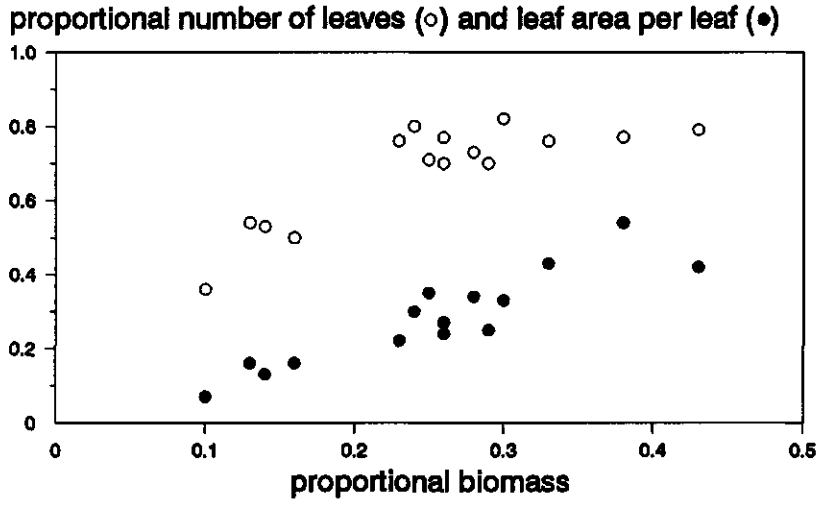


Figure 5.5. Relationship between proportional number of leaves and proportional leaf area per leaf in *G. pallida* infested soil, compared to *G. pallida* free soil, and proportional biomass 35 days after planting in the greenhouse, for fifteen *G. pallida* resistant genotypes.

Some resistant genotypes, at least as tolerant as the standard cultivars were found, indicating that high levels of resistance and tolerance can be combined. The low tolerance of some of the resistant genotypes emphasizes the need for selection against intolerance in a *G. pallida* resistance breeding programme (Evans & Haydock, 1990).

A significant correlation between differences in biomass production of infested and uninfested plants was found after 35 days, but not after 70 days. Thus, the contribution of plant vigour to tolerance remains unclear. The root system size of uninfested plants was apparently not associated with tolerance, as no high correlations for root weights of infested and uninfested plants were found after 35 and 70 days. In the field, tuber yield in nematicide treated strips was highly correlated with tuber yield in untreated plots, which was also found by Dale *et al.* (1988). This correlation may be due to some PCN damage in the treated plots, as nematicide treatment does not completely control PCN.

The maturity score was found to be related to the amount of tubers after 10 weeks, but not to tolerance. Indications were obtained from grafting studies with a tolerant and an intolerant cultivar that late maturity may contribute to tolerance (Trudgill, 1987a). However, with the set of genotypes tested here, late maturity thus appeared to be not a major factor of tolerance. The results suggest that early and tolerant cultivars may be obtained through selection.

Shoot weight and root weight were about equally affected by PCN. However, Trudgill and Cotes (1983) reported that PCN decreased the shoot/root ratio. The results reported here show that PCN damage is not necessarily associated with a lower shoot/root ratio. Differences in reduction of shoot weight thus may be explained as an effect of differences in reduction of root weight, since the roots are primarily affected by PCN. As a consequence of the reduced root system, intolerant genotypes may exploit less volume of soil for mineral acquisition (Evans & Trudgill, 1992). Mineral content was reduced more in intolerant than in tolerant genotypes (Trudgill, 1987b). The results on root growth are in accordance with those of Stanton and Fisher (1988), who found that a high reduction of root length was correlated with intolerance of wheat genotypes to *Heterodera avenae*. Research on mechanisms of PCN tolerance should be directed first to factors explaining the observed differences in root growth. Evans & Haydock (1990) suggested that the amount of necrosis around invading juveniles in roots of genotypes with resistance from *S. vernei* may be the cause of differences in tolerance, but no experimental evidence for this hypothesis has been reported yet.

The correlation between tolerance in the field and the reduction of leaf area in the greenhouse confirmed results of Dale & Brown (1989), who found a high correlation between foliage score and tolerance data in the field. The reduction of total leaf area was mainly caused by a reduction of leaf area per leaf, and less by a reduction in number of leaves. The effect of PCN on the number of leaves reported here might even be overestimated, because only leaves longer than 1 cm were counted. Infested plants presumably had more leaves with length less than 1 cm. A relatively slow development of infested plants, a possible nematode damage mechanism (Seinhorst, 1979), thus appeared not to be a main factor for damage in our experiment. The results presented here largely confirm earlier findings by Schans and Arntzen (1991), who found a large effect of PCN on the total leaf area of four cultivars in pots, but no effect of PCN on the number of leaves formed.

The results presented here show a good relationship between field and pot tolerance assessments, for a set of genotypes, which differed much for tolerance. Because of the

observed large differences in tolerance between resistant genotypes, selection for tolerance in *G. pallida* resistance breeding programmes is advised. The results presented here indicate that tolerance assessment may be carried out in the field, but also in the greenhouse, during early growth. The causes of the differences in tolerance, however, remain to be investigated.

CHAPTER 6

Inheritance of tolerance of *Globodera pallida* and the relationship between tolerance of and resistance to *G. pallida* in potato genotypes

Abstract

Tolerance of and resistance to *Globodera pallida* of two potato progenies, segregating for these characteristics, were assessed in pots in the greenhouse. The level of tolerance varied widely between genotypes of the progenies, with a considerable number of genotypes being significantly more tolerant than the intolerant parent genotype. The levels of resistance and tolerance in the progenies were not significantly correlated. No indications were obtained that the ranking of genotypes for tolerance is different at different *G. pallida* densities in the soil. The results confirm that screening for tolerance in a *G. pallida* resistance breeding programme is useful and feasible.

Introduction

Potato cyst nematodes (PCN), *Globodera rostochiensis* and *G. pallida*, are major pests of the potato crop. Resistance of potato cultivars is important to control PCN, but resistance should preferably be combined with tolerance (van der Wal, 1978). Large differences in tolerance occur between potato genotypes, resistant to *G. pallida* (Chapter 5).

In potato breeding, selection for specific characteristics should take place early in the course of the breeding programme (Neele, 1991). Then, however, only a few tubers are available per genotype. Screening for tolerance should consequently require little tuber material, space and labour. Tolerance tests may be carried out in large pots in the greenhouse, with less tuber material needed than in the field (Chapter 5). The initial screening for tolerance should preferably be done at a single inoculum density of PCN, as screening at various densities would require considerably more tuber material. Then, ranking of genotypes for tolerance should not change at different densities of PCN. Furthermore, tolerance has to be sufficiently heritable and not linked with undesirable traits.

Resistance to *G. pallida* generally does not confer tolerance, as resistance does not prevent invasion by juveniles (Trudgill, 1986), and resistant plants have been found to suffer considerable damage by *G. pallida* (Elston *et al.*, 1991; Chapter 5). Evans and Haydock (1990), however, hypothesized that intolerance as found in resistant potato genotypes, may be due to pronounced necrosis around invading juveniles in the resistant roots. In that case, resistance might confer intolerance to nematodes.

Reese *et al.* (1988) found a range of tolerance levels to soybean cyst nematode among offspring of crosses between intolerant and tolerant soybean genotypes. No reports about the inheritance of tolerance of *G. pallida*, however, have been published yet. For breeding for resistance to and tolerance of *G. pallida*, knowledge about the inheritance of tolerance and the relationship of tolerance with resistance is useful.

In this study, first the efficacy of screening genotypes for tolerance in relatively small pots with 0.5 kg soil in the greenhouse has been investigated. Then, the tolerance and resistance of progenies of two crosses between a *G. pallida* resistant, but intolerant potato genotype and tolerant, but susceptible potato genotypes were assessed. Finally, the tolerance of the parent genotypes was investigated at various densities of *G. pallida* in the soil.

Materials and methods

In four pot experiments, samples of the same stock of *G. pallida* cysts were used. This stock was also used in previous experiments (Chapter 5).

Experiment 6.1. Aim was to test the efficacy of screening for tolerance in small pots. Fifteen genotypes were used, resistant to *G. pallida*, which had already been tested before for tolerance of *G. pallida* in the field and in pots with 3 kg soil (Chapter 5). Another three genotypes, Cara, Multa and AM78-3778, parents of the progenies in Expts 6.2 and 6.3, were included also. The genotypes were grown in clay pots containing 0.5 kg sandy loam soil, either infested with *G. pallida* cysts, equivalent to a density of 275 eggs/g soil, or uninfested. The soil was fertilized with 100 ml/kg soil Steiner nutrient solution (Steiner, 1968) and 1 g/kg soil NPK 12-18-10. Eye pieces were planted and plants grew with a single stem. The pots were spaced 25 x 25 cm in the greenhouse, and watered twice daily. After 35 days, stem length was measured and plants were harvested. Dry weight of sprouts, including stolons, dry weight of roots and of tubers were determined separately. A split plot design was used, with infested and uninfested treatments as main plots and genotypes as subplots, in four replicates.

Tolerance was assessed either by the absolute dry weight of infested plants or by the dry weight of infested plants proportional to the dry weight of uninfested plants. Correlations with previously obtained tolerance data were determined by the non-parametric Spearman's rank correlation coefficient (r_s).

Experiment 6.2. The tolerance of two progenies of AM78-3778 and two cultivars, Cara and Multa, was tested. The potato genotype AM78-3778 has a high level of resistance to a range of *G. pallida* populations. The resistance of AM78-3778 is derived from various *Solanum vernei* genotypes (Chapter 2). This genotype is an important progenitor of resistance in potato breeding in the Netherlands. AM78-3778 is, however, also highly intolerant to *G. pallida* (Mulder *et al.*, 1991). Cara is not known to have resistance to *G. pallida*, and Multa has been found to possess only a low level of resistance to various *G. pallida* populations (Chapter 3). Both cultivars have been reported to be highly tolerant (Trudgill *et al.*, 1990; Huijsman *et al.*, 1969). Progenies of these crosses were tested for resistance in a preliminary container test (Phillips *et al.*, 1980) with *G. pallida* populations P2-22 and Rookmaker, and sets of genotypes were chosen which included many highly resistant and many highly susceptible genotypes. Thus, 48 genotypes derived from the cross AM78-3778 x Cara, and 47 genotypes derived from the cross AM78-3778 x Multa, and the parent genotypes, were tested for tolerance in an experiment under similar

conditions as described for Expt 6.1. The four replicates of the experiment were planted at weekly intervals.

Experiment 6.3. The resistance of the same genotypes, also tested in Expt 6.2, was tested as described in Chapter 3, in 240 ml pots. With the small amount of inoculum used, damage to the plants affecting the level of resistance (Trudgill, 1991) was avoided. The number of cysts, visible on the rootball, was counted, with the assumption that they are representative of the total number of newly formed cysts (Forrest & Holliday, 1979). The experiment was carried out with three replicates. The numbers of cysts were transformed to the logarithm with base 10 of the numbers plus 1.

Experiment 6.4. The tolerance of the parent genotypes, used in Expts 6.2 and 6.3, was investigated at various densities of PCN. Pots with a volume of 2 liters were filled with 1.5 kg of a mixture of 3:1 peat and sand. Soil was either uninfested or infested with each of nine densities of *G. pallida*, which were 2.1, 4.1, 8.3, 16.6, 33.1, 66.3, 132.5, 265 and 530 eggs/g soil. Pots were spaced 30 x 75 cm in the greenhouse. After 35 and 70 days, dry weight of sprouts including stolons, and dry weight of tubers were determined. The experiment was carried out in a randomized block design, with harvest time as blocks, in two replicates. Other experimental conditions were the same as in Expt 6.1. Regression analysis was conducted, using an inverse linear model, proposed by Elston *et al.* (1991), for regression of tuber yield on density of *G. pallida*. In this way, dry weight of sprouts and tubers together, was expressed by the equation:

$$DW = DW_{max} (1-(1-m)P_i/(c+P_i))$$

in which:

DW = dry weight of sprouts and tubers (g)

DW_{max} = parameter, representing DW without *G. pallida* infestation (g),

P_i = density of *G. pallida* (eggs/g soil)

c = tolerance parameter, representing the slope of the curve (eggs/g soil)

m = tolerance parameter, expressing the minimum fraction of dry weight, found with high densities of PCN

The percentage variance explained by curve fitting was determined for the above mentioned three parameter equation and for the two parameter equation with m=0 (Elston *et al.*, 1991). Analysis of variance was carried out on the estimates of the

parameters (Madden, 1986).

Results

Considerable damage due to *G. pallida* and large differences in tolerance between genotypes were found in small pots (Expt 6.1). Mean total dry weights per genotype after 35 days varied from 0.1 to 2.0 g for infested plants and from 2.0 to 3.8 g for uninfested plants. The correlation with tolerance assessments of the same genotypes in the field and in large pots was high for both dry weights of infested plants and these dry weights proportional to dry weights of uninfested plants (Table 6.1). Thus, even tolerance assessed in this way in small pots, appears to be related to tolerance in the field.

The correlation between dry weight of infested and uninfested plants was low in Expt 6.1 ($r=0.09$; n.s.). Very few genotypes had formed tubers after 35 days, and for all genotypes in both treatments, tubers contributed less than 5% to the total dry weight. *G. pallida* reduced both sprout and root weight considerably. Sprout and root weights of plants, infested with *G. pallida*, were highly correlated ($r=0.96$; $P<0.01$), but not with uninfested plants ($r=-0.10$). Stem length data of infested plants after 35 days were also significantly correlated with tolerance data in large pots and in the field ($r_s>0.70$).

Dry weights of infested plants and proportional dry weights gave largely similar results in Expt 6.2. Differences in dry weights of infested plants for both progenies are presented in Figure 6.1. Tolerance, expressed in this manner, seems to be inherited in a quantitative way, as shown by the frequency distributions in Figure 6.1. In both progenies, a considerable number of genotypes was found, which were significantly more tolerant than the intolerant parent AM78-3778. With AM78-3778 x Cara, genotypes were found with a higher tolerance than Cara, but the differences were not significant (Figure 6.1). With AM78-3778 x Multa, nearly all genotypes had a level of tolerance between that of the parents.

The tolerance of the parent genotypes Multa and Cara and the intolerance of AM78-3778, as indicated by the weight of infested plants, were confirmed in both Expt 6.1 and Expt 6.2, and appeared not to be related to the weight of uninfested plants (Table 6.2). A significant difference in dry weight of infested plants between Multa and Cara was only found in Expt 6.2.

The mean number of cysts of the parents (Table 6.2) confirmed the high level of

Table 6.1. Rank correlations between dry weight of potato genotypes after 5 weeks in small pots with 0.5 kg soil, heavily infested with *G. pallida*, or the same dry weight, proportional to the dry weight in control pots, in Expt 6.1, and tolerance measured previously by proportional yield in infested fields in two years and proportional biomass in large pots with 3.0 kg infested soil after 35 and 70 days (Chapter 5).

	Number of genotypes	Dry weight infested small pots	Proportional dry weight small pots
Field 1989	15	0.76 **[1])	0.76 **
Field 1990	13	0.75 **	0.63 *
Large pots 35 days	15	0.76 **	0.72 **
Large pots 70 days	15	0.77 **	0.71 **

[1]) * = significant (P<0.05)
** = significant (P<0.01)

Table 6.2. Mean dry weight (g) of parental potato genotypes, tested in Expts 6.1 and 6.2 in 0.5 kg pots after 35 days, and number of cysts on these parental genotypes and the susceptible standard cultivar Maritta in Expt 6.3. Means within each column, carrying different letters, are significantly different from each other (LSD;P<0.05)

Potato genotype	Dry weight Expt 6.1		Dry weight Expt 6.2		Number of cysts in Expt 6.3
	infested	uninfested	infested	uninfested	
Multa	1.91 b	2.95 ab	1.54 c	2.05	171 b
Cara	1.65 b	2.39 a	1.03 b	1.83	317 bc
AM78-3778	0.39 a	3.30 b	0.63 a	2.25	0 a
Maritta					444 c

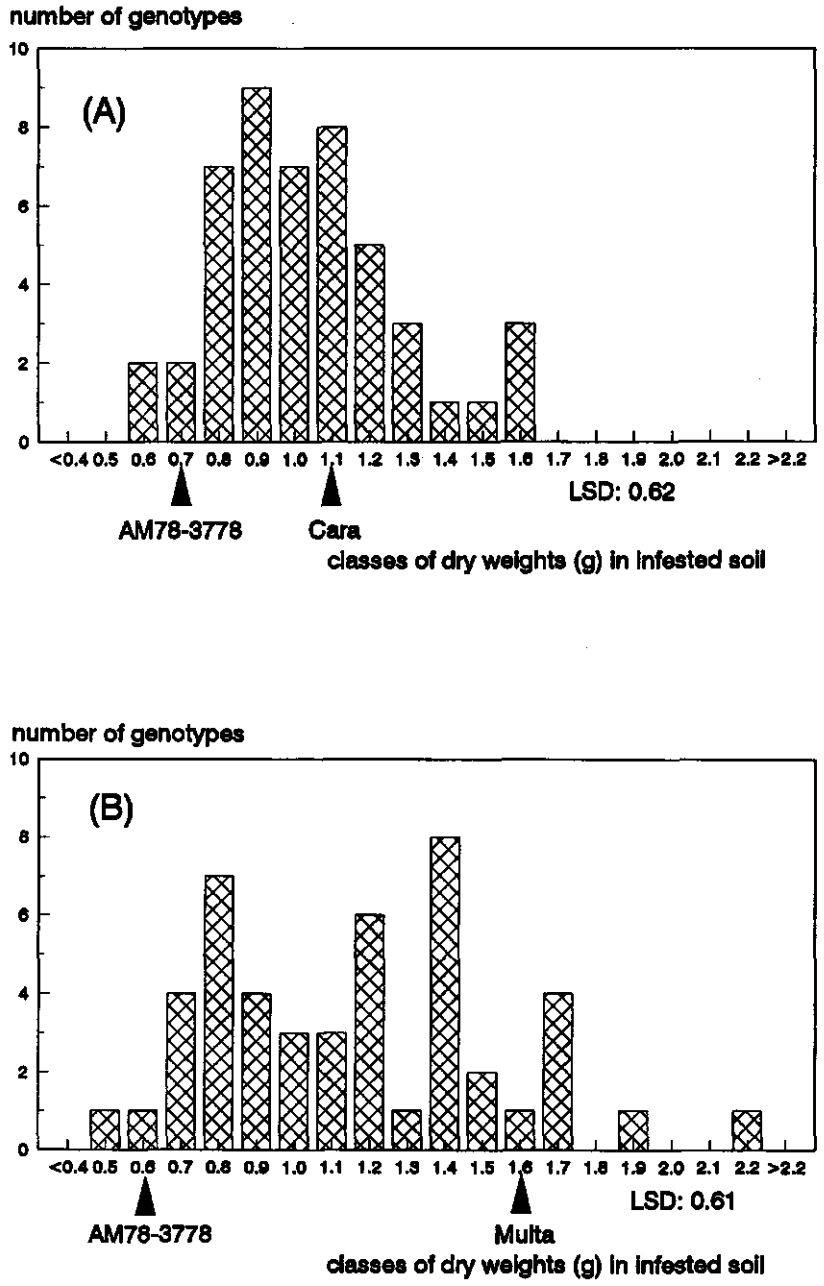


Figure 6.1. Frequency distributions of number of genotypes in classes of mean dry weight after 35 days in infested pots in Expt 6.2, for (A) the progeny of AM78-3778 x Cara and (B) the progeny of AM78-3778 x Multa.

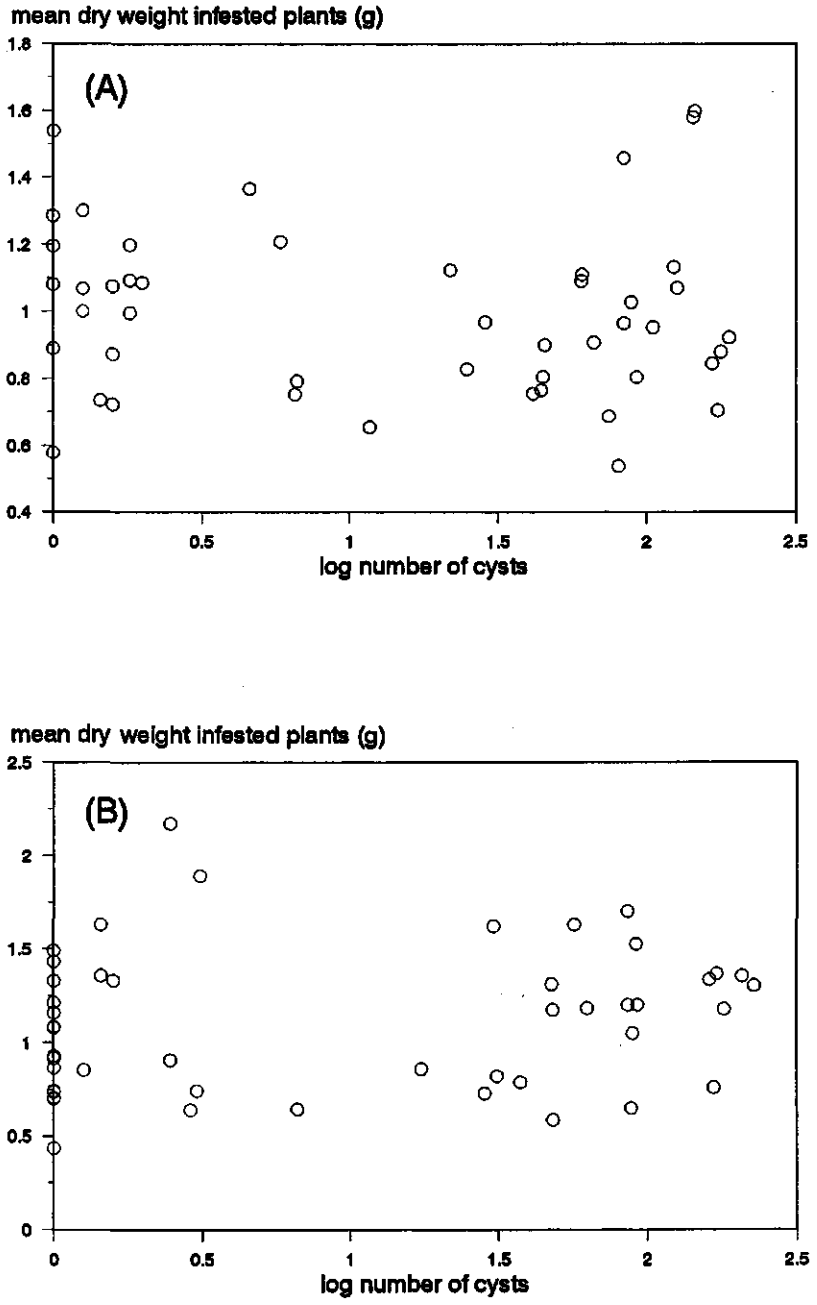
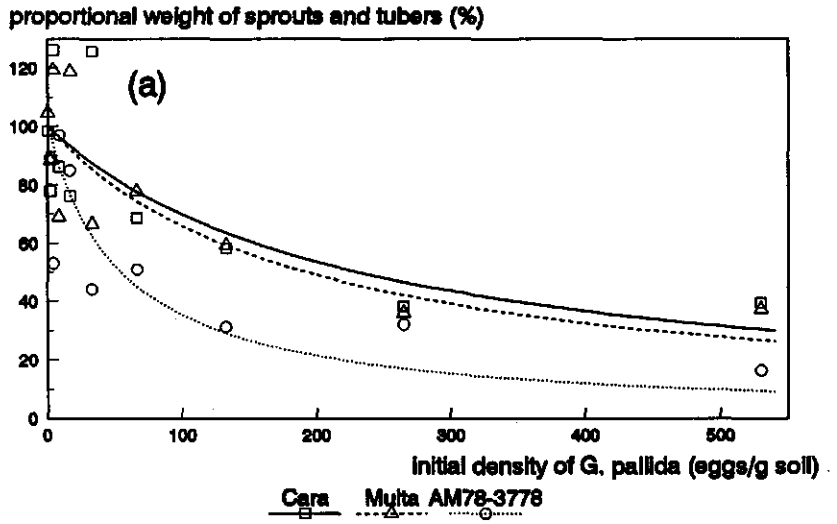


Figure 6.2. Relationship between the mean log number of cysts of genotypes in the resistance test (Expt 6.3) and the dry weight after 35 days with heavy *G. pallida* infestation in the tolerance test (Expt 6.2), for (A) progeny of AM78-3778 x Cara and (B) progeny of AM78-3778 x Multa.

resistance of AM78-3778 and the lower level of resistance of Multa. The mean number of cysts, found on genotypes of progenies in Expt 6.3 varied widely, from 0 to 209 for AM78-3778 x Cara and from 0 to 252 for AM78-3778 x Multa. Analysis of variance of numbers of cysts could not be conducted, even after log transformation, since many genotypes without cysts were found, which resulted in lack of homogeneity of variances and normality. Among the more resistant and more susceptible genotypes (Expt 6.3), similar variation in dry weights of infested plants (Expt 6.2) appeared to occur in both progenies tested (Figure 6.2). Rank correlations between log number of cysts and dry weight of infested plants were low and not significant for both the AM78-3778 x Cara progeny (r_s= -0.10) and the AM78-3778 x Multa progeny (r_s=0.19). The comparison of log number of cysts and proportional dry weights gave very similar results. These results suggest that tolerance of *G. pallida* is inherited independently from resistance to *G. pallida*.

Damage due to *G. pallida* was clearly found at higher densities of *G. pallida* in Expt 6.4 (Figure 6.3). Curve fitting was carried out with both the three parameter and the two parameter inverse linear model (with m=0) and percentages of explained variance were not higher with the former than with the latter. Therefore, the latter, simplified model (with m=0) was used for further analysis. In some cases, a low percentage of explained variance was found (Table 6.3), which may be attributed to the occurrence of relatively little PCN damage in these cases and to the low number of replicates of the experiment. Significant differences between the parameter c of the inverse linear curve were found at 35 days and 70 days after planting, and indicated that AM78-3778 suffered more damage by *G. pallida* than the other genotypes. After 70 days, Multa was found to be significantly more tolerant than Cara, as expressed by the parameter c (Table 6.3). The percentage tuber weight after 70 days was highest for AM78-3778 (Table 6.3), which indicates that this genotype may be earlier than the other genotypes. No significant genotype-*G. pallida* density interaction effect on percentage tuber weight was found. Proportional dry weight at each harvest date and density were also analyzed separately, and no significant change in tolerance ranking was found at any harvest date and *G. pallida* density.



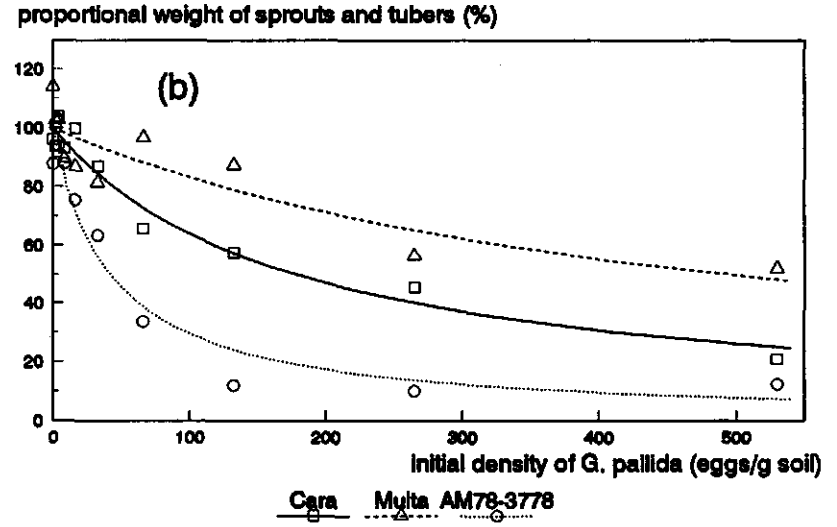


Figure 6.3. Relationship between percentage of dry weight of sprouts and tubers, proportional to DW_{max}, of three potato genotypes and density of *G. pallida* (eggs/g soil), with curves following the fitted simplified inverse linear model (m=0), for (a) 35 days after planting, and (b) 70 days after planting (Expt 6.4).

Discussion

The data presented here show that tolerance of *G. pallida* may be improved by crossing and selection. No relationship in the progenies was found between the level of resistance and tolerance, thus indicating that tolerance and resistance can be combined in one cultivar. This is in accordance with earlier findings of large differences in tolerance between resistant genotypes (Chapter 5) and differences in tolerance between genotypes, not related to their level of resistance (Dale *et al.*, 1988; Elston *et al.*, 1991). These results suggest that the resistance mechanism or associated changes do not confer intolerance.

The assessment of tolerance by measuring dry weight after 35 days in pots in Expt 6.1 appeared to be strongly correlated with tolerance data, obtained by measuring yield in the field. Thus, the tolerance data, as presented here, may be considered relevant for the field situation. The correlations between sprout and root weight of infested plants and between stem length and total dry weight confirm earlier findings in Chapter 5.

No indications were obtained that the ranking for tolerance is influenced by the density of *G. pallida*. The effect of density on dry weight of plants could be described by a simple two parameter inverse linear model, as proposed by Elston *et al.* (1991). Much research work has been carried out on the influence of PCN density on tolerance of genotypes (Seinhorst & den Ouden, 1971; Brown & Sykes, 1983; Trudgill *et al.*, 1985; Trudgill, 1987b; Elston *et al.*, 1991) and no clear indications have been described that ranking of potato genotypes for tolerance may be different at different densities of PCN. Therefore, there appears to be no reason to assess tolerance of large numbers of genotypes, as necessary in a breeding programme, at more than one density of *G. pallida*. For tolerance tests a high *G. pallida* density is preferred, in order to create large differences in damage (van der Wal, 1981).

A continuous range of intolerance to tolerance of *G. pallida* was found in the progenies examined, suggesting quantitative inheritance. This is in accordance with earlier observations, in which a range from tolerant to intolerant genotypes was also found, although the tested genotypes did not originate from the same crosses as described here (Chapter 5). No relationship between the growth of uninfested plants and tolerance was found, which is consistent with findings in Chapter 5 and by Reese *et al.* (1988) in soybean. For selection of tolerant genotypes, growing of uninfested plants is apparently not necessary.

Screening for tolerance in a *G. pallida* resistance breeding programme is advised

Table 6.3. Means of parameters, DW_{max} (g) and c (eggs/g soil), of the regression lines of dry weight of sprout and tubers of three genotypes on density of *G. pallida*, 35 and 70 days after planting (Expt 6.4), the percentage explained variance by curve fitting, and dry weight of tubers, proportional to dry weight of sprouts and tubers (%), after 35 and 70 days. Parameters within each column, carrying different letters, are significantly different from each other (LSD;$P<0.05$)

Potato genotype	35 days after planting				70 days after planting			
	DW_{max}	c	%var exp.	% tubers	DW_{max}	c	% var exp.	% tubers
Cara	2.7 a	308 b	44	0	28.7 a	177 b	88	42
Multa	4.6 b	257 b	44	0	32.3 a	493 c	57	51
AM78-3778	5.0 b	56 a	62	7	43.6 b	39 a	95	72

and can be carried out in the greenhouse, using small pots. Field trials (Phillips *et al.*, 1988a) are for that purpose not necessary. The results, presented here, confirm that resistance to and tolerance of *G. pallida* can be combined.

CHAPTER 7

Hatching of *Globodera pallida* juveniles by diffusate of potato genotypes, differing in tolerance to *G. pallida*

Abstract

Hatching induced by root diffusate, obtained from various potato genotypes, and by standard potato root diffusate, was determined *in vitro*. The tested potato genotypes differed considerably in tolerance to *Globodera pallida*. A three parameter logistic model was used to describe the numbers of hatched juveniles in relation to time of exposure to root diffusate. Clear differences in hatching characteristics of genotypes were found. Some tolerant genotypes induced hatching of *G. pallida* juveniles relatively late, compared to intolerant genotypes. Other tolerant genotypes, however, induced hatching as early as intolerant genotypes.

Introduction

Juveniles of potato cyst nematodes (PCN), *Globodera pallida* Stone and *G. rostochiensis* (Woll.) Skarbilovich, are known to hatch in potato root diffusate (Triffitt, 1930). Until now, only partial purification of hatching factors for PCN has been reported, and indications were obtained that various chemical compounds may be involved (Atkinson *et al.*, 1987). Recently, Heinicke *et al.* (1990) suggested that the solanidine glycosides α-solanine and α-chaconine may play a role in hatching of PCN.

The relationship between hatching and resistance to PCN of potato genotypes has been the subject of several investigations (Farrer & Phillips, 1983; Turner & Stone, 1981; Balandras, 1988). Differences in hatching activity of diffusate of genotypes were found, but their role in resistance appeared to be only of minor importance. The relationship between tolerance and hatching activity has been given hardly any attention. Evans (1983) investigated the hatching activity of diffusates of 25 cultivars. Some cultivars stimulated only poorly hatching of *G. pallida* juveniles. Evans suggested that poor hatching might contribute to tolerance of these cultivars. However, the tolerance of only one cultivar was actually known in that experiment.

Large differences in tolerance to *G. pallida* were observed between *G. pallida* resistant genotypes (Chapter 5) and were associated with differences in root growth of infested plants, suggesting that tolerance may be caused by relatively little damage to the roots. Initial damage to potato plants is caused by juveniles penetrating the roots, which occurs in both susceptible and resistant plants (Trudgill, 1986). Therefore, it was decided to investigate the possible relationship between the differences in tolerance, as observed before, and hatching of juveniles with these genotypes. Since late hatching may allow longer root growth before large numbers of juveniles penetrate, not only numbers of juveniles hatched by host genotypes should be studied (Evans, 1983), but also differences in time of hatching (Oostenbrink, 1967; Robinson *et al.*, 1987).

In this study, the possible relationship between tolerance and hatching was investigated, using a bioassay, in which number of juveniles hatched and time of hatching could be determined separately. Furthermore, the relationship between hatching and root system development and the α-solanine and α-chaconine content of roots and tubers was investigated.

Materials and methods

Two hatching experiments were carried out, with different potato root diffusates. Hatching procedures in these experiments were mostly similar. Cysts of a *G. pallida* batch were used, consisting of a mixture of *G. pallida* populations. The batch was the same as used in the greenhouse tolerance test, from which tolerance data of the potato genotypes were obtained (Chapters 5 and 6), and had been stored for two and a half years at 4° C. The batch was sieved in order to avoid large variation in size of cysts, and only cysts with a diameter between 0.56 and 0.65 mm were used. The mean number of eggs per cyst was found to be 450. Samples of five cysts were taken and each sample was placed in a small sieve with pore diameter <0.2 mm, through which juveniles could pass, which was placed in a small cup. The cysts were presoaked in the cups for a week in demineralized, filtered water, here after referred to as water. Subsequently, the cysts were exposed to 1 ml of the various diffusates, and kept at 20° C in the dark. The diffusates were renewed initially three and later two times per week. The number of juveniles hatched in each cup was counted at each renewal of the diffusate. As controls, solutions of dried standard potato root diffusate (SPRD), described by Janzen & van der Tuin (1956), at a concentration of 12 mg/l were used.

The experiments were terminated, when for each treatment less than ten hatched juveniles per cup were observed. Afterwards, the cysts were crushed and the remaining eggs with juveniles were counted (Seinhorst & den Ouden, 1966).

Experiment 7.1. Presprouted tubers of two genotypes, Ve8550 and Ve8590, differing in tolerance to *G. pallida* (Chapter 5), were held with wooden pins just above 400 ml water in glass beakers, and covered with black plastic. A single tuber was placed in each beaker. The beakers were placed in a randomized block design in a greenhouse, in five replicates. Roots grew in the water and stems were allowed to grow through holes in the plastic. Plants grew for four days on a beaker with water, from which twice a week a few ml root diffusate was sampled, and the water was then replaced by Steiner nutrient solution (Steiner, 1968) for three days. This procedure was repeated weekly. Cysts were exposed to undiluted root diffusate and 1:4 diluted root diffusate, sampled from each beaker. As controls, SPRD, 1:4 diluted SPRD, Steiner nutrient solution and water were used. Hatching was observed in two replicates, for each beaker. A score was given for the relative root system size (1-5) of each plant at each sampling date. Plants were harvested after 44 days, when the production of hatching factors presumably had decreased (Widdowson, 1958a; Rawsthorne & Brodie, 1986). Root tips, remaining roots,

sprouts and the original tubers, from which the plants had grown, were collected separately. Dry weight was determined and the content of the solanidine glycosides α-solanine and α-chaconine was determined with the method described by Jonker *et al.* (1992). The hatching experiment was continued with the root diffusate, collected at harvest and stored in a refrigerator at 4° C, until 81 days after planting.

Experiment 7.2. Eleven genotypes, all with a high level of resistance to *G. pallida*, mainly derived from *Solanum vernei* (Chapters 5 and 6), and differing in tolerance to *G. pallida* were used. Tolerance had been assessed by the yield on a heavily *G. pallida* infested field in 1989 and 1990, and by biomass in heavily infested large pots with 3 kg soil, 35 and 70 days after planting in the greenhouse (Chapter 5), and by the biomass in infested small pots, containing 0.5 kg soil (Chapter 6). In addition, the tolerant cultivars Multa and Cara (Chapter 6) were included. Cara has no resistance to *G. pallida* (Phillips *et al.*, 1988b) and Multa has a low level of resistance to various *G. pallida* populations (Chapter 3). Eyepieces of presprouted tubers of these genotypes were placed in 400 ml glass beakers with clean gravel and about 100 ml half concentration Steiner nutrient solution, just above the level of the liquid. The beakers were fitted into holes in a wooden plate in the greenhouse, thus allowing the stems to grow in the light and the roots to develop in the dark. The beakers were placed in a randomized block design with four replicates. Plants grew with a single stem. At regular intervals, additional 1:2 nutrient solution was added and sampling of a few ml of solution with root diffusate was done initially three times a week, and towards the end of the experiment two times a week. A score (1-5) for the relative size of the root system, as visible from the outside of the beaker, was given at each sampling date. Cysts were exposed to sampled root diffusate, diluted to an equivalent of 400 ml diffusate per plant, the same volume as in Expt 7.1. As controls, SPRD, half concentration nutrient solution, water and SPRD diluted 1:2 with nutrient solution or water, were used. The hatching experiment was carried out in two replicates. Plants were harvested after 49 days. Dry weight of sprouts, roots, stolons and tubers was determined. The hatching experiment was continued with the root diffusate, collected at harvest and stored in a refrigerator at 4° C, until 63 days after planting.

Statistical analysis. Logistic curves were fitted to numbers of hatched juveniles in relation to days of exposure for each experimental unit. The logistic expression is characterized by:

$$J = J_{max} / (1 + e^{-c(t-t_{50})}),$$

in which:

J = percentage of hatched juveniles
t = number of days of exposure to diffusate
J_{max}, c, t_{50} = parameters

In this expression, the lower limit of the logistic curve is assumed to be zero. The parameter c indicates the steepness of the slope, J_{max} the upper limit of the percentage of hatched juveniles, and t_{50} (in days) the point of inflection, at which half of the value of J_{max} is reached. Analysis of variance was carried out on the estimated parameters of the fitted logistic curves (Madden, 1986). When data did not fit a normal distribution, correlations were determined by calculation of the non-parametric Spearman's rank correlation coefficient (r_s).

Results

The percentage of hatched juveniles followed in all experiments the logistic model (Figures 7.1 and 7.2). Only in some cases, sudden small increases in the percentages of hatched juveniles were still found after a long time of exposure to diffusate, which did not fit the used logistic model. In all experiments regression analysis, using the logistic model, explained more than 95% of the total variance.

Hatching by diffusates of genotypes started later than by SPRD (Figure 7.1). Clear differences in hatching between the genotypes were found in Expt 7.1 (Figure 7.1), and significant differences between genotypes and dilutions were found for the hatching parameters c, t_{50} and J_{max} (Table 7.1). The data for t_{50} show that even diluted Ve8550 diffusate hatched significantly faster than the undiluted Ve8590 diffusate. The parameters c and J_{max} had the lowest values for diluted Ve8590 diffusate. Increased rate of hatching could not be related to an increase in root weight, as Ve8590 had a higher root weight than Ve8550 (Table 7.1). Also, no significant correlations (r_s) were found between root score at various days after planting and any of the hatching parameters. In undiluted nutrient solution 7% of the juveniles hatched, in diluted nutrient solution 4%. In water, only 2% of the juveniles hatched.

The solanidine glycoside content (α-solanine and α-chaconine) of the two genotypes did not differ largely (Table 7.2). The total amount of solanidine glycosides per root

Table 7.1. Parameters of hatching curves of diffusates, undiluted and diluted 1:4, of two genotypes in Expt 7.1, with c representing the slope, t_{50} the point of inflection and J_{max} the upper limit. Root dry weight and total plant dry weight after harvest are also given. Means, carrying different letters, are significantly different (LSD;$P<0.05$).

Genotype	c		t_{50} (days)		J_{max} (% juv.)		Root weight (g)	Plant weight (g)
	undiluted	diluted	undiluted	diluted	undiluted	diluted		
Ve8550	0.27 ab	0.29 b	17.4 a	22.1 a	84.1 ab	74.0 ab	0.35 a	3.60
Ve8590	0.23 ab	0.19 a	33.3 b	36.5 b	88.2 a	61.2 b	0.64 b	4.24

Table 7.2. The α-solanine and α-chaconine content (mg/g dry weight) of root tips, of remaining roots, and of seed tubers, from which the plants had grown. Contents carrying different letters are significantly different from each other (LSD;P<0.05).

Genotype	α-solanine (mg/g)	α-chaconine (mg/g)	dry weight (g)
root tips:			
Ve8550	3.9 a	5.2	0.05
Ve8590	5.9 b	5.4	0.09
remaining roots:			
Ve8550	2.1	3.3	0.30
Ve8590	2.7	2.8	0.55
tubers:			
Ve8550	0.3	0.5	12.80
Ve8590	0.3	0.4	10.70

system was higher for Ve8590 (4.0 mg) than for Ve8550 (2.1 mg). The differences in hatching, as found between Ve8550 and Ve8590, were apparently neither positively related to the α-solanine nor to the α-chaconine content of the roots.

In Expt 7.2, again clear differences in hatching between genotypes were found (Figure 7.2). This resulted in significant differences between genotypes for all hatching parameters (Table 7.3). The difference between hatching induced by Ve8550 and Ve8590, as found in Expt 7.1, was found again. The tolerant cultivar Multa induced hatching most slowly, as expressed by a high value of t_{50} and the low value of J_{max}. The tolerant genotype Ve8515 also induced hatching also slowly. However, some tolerant genotypes were found to induce hatching rapidly. The correlations between tolerance data and hatching parameters for genotypes were in most cases low and not significant (Table 7.4).

In Expt 7.2, the control treatment nutrient solution (diluted 1:2) hatched in total 6% of the juveniles, and water 3%. No significant differences for hatching parameters were found between SPRD, diluted 1:2 with either nutrient solution or water. The final percentage of juveniles hatched was 96% and 87% respectively.

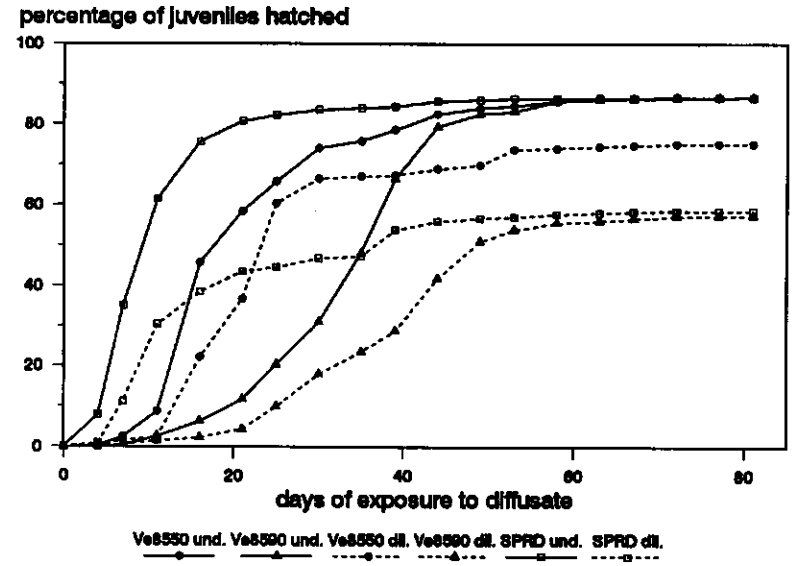


Figure 7.1. The percentage of juveniles hatched in relation to the time of exposure to root diffusates of two genotypes, Ve8550 and Ve8590, and to standard potato root diffusate (SPRD), undiluted and diluted 1:4, in Expt 7.1.

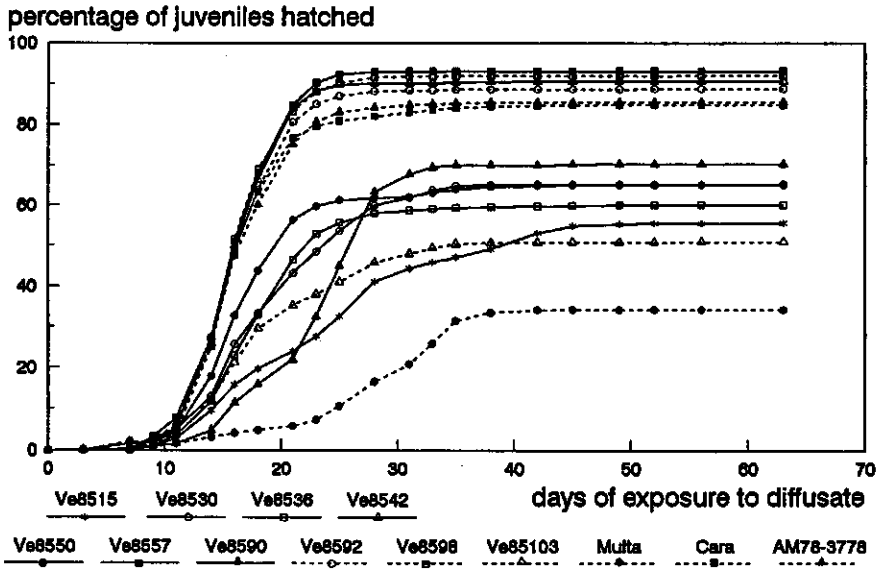


Figure 7.2. The percentage of juveniles hatched in relation to the time of exposure to root diffusates of 13 genotypes, in Expt 7.2.

Table 7.3. Mean values of parameters of hatching curves of diffusates of 13 genotypes, differing in tolerance, in Expt 7.2, with c representing the slope, t_{50} the point of inflection and J_{max} the upper limit, and root dry weight and total plant dry weight after 49 days. Tolerance rankings are based on the biomass of infested plants after 35 days in small pots (Chapter 6), the biomass of infested plants after 70 days in large pots and the yield in a heavily *G. pallida* infested field in 1989 (Chapter 5). The lowest ranking indicates the highest tolerance.

Genotypes	Tolerance rankings			Hatching parameters			Biomass	
	small pots 35 d.	large pots 70 d.	field 1989	c	t_{50} (days)	J_{max} (% juv.)	root weight (g)	plant weight (g)
Ve8515	4	4	1.5	0.38	25.5	55.4	0.15	1.53
Ve8530	11	7.5	8	0.41	20.1	65.3	0.14	1.87
Ve8536	9	9	9	0.40	17.5	58.2	0.22	1.64
Ve8542	12	7.5	7	0.53	15.8	90.6	0.19	1.70
Ve8550	13	10	10	0.45	16.8	64.6	0.17	1.61
Ve8557	8	5	5	0.51	15.9	93.3	0.21	2.48
Ve8590	1	1	4	0.52	23.0	70.2	0.27	2.51
Ve8592	7	3	3	0.56	16.0	88.6	0.15	1.15
Ve8598	5	2	1.5	0.57	15.8	91.7	0.12	1.63
Ve85103	6	6	6	0.32	19.1	50.7	0.18	1.34
Multa	2	-	-	0.33	27.7	35.0	0.39	1.80
Cara	3	-	-	0.54	15.9	84.0	0.25	2.08
AM78-3778	10	-	-	0.50	16.0	85.2	0.18	1.67
LSD ($P<0.05$)				0.13	5.0	19.0	0.05	0.52

Table 7.4. Correlations between parameters of the hatching curve (Table 7.3), and tolerance, assessed by the yield in a heavily *G. pallida* infested field and by biomass of infested plants in the greenhouse (Chapters 5 and 6).

Tolerance data	Number of genotypes considered	Hatching parameters		
		c	t_{50}	J_{max}
Field 1989	10	0.33	0.34	0.24
Field 1990	9	-0.01	0.70 *[1]	-0.09
Large pots, 35 days	10	0.19	0.61	0.05
Large pots, 70 days	10	0.46	0.28	0.28
Small pots, 35 days	13	0.03	0.59 *	0.29

[1] * = significant ($P<0.05$)

Significant correlations were found between the parameters c and t_{50} ($r= -0.65$; $P<0.05$), between c and J_{max} ($r=0.93$; $P<0.01$) and between t_{50} and J_{max} ($r= -0.80$; $P<0.01$). No correlations of root weight and total biomass after 49 days with c and J_{max} were found higher than 0.30 (not significant). However, root weight was significantly ($P<0.05$) correlated with t_{50} ($r=0.55$). Root scores and hatching parameters were significantly negatively correlated (r_s) with c and J_{max} only after 28 days after planting and later.

Discussion

Clear differences between genotypes for hatching characteristics were found. For the genotypes Ve8550 and Ve8590, these differences were found in both Expts 7.1 and 7.2, using diffusate in water and nutrient solution respectively. The genotypes Multa, Ve8515 and Ve8590, which have been found to be tolerant, appeared to induce hatching poorly, as indicated by a late point of inflection t_{50} of the hatching curve (Expt 7.2). Multa moreover had a lower percentage of juveniles hatched. No intolerant genotypes, inducing PCN to hatch as slowly as these three genotypes, were found. However, some tolerant genotypes were found, which hatched juveniles as fast as intolerant genotypes. Thus, no generally high correlations were found between hatching characteristics and tolerance. Apparently, poor hatching can explain some but not all differences in tolerance, observed

in the tested genotypes. The poor hatching of the tolerant cultivar Multa, as found here, confirms earlier results of Evans (1983). All genotypes, except Cara and Multa, were highly resistant, and thus also considerable differences in hatching between highly resistant genotypes were demonstrated.

Differences in hatching parameters appeared not to be related to root score or root weight. However, Rawsthorne & Brodie (1986) found a clear relationship between root weight or length up to three weeks after plant emergence and the hatching activity of the diffusate, when comparing different plants of the same genotypes. Thus, although differences in root growth can affect hatching, the differences in hatching, found in our experiments, were apparently not related to differences in root growth.

The hatching parameters here are determined *in vitro*. It is important to notice that the measured parameters are not affected by a possible effect of penetration of juveniles in the roots on the production and/or exudation of hatching factors by these roots, which may occur *in vivo*.

Hatching factors persist less than two months in soil, after removal of potato plants (Perry *et al.*, 1981). Also in the beakers used in Expts 7.1 and 7.2, presumably not only exudation of hatching factors, but decline of these factors (Widdowson, 1958b) may have played a role too. However, hatching of *G. pallida* juveniles is induced already after brief exposure to potato root diffusate (Forrest & Perry, 1980). In the experiments, the cysts were exposed at regular intervals to fresh diffusate, and our results thus presumably reflect well the hatching activity of the diffusate at various stages of plant growth. Diffusates, stored after harvest of the potato plants, contributed relatively little to the hatching results (Figures 7.1 and 7.2).

The bioassay used here demonstrated differences in the time after planting at which juveniles were hatched, which could not have been demonstrated with the bioassay of Fenwick (1952). The hatching parameters, as determined in Expt 7.2, however, were highly correlated. Therefore, it has to be noted that the differences in hatching between genotypes may be caused by the exudation of different amounts of the same hatching factors by the various genotypes.

No indication for an influence of solanidine glycoside content of roots or tubers on hatching were found. These substances are known to destabilize lipid membranes (Roddick & Rijnenberg, 1986). A lipid layer is present in the eggshell of PCN eggs (Perry, 1986). It is, however, not known whether the concentration of solanidine glycosides in roots is related to the concentration in the root diffusate. Differences in exudation of these substances could not be investigated, as their concentrations in the diffusate were

too low for detection.

The results presented here clearly show differences in hatching activity between diffusates of potato genotypes. Selection for slow and decreased induction of hatching may lead to increased tolerance, but in a commercial breeding programme, such selection would be far too laborious with the hatching test used here. Only chemical identification of substances, responsible for hatching would offer the opportunity to develop faster screening methods for this trait.

CHAPTER 8

The effect of the potato cyst nematode *Globodera pallida* on *in vitro* root growth of potato genotypes, differing in tolerance

Abstract

Roots of eighteen potato genotypes, differing in tolerance to *G. pallida*, grew from tuberpieces on agar in petridishes. Juveniles of *G. pallida* were inoculated directly on root tips. The root length was measured at various days after inoculation. Inoculation reduced root growth within one day. At later stages, genotypes differed strongly in growth of inoculated roots. Between four and seven days after inoculation, growth of inoculated roots was not associated with growth of untreated roots. Correlations between growth of inoculated roots, four to seven days after inoculation, and tolerance assessed in the greenhouse and in the field, were generally low. However, multiple regression analysis revealed that the tolerance of the tested genotypes was associated with both the induced rate of hatching and the growth of roots after inoculation. The combination of these two explanatory variables gave high percentages of explained variance in the analysis.

Introduction

Resistance to potato cyst nematodes (PCN), *Globodera pallida* and *G. rostochiensis*, in potato cultivars is important for the control of PCN, and reduces the need of nematicides. Intolerance of cultivars with resistance to *G. pallida* should be avoided (Trudgill, 1991). Potato genotypes with resistance to *G. pallida* derived from *Solanum vernei*, appeared to differ strongly in tolerance of *G. pallida* (Chapters 5 and 6). The differences in tolerance occurred early in growth. They were associated with differences in root weight of these genotypes in infested pots. Various factors conferring tolerance have been suggested (Evans & Haydock, 1990; Trudgill, 1991), but the relative importance of these factors for tolerance is not yet known. Potato genotypes appeared to differ in rate of hatching of *G. pallida* juveniles from cysts (Chapter 7), which may result in exposure of roots to different amounts of juveniles and thus explain some of the differences in tolerance found (Schans, 1993). However, although slow hatching was found with some tolerant genotypes, no generally high correlations were found between this trait and tolerance data (Chapter 7).

Differences in tolerance in oats infested with *Heterodera avenae* appeared to occur also early in growth (Stanton & Fisher, 1988; Volkmar, 1990). Root growth of host plants was strongly reduced directly after inoculation with juveniles of *H. avenae* (Price *et al.*, 1983; Rawsthorne & Hague, 1986; Davy de Virville & Person-Dedryver, 1989). Some tolerant genotypes resumed root growth earlier than intolerant genotypes (Davy de Virville & Person-Dedryver, 1989).

Research on direct effects of inoculation of PCN juveniles on root growth of potato genotypes has only been reported by Seinhorst and den Ouden (1971). Two susceptible potato cultivars appeared to differ considerably in root growth after inoculation with hatched *G. rostochiensis* juveniles.

The aim of this study was to investigate whether root growth differs between *G. pallida* resistant potato genotypes with different levels of tolerance after inoculation of roots with hatched *G. pallida* juveniles and whether differences in root growth are associated with differences in tolerance in pot and field tests.

Materials and methods

Sprouted tubers of eighteen genotypes were used, differing strongly in tolerance of *G. pallida*. Tolerance of fifteen of these genotypes had been assessed by measuring the tuber yield in heavily *G. pallida* infested fields. Tolerance of these genotypes had also been assessed by measuring the biomass of plants in pots heavily infested with *G. pallida* (Chapters 5 and 6). Some tolerance data are presented in Table 8.1. Sixteen genotypes were derived from a *G. pallida* resistance breeding programme and were highly resistant to *G. pallida* (Chapters 5 and 6). Two tolerant cultivars, Multa and Cara, were also included. Multa has intermediate resistance to *G. pallida* (Chapters 3 and 6) and Cara is susceptible (Chapter 6). The rate of hatching of thirteen genotypes was determined before (Chapter 7). The values of the hatching parameter t_{50}, indicating the time at which half of the finally hatched number of juveniles was observed, are presented in Table 8.1.

Root growth was assessed in petridishes (Mugniéry & Person, 1976). The petridishes with a diameter of 8.5 cm contained a layer of 20 ml solid agar (2% Bacto-agar in water). In each petridish, a single sprout with a piece of tuber was placed. The petridishes were stored at 20 °C in the dark, and roots grew over the agar. Half of the petridishes were used for inoculation of roots, in the other half roots grew without inoculation. Juveniles for inoculation were hatched with standard potato root diffusate (Janzen & van der Tuin, 1956). Cysts were obtained from the same stock of a mixture of *G. pallida* populations, used previously for pot tests of tolerance and hatching tests (Chapter 5, 6 and 7). At most four roots with a length of 1-3 cm were inoculated on the root tips with droplets of 0.01 ml water, containing on average 80 juveniles. The droplet evaporated soon after inoculation. The point of inoculation was marked on the bottom of the petridish. Root length of the main root from the point of inoculation was measured at one, four and seven days after inoculation. Brown discoloration of the roots at the point of inoculation was scored (0-3) at one, two and three days after inoculation. A score of 0 indicated no discoloration, and 1-3 light to heavy brown discoloration. Roots in the control petridishes were chosen with a length between 1 and 3 cm. The position of the root tips was marked, and the increase in length from that point was measured. The length of the roots at seven days was measured after removal of the roots from the petridishes. The experiment was carried out with 24 inoculated and 24 untreated roots for each genotype.

Correlations between data which were not normally distributed, were determined by

Table 8.1. Increase in length of inoculated roots, 0-1, 1-4 and 4-7 days after inoculation with *G. pallida*, and the same increase of untreated roots, the score for brown discoloration, visible three days after inoculation on inoculated roots, for 18 potato genotypes. The genotypes differed in tolerance, as shown by their tuber yield (kg fresh weight/plant) in a heavily *G. pallida* infested field and biomass (g dry weight/plant) after 35 days in large and small pots, heavily infested with *G. pallida* (Chapters 5 and 6) and their rate of hatching, as represented by t_{50} (Chapter 7).

Genotype	Tolerance data			Hatching parameter t_{50}	Length increase inoculated and untreated roots						Score (0-3) for discoloration of infested roots
	field 1989	small pots	large pots		0-1 day		1-4 days		4-7 days		
					inoc.	untr.	inoc.	untr.	inoc.	untr.	
Ve8515	1.08	1.26	1.47	25.5	1.0	2.1	1.7	5.4	0.4	3.0	1.9
Ve8530	0.33	0.27	0.63	20.1	1.0	1.9	1.1	6.3	0.2	5.5	2.1
Ve8536	0.26	0.60	0.57	17.5	1.0	2.1	2.5	5.6	1.1	6.3	1.8
Ve8538	1.09	0.64	0.79	-	0.5	1.2	0.3	3.0	0.1	2.5	1.9
Ve8542	0.36	0.18	0.42	15.8	0.9	1.5	1.2	4.9	0.4	4.2	1.6
Ve8550	0.16	0.14	0.30	16.8	0.4	1.1	0.6	4.1	0.2	3.4	2.5
Ve8556	0.70	0.52	1.12	-	1.7	2.2	3.8	6.0	2.2	3.3	1.5
Ve8557	0.73	0.64	1.14	15.9	1.1	2.4	2.7	6.4	2.1	6.2	1.8
Ve8567	0.80	0.79	0.90	-	1.0	2.1	1.7	6.9	1.0	7.2	1.9
Ve8571	0.87	1.13	0.95	-	1.2	2.5	2.1	6.7	0.5	4.4	2.0
Ve8577	0.47	0.45	0.99	-	1.2	1.8	2.2	5.4	0.8	4.3	1.8
Ve8590	0.99	2.00	1.75	23.0	1.2	2.5	2.5	6.0	1.0	4.9	1.8
Ve8592	1.01	0.70	0.92	16.0	1.2	1.9	2.3	6.3	1.8	3.2	1.5
Ve8598	1.08	1.23	1.15	15.8	1.2	2.3	2.6	6.1	2.2	4.9	1.7
Ve85103	0.69	0.74	0.79	19.1	1.0	1.7	1.8	4.2	0.7	3.8	1.8
AM78-3778	-	0.39	-	16.0	1.0	1.4	1.8	5.8	0.9	7.5	2.1
Cara	-	1.65	-	15.9	0.9	1.8	4.6	5.1	4.3	5.4	1.8
Multa	-	1.91	-	27.7	1.2	2.0	4.0	5.5	3.2	4.7	1.6
Mean	0.71	0.85	0.93	18.9	1.0	1.9	2.2	5.6	1.3	4.8	1.8
LSD(0.05)	0.17	0.65	0.44	5.0	0.3	0.4	1.0	1.2	1.0	1.7	not det.

Table 8.2. Correlations for genotypes between increase in length of inoculated roots in petridishes, 4-7 days after inoculation (Table 8.1) and biomass of plants in heavily *G. pallida* infested pots of differing size after 35 and 70 days, and yield in heavily *G. pallida* infested fields (Chapters 5 and 6).

Tolerance data	Correlation with increase in root length	Number of genotypes
Biomass infested small pots, 35 d.	0.55 *[1]	18
Biomass infested large pots, 35 d.	0.39	15
Biomass infested large pots, 70 d.	0.17	15
Yield infested field, 1989	-0.08	15
Yield infested field, 1990	0.30	13

[1] * = significant (P<0.05)

Spearman's rank correlation coefficient (r_s).

Results

A reduction in growth of inoculated roots compared to untreated roots was found already one day after inoculation for all genotypes (Table 8.1). Later, the differences in increase in root length between inoculated and untreated roots became larger (Table 8.1). At that stage, large and significant differences occurred between the growth of inoculated roots of potato genotypes, as well as between the growth of untreated roots. The growth of inoculated roots was one day after inoculation significantly correlated with the growth of untreated roots (r=0.73; P<0.01). Between one and four days and between four and seven days after inoculation, no significant correlation was found anymore (r=0.38 and 0.19 respectively). Growth of inoculated roots, four to seven days after inoculation, was thus least associated with the growth of untreated roots and these data were used in further analyses. However, the growth of inoculated roots from one to four days was highly correlated with the growth from four to seven days after inoculation (r=0.92; P<0.01).

Table 8.3. The t-values of regression coefficients and the percentage explained variance with multiple regression analysis of tolerance data with the explanatory variables increase in length of inoculated roots, 4-7 days after inoculation, and rate of hatching, as represented by the parameter t_{50}.

Tolerance data	Values of t of regression coefficients		Percentage explained variance	Number of genotypes
	Root growth	Rate of hatching		
Biomass infested small pots, 35 d.	3.96 *[1]	3.83 *	69.8	13
Biomass infested large pots, 35 d.	6.27 *	7.50 *	87.7	10
Biomass infested large pots, 70 d.	4.02 *	3.36 *	65.2	10
Yield infested field, 1989	4.09 *	3.45 *	66.4	10
Yield infested field, 1990	1.94	3.59 *	58.3	9

[1] * = significant ($P<0.01$)

All inoculated roots showed some degree of brown discoloration, while untreated roots showed none. The discoloration of inoculated roots was visible after one day. The daily scores for brown discoloration during the first three days after inoculation were highly correlated (r_s>0.85; P<0.01). The scores at three days after inoculation are presented in Table 8.1, and did not indicate large differences between genotypes. No further changes in discoloration were observed afterwards.

The correlations between the growth of inoculated roots between four and seven days after inoculation and scores for brown discoloration on the one hand and tolerance data from the field and the greenhouse on the other hand were generally low (Table 8.2).

Multiple regression analysis was carried out with five sets of tolerance data (Table 8.3). Possible explanatory variables were maturity score (Chapter 5), rate of hatching, represented by t_{50}, the final number of juveniles hatched (J_{max}), which had both been determined before (Chapter 7) and the increase in length of inoculated roots four to seven days after inoculation, determined here. For none of the sets, maturity score or final number of juveniles hatched appeared to give a significant explanation, independent of the order of declaration of the variables in the regression analysis. However, root growth and rate of hatching appeared to be very significant explanatory variables (Table 8.3). The percentages of explained variances were generally high.

Discussion

The results indicate clearly a growth reduction of roots, directly after inoculation with *G. pallida* juveniles, which has also been found with *G. rostochiensis* (Seinhorst & den Ouden, 1971) and *H. avenae* on host plants (Price et al., 1983; Rawsthorne & Hague, 1986; Davy de Virville & Pe'rson-Dedryver, 1989). The differences between genotypes for this growth reduction after one day were relatively small. Moreover, the growth of inoculated roots, this early after inoculation, was still highly correlated with the growth of untreated roots, indicating that these differences may be mainly due to differences in root growth vigour. Vigorous root growth may be a factor conferring tolerance to nematodes (Evans & Haydock, 1990). However, at later stages, the growth of inoculated and untreated roots were no longer significantly correlated, suggesting that other traits of genotypes may be more important for the growth of inoculated roots. The causal factors affecting the ability of genotypes to resume growth after inoculation with *G.*

pallida are still unclear. Volkmar (1990) suggested that measurements of phytohormone concentrations can provide an explanation. Further research is necessary to reveal the underlying mechanisms of the observed differences in root growth after infection.

Some genotypes were clearly able to continue root growth much better than other genotypes. However, only a low, and only in one case significant correlation between this trait and tolerance in the greenhouse at early stages was found, while no significant correlation was found with tolerance data in the field. Apparently, the observed genotypic differences in *in vitro* root growth after inoculation with *G. pallida* do not explain solely the observed differences in tolerance to a large extent.

In earlier experiments, also no high correlations of tolerance with maturity class (Chapter 5) or hatching parameters (Chapter 7) were found. The results of the multiple regression analysis confirmed that maturity class is not a major factor determining tolerance of the genotypes tested. However, this analysis indicated that the rate of hatching and differences in root growth after infection are associated with the observed differences in tolerance between these genotypes. The observed differences in tolerance occurred already early in growth (Chapter 5), which is in accordance with these findings. However, different results have been obtained with *H. avenae*, where the effect of inoculation of juveniles on root growth is likely to be the major factor associated with tolerance of genotypes (Davy de Virville & Person-Dedryver, 1989). Hatching of *H. avenae* is presumably not induced by root diffusate (Clarke & Shepherd, 1966), whereas hatching of *G. pallida* juveniles occurs mainly in response to root diffusate (den Nijs & Lock, 1992). This may explain the relative importance of hatching for the tolerance of genotypes to *G. pallida*.

GENERAL DISCUSSION

Resistance

Currently only high levels of PCN resistance, which reduce the PCN density in pot trials, are distinguished in the Dutch Cultivar List (*Anon.*, 1992c). The results of Chapters 2, 3 and 4 provide additional evidence for the occurrence of various degrees of PCN resistance in potato cultivars and in genotypes, used in potato breeding. A lower rate of PCN multiplication, compared to fully susceptible cultivars, may contribute to the control of PCN. The results presented here confirm that resistance should be expressed in a quantitative way, distinguishing various degrees of resistance (Mugniéry *et al.*, 1989; Nijboer & Parlevliet, 1990). However, major interactions were found between potato genotypes and PCN populations within *G. rostochiensis* Ro2/Ro3/Ro5 and within *G. pallida* Pa2/Pa3, showing that these groups are heterogeneous for virulence characteristics (Chapter 2). Tests for quantitatively expressed resistance for statutory purposes should be carried out with PCN populations, representative of the various virulence groups. If this condition is fulfilled, the importance of the quantitative expression of PCN resistance cannot easily be overemphasized.

In Chapter 2, the extent of variation of the virulence spectrum within the pathotypes Pa2/Pa3 was studied with five populations and already three virulence groups could be distinguished. Afterwards, research in Chapters 3 and 4 showed additional differences in virulence between *G. pallida* populations. The results presented in this thesis indicate that the extent of variation for virulence in *G. pallida* is considerably larger than previously known.

The pathotype scheme of Kort *et al.* (1977) does inadequately classify differences in virulence between PCN populations (Nijboer & Parlevliet, 1990) and ought to be replaced. The qualitative nature of the scheme, only distinguishing susceptibility or resistance, does not account for quantitative differences in virulence. The proposal for the distinction of 'virulence groups' in Chapter 2 provides a better basis for the classification of PCN populations in this respect.

The distinction of virulence groups would be facilitated by increased knowledge of the genetics of PCN resistance in potato. The use of molecular markers (Gebhardt *et al.*, 1989; Williams *et al.*, 1990) may lead to the identification of quantitative trait loci for resistance (Paterson *et al.*, 1988), which cannot be identified with classical methods. Kreike (pers. comm.) found two loci in *S. spegazinii*, associated with decreased numbers

of newly formed cysts of *G. rostochiensis*.

The knowledge about the frequency of PCN populations with specific virulence characteristics in the field is still insufficient. Biochemical and molecular data of collected PCN populations (Bakker, 1987; Phillips *et al.*, 1992; Stratford *et al.*, 1992) can reveal genetic relationships, which are likely to be associated with specific virulence characteristics. These data may be useful tools to select populations for resistance testing, which are representative of the variation in virulence (Bakker *et al.*, 1992). The feasibility of this approach was further confirmed here, as differences in the protein pattern between D234 and D236 and other *G. pallida* populations (Bakker *et al.*, 1992), appeared to be reflected by clear differences in virulence (Chapters 3 and 4).

The discovery of a monogenic resistance to some *G. pallida* populations (Chapter 3) may stimulate further research into finding other sources of monogenic resistance, effective to a larger number of *G. pallida* populations. Such research was strongly advocated by Bakker (Agric. Univ. Wageningen, pers. comm.). Apart from the H_2 resistance gene, effective to the *G. pallida* pathotype Pa1, no single genes for resistance to *G. pallida* had been found. The inheritance of resistance to *G. pallida* from several sources is reported to be polygenic (Dale & Phillips, 1982; Dellaert *et al.*, 1988). Monogenic resistance has important advantages over the presumably polygenically inherited resistance from *S. vernei*, which is currently used. (1) Monogenic resistance can be incorporated in cultivars more easily (Huijsman, 1974). The presence of monogenic resistance in offspring of crosses can be tested with a single PCN population, and the test results are highly reproducable. With polygenic resistance, it is very difficult to maintain a high level of resistance in successive backcross-generations (Huijsman, 1974). (2) Tests of the resistance levels to various PCN populations of different cultivars with the same single gene resistance would not be necessary. Various cultivars with resistance from *S. vernei* show different resistance spectra to PCN (Chapter 2), which may require much testing.

Possibly, more research will lead to the discovery of additional sources of monogenic resistance to *G. pallida*. The inheritance of several sources of resistance to *G. pallida* (van Soest *et al.*, 1983; Dellaert & Hoekstra, 1987) has not been reported yet and may be assessed. Research with Mexican wild *Solanum* species may be especially interesting. Some accessions of some of these species have been reported to be resistant to *G. pallida* populations (van Soest *et al.*, 1983; Dellaert & Hoekstra, 1987). *G. pallida* is believed to originate from South-America (Evans *et al.*, 1975). Thus, virulence in *G. pallida* may not have evolved to the resistance in these *Solanum* species, in the way

described by Stone (1985).

The durability of monogenic resistance presumably depends on the presence of the corresponding virulence in PCN populations. The multiplication rate of PCN is low and mutations are not believed to have noticeably contributed to genetic variation (Bakker *et al.*, 1992). The cultivar Maris Piper with the H_1 gene, conferring resistance to *G. rostochiensis* pathotype Ro1, has been used for over 30 years in the United Kingdom. There is however no evidence that this has led to the appearance of populations of this species with virulence to the H_1 gene (Jones, 1985). Similarly, a major gene to *Meloidogyne incognita* and *M. javanica* in a peach rootstock has proved to be durable for more than 35 years now (Roberts, 1992). When, however, virulence is present at a low frequency in populations, selection for virulence may occur. A breakdown of the H_1 gene resistance was reported by Huijsman (1963). However, selection for virulence to the polygenic resistance from *S. vernei* has also been found (van der Wal, 1987; Turner, 1990). Stone (1985) suggested that the actual number of genes involved in this resistance may be small.

The resistance to *G. pallida* population D236 occurred in several potato cultivars, which have not been intentionally selected for it (Chapter 3). The unexpectedly high frequency of this resistance in potato cultivars was likely to be due to linkage of the resistance to the H_1 gene or possibly to some other trait, for which these cultivars had been selected. Possibly, more potato cultivars may be found to contain not yet identified PCN resistance genes. The number of backcrosses of wild donor species with *S. tuberosum* ssp. *tuberosum* genotypes is usually low. Introduced genes for disease resistance may be linked to PCN resistance. Potato breeders have used several primitive and wild *Solanum* species for the development of new cultivars, such as for instance *S. phureja*, *S. acaule*, *S. gourlayi* for virus resistance and *S. vernei* and *S. verrucosum* for late blight resistance (Ross, 1986). In these species, PCN resistance has been reported to occur (Hoekstra & Seidewitz, 1987). Genes for resistance to diseases appear to be linked quite often (Hooker & Saxena, 1971; Hulbert & Michelmore, 1985; Jörgensen, 1992). The same may occur for resistance to nematodes and fungi. The *Cladosporium* leaf mold resistance gene *Cf2*, the powdery mildew resistance gene *O1* and the *Meloidogyne* nematode resistance *Mi* gene are closely linked on chromosome 6 of tomato (Kerr *et al.*, 1980; van der Beek, pers. comm.). Valuable potato cultivars, which however have not been intentionally selected for PCN resistance, are currently not tested for the presence of such resistance (Bakker, Phytosanitary Service, pers. comm.) and thus implicitly regarded as susceptible in the Dutch Cultivar List (*Anon.*, 1992c). It may therefore be worthwhile to screen all

potato cultivars, derived from crosses with wild or primitive species, for possible additional resistance to PCN.

Tolerance

Schafer (1971) proposed to compare tolerance between genotypes relative to disease severity or pathogen development. In this thesis, tolerance of a potato genotype to PCN has been compared to other genotypes at the same PCN infestation level in the soil (Trudgill, 1986). This is a practical and a useful conception of tolerance, when considering the life-cycle of PCN. For comparison of the tolerance of potato genotypes, it is thus not necessary to assess infection levels of roots with various developmental stages of the nematodes at regular intervals during crop growth.

This research revealed that large differences occur in tolerance between genotypes with resistance to *G. pallida*, derived mainly from *S. vernei* (Chapters 5 and 6). By further crossing, potato cultivars with *G. pallida* resistance are being developed from these genotypes. These findings demonstrate that the use of newly developed cultivars with PCN resistance may be severely limited by intolerance to PCN, when no selection for tolerance was carried out during the breeding programme.

Tolerance in the field was assessed by measuring the absolute yield in heavily PCN infested fields and by this yield, proportional to the yield on adjacent, nematicide-treated strips. The absolute biomass of genotypes in heavily infested pots, as well as this biomass, proportional to the biomass in uninfested pots, was well correlated with the field tolerance data (Chapter 5). However, the correlations (Chapter 5) were slightly lower with proportional data than with absolute data. In the field, proportional data may be inaccurate, due to damage to plants by remaining PCN on the nematicide-treated strips. Moreover, in general ratios are more variable as their constituencies. When screening for tolerance, control treatments are presumably not necessary.

The large differences in tolerance between resistant genotypes (Chapter 5) suggest that tolerance and resistance are different traits. Also, no relationship between the levels of resistance and tolerance in progenies was found (Chapter 6). The infection process in resistant genotypes is very different, compared to susceptible genotypes, which may affect the extent of damage by PCN. Resistant genotypes are invaded by nematodes, but in roots of resistant genotypes, many penetrated juveniles leave the roots after some time (Forrest *et al.*, 1986; Mullin & Brodie, 1988). Differences between potato genotypes for

the distance in the roots travelled by PCN juveniles after invasion have been found, presumably related to resistance or susceptibility of the roots (Robinson *et al.*, 1988). In resistant genotypes, the hypersensitivity reaction may cause damage to the plant (Evans & Haydock, 1990). Hypersensitivity, however, is not confined to non-compatible host-nematode interactions (Robinson *et al.*, 1988). The amount of newly formed cysts is less in resistant genotypes, but the number of males formed is higher (Mugniéry & Fayet, 1984; Janssen *et al.*, 1992). The total food consumption of female nematodes is much higher than that of males (Müller *et al.*, 1981). However, the total withdrawal of cell sap by nematodes is small and thought not to contribute much to plant damage (Müller *et al.*, 1981). Despite the mentioned differences, no relationship between tolerance and resistance was found (Chapter 6). These results confirm earlier findings of Dale *et al.* (1988) and Elston *et al.* (1991), based on less genotypes. The results demonstrate clearly that tolerance and resistance may be treated as separate plant characteristics, and can be combined in genotypes.

The continuation of root growth after infection with *G. pallida* juveniles and the rate of hatching appeared to be two important variables, associated with tolerance, for the set of genotypes tested. With these explanatory variables, high percentages explained variance were obtained in multiple regression analysis (Chapter 8). Research on the induction of hatching by potato genotypes has been reported before by several authors (Turner & Stone, 1981; Evans, 1983; Farrer & Phillips, 1983; Forrest & Phillips, 1984; Balandras, 1988), and mostly concerned the final numbers of juveniles hatched. Some resistant genotypes were found to induce less hatching, but the observed differences in hatching were small and were assumed not to contribute much to resistance (Turner & Stone, 1981; Forrest & Phillips, 1984). Hatching was thought only to play a minor role in determining the severity of invasion damage (Evans & Haydock, 1990). In Chapter 7, the hatching process was described by three parameters, and the rate of hatching could be distinguished from the final numbers of juveniles hatched. The rate of hatching appeared to have considerably more explanatory value than the final numbers of juveniles hatched for the tolerance of the genotypes tested. A reduced rate of hatching may allow the root system to grow longer before PCN damage occurs. Simulation studies of population dynamics of PCN and damage by PCN support this hypothesis (Schans, 1993). Differences in hatching behaviour between *G. rostochiensis* and *G. pallida* have been found (den Nijs & Lock, 1992), and it is possible that tolerance conferred by slow hatching is at least species-specific.

The underlying mechanisms affecting root growth after infection are still unclear.

Huijsman *et al.* (1969) observed differences in extent of necrosis between potato genotypes, but these differences occurred only at later stages of infection. No significant correlations between root weight of uninfested and infested plants were found (Chapters 5 and 6). Also, no significant correlation of growth of inoculated and untreated roots was found, more than one day after inoculation (Chapter 8). Thus, no relationship of the observed differences in root growth of infected roots and the vigour of growth of uninfected roots could be demonstrated. The differences in growth of infected roots may explain the different extent of damage by the nematodes, but may also be associated with other mechanisms of damage (Dorhout, 1992; Schans, 1993).

Wallace (1987) suggested that tolerance to nematodes has an aspecific nature. The response of the plant is supposedly not specific to the nematodes but to the injury caused by them, which includes nutrient deficiency, water stress and disruption of the hormonal balance in plants. Tolerance would thus not exert any selection pressure on the nematodes (Wallace, 1987). Trudgill (1987b) found that nutrient deficiency may enhance PCN damage. However, no differences in tolerance to nutrient stress (Gerloff, 1987) have been documented in potato. No interaction between drought stress and nematode damage was reported (Haverkort *et al.*, 1992). It is very likely that hormone production is affected by nematodes, as tissue injury generally leads to at least an increased production of ethylene (Hale & Orcutt, 1987). Cytokinins are mainly produced in the root tips, which may be invaded by PCN. The effects of nematode inoculation within a few days on root growth *in vitro* (Chapter 8) and on photosynthesis and transpiration (Schans, 1991) may be caused by the production of growth inhibiting hormones as a reaction to injury, caused by the nematodes. Photosynthesis by PCN was less reduced in later stages of infection (Schans & Arntzen, 1991). The disruption of the hormonal balance in plants by cyst nematodes has not been studied often. Fatemy *et al.* (1985) found a ninefold difference in the abscisic acid (ABA) content of leaves of uninfested plants of the PCN tolerant potato cultivar Cara and the intolerant cultivar Pentland Dell. PCN did not increase significantly the ABA content in both cultivars. Volkmar (1991) measured levels of the root growth inhibiting hormones ABA and ethylene in cultured root segments of oats, either uninfected or infected with the cereal cyst nematode *H. avenae*. ABA and ethylene levels increased considerably, directly after infection. No difference in these levels between two cultivars, differing in tolerance, was found.

In a breeding programme for *G. pallida* resistance, it is advised to select against extreme intolerance, which occurs in parts of the resistant material. Screening methods have been indicated in this thesis. However, with some speculation alternative strategies can be

mentioned, possibly suitable to achieve high levels of tolerance. (1) Direct selection against the production of hatching agents in plants may be feasible, as the chemical structure of hatching agent is said to be identified in research by a private company (*Anon.*, 1991b). Genetic variation for hatching in potato has been demonstrated by several authors and also in this thesis (Chapter 7). (2) Further research into the mechanism of continued root growth after PCN infection may be useful, especially into the role of the disruption of the phytohormone balance by the nematodes (Wallace, 1987; Volkmar, 1991). These studies may include the effects of exogenous applications of phytohormones on root growth after PCN infection. Transformation with cytokinin-synthesizing genes is possible (Spena *et al.*, 1992) as well as with wound-inducible promoters (Vierling & Kimpel, 1992).

Potato breeding and breeding research has been mainly focused on PCN resistance and not on tolerance. The combination of resistance to and tolerance of PCN in potato, however, can be achieved rather easily, and is therefore strongly advocated.

SUMMARY

Potato cyst nematodes, *Globodera rostochiensis* and *G. pallida*, are major pests of the potato crop. Control of potato cyst nematodes (PCN) can be obtained by crop rotation, application of nematicides and growing resistant cultivars. When suitable cultivars with PCN resistance are available, the growth of such cultivars is the most attractive way to control PCN. However, resistance in most potato cultivars is specific to PCN populations. Moreover, resistant cultivars may be very intolerant, resulting in a low yield in heavily PCN infested fields. In this thesis, results of research are presented, relevant for the breeding and use of PCN resistant cultivars.

Some initial methodological research revealed that the weight of dry cysts measured with a highly sensitive balance, is highly representative of the number of eggs in cysts, reared in the greenhouse (Chapter 1). Usually, this number is determined by crushing the cysts and counting the eggs. The number of eggs in such cyst samples can be assessed in a rapid and non-destructive way by weighing.

In Chapter 2, in two experiments the number of newly formed cysts was determined of ten PCN populations with nine potato genotypes with resistance from various sources. Various differences in levels of virulence and resistance were found. Several 'virulence groups' could be identified within *G. rostochiensis* as well as within *G. pallida* by the use of a simultaneous hierarchical clustering procedure. The distinction of such virulence groups is a good alternative for the present distinction of pathotypes. The expression of resistance in potato cultivars in a quantitative way is advised, tested against PCN populations representative of virulence groups.

Previously unknown resistance to *G. pallida* was found in the cultivar Multa (Chapter 3). Crosses were made and the progenies were tested for resistance. The frequency distributions of genotypes for number of cysts in the progenies indicated clear segregations. Multa appeared to carry a single gene, conferring high level resistance to the *G. pallida* population D236 and presumably also to D234. The low level resistance to various other *G. pallida* populations, as found in Multa, appeared to have a separate genetic basis. This is only the second report of monogenic resistance to *G. pallida*. This type of resistance is easier to incorporate in new cultivars than the currently used resistance from *S. vernei*, with polygenic inheritance. This finding may stimulate further research for sources of monogenic resistance to G. pallida, effective to a larger number of populations.

The reported monogenic resistance presumably originates from *S. tuberosum* ssp. *andigena* CPC 1673, widely known as the source of the H_1 resistance gene to G. *rostochiensis* (Chapter 3). Eight cultivars with the H_1 gene, out of the eleven tested, appeared to have also the resistance to D236. This is likely to be due to linkage of the D236 resistance to some other trait, for which these cultivars have been selected, presumably the H_1 gene.

Chapter 4 describes the comparison of the resistance in Multa with the earlier found monogenic resistance, derived from *S. multidissectum*. Differential interaction between the resistance and PCN populations revealed that the two genes are distinct. The results presented in Chapters 2, 3 and 4 indicate that the extent of variation for virulence in *G. pallida* is considerably larger than previously known.

In Chapter 5, tolerance of genotypes was assessed in the field, by the yield on a heavily *G. pallida* infested field, and by this yield, proportional to the yield in a nematicide-treated strip in the same field, in two years. The tolerance of fifteen genotypes, with resistance to *G. pallida* from *S. vernei*, appeared to differ largely. Some genotypes showed extreme intolerance, compared to three standard cultivars. Selection for tolerance in a *G. pallida* resistance breeding programme is advised, in order to avoid the development of resistant, but highly intolerant potato cultivars. Tolerance of these genotypes was also assessed in pots in the greenhouse by the total biomass after 35 and 70 days growing in heavily *G. pallida* infested soil and in uninfested soil (Chapter 5). High correlations for tolerance assessments were found between the two field experiments and between field and pot experiments. Differences in damage occurred already early in growth of the plants in the greenhouse. Shoot and root dry weight were about equally affected by the nematodes. No relationship between tolerance and maturity score was found.

Crosses were made between a *G. pallida* resistant, intolerant genotype and two tolerant genotypes, with only a low level of resistance or susceptibility. Tolerance of and resistance to *G. pallida* of the progenies, segregating for these characteristics, were assessed in pots in the greenhouse (Chapter 6). A considerable number of genotypes was significantly more tolerant than the intolerant parent genotype. The levels of resistance and tolerance in the progenies were not significantly correlated. Resistance and tolerance may be regarded as separate plant characteristics, which can be combined in genotypes.

Slow hatching of PCN juveniles by root diffusate may allow longer root growth before large numbers of juveniles penetrate and thus confer tolerance. To investigate a possible relationship between slow hatching and tolerance, hatching was induced *in vitro* by root

diffusate, obtained from various potato genotypes with differing tolerance, and compared to standard potato root diffusate (Chapter 7). A three parameter logistic model was used to describe the numbers of hatched juveniles in relation to time of exposure to root diffusate. Some tolerant genotypes hatched *G. pallida* juveniles relatively late, compared to intolerant genotypes. Other tolerant genotypes, however, hatched juveniles about as early as intolerant genotypes.

The effect of direct inoculation of juveniles of *G. pallida* was studied on root growth in vitro of eighteen potato genotypes, differing in tolerance (Chapter 8). Four to seven days after inoculation, genotypes differed strongly in increase of length of inoculated roots. This growth was not associated with growth of untreated roots at that stage. Only low correlations were observed between the growth of inoculated roots and tolerance assessed in the greenhouse and in the field. Multiple regression analysis, however, revealed that the tolerance of the tested genotypes was associated with both the induced rate of hatching and the growth of roots after inoculation. The combination of these two explanatory variables gave high percentages of explained variance in the analysis.

SAMENVATTING

Aardappelcysteaaltjes, *Globodera rostochiensis* en *G. pallida*, vormen een belangrijke plaag in het aardappelgewas. Ze veroorzaken aardappelmoeheid. Bestrijding van aardappelcysteaaltjes (ACA) kan plaatsvinden door vruchtwisseling, gebruik van chemische bestrijdingsmiddelen en de teelt van resistente rassen. Resistentie in planten leidt tot minder of geen vermeerdering van de aaltjes. Als goede resistente rassen beschikbaar zijn, is de teelt daarvan het meest aantrekkelijk ter beheersing van aardappelmoeheid. Helaas werkt de resistentie in de meeste rassen slechts tegen een gedeelte van de aaltjespopulaties, die in het veld voorkomen. Tegen andere, meer virulente populaties is die resistentie minder of niet effectief. Bovendien kunnen resistente rassen intolerant voor aaltjes zijn, dat wil zeggen dat ze relatief zwaar beschadigd worden en weinig opbrengen bij zware besmetting van de grond. In dit proefschrift is onderzoek beschreven, dat relevant is voor verbetering van de ontwikkeling van ACA resistente rassen en van het gebruik van deze rassen.

Methodologisch onderzoek aan in de kas verkregen cysten gaf aan dat het gewicht van die cysten, gemeten met een zeer gevoelige balans, representatief is voor het aantal eieren dat deze cysten bevatten (Hoofdstuk 1). Het aantal eieren wordt doorgaans bepaald door de cysten te vermalen in water en het aantal vrijgekomen eieren te tellen. Wegen is een snelle methode om het aantal eieren in cysten te schatten. De methode is niet-destructief en de cysten kunnen zo voor verder onderzoek gebruikt worden.

In Hoofdstuk 2 zijn experimenten beschreven, waarin het aantal nieuwgevormde cysten is bepaald op negen aardappelgenotypen met tien verschillende aaltjespopulaties. De niveaus van resistentie van de genotypen, en die van virulentie van de populaties verschilden sterk. Verschillende virulentiegroepen konden worden onderscheiden binnen zowel *G. rostochiensis* als *G. pallida*, met gebruikmaking van een simultane hiërarchische clustering procedure. Het onderscheid van virulentiegroepen is een goed alternatief voor het onderscheid van pathotypen, zoals nu gebruikelijk is. Resistentie in aardappelrassen kan het beste kwantitatief worden uitgedrukt, getoetst met ACA populaties die representatief zijn voor virulentiegroepen.

Een tot nu toe onbekende resistentie werd gevonden in het aardappelras Multa (Hoofdstuk 3). Er werden kruisingen met dit ras gemaakt en de nakomelingsschappen werden getoetst op resistentie. De frequentieverdelingen van genotypen voor aantallen cysten in deze nakomelingsschappen gaven duidelijke uitsplitsingen te zien. Multa bleek

een door één gen bepaald hoog niveau van resistentie te bezitten tegen *G. pallida* populatie D236, en vermoedelijk ook tegen D234. Het lagere niveau van resistentie tegen diverse andere *G. pallida* populaties in Multa bleek een aparte genetische basis te hebben. Dit is pas de tweede keer dat monogene resistentie tegen *G. pallida* wordt beschreven. Monogene resistentie (één resistentiegen) kan makkelijker in rassen ingekruist worden dan de tot nu toe gebruikte resistentie uit *S. vernei*, die op meerdere genen berust. Dit resultaat kan onderzoek stimuleren naar andere bronnen van monogene resistentie tegen *G. pallida*, die werkzaam zijn tegen een groter aantal populaties.

De beschreven monogene resistentie komt waarschijnlijk oorspronkelijk uit de primitieve aardappelsoort *S. tuberosum* ssp. *andigena* CPC 1673, zeer bekend als de bron van het H_1 resistentiegen tegen *G. rostochiensis*. Acht van de elf getoetste rassen met het H_1 gen bleken ook de resistentie tegen D236 te bezitten. Dit kan waarschijnlijk verklaard worden door genetische koppeling van deze resistentie met een andere eigenschap, waarvoor deze rassen wèl geselecteerd zijn, vermoedelijk het H_1 gen.

Hoofdstuk 4 beschrijft de vergelijking van de resistentie in Multa met de eerder gevonden monogene resistentie, afkomstig van *S. multidissectum*. De verschillende interactie tussen aaltjespopulatie en de resistentie gaf aan dat het om twee verschillende genen gaat. De resultaten in de hoofdstukken 2, 3 en 4 geven aan dat de variatie in virulentie binnen *G. pallida* aanzienlijk groter is dan tot nu toe aangenomen.

In Hoofdstuk 5 werd de tolerantie van genotypen bepaald in het veld. De opbrengst op een zwaar met *G. pallida* besmet veld werd gemeten, en vergeleken met de opbrengst op een met nematicide ontsmet gedeelte van hetzelfde veld. De tolerantie van vijftien genotypen met resistentie tegen *G. pallida* uit *S. vernei* bleek sterk te verschillen. Sommige genotypen waren extreem intolerant, vergeleken met enkele standaardrassen. Het is aanbevelenswaardig selectie voor tolerantie van genotypen in een veredelingsprogramma voor *G. pallida* resistentie uit te voeren, om de ontwikkeling van nieuwe resistente, maar zeer intolerante rassen te voorkomen. Tolerantie van dezelfde genotypen werd ook bepaald in potten in de kas door meting van de totale biomassa op enkele tijdstippen in zwaar met *G. pallida* besmette grond (Hoofdstuk 5). Tolerantiebepalingen in het veld gedurende twee jaar waren sterk gecorreleerd, en ook werden hoge correlaties gevonden tussen tolerantiebepalingen in het veld en in de kas. De verschillen in schade ontstonden al vroeg in de groei van de planten in de kas. Spruit- en wortelgewicht werden ongeveer evenredig gereduceerd door de nematoden. Er was geen verband tussen tolerantie en rijptijdscore.

Er werden kruisingen gemaakt tussen een *G. pallida* resistent, intolerant genotype en

twee tolerante genotypen, met slechts een laag nivo van resistentie of vatbaar. De tolerantie van en resistentie tegen *G. pallida* van de nakomelingsschappen werd bepaald in potten in de kas (Hoofdstuk 6). Een aanzienlijk aantal genotypen in de nakomelingsschappen was significant toleranter dan de intolerante ouder. Er was geen aantoonbaar verband tussen de niveaus van resistentie en tolerantie in de nakomelingsschappen. Resistentie en tolerantie kunnen het beste als aparte eigenschappen worden beschouwd, die kunnen worden gecombineerd in genotypen.

Langzame wekking van ACA juvenielen door wortelexudaat kan langere wortelgroei tot gevolg hebben voordat grote aantallen juvenielen penetreren en zou zo tot tolerantie kunnen leiden. Om het eventuele verband tussen snelheid van wekking en tolerantie te onderzoeken, werd de wekking *in vitro* bepaald door exudaat van genotypen met verschillende niveaus van tolerantie (Hoofdstuk 7). Een logistisch model met drie parameters werd gebruikt om de aantallen gewekte juvenielen in relatie tot de tijd van blootstelling aan exudaat te beschrijven. Enkele tolerante genotypen wekten *G. pallida* juvenielen relatief laat, vergeleken met intolerante genotypen. Andere tolerante genotypen echter wekten de juvenielen ongeveer even vroeg als intolerante genotypen.

Het effect van directe inoculatie van *G. pallida* juvenielen op de wortelgroei *in vitro* werd bepaald met achttien genotypen, met verschillende tolerantie (Hoofdstuk 8). Vier tot zeven dagen na inoculatie verschilde de lengtegroei van de genotypen sterk. Deze groei was niet gerelateerd aan de groei van onbehandelde wortels in die periode. Er werden slechts lage correlaties gevonden tussen deze lengtegroei van geïnoculeerde wortels en tolerantie, zoals bepaald in het veld en in de kas. Multipele regressie analyse gaf echter aan dat de snelheid van wekking en de groei van wortels na inoculatie gezamenlijk goede verklarende variabelen voor tolerantie zijn, met in alle gevallen hoge percentages verklaarde variantie.

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Curriculum vitae

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