Resistance to root-knot nematodes, *Meloidogyne* spp., in potato.

Geert J.W. Janssen

Resistance to root-knot nematodes, *Meloidogyne* spp., in potato.

Resistentie tegen wortelknobbelaaltjes, *Meloidogyne* spp., in aardappel.

Promotor: dr. ir. E Jacobsen
hoogleraar in de plantenveredeling
in het bijzonder de genetische variatie en reproductie

Resistance to root-knot nematodes, *Meloidogyne* spp., in potato.

Geert J.W. Janssen

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Bibliographic abstract
This thesis describes the identification and evaluation of sources of resistance to the root-knot nematodes *Meloidogyne chitwoodi*, *M. fallax* and *M. hapla* in potato. Resistance has been identified in numerous wild tuber-bearing *Solanum* species and tested against an array of nematode populations to investigate the level of resistance and working spectrum. The effectivity of resistance is also evaluated under natural circumstances in the field. The inheritance of resistance to *M. chitwoodi* and *M. fallax* in *S. fendleri* and *S. hougasii* is studied and revealed the likely presence of one dominantly inherited gene in both species, whereas in the case of resistance in *S. stoloniferum* several additive genes are involved. The first steps of introgression of resistance from these wild species into cultivated potato have been made.

Key words: genetic variation, inheritance, introgression, *Meloidogyne chitwoodi*, *M. fallax*, *M. hapla*, resistance, wild tuber-bearing *Solanum* species

Stellingen

1. Er is voldoende genetische variatie in wilde knoldragende *Solanum* soorten voor resistentie tegen wortelknobbelaaltjes aanwezig.
   Dit proefschrift

2. De koppelling tussen de aanwezigheid van *M. chitwoodi* resistentie en de geografische verspreiding van bepaalde *Solanum* soorten kan verklaard worden door het optreden van co-evolutie tussen plant en pathogeen.
   Dit proefschrift

3. Voor een duurzaam gebruik van nematode resistentie dienen meerdere resistentie-bronnen geïntroduceerd te worden in nieuwe aardappelrassen. Hierdoor kan een hoge selectie-druk op virulentie vermeden worden.
   Dit proefschrift

4. De kruisingsbarriëre tussen *Solanum* soorten, die verklaard wordt door de "endosperm balance number" theorie (Johnston *et al.* 1980), is geen limiterende factor voor de introgressie van nuttige eigenschappen vanuit deze wilde soorten naar de cultuuraardappel.
   Johnston SA et al. *(1980) The significance of genic balance to endosperm development in interspecific crosses. Theor Appl Genet 57: 5-9*
   Dit proefschrift

5. De benaming "waardplant-ras" ter karakterisatie van stengelaaltjes populaties leidt tot een verkeerde benadering van het *Ditylenchus dipsaci* complex.

6. De meeste *Meloidogyne* soorten zullen nog beschreven moeten worden.

7. De recente overnames van biotechnologische bedrijven voor exorbitant hoge bedragen geeft de indruk dat planten en dieren niets meer zijn dan zakken met (te patenteren) genen.

8. Over 25 jaar zal de braak-regeling vermeld gaan worden als een voorbeeld van het onbenut laten van milieu-vriendelijke en duurzame energie-bronnen in Nederland.

9. De "Melkert-banen" zouden eigenlijk "Deetman-banen" genoemd dienen te worden, omdat laatstgenoemde minister als eerste de voordelen heeft uitgebuit van het ondermaats betalen van AIO's voor het uitvoeren van kwalitatief hoogwaardige arbeid.

10. Onderzoek simpel beschrijven is iets anders dan simpel onderzoek beschrijven.

11. AIO's, die werknemer zijn bij een universiteit, gefinancierd worden via externe gelden, gestationneerd op een DLO-instituut en ondergebracht bij een onderzoeksschool hebben een grote kans op een identiteitscrisis.

12. Er gaat niets boven Noorwegen.

Stellingen behorende bij het proefschrift, getiteld "Resistance to root-knot nematodes, Meloidogyne spp., in potato.", door G.J.W. Janssen

Wageningen, 18 april 1997

Contents

Chapter 1

General introduction.

This thesis presents research on the resistance to the root-knot nematodes, *Meloidogyne* spp., in potato and wild *Solanum* spp. In this chapter, first the genetic resources of potato and characteristics of *Meloidogyne* - in particular *M. chitwoodi*, *M. fallax* and *M. hapla* - will be described. Subsequently the interaction of root-knot nematodes with potato and the scope of this thesis.

Genetic resources of potato

The potato, *Solanum tuberosum* ssp. *tuberosum*, is a widely grown field crop with an estimated annual production of approximately 2.6 x 10^{11} kg following wheat, maize and rice as fourth crop in global food production (Anonymous 1992). Potato has one of the richest genetic resources of any cultivated plant. At present, about 230 wild and primitive species are recognised in the section *Petota* of the genus *Solanum* (Hawkes 1990). Most wild species are tuber-bearing and directly crossable with potato and, therefore, useful for the enrichment of germ plasm into cultivars (Ross 1986). They possess a broad spectrum of resistances to pests and diseases, tolerances to frost and drought and many other valuable traits. The tuber-bearing *Solanum* species are very widely distributed in the Americas from South Western USA to Southern Chile and Argentina, from sea level to the highlands of the Andes mountains and a wide genetic gene pool has been created through evolution.

The centre of origin, from which *Solanum* species have evolved, is thought to be in Mexico. The diploid Central American *Solanum* species from series *Bulbocastana*, *Morelliformia*, *Polyadenia* and *Pinnatisecta* are presumed to be closely related to these ancestors of wild *Solanum* spp. and therefore considered as most primitive species. From Mexico, species migrated southwards and evolved into a geographically and genetically separate gene pool of diploid and polyploid *Solanum* species in South America. The Central American polyploid species from series *Demissa* and *Longipedicellata* are thought to have evolved later from amphidiploidisations of primitive Mexican ancestors with the more advanced South American species (Hawkes 1990, 1992). This hypothesis of evolution of tuber-bearing *Solanum* species is supported with phylogenetic analyses using morphological and molecular markers (Hosaka *et al.* 1984; Debener *et al.* 1990) and genetic studies (*e.g.* Matsubayashi 1991; Hawkes & Jackson 1992).

The cultivated potato, *S. tuberosum*, evolved in South America, probably from a complex of ancient domesticated species with *S. stenotomum* as basic ancestor and

related *S. leptophyes, S. sparsipilum, S. megistacrolobum* and *S. phureja* as other genetic sources (Hawkes 1990; Matsubayashi 1991; Hosaka 1995). *Solanum tuberosum* ssp. *tuberosum* has been the basis of all currently used potato cultivars. Nowadays, as a result of breeding efforts, potato cultivars contain genes from one or more wild *Solanum* spp., but the introgressed part remains in general small. Moreover, the number of frequently used wild species for germ plasm enrichment is limited to six, indicating that the genetic resources of *Solanum* spp. have not been exploited extensively (Ross 1986; Hawkes 1990).

Root-knot nematodes, *Meloidogyne* species

Root-knot nematodes, *Meloidogyne* species, are the most damaging plant-parasitic nematodes causing an estimated yield loss of over 10 % world-wide (Sasser & Freckman 1987). They are obligate parasites able to reproduce on monocotyledons and dicotyledons, herbaceous and woody plants, but the host ranges vary largely between the nematode species. There are over 60 species identified, but only four of them - *M. incognita, M. hapla, M. arenaria* and *M. javanica* - are world-wide of major economic importance. Some other species, like *M. chitwoodi*, are also of known economic importance, but have a more restricted distribution (Jepson 1987; Eisenback & Triantaphyllou 1991). In general, root-knot nematodes are more persistent and show higher infectivity levels in sandy and sandy-loam soils due to a reduced ability of migration in soils with a high clay content (Prot & Van Gundy 1981; Mojtahedi *et al.* 1991).

Identification

It is essential to have a reliable identification procedure for *Meloidogyne* species, both for research purposes as for decisions on control measures of root-knot nematodes, such as choosing a suitable crop rotation or resistant cultivar. The identification of root-knot nematodes and the description of new species is usually based on morphological features, but some of these features show considerable variation and can be influenced by the host and environmental conditions (Jepson 1987). Moreover, morphological identification requires a lot of skill and is often inconclusive for individuals. The use of a differential host plant test, as developed for the major *Meloidogyne* species (Sasser 1979; Sasser & Carter 1985), is time consuming and restricted to practical purposes only. Even the feasibility of such a differential test to distinguish the subtropical and tropical nematode species is questioned (Netscher 1978, 1983). Therefore, species identification and characterisation using biochemical

methods and DNA polymorphisms are increasingly becoming important (Baldwin & Luc 1995).

Dickson *et al.* (1971) first described specific enzymes useful for the identification of *Meloidogyne* species. The possibilities of using these specific isozymes for species identification have been further exploited by others (Dalmasso & Bergé 1983; Esbenshade & Triantaphyllou 1985, 1987, 1990). To date, isozyme analysis is an important diagnostic tool due to the stability of the patterns and the ease to carry out this technique. Other techniques have been developed or are in development for species identification, which are based on polymorphisms of nuclear DNA, such as RAPD's (Cenis 1993; Castagnone-Sereno *et al.* 1994*a*), RFLP's (Curran *et al.* 1986; Cenis *et al.* 1992; Fargette *et al.* 1996) and satellite DNA (Piotte *et al.* 1995), mitochondrial DNA (Harris *et al.* 1990) or ribosomal DNA (Zijlstra *et al.* 1995; Petersen & Vrain 1996). Some of these techniques have the potential to become diagnostic tools for the assessment of species characterisation and, possibly, intraspecific variation.

Meloidogyne hapla

The Northern root-knot nematode, *M. hapla*, has been described by Chitwood (1949) together with a revision of the genus *Meloidogyne*. It is known as one of the four major *Meloidogyne* species and occurs on all continents. The enormous list of host plants include most dicotylous food crops and several angiosperms, like woody ornamentals and fruit trees (Bernard & Witte 1987; Jepson 1987). *M. hapla* is generally found in the cooler regions of the world and in subtropical and tropical areas at higher altitudes (Eisenback & Triantaphyllou 1991). Eggs and juveniles of *M. hapla* can survive low temperatures down to -15 °C in the soil for a prolonged period in contrast to the tropical and subtropical *Meloidogyne* species (Dao 1970; Belair 1985).

The base threshold temperature for hatching and development of *M. hapla* is estimated to be 9 °C (Inserra *et al.* 1983; Lahtinen *et al.* 1988) and the optimum reproduction occurs at 25 °C (Griffin & Jorgenson 1969). The thermal time required to complete the first generation was found to vary between 550 and 650 day ° (Lahtinen *et al.* 1988). Under natural cropping circumstances in North Western Europe, up to 3 generations will therefore be possible on susceptible crops.

There are two cytological races - A and B - known, of which race A is most common. Populations of race A reproduce by amphimixis and/or meiotic parthenogenesis and have chromosome numbers varying from 14 to 17. Race B populations have chromosomes up to 48 and reproduce by obligatory mitotic parthenogenesis. Race B populations are presumed to have evolved as polyploids from race A (Triantaphyllou 1966, 1985). There are no host races described of *M. hapla*, although several studies have indicated large differences between *M. hapla* populations on host

plants, like strawberry (Sasser 1966, 1972), cowpea and rose stocks (Dalmasso & Bergé 1975).

Meloidogyne chitwoodi

In 1980, *M. chitwoodi* has been described as a new nematode species (Golden *et al.* 1980). The host range of this nematode species include not only numerous dicotylons, but also cereals and grasses (O'Bannon *et al.* 1982). The occurrence of *M. chitwoodi* populations capable of reproducing on lucerne led to the designation of a second race (Santo & Pinkerton 1985), which could also be distinguished from race 1 populations on carrot, which is a good host to race 1, but non-host to race 2 (Mojtahedi *et al.* 1988). *M. chitwoodi* has 14 to 18 chromosomes and reproduces by facultative meiotic parthenogenesis (Triantaphyllou 1985).

The infectivity of *M. chitwoodi* starts with a base threshold temperature of 5 - 6 °C and a thermal time requirement to complete a generation of approximately 600 - 1000 day ° (Pinkerton *et al.* 1991). On potato, the rate of reproduction is therefore higher at temperatures lower than 30 °C compared to *M. hapla* due to this low base threshold temperature (O'Bannon & Santo 1984) and up to 5 generations can be obtained during one growing season in North Western Europe.

The presence of *M. chitwoodi* has been overlooked in the past and the economic damage underestimated due to the morphological resemblances with *M. hapla* (Santo 1994). Analysis of nematological collections showed that *M. chitwoodi* has been present in the Netherlands since at least 1930 (Anonymous 1991). Also re-identification of preserved potato tubers from Argentina revealed that *M. chitwoodi* has been present there since 1969 (Van Halteren 1996). In 1955 in Belgium, a population of *M. hapla* was found able to multiply on barley (Gillard 1961), while cereals are strict non-hosts for *M. hapla* (*e.g.* Eisenback & Triantaphyllou 1991), suggesting that this might have been *M. chitwoodi*.

To date, the exact distribution area of *M. chitwoodi* is still unclear. So far, populations of *M. chitwoodi* have officially been found in USA, Mexico, the Netherlands, Belgium, Germany, Portugal, South Africa and Argentina (Golden *et al.* 1980; Esbenshade & Triantaphyllou 1985; Kleynhans & Van den Berg 1988; Cuevas & Sosa Moss 1990; Anonymous 1991; Eisenback & Triantaphyllou 1991; Müller *et al.* 1996).

Meloidogyne fallax

In 1994, a deviating group of populations of *M. chitwoodi* was distinguished on the basis of isozyme pattern and host- and morphological differences (Van Meggelen *et al.* 1994). Recently, this group has been characterised as a new species, *M. fallax*

(Karssen 1996). In pot experiments, a differential behaviour of *M. fallax* compared to *M. chitwoodi* was found on maize, French bean and valerian, being poor hosts to *M. fallax* and good hosts to *M. chitwoodi*. However, large differences in reproduction were found between cultivars of French bean, indicating the possible action of resistance genes (Brinkman *et al.* 1996). The temperature requirements of *M. fallax* are supposed to be similar to *M. chitwoodi*. Populations of *M. fallax* are found in the south-eastern part of the Netherlands near the borders of Germany and Belgium (Karssen 1996). Besides the Netherlands, a population of *M. fallax* has been located in France (Daher *et al.* 1996). The distribution of *M. fallax* seems to coincide largely with the *M. chitwoodi* distribution pattern (Karssen 1996) and therefore other countries, as described above for *M. chitwoodi*, may have this newly described species as well.

Control

Crop rotation is still the most widely used control measure to suppress damage by and population build-up of *Meloidogyne* spp. However, the wide host range of root-knot nematodes and the low profits of various poor- and non-host crops make alternative measurements necessary for intensive agricultural cropping systems. Nematicides have proven their economic value, but the environmental pollution and risks to human health through residuals in soil and water have led to regulatory restrictions and the use of nematicides should be avoided, if possible.

Plant resistance is an effective, economical and environmentally safe alternative to control root-knot nematodes (Fassuliotis 1979; Boerma & Hussey 1992; Roberts 1995). The number of cultivars of certain crops with resistance to *Meloidogyne* spp. is extensive (*e.g.* Fassuliotis 1979; Sasser & Kirby 1979), but resistance is still lacking in cultivars of some important crops such as the potato (Sasser & Freckman 1987; Roberts 1992).

The interaction of root-knot nematodes on potato

Several *Meloidogyne* spp. are able to parasitise potato and give rise to considerable yield losses and quality damage of tubers. In warm climates, *M. incognita*, *M. arenaria*, *M. javanica* and *M. mayaguensis* are the most prevalent species, whereas in temperate climates *M. chitwoodi*, *M. fallax* and *M. hapla* are major damaging species. Damage threshold studies have shown that one *M. chitwoodi* juvenile per 250 cm^3 of soil already can cause economic damage (Santo 1994). Besides the losses as a direct result of nematode infection, *Meloidogyne* spp. can also influence the severity of other diseases, like *Verticillium* spp. and *Pseudomonas solanacearum*, leading to a synergistic interaction with respect to yield losses (Jacobsen *et al.* 1979; Webster

1985; Wheeler *et al.* 1994; Roberts *et al.* 1995). Last but not least, seed potato production is threatened in areas where *Meloidogyne* spp. are detected, since infected potato tubers are hearths for the infestation of *Meloidogyne*-free fields. In 1997, a quarantine legislation will be in force for *M. chitwoodi* and *M. fallax* on seed potatoes in the countries of the European Community (Gussekloo 1996).

The infection process

In a compatible interaction, second stage *Meloidogyne* juveniles penetrate the roots directly behind the root cap or at branching points of lateral roots and induce the development of so-called 'giant cells' in the differentiating vascular tissue by secreting saliva proteins into the penetrated host cell (Huang 1985; Hussey 1989*a*). These giant cells are multinucleate cells, formed by repeated mitotis without cytokinesis. The cells contain increased numbers of mitochondria, plastids, ribosomes and there is a proliferation of cytoplasm and rough endoplasmic reticulum (Huang & Maggenti 1969; Huang 1985). The formation and organisation of giant cells has been reviewed in detail by several authors (*e.g.* Bird 1979; Jones 1981; Huang 1985). The nematodes are completely dependent on these giant cells, since they function as transfer cells for large quantities of nutrients from the adjacent vascular tissue during the development of the nematodes.

Root-knot nematodes are also able to penetrate developing potato tubers and reproduce inside the tuber. Juveniles enter through natural openings ('eyes'), woundings caused by other pathogens or through the epidermis (Finley 1981). Heavy infestations are regularly accompanied with malformations of the tuber tissue, which is visible on the outside as galls. In the case of *M. hapla*, these external symptoms are hardly pronounced under temperate conditions. Inside the tuber, the infected sites are visible as brown spots, mostly around vascular traces in the cortex (Finley 1981), but artificial inoculations have shown that nematodes can reproduce apparently random inside the tuber (Janssen *et al.* 1995).

Resistance to root-knot nematodes

In potato cultivars, resistance to root-knot nematodes appears to be lacking (Iwanaga *et al.* 1989; Brown *et al.* 1994; Janssen *et al.* 1995). In Figure 1.1, the reproduction of *M. fallax* and *M. hapla* on ten frequently used cultivars in the Netherlands is shown. All genotypes show good reproduction of either nematode species (adapted from Janssen *et al.* 1995).

Resistance to *M. hapla* has been reported in wild *Solanum* spp. by Brücher (1967), but he only indicated resistance by rating infections from slight to heavy. Hoyman

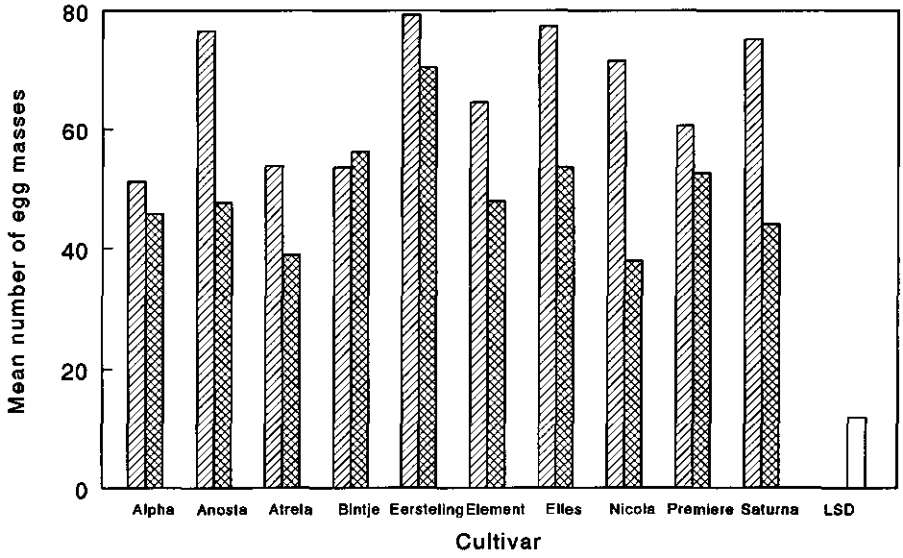


Figure 1.1. Reproduction of *M. fallax* (striped) and *M. hapla* (cross-hatched) on ten Dutch potato cultivars, seven weeks after inoculation of 260 juveniles (adapted from Janssen *et al.* 1995).

(1974) tested 218 clones of *S. tuberosum* and 238 wild *Solanum* accessions, but the field was probably also infected with *M. chitwoodi*, which at that time had not yet been described (Brown *et al.* 1994). Still, 81 % of the accessions tested remained free from root galls, indicating poor selective conditions. More recently, Brown *et al.* (1989, 1991) selected resistance to *M. chitwoodi* and/or *M. hapla* in *S. bulbocastanum* and *S. hougasii.* The resistance of *S. bulbocastanum* to *M. chitwoodi* race 1 appeared to be based on a single dominant gene located at chromosome 11 (Brown *et al.* 1996).

Resistance to tropical root-knot nematode species has also been reported in various wild *Solanum* species (Brücher 1967; Nirula *et al.* 1967, 1969; Jatala & Mendoza 1978). The inheritance of the resistance from *S. sparsipilum* appeared to be oligogenic and the genes involved were effective to *M. incognita*, *M. javanica* as well as *M. arenaria* (Gomez *et al.* 1983*a*), but not to *M. chitwoodi* (Brown *et al.* 1994).

Resistance mechanisms

Resistance to root-knot nematodes is most commonly reported as a hypersensitive reaction leading to localised cell necrosis at the infection site (Fassuliotis 1979; Kaplan & Keen 1980; Bingefors 1982). This phenomenon has also been observed in the incompatible reactions of *M. chitwoodi* and *M. incognita* on different resistant *Solanum* genotypes (Canto-Saenz & Brodie 1987; Mojtahedi *et al.* 1995). The time

between penetration and the collapsing of cells may vary between crops from 6 hours in tomato (Dropkin 1969) to up to 15 days in peaches (Malo 1967; Marull *et al.* 1994). Necrosis may not always cause the resistance, but also appear as a consequence of an incompatible reaction (Kaplan & Keen 1980; Bingefors 1982). The absence of necrosis in resistant cotton and failure of nematodes to initiate nuclear divisions in putative giant cells (McClure *et al.* 1974) is illustrative for the existence of other resistance mechanisms. Post-infectional production of phytoalexins, which can inhibit nematode development, has often been associated with the hypersensitive response and might be regarded as a defence mechanism (Kaplan & Keen 1980; Veech 1981). More recent research on hypersensitive reactions to pathogens suggests the activation of a programmed cell death (Greenberg *et al.* 1994; Mittler & Lam 1996), but this genetically controlled mechanism has not yet been investigated in plant-nematode interactions. Another different response leading to (incomplete) resistance is the inhibition of growth of giant cells and retarded growth of the nematode (Fassuliotis 1970; McClure *et al.* 1974; Balhadere & Evans 1995). In some occasions the poor nutritional circumstances give rise to sex reversal in developing nematodes favouring male development and thus resulting in a low reproduction rate (Triantaphyllou 1973).

Scope of this thesis

The aims of this thesis were i) to identify and evaluate sources of resistance to the root-knot nematodes *M. chitwoodi*, *M. fallax* and *M. hapla*, ii) to investigate the inheritance of resistance and iii) to initiate the introgression of the resistance into cultivated potato by interspecific hybridisations followed with backcrosses. In Chapter 2, the results of screening trials of accessions of 64 wild tuber-bearing *Solanum* species are described and the presence of resistance is discussed in relation to the natural distribution of the *Solanum* species. Before resistance will be incorporated into new potato cultivars, it is desired that resistance is effective against a broad spectrum of populations within a species and other *Meloidogyne* species as well. Therefore, resistant and susceptible genotypes of various *Solanum* species were evaluated in a glasshouse against an array of *Meloidogyne* populations to determine the level of resistance and the possible presence of virulence in nematode populations (Chapter 3). The effectivity of the resistance of some selected resistant genotypes was also evaluated under natural field conditions in two *Meloidogyne* infested fields in the Netherlands (Chapter 4). In Chapter 5, the inheritance of resistance of different *Solanum* sources was investigated by analysing progenies of crosses within a *Solanum* species. The first steps of introgression of resistance into the cultivated potato have been initiated by interspecific crosses and first backcrosses and are described in Chapter 6. The implications of the results described in this thesis for potato breeding and some future perspectives are discussed in Chapter 7.

Chapter 2

Resistance to *Meloidogyne chitwoodi*, *M. fallax* and *M. hapla* in wild tuber-bearing *Solanum* spp.[1]

Summary

Over 5000 plants of 64 tuber-bearing wild *Solanum* spp. have been individually screened for resistance to *Meloidogyne chitwoodi*, *M. fallax* and *M. hapla*. Seedlings were analysed by means of counting number of egg masses and resistance was verified by re-testing low-scoring plants using stem cuttings. Resistance to both *M. chitwoodi* and *M. fallax* was observed in *S. bulbocastanum*, *S. cardiophyllum*, *S. brachistotrichum*, *S. fendleri* and *S. hougasii*. Only in *S. chacoense* and to a lesser extent in *S. stoloniferum* and *S. gourlayi* differential results between *M. chitwoodi* and *M. fallax* were observed. Resistance to *M. hapla* was found in *S. bulbocastanum*, *S. brachistotrichum*, *S. cardiophyllum*, *S. arnezii*, *S. chacoense*, *S. tarijense*, *S. boliviense*, *S. gourlayi*, *S. microdontum*, *S. sparsipilum*, *S. spegazzinii*, *S. sucrense*, *S. acaule* and *S. hougasii*. The occurrence of resistance in wild *Solanum* species in relation to their taxonomic status and the implications for introgression of resistance into *S. tuberosum* are discussed.

[1]) This chapter is based on: Janssen GJW, Van Norel A, Verkerk-Bakker B & Janssen R (1996) Resistance to *Meloidogyne chitwoodi*, *M. fallax* and *M. hapla* in wild tuber-bearing *Solanum* spp. Euphytica 92: 287-294

Introduction

In North Western Europe root-knot nematodes, *Meloidogyne* spp., are expected to become a major problem in potato growing areas as a result of recent changes in crop rotation, that now include highly profitable host crops like vegetables, and a reduced use of nematicides in potato. Resistance is known to be very effective to control root-knot nematodes, but is still lacking in most host crops for *M. hapla* (Roberts 1992) and probably also for *M. chitwoodi*, which has been characterised since 1980 (Golden *et al.* 1980). More recently, in the Netherlands a deviating group of populations of *M. chitwoodi* was distinguished on the basis of isozyme pattern and preliminary host- and morphological differences (Van Meggelen *et al.* 1994). Detailed morphological and morphometrical studies have now lead to the description of a new *Meloidogyne* species; *M. fallax* n.sp. (Karssen 1996).

For potato, resistance to root-knot nematodes appears to be lacking in the currently used cultivars (Brown *et al.* 1994; Janssen *et al.* 1995). In wild *Solanum* spp. resistance seems to be present. Resistance to *M. hapla* has been reported by Brücher (1967) and Hoyman (1974). However in both cases experiments were carried out with infested soil or fields. Plots might have been infested with *M. chitwoodi* or *M. fallax*, which at that time had not been described taxonomically (Brown *et al.* 1994). In search for resistance to *M. chitwoodi*, *S. hougasii* and *S. bulbocastanum* were identified as sources of resistance (Brown *et al.* 1989, 1991). Due to the very recent characterisation no resistance screening has been reported with *M. fallax*.

The present report describes a large screening of wild tuber-bearing *Solanum* species for resistance to *M. chitwoodi*, *M. fallax* and *M. hapla*. The occurrence of resistance in wild species is then considered in relation to their taxonomic status and the implications this has for introgression of resistance into *S. tuberosum*.

Materials & Methods

Origin of true potato seed

Seed populations of *Solanum* spp. were kindly provided by the Dutch-German Curatorium for Plant Genetic Resources, Wageningen, the Netherlands. Few other populations are originating from the CPRO-DLO seed collection. Abbreviations for *Solanum* spp. will be used as listed by Hawkes (1990).

Supply of inoculum

Field populations of *M. hapla*, *M. fallax* and *M. chitwoodi* were collected by means of infested roots and used as an inoculum for tomato plants growing in silver sand. Approximately 10 weeks after inoculation single egg masses were taken from the tomato roots and labelled, while the female itself was carefully extracted from the root and identified biochemically using esterase and malate dehydrogenase isozyme patterns (Esbenshade & Triantaphyllou 1990; Van Meggelen *et al.* 1994). After identification approximately 40 egg masses from a *Meloidogyne* infested field with the same isozyme pattern were used as inoculum for young tomato plants in silver sand in large clay pots. Eggs were harvested approximately three months after inoculation by dissolving egg masses with 0.5 % NaOCl-solution (Hussey & Barker 1973). Second stage juveniles were hatched in water and stored at 4 °C until further use. Contamination during multiplication was prevented by the use of sterilised sand and pots, spatial isolation with borders and saucers and growing of *M. incognita*-resistant tomato plants (cv Nematex). Tomato plants were grown in a temperature-conditioned glasshouse (22 ± 2 °C).

Resistance tests

Seeds of *Solanum* spp. were sown in clay pots (diameter 7 cm) in moist silver sand with additional slow release NPK fertiliser and the pots were placed in plastic trays (Janssen *et al.* 1995). When plants showed a vigorous growth - in most cases three to five weeks after sowing -, approximately 600 nematode juveniles suspended in 1 ml water were supplied around the base of the seedlings with an automatic syringe. Seven weeks later plants were individually harvested, roots stained with Phloxine B solution (Dickson & Strubble 1965) and the egg masses counted. Each test contained tomato plants (cv Money Maker) to serve as check for possible miscellaneous nematode conditions.

From every seedling with less than 6 egg masses, a stem cutting was taken and planted in silver sand to repeat the resistance test. Pots were placed in trays with a cover during two weeks to minimise water uptake. When plants showed vigorous growth the resistance test was carried out as previously described for seedlings.

Resistance tests with further stem cuttings were performed, depending on the health condition of the shoots, and were also done to analyse the level of resistance to the other *Meloidogyne* spp. As a control, cuttings were taken of some susceptible seedlings from accessions in which resistance had been found and also tested. All tests were performed in a temperature-conditioned glasshouse (22 ± 2 °C) from July 1992 until June 1994.

Results

Over 5000 plants of 64 tuber-bearing *Solanum* spp. were individually analysed for resistance to *Meloidogyne chitwoodi*, *M. fallax* and *M. hapla*. An arbitrary infection level of 6 egg masses, representing approximately 1 % successful reproduction of the total number of inoculated juveniles, was used to distinguish low- and high-scoring plants and for this survey we defined plants being resistant, when roots had shown less than 6 egg masses in both seedling- and cutting-test (Table 2.1). Only a few accessions with low-scoring plants could not be re-tested due to poor condition of the cuttings or the inability of stem cuttings to develop a proper root system.

Large differences in resistance were observed between *Solanum* species, between accessions of a *Solanum* species and within accessions. Table 2.2 shows mean number of egg masses of *M. chitwoodi*, *M. fallax* and *M. hapla* on a selected number of *Solanum* genotypes, which were repeatedly tested with cuttings in time. Resistance to *M. chitwoodi* and *M. fallax* was observed in series *Bulbocastana*, *Pinnatisecta*, *Yungasense*, *Tuberosa*, *Longipedicellata* and *Demissa*. The resistant accessions involved are diploid (*S. bulbocastanum*, *S. brachistotrichum*, *S. cardiophyllum*, *S. chacoense*), tetraploid (*S. gourlayi*, *S. fendleri*, *S. stoloniferum*) and hexaploid (*S. hougasii*). The observed resistance in *S. hougasii* and *S. bulbocastanum* is in line with results of Brown *et al.* (1989, 1991). The selected resistant genotypes of *S. bulbocastanum*, *S. brachistotrichum*, *S. cardiophyllum*, *S. fendleri* and *S. hougasii* were resistant to both *M. chitwoodi* and *M. fallax* and roots were mostly free of any egg masses, while on susceptible plants over 100 egg masses could be observed (Table 2.2). Seedlings of *S. gourlayi* showed only resistance to *M. chitwoodi*, whereas *S. stoloniferum* and *S. chacoense* had only resistant plants to *M. fallax* (Table 2.1). The preliminary re-testing with cuttings to the other *Meloidogyne* species confirmed these differentiating results, but only in the case of *S. chacoense* the difference was very clearly expressed (Table 2.2).

More *Solanum* spp. from various series showed resistance to *M. hapla*. Resistant plants were observed in series *Bulbocastana* (*S. bulbocastanum*), *Pinnatisecta* (*S. brachistotrichum*, *S. cardiophyllum*), *Yungasense* (*S. arnezii*, *S. chacoense*, *S. tarijense*), *Tuberosa* (*S. sparsipilum*, *S. spegazzinii*, *S. gourlayi*, *S. sucrense*), *Acaulia* (*S. acaule*) and *Demissa* (*S. hougasii*). However, in general, the expression of resistance was not as high as the level of resistance to *M. chitwoodi* and *M. fallax* in the Central American *Solanum* spp. and re-testing with cuttings confirmed the non-absolute level of resistance in most cases (Table 2.2).

Table 2.1. Results of resistance screening to *Meloidogyne chitwoodi*, *M. fallax* and *M. hapla* in wild tuber-bearing *Solanum* spp. Reported are number of plants showing less than 6 egg masses on roots after 7 weeks inoculation with approximately 600 juveniles in seedling-test and subsequent stem cutting-test (res). Seedlings with more than 5 egg masses are scored as susceptible (sus).

Series	*M. chitwoodi*		*M. fallax*		*M. hapla*	
Solanum spp. (accession number)	res	sus	res	sus	res	sus
Bulbocastanana						
S. bulbocastanum (blb 7999)	3	0	3	0	3	0
S. bulbocastanum (blb 8002)	7	0	7	0	4	0
S. bulbocastanum (blb 8006)	5	3	10	11	3	1
S. bulbocastanum (blb 8008)	0	5	0	5	4	1
S. bulbocastanum (blb 8009)	0	17	0	19	5	0
Pinnatisecta						
S. brachistotrichum (bst 7986)	16	1	17	1	16	19
S. cardiophyllum (cph 8024)	4	0	18	0	11	7
S. jamesii (jam 10054)	0	8	0	11	0	9
S. pinnatisectum (pnt 8168)	0	17	0	17	0	34
Polyadenia						
S. polyadenium (pld 8182)	0	16	n.t.[a]	n.t.	0	24
Circaeifolia						
S. capsicibaccatum (cap 53016)	0	7	0	18	0	24
S. circaeifolium (crc 27163)	0	5	n.t.	n.t.	0	27
Lignicaulia						
S. lignicaule (lgl 8106)	0	10	0	7	0	17
Yungasensa						
S. arnezii (arz 27309)	0	17	0	17	19	14
S. chacoense (chc 58[b])	0	5	0	20	0	17
S. chacoense (chc 16993x58[b])	0	4	0	18	14	42
S. chacoense (chc 16998)	0	22	13	2	23	20
S. chacoense (chc 55191)	0	20	3	15	2	18
S. tarijense (tar 16853)	0	1	0	3	11	5
S. tarijense (tar 17423)	0	14	0	10	0	34
Megistacroloba						
S. astleyi (ast 27382)	0	13	0	13	3[c]	26
S. boliviense (blv 7985)	n.t.	n.t.	0	20	4	10
S. megistacrolobum (mga 8113)	0	16	0	12	0	25
S. megistacrolobum (mga 17464)	0	19	0	19	0	36
S. megistacrolobum (mga 27262)	0	31	0	25	0	47
S. sancta-rosae (sct 15454)	0	14	0	12	0	36
S. toralapanum (tor 27119)	0	15	0	16	1[c]	33
Cuneoalata						
S. infundibuliforme (ifd 16842)	0	19	0	19	0	19
Conicibaccata						
S. agrimonifolium (agf 53004)	0	20	0	20	0	44
S. chomatophilum (chm 8078)	0	18	0	19	0	38
S. colombianum (col 53006)	n.t.	n.t.	0	25	0	25
Maglia						
S. maglia (mag 23571)	0	12	0	22	0	35

Table 2.1. Continued.

Series	*M. chitwoodi*		*M. fallax*		*M. hapla*	
Solanum spp. (accession number)	res	sus	res	sus	res	sus
Tuberosa						
S. achacachense (ach 29617)	0	14	0	9	0	22
S. alandiae (aln 31187)	0	19	0	19	0	24
S. berthaultii (ber 24578)	2[c]	31	n.t.	n.t.	0	24
S. berthaultii (ber 28033)	0	25	0	18	0	19
S. brevicaule (brc 28038)	0	17	0	13	0	31
S. bukasovii (buk 7992)	0	17	0	10	0	23
S. canasense (can 8012)	0	14	0	19	0	26
S. canasense (can 15480)	1[c]	16	0	19	0	32
S. candolleanum (cnd 27078)	0	17	0	14	0	33
S. chancayense (chn 18517)	0	3	0	12	0	26
S. gandarillasii (gnd 7174)	0	19	0	20	0	20
S. gourlayi (grl 7180)	0	20	0	29	0	20
S. gourlayi (grl 27257)	4	15	0	16	4	14
S. gourlayi ssp. vidaurrei (vid 16828)	0	15	0	13	0	27
S. hannemannii (16843)	0	12	0	13	0	28
S. hawkesianum (16955)	0	17	0	14	2[c]	28
S. incamayoense (inm 16904)	0	18	0	11	0	30
S. kurtzianum (ktz 17571)	0	3	n.t.	n.t.	1[c]	9
S. kurtzianum (ktz 17578)	0	7	0	20	0	18
S. leptophyes (lph 7184)	0	20	0	19	0	30
S. leptophyes (lph 27211)	0	20	0	20	0	25
S. marinasense (mrn 7191)	0	17	0	20	0	38
S. medians (med 18554)	0	15	0	17	0	34
S. microdontum (mcd 18302)	0	9	0	9	2	7
S. mochicense (mcq 32672)	0	18	0	21	0	18
S. multidissectum (mlt 8145)	1[c]	36	n.t.	n.t.	0	19
S. neorossii (nrs 7211)	0	13	n.t.	n.t.	0	20
S. okadae (oka 17551)	0	19	0	19	0	37
S. oplocense (opl 24655)	n.t.	n.t.	0	15	0	10
S. pampasense (pam 8161)	0	15	0	13	0	16
S. sparsipilum (spl 15455)	0	38	0	17	2	17
S. sparsipilum (spl 28027)	0	17	0	17	0	33
S. spegazzinii (spg 15458)	0	20	0	27	3	16
S. spegazzinii (spg 16943)	0	11	n.t.	n.t.	0	25
S. stenotomum (stn 27166)	0	8	0	12	3[c]	20
S. stenotomum ssp. *goniocalyx* (gon 7227)	0	19	0	28	0	20
S. sucrense (scr 27290)	0	13	0	16	14	14
S. verrucosum (ver 8249)	0	6	0	4	4[c]	1
S. virgultorum (vrg 8265)	0	17	0	15	5[c]	25
Acaulia						
S. acaule (acl 27361)	0	16	0	17	0	34
S. acaule (acl 15465)	0	4	0	4	0	4
S. acaule (acl 15466)	0	8	0	8	0	9
S. acaule ssp. *acaule* (acl 7139)	0	5	0	12	0	7
S. acaule ssp. *aemulans* (aem 17180)	n.t.	n.t.	0	14	15	12

Table 2.1. Continued.

Series	*M. chitwoodi*		*M. fallax*		*M. hapla*	
Solanum spp. (accession number)	res	sus	res	sus	res	sus
Longipedicellata						
S. fendleri (fen 8088)	n.t.	n.t.	0	15	3[c]	15
S. fendleri (fen 8090)	0	16	0	16	0	18
S. fendleri ssp. *fendleri* (fen 8083)	15	0	15	0	0	15
S. fendleri ssp. *arizonicum* (azn 23568)	4	0	20	0	0	30
S. hjertingii (hjt 8089)	0	14	0	15	0	15
S. hjertingii (hjt 32671)	n.t.	n.t.	0	10	0	25
S. papita (pta 15444)	0	14	0	19	0	20
S. polytrichon (plt 53650)	0	20	0	20	0	20
S. stoloniferum (sto 72291)	0	5	5	0	0	4
S. stoloniferum (sto 7230)	0	17	0	29	0	17
Demissa						
S. brachycarpum (bcp 8100)	0	18	0	19	0	19
S. demissum (dms 10000)	0	7	0	10	0	9
S. demissum (dms 10036)	0	4	0	4	0	10
S. guerreroense (grr 7186)	0	18	0	22	0	24
S. hougasii (hou 55203)	35	0	37	0	18	16
S. iopetalum (iop 8101)	0	11	0	12	0	22
S. iopetalum (iop 8102)	0	2	0	15	0	15
S. iopetalum (iop 8103)	0	2	0	15	0	7
S. schenckii (snk 62749)	0	14	n.t.	n.t.	0	14

[a]) Not tested.
[b]) From CPRO-DLO collection.
[c]) Not re-tested with cuttings.

In Table 2.3 the occurrence of resistance to root-knot nematodes is described in relation to the geographical distribution of wild *Solanum* spp. The observed resistance to *M. chitwoodi* and *M. fallax* was predominantly found in Central American *Solanum* spp., while resistance to *M. hapla* was present in numerous Central- and South American species. In total approximately 10 and 13 % of the total number of accessions showed resistance to *M. chitwoodi* and *M. fallax* respectively, whereas for *M. hapla* this percentage was 23 %.

Discussion

The screening of wild tuber-bearing *Solanum* spp. has revealed several sources with resistance to *Meloidogyne chitwoodi*, *M. fallax* and/or *M. hapla*. In most *Solanum* spp. with resistant plants, the resistance was not present in all tested genotypes of the accession or the species indicating genetic variation for resistance within the *Solanum* species in those cases.

Table 2.2. Number of egg masses of *Meloidogyne* spp. on roots of the original seedling and mean number of egg masses on roots of stem cuttings on some selected genotypes of wild *Solanum* spp. (based on total number of tested cuttings).

Series	*M. chitwoodi*		*M. fallax*		*M. hapla*	
genotype (accession)	seedling	cutting	seedling	cutting	seedling	cutting
Bulbocastanana						
93-60-1 (blb 8006)		0.0 (3)	0	0.0 (8)		1.2 (6)
93-60-2 (blb 8006)		0.0 (3)	0	0.0 (8)		0.0 (4)
93-60-10 (blb 8006)		117.5 (2)	120	112.5 (2)		15.0 (3)
93-60-13 (blb 8006)		118.0 (1)		122.5 (2)	1	2.5 (2)
93-57-5 (blb 8009)		n.t.[a]		70.0 (2)	0	3.5 (4)
Pinnatisecta						
93-116-1 (bst 7986)	0	0.0 (1)		0.0 (2)		21.0 (1)
93-116-10 (bst 7986)	0	0.0 (1)		0.0 (1)		75.0 (1)
93-79-1 (cph 8024)	0	0.0 (3)		0.5 (4)		22.0 (1)
93-79-7 (cph 8024)		0.0 (2)	0	0.0 (2)		110.0 (2)
93-79-33 (cph 8024)		0.0 (2)		0.0 (1)	2	4.0 (2)
Yungasensa						
93-24-3 (chc 16993x58)		n.t.		65.0 (3)	1	2.1 (8)
93-68-1 (chc 55191)		121.0 (3)		7.3 (3)	1	1.7 (6)
93-68-2 (chc 55191)		117.0 (3)	1	0.4 (5)		17.8 (4)
93-68-6 (chc 55191)		127.0 (2)	156	115.0 (3)		18.5 (4)
93-113-1 (chc 16998)		41.0 (1)	0	1.8 (4)		17.0 (2)
93-113-2 (chc 16998)		31.0 (1)	0	0.7 (3)		18.8 (4)
93-119-9 (tar 15853)		60.0 (1)		28.0 (1)	0	1.4 (5)
93-119-10 (tar 15853)		30.0 (1)		24.7 (3)	0	1.8 (4)
Megistacroloba						
93-74-4 (blv 7985)		n.t.		26.0 (1)	4	2.4 (5)
Tuberosa						
93-94-6 (grl 27257)	2	2.5 (2)		10.0 (1)		0.0 (3)
93-94-8 (grl 27257)		8.0 (1)		59.0 (1)	3	2.0 (2)
93-MCD-4 (mcd 18302)		30.0 (1)		11.0 (1)	1	1.0 (3)
93-58-1 (spg 15458)		90.0 (1)		68.0 (1)	1	2.8 (4)
93-107-1 (spl 15455)		33.0 (1)		44.0 (1)	2	0.9 (6)
93-107-8 (spl 15455)		63.0 (1)		31.0 (1)	80	81.8 (4)
93-146-2 (scr 27290)		n.t.		n.t.	1	4.0 (3)
Acaulia						
93-104-7 (aem 17180)		n.t.		n.t.	0	1.5 (3)
Longipedicellata						
93 114-5 (fen 8083)	0	0.0 (3)		0.3 (4)		54.0 (2)
93-114-12 (fen 8083)	0	0.0 (3)		0.0 (3)		25.0 (2)
93-115-7 (fen 8090)	111	122.7 (3)		124.0 (3)		90.7 (3)
93-STOL-2 (sto 7229)		5.3 (3)	0	0.7 (3)		61.0 (2)
93-STOL-4 (sto 7229)		11.5 (2)	0	0.5 (2)		19.0 (2)
Demissa						
93-71-3 (hou 55203)	0	0.0 (2)		0.0 (2)		4.0 (1)
93-71-5 (hou 55203)	0	0.0 (2)		0.0 (2)		4.0 (4)
93-71-33 (hou 55203)	0	0.0 (2)		0.0 (1)		3.5 (2)

[a]) not tested.

Table 2.3. Occurrence of resistance to *Meloidogyne* spp. in wild *Solanum* spp. in relation to their original geographical distribution. Reported are number of accessions with one or more resistant (res) seedlings, only susceptible (sus) seedlings, and possible resistant, but not re-tested (nr) seedlings respectively.

	M.chitwoodi			*M. fallax*			*M. hapla*		
geographical distribution	res	sus	nr	res	sus	nr	res	sus	nr
North and Central America	8	21	0	9	20	0	8	21	1
South America	1	56	3	2	56	0	12	46	7
total number of accessions	9	77	3	11	76	0	20	67	8
representing number of *Solanum* sp.	6	57	3	7	56	0	14	51	8

The occurrence of resistance to *M. chitwoodi* and *M. fallax* was the same for most *Solanum* spp. Only *S. chacoense* and to a lesser extent *S. stoloniferum* and *S. gourlayi* showed differentiating results to either *Meloidogyne* species. Originally a deviating isozyme pattern has lead to closer examination of this alternate group of root-knot nematodes (Van Meggelen *et al.* 1994), which has resulted in the description of *M. fallax* (Karssen 1996). However, the observed similarity in these resistance trials indicates that these *Meloidogyne* spp. are likely to be strongly related to each other.

The presence of resistance to *M. chitwoodi* and *M. fallax* in mostly Central American *Solanum* spp. is noteworthy. *M. chitwoodi* has been reported in Mexico (Cuevas & Sosa Moss 1990) and South Western USA (Pinkerton & McIntyre 1987; Griffin 1988; Walters SA & Barker KR 1994) and co-evolution could have taken place. The distribution of resistance genes in association with the (original) natural distribution of the parasite has lead earlier to the hypothesis of co-evolution of *Globodera* spp. and wild *Solanum* spp. in the Andes region of South America (Stone 1985). This is clearly supported by the existence of a gene-for-gene relationship of *Globodera rostochiensis* and *S. tuberosum* ssp. *andigena* (Janssen *et al.* 1991). Further investigations regarding the natural occurrence of *M. chitwoodi* and *M. fallax* will be necessary to confirm whether co-evolution could have occurred.

The question also arises whether it is possible that resistance to *M. chitwoodi* and *M. fallax* in various Central American *Solanum* spp. is related. The series *Bulbocastana* and *Pinnatisecta* are supposed to be closely related and belong to the primitive *Stellata* group of tuber-bearing *Solanum* spp. The series *Longipedicellata* and *Demissa* belong to the more advanced *Rotata* group of *Solanum*, but there are strong indications that these polyploid species have arisen through hybridisations between *Stellata* species and advanced South American species (Hawkes 1990). Presence of broad genetic diversity for resistance to root-knot nematodes will probably have major impact on the selection pressure of nematode populations and hence on the durability of resistance.

With regard to *M. hapla* resistance was present in numerous Central- and South American *Solanum* spp. Especially the resistance in *Solanum* spp. of series *Tuberosa* seems promising for further investigations, since they are closely related to *S. tuberosum*. Some *Solanum* spp. showed resistance to *M. hapla*, as well as to *M. chitwoodi* and *M. fallax*. However, resistance is not expected to be located at the same locus or to be closely linked in most cases due to the clear differences in segregation of low- and high-scoring plants (Table 2.1) and the differential reactions of some selected *Solanum* genotypes to either *Meloidogyne* spp. (Table 2.2).

Several *Solanum* sources have the potential of being used in plant breeding programmes aiming at resistance to root-knot nematodes. However the introgression of resistance from most Central American *Solanum* spp. into the cultivated *S. tuberosum* gene pool is complicated due to the occurrence of structural differences between genomes (Matsubayashi 1991) and dysfunctional endosperm of interspecific hybrid combinations (Johnston *et al.* 1980). In the case of *S. bulbocastanum* sexual crossings with *S. tuberosum* have failed until now (Novy & Hanneman 1991; Brown *et al.* 1994) and the introgression of resistance from *S. bulbocastanum* is being carried out through somatic hybridisation with *S. tuberosum* (Austin *et al.* 1993). The prospects for using resistance from South American *Solanum* spp. are much better, but in this survey the resistance found in these species was mostly restricted to *M. hapla*. The inheritance of resistance however will be one of the most important features for a successful introduction of resistance to *Meloidogyne* spp. into the cultivated potato.

It can be concluded that numerous sources of resistance to *M. chitwoodi*, *M. fallax* and *M. hapla* are present in wild tuber-bearing *Solanum* spp. Future studies on the described resistant *Solanum* sources will be carried out to evaluate the level of resistance and to analyse the inheritance of resistance for the introgression into the potato gene pool.

Chapter 3

Intra- and interspecific variation of root-knot nematodes, *Meloidogyne* spp., for resistance in wild tuber-bearing *Solanum* species.[1]

Summary

Genotypes of wild *Solanum* species were tested to determine the level of resistance to root-knot nematodes and to detect the presence of virulent populations within *Meloidogyne chitwoodi*, *M. fallax* and *M. hapla*. High resistance to all tested populations of both *M. chitwoodi* and *M. fallax* was observed in genotypes of *Solanum bulbocastanum*, *S. hougasii*, *S. cardiophyllum* and *S. fendleri*. Some genotypes of *S. chacoense* and *S. stoloniferum* showed moderate resistance to *M. fallax*, but not or in a lesser extent to *M. chitwoodi*. There was little variation in virulence found between populations of *M. chitwoodi* and of *M. fallax* on resistant plants. In contrast, large differences in virulence were observed between four populations of *M. hapla* on resistant genotypes of *S. bulbocastanum*, *S. hougasii*, *S. chacoense*, *S. gourlayi*, *S. sparsipilum* and *S. spegazzinii*. The resistance to *M. chitwoodi*, *M. fallax* and/or *M. hapla* was found not to correspond with resistance to the high temperature adapted nematode species *M. incognita*, *M. arenaria* and *M. javanica*.

[1]) This chapter is based on: Janssen GJW, Van Norel A, Verkerk-Bakker B & Janssen R (1997) Inter- and intraspecific variation of root-knot nematodes, *Meloidogyne* spp., for resistance in wild tuber-bearing *Solanum* species. Fund Appl Nematol (in press)

Introduction

In North Western Europe root-knot nematodes, *Meloidogyne* spp., are expected to become serious pests in arable farming due to the reduced use of nematicides and change in crop rotation, which favours highly profitable host crops like vegetables. The predominant *Meloidogyne* species attacking potato are *M. hapla* Chitwood, *M. chitwoodi* Golden *et al.* and *M. fallax* Karssen. These nematodes can cause serious economic losses due to reduction in yield and deteriorating quality of the tubers. Especially *M. chitwoodi* and *M. fallax* cause severe external malformations of tubers and internal necrotic spotting, which renders tubers unsuitable for consumption or processing. Resistance would be very effective in controlling root-knot nematodes, but is not available in currently used potato cultivars (Brown *et al.* 1994; Janssen *et al.* 1995).

Resistance screening trials in glasshouses have revealed genotypes from various wild tuber-bearing *Solanum* spp. with resistance to *M. chitwoodi* (Brown *et al.* 1989, 1991; Janssen *et al.* 1996), *M. fallax* and/or *M. hapla* (Janssen *et al.* 1996). However, these screening trials have been performed with only one or a few populations of a *Meloidogyne* species and virulence towards supposedly resistant *Solanum* species might already exist. So far, one virulent population of *M. chitwoodi* on resistant genotypes of *S. bulbocastanum* has been reported (Mojtahedi & Santo 1994). By definition, virulent populations are able to reproduce significantly on resistant host plants that prevent or suppress reproduction of a-virulent populations of the parasite species (Roberts 1995).

Conversely, it has been shown that resistance in some wild *Solanum* spp. can be effective to a broader spectrum of *Meloidogyne* spp. Preliminary results indicated that resistance to both *M. chitwoodi* and *M. fallax* occurs in genotypes of several Central American *Solanum* spp. (Janssen *et al.* 1996). In subtropical and tropical climates, *M. incognita* (Kofold & White) Chitwood, *M. arenaria* (Neal) Chitwood and *M. javanica* (Treub) Chitwood, cause serious economic problems for potato growers (Iwanaga *et al.* 1989). Resistance to these *Meloidogyne* spp. has been identified in various wild *Solanum* species (Brücher 1967; Nirula *et al.* 1967, 1969; Jatala & Mendoza 1978) and some of these resistant species have also been selected for resistance against *M. chitwoodi*, *M. fallax* and/or *M. hapla* (Janssen *et al.* 1996).

The occurrence of virulent individuals or related (virulent) nematode species, already present in fields, can result in a rapid decay of the effectiveness of a resistant cultivar. In some areas in the Netherlands and UK, the extensive use of cultivars with resistance to *Globodera rostochiensis* has lead to a great increase in the incidence of fields, infected with *G. pallida* (Evans 1993; Mulder 1994). Therefore, the evaluation of the resistance of wild *Solanum* spp. against an array of *Meloidogyne* populations, before its introduction into new potato cultivars, will lead to a better prediction of the

durability of resistance and will help to determine better management strategies for resistance genes.

This study describes the results of glasshouse experiments carried out to determine the level of resistance of wild *Solanum* genotypes and the variation in virulence against the resistance of several *Meloidogyne* populations. The occurrence of intra- and interspecific variation for resistance is discussed and the implications this may have for the development and use of new resistant cultivars.

Materials & Methods

Plant material

Resistant and susceptible genotypes of the wild *Solanum* species *S. bulbocastanum*, *S. hougasii*, *S. iopetalum*, *S. fendleri*, *S. stoloniferum*, *S. microdontum*, *S. gourlayi*, *S. cardiophyllum*, *S. sparsipilum*, *S. gourlayi*, *S. spegazzinii*, *S. brachistotrichum* and *S. arnezii* had been identified in resistance screening trials (Janssen *et al.* 1996). Some resistant genotypes were crossed with susceptible ones to provide progenies in order to investigate the inheritance of resistance (Janssen *et al.* 1997*a*). The genotypes were multiplied under short day conditions in a glasshouse to induce tubers and the tubers were stored at 5 °C until use.

Where possible, *in vitro* multiplication was used for rapid multiplication of genotypes. Young growing shoots were sterilised for 10 min in 4 % NaOCl-solution and then transferred into sterile tubes containing MS 30 medium (Murashige & Skoog 1962) with Cefotaxime sodium. Shoots were cut every two to three weeks and transferred into new tubes with MS 30 medium, until the required number of plants per genotype was obtained. An experiment was carried out with several resistant and susceptible genotypes to compare the relative level of infection between plants originating from tissue culture and those obtained from tubers. No significant difference was found in number of egg masses between the two type materials used.

Inoculum

Field populations of *M. hapla*, *M. chitwoodi* and *M. fallax* were derived from infested soil and infected plant material, collected from several areas in the Netherlands from 1991 to 1993 (Table 3.1). To obtain species specific populations the infested soil or infected roots were added to young growing tomato plants and after ten weeks 30 to 40 females with egg masses were individually identified to the species level by isozyme pattern of esterase and malate dehydrogenase (Esbenshade & Triantaphyllou 1990; Van Meggelen *et al.* 1994). The egg masses were used to inoculate young tomato

plants (cv Nematex) and from 12 weeks onwards, plants were used for obtaining inoculum. To maintain the purity of populations the procedure of identification of females and inoculation with egg masses was repeated approximately every 3 to 4 months. Contamination with other nematode populations during propagation was prevented by using sterilised sand and pots and by spatial isolation. All populations were maintained on tomato plants in a temperature-controlled glasshouse (22 ± 2 °C).

Isolates of *M. incognita*, *M. javanica* and *M. arenaria*, originating from single egg masses, were kindly provided by dr M Fargette (ORSTOM, Montpellier France) (Table 3.1) and were multiplied for one generation on potato (cv Première). Subsequently ten to twenty females per isolate were used for isozyme analysis and no contamination with other species was found.

To obtain juveniles for inoculation, eggs were harvested from roots of tomato and potato plants approximately three months after inoculation by dissolving egg masses with 0.5 % NaOCl-solution (Hussey & Barker 1973). Second stage juveniles were hatched in water and stored at 4 °C for up to one month until use.

Table 3.1. Origin of *Meloidogyne* populations and isolates used in this study.

Area	Country	*Meloidogyne* sp.	last field-grown crop (year)	designation
Smilde	The Netherlands	*M. hapla*	carrot (1992)	HSMA
Smilde	The Netherlands	*M. hapla*	everlasting flowers (1992)	HSMB
Roggel	The Netherlands	*M. hapla*	hemp (1992)	HRO
Slochteren	The Netherlands	*M. hapla*	chicory (1992)	HSL
Rips	The Netherlands	*M. chitwoodi*	maize (1991)	CHR
Heide	The Netherlands	*M. chitwoodi*	scorzonera (1993)	CHE
Middenmeer	The Netherlands	*M. chitwoodi*	potato (1992)	CHW
Baexem	The Netherlands	*M. fallax*	potato (1992)	FB
Vreedepeel	The Netherlands	*M. fallax*	scorzonera (1992)	FVR
Kempen	The Netherlands	*M. fallax*	scorzonera (1992)	FK
Unknown	Burkina Faso	*M. incognita*	unknown	Mi67
Unknown	Russia	*M. incognita*	unknown	Mi82
Unknown	Russia	*M. arenaria*	unknown	Ma84
Ste Anne	French W. Indies	*M. arenaria*	unknown	Ma29
Queensland	Australia	*M. javanica*	unknown	Mj44
Unknown	Marocco	*M. javanica*	unknown	Mj57

Resistance tests

1. Resistance to *M. chitwoodi*, *M. fallax* and/or *M. hapla*.

Genotypes of a *Solanum* species were tested with two to four populations of each *Meloidogyne* species for which resistance had been observed, and with one population of the *Meloidogyne* species for which they originally had been found to be susceptible.

Plantlets from tissue culture or tubers were grown in clay pots of 350 cm^3, filled with moist silver sand and additional NPK nutrients, in a temperature-controlled glasshouse (22 ± 2 °C). The ten *Meloidogyne* populations were represented as main plots in four replicates, physically separated by splashing boards to avoid cross contamination. Genotypes were randomly assigned as subplots to three up to ten *Meloidogyne* populations, depending on the observed resistance of the *Solanum* species (Janssen *et al.* 1996). Two to four weeks after planting, as soon as plants showed vigorous growth, they were inoculated with 400 juveniles of *M. chitwoodi*, *M. fallax* or *M. hapla* per pot using an automatic syringe. Plants were individually harvested eight weeks after inoculation. Roots were washed clean of sand, stained with a Phloxine B solution (Dickson & Strubble 1965) and egg masses were counted. The potato cvs Darwina and Nicola and the tomato cv Money Maker were included as controls.

Due to the large differences in levels of resistance of *Solanum* genotypes and the unbalanced design of the experiment no analysis of variance or equivalent statistical analysis was used. Instead, for each genotype two standard errors of means were calculated, one combined over populations of *M. hapla* ($s.e.^{h}$) and one combined over populations of *M. chitwoodi* together with *M. fallax* ($s.e.^{cf}$). Data were square root transformed to obtain a normal distribution of variance. Infection levels were compared using a t-test based on the standard errors of means described.

2. Resistance to *M. incognita*, *M. arenaria* and *M. javanica*.

A second experiment was set up to investigate whether resistance to the temperate *Meloidogyne* spp. in various *Solanum* spp. was also effective against some high temperature adapted *Meloidogyne* spp. Besides populations of *M. chitwoodi*, *M. fallax* and *M. hapla*, two isolates each of *M. incognita*, *M. arenaria* and *M. javanica* were used. The experiment was carried out similarly as previously described. Genotypes were represented by four randomly placed replicates for each nematode population/isolate. Nematode populations/isolates were analysed separately with ANOVA after square root transformation of data using Genstat (Payne *et al.* 1987).

Results

In the first experiment large differences in resistance of genotypes of various *Solanum* species were observed between *M. chitwoodi*, *M. fallax* and *M. hapla*, as well as between populations of the same *Meloidogyne* species. Mean square root of number of egg masses of all genotype/*Meloidogyne* population combinations tested are shown in Table 3.2. The results of the earlier screening experiments are also presented as reference.

Table 3.2. Mean square root number of egg masses of populations of *Meloidogyne fallax* (mf), *M. chitwoodi* (mc) and *M. hapla* (mh) on roots of genotypes of various *Solanum* spp. The resistance rating of earlier screening experiments are indicated as reference.

	Resistance rating[a]			*M. fallax*			*M. chitwoodi*				*M. hapla*				
	mf	mc	mh	FB[b]	FVR	FK	CHW	CHE	CHR	s.e.[cf,c]	HRO	HSL	HSMB	HSMA	s.e.[h,c]
S. bulbocastanum															
93-57-5	sus	sus	res	6.80	7.59	7.71	8.39	6.39	1.63	0.57	6.07	0.50	0.60	--[d]	0.81
93-60-2	res	res	res	0.00	0.00	--	0.25	0.00	0.00	0.05	5.90	0.71	0.25	--	0.79
93-D26-3	res	res	res	0.00	0.00	--	--	0.50	0.00	0.09	4.01	--	0.25	--	0.81
M94-98-1	--	res	--	4.93	3.49	4.16	5.10	4.13	0.00	0.42	4.07	0.35	0.60	--	0.56
M94-106-2	--	res	--	0.00	0.00	0.00	0.00	0.25	0.25	0.06	--	0.00	0.00	--	0.00
M94-106-4	--	res	--	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.30	0.00	0.00	--	0.75
S. hougasii															
93-71-6	res	res	res	0.00	0.00	--	0.00	0.00	0.00	0.00	6.30	3.88	2.45	0.58	0.57
93-71-28	res	res	res	0.00	0.00	--	0.00	0.25	0.00	0.05	5.94	3.31	2.38	--	0.54
S. iopetalum															
93-108-1	sus	sus	sus	8.51	7.08	--	7.91	7.95	7.33	0.30	--	6.85	7.48	6.15	0.45
93-108-11	sus	sus	sus	8.44	7.18	--	7.25	6.94	8.29	0.32	7.32	6.70	8.40	6.37	0.43
S. cardiophyllum															
93-CAR-1	res	res	sus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	--	--	4.09	--	0.41
93-79-10	res	res	sus	0.00	0.50	0.00	0.00	0.00	0.00	0.08	--	--	3.49	--	0.29
93-79-13	res	res	sus	0.33	0.00	0.00	0.25	0.00	0.00	0.07	--	--	4.94	--	0.68
S. fendleri															
93-114-5	res	res	sus	0.00	0.00	0.25	0.71	0.00	0.25	0.10	--	--	3.35	--	0.66
93-114-11	res	res	sus	0.25	0.25	0.75	0.00	0.00	0.00	0.08	--	--	4.95	--	0.50
93-114-12	res	res	sus	0.00	0.00	0.35	0.00	0.00	0.00	0.06	--	--	4.64	--	0.23
93-115-7	sus	sus	sus	--	7.83	8.47	7.13	9.25	8.90	0.37	--	--	6.75	--	0.49
93-115-14	sus	sus	sus	9.89	--	9.16	8.61	8.33	8.34	0.30	--	--	7.62	--	1.03
93-115-18	sus	sus	sus	8.97	9.56	7.96	10.48	8.58	7.66	0.34	--	--	8.72	--	0.59
M94-33-3	--	res	--	0.00	0.00	0.50	0.00	0.00	0.00	0.11	--	--	5.24	--	0.33
M94-33-5	--	res	--	0.00	0.00	0.25	0.25	0.00	.0.00	0.06	--	--	6.47	--	0.25
M94-51-1	--	res	--	0.00	0.33	0.00	0.00	0.33	0.00	0.07	--	--	--	--	--

Table 3.2. Continued.

	Resistance rating[a]			*M. fallax*			*M. chitwoodi*				*M. hapla*				
	mf	mc	mh	FB[b]	FVR	FK	CHW	CHE	CHR	s.e.[cf,c]	HRO	HSL	HSMB	HSMA	s.e.[h,c]
S. stoloniferum															
93-STOL-2	res	res	sus	0.79	1.14	0.75	3.28	2.34	2.38	0.27	--	--	7.31	--	0.96
93-STOL-3	res	res	sus	1.04	1.57	1.21	1.82	1.85	1.16	0.17	--	--	7.26	--	0.52
93-STOL-4	res	res	sus	0.25	0.00	1.00	3.02	1.82	1.06	0.26	--	--	6.49	--	0.30
93-STOZ-1	sus	sus	sus	9.93	10.14	8.47	10.87	10.54	--	0.32	--	--	8.24	--	0.27
M94-131-5	res	--	--	1.47	2.21	3.80	4.07	5.79	6.20	0.47	--	--	6.37	--	1.64
S. chacoense															
93-24-3	sus	sus	res	4.85	6.84	--	--	9.15	--	0.73	0.25	0.50	0.33	0.60	0.14
93-24-27	sus	sus	res	6.31	5.12	--	--	7.08	--	0.36	0.50	1.21	0.60	0.60	0.18
93-68-1	res	sus	res	0.33	0.00	--	--	--	--	0.20	--	0.80	1.88	2.44	0.38
93-68-2	res	sus	res	0.00	0.25	--	--	6.70	--	0.95	0.60	0.60	5.69	--	0.75
93-68-4	res	sus	res	1.93	1.04	--	--	8.80	--	1.09	1.01	0.96	1.66	0.85	0.25
93-68-6	sus	sus	sus	8.83	7.45	--	--	8.13	--	0.46	1.65	1.62	5.88	5.36	0.57
93-68-11	sus	sus	res	4.82	2.73	--	--	8.84	--	0.82	0.60	0.67	0.94	1.65	0.24
93-113-1	res	sus	res	0.97	0.35	--	--	5.23	--	0.70	0.33	0.25	0.00	0.00	0.10
93-113-2	res	sus	res	0.80	0.33	--	--	8.15	--	1.32	2.03	0.71	2.16	--	0.45
M94-28-1	--	--	res	2.32	2.52	--	--	6.88	--	0.83	1.06	1.87	5.86	5.66	0.63
M94-28-4	--	--	res	4.69	4.08	--	--	8.06	--	0.73	0.60	1.43	1.56	2.06	0.32
M94-115-2	--	--	res	6.06	7.21	--	--	8.48	--	0.37	0.50	1.40	0.33	1.79	0.29
M94-93-5	--	--	res	7.63	6.70	--	--	5.90	--	0.65	0.25	0.43	0.25	0.00	0.13
S. microdontum															
93-MCD-4	sus	sus	res	7.89	--	--	--	6.20	--	0.76	0.81	0.79	0.33	--	0.25
93-MCD-17	sus	sus	res	--	--	--	--	6.24	--	0.38	0.80	1.62	0.87	--	0.32
S. gourlayi															
93-94-7	sus	sus	res	6.25	--	--	--	5.65	--	0.61	2.12	1.55	4.76	--	0.61
93-94-8	sus	sus	res	6.98	--	--	--	5.46	--	0.51	0.71	1.04	3.67	--	0.51

Table 3.2. Continued.

	Resistance rating[a]			*M. fallax*			*M. chitwoodi*				*M. hapla*				
	mf	mc	mh	FB[b]	FVR	FK	CHW	CHE	CHR	s.e.[cf,c]	HRO	HSL	HSMB	HSMA	s.e.[h,c]
S. sparsipilum															
93-107-1	sus	sus	res	2.69	--	--	--	6.65	--	0.92	1.75	1.61	4.52	--	0.48
93-107-5	sus	sus	sus	--	--	--	--	--	--	--	1.52	1.14	4.05	--	0.49
S. spegazzinii															
93-58-1	sus	sus	res	5.47	--	--	--	--	--	0.42	0.81	1.08	4.43	--	0.62
S. tuberosum															
Darwina	sus	sus	sus	10.09	10.14	8.49	8.91	8.51	7.57	0.66	6.93	6.50	6.83	6.96	0.37
Nicola	sus	sus	sus	10.33	10.06	9.67	10.42	9.86	8.40	0.45	7.91	8.29	9.08	--	0.33
L. esculentum															
Money Maker	sus	sus	sus	7.18	8.20	7.01	7.22	5.71	5.49	0.76	7.27	7.79	8.83	9.24	0.90

[a]) Genotypes had been selected as resistant if roots had shown less than 5 ($\sqrt{5} \approx 2.2$) egg masses and as susceptible if they had more (Janssen *et al.* 1996)

[b]) See table 3.1 for code of population.

[c]) s.e.[cf] and s.e.[h] are standard errors calculated for each genotype combined over populations of *M. chitwoodi* and *M. fallax*, and over populations of *M. hapla*, respectively.

[d]) -- = not tested.

Table 3.3. Mean square root number of egg masses of isolates of *Meloidogyne incognita*, *M. arenaria* and *M. javanica* on roots of *Solanum* genotypes with resistance to *M. hapla*, *M. chitwoodi* and/or *M. fallax*.

	M. fallax	*M. chitwoodi*	*M. hapla*		*M. incognita*		*M. arenaria*		*M. javanica*	
Genotype	FVR[a]	CHW	HSL	HSMB	Mi67	Mi82	Ma84	Ma29	Mj44	Mj57
S. bulbocastanum										
93-60-2	0.00	0.00	0.00	0.00	8.89	5.66	5.55	7.48	4.41	3.17
S. brachistotrichum										
93-7986	0.00	0.00	4.06	1.18	3.49	--[b]	7.68	6.94	3.01	5.71
S. cardiophyllum										
93-CAR-1	0.00	0.00	0.50	1.18	3.57	4.34	4.43	3.33	3.57	--
S. hougasii										
93-71-6	0.00	0.00	3.88	0.96	2.84	3.73	5.70	3.99	3.46	3.05
S. fendleri										
93-114-11	0.25	0.00	4.94	6.61	5.23	6.54	6.31	6.46	6.36	5.47
S. stoloniferum										
93-STOL-2	0.60	3.41	2.28	6.77	3.31	3.02	0.80	1.60	1.56	3.39
S. arnezii										
93-138-1	8.30	10.08	0.00	2.39	4.10	4.01	2.87	5.61	7.68	4.78
S. chacoense										
93-24-3	4.18	7.90	0.00	0.00	2.59	3.19	4.23	6.47	4.63	4.18
S. tuberosum										
Nicola	9.22	9.83	6.68	--	8.16	11.18	6.87	8.30	8.54	8.53
LSD (P<0.05)	1.30	1.22	1.12	1.85	2.23	2.02	2.04	2.56	2.57	2.70

[a]) See table 3.1 for code of population c.q. isolate.
[b]) -- = not tested.

High resistance to all populations of both *M. chitwoodi* and *M. fallax* was present in most genotypes of *S. bulbocastanum*, *S. hougasii*, *S. fendleri* and *S. cardiophyllum*. Only the genotypes 93-57-5 and M94-98-1 of *S. bulbocastanum* showed significant differences between populations, *i.e.* being susceptible to all populations of *M. chitwoodi* and *M. fallax* except CHR. Genotypes of *S. stoloniferum* showed moderate resistance to *M. fallax*, but to a lesser extent or not to *M. chitwoodi*. The difference between *M. fallax* and *M. chitwoodi* was most clearly expressed by some genotypes of *S. chacoense*, which were resistant to *M. fallax*, but susceptible to *M. chitwoodi*.

The effectivity of resistance to *M. hapla* varied greatly between the nematode populations used. All genotypes of *S. bulbocastanum* were resistant to the *M. hapla* populations HSL and HSMB, but susceptible to HRO. The level of resistance of genotypes of *S. hougasii* varied between populations from resistant (HSMA) via intermediate (HSMB, HSL) to susceptible levels (HRO). Plants of *S. chacoense* expressed resistance to *M. hapla*, but some genotypes were resistant to only some populations of *M. hapla* (*e.g.* 93-68-6), others to all populations of *M. hapla* (*e.g.* 93-24-3). Some genotypes were resistant to both *M. hapla* and *M. fallax* (*e.g.* 93-113-1). Genotypes of *S. microdontum* were resistant to all three tested populations of *M. hapla*, whereas genotypes of *S. gourlayi*, *S. sparsipilum* and *S. spegazzinii* were only resistant to HRO and HSL, not to HSMB. Genotypes of *S. tuberosum* and tomato exhibited little or no differences in susceptibility.

In the second experiment, genotypes of the wild *Solanum* sources with resistance to *M. hapla*, *M. chitwoodi* and/or *M. fallax* were tested against isolates of *M. incognita*, *M. javanica* and *M. arenaria* to investigate whether the resistance was also effective against these species. As a control, populations of *M. chitwoodi*, *M. fallax* and *M. hapla* were included. The results (Table 3.3) with *M. chitwoodi*, *M. fallax* and *M. hapla* confirmed results from the first explained (Table 3.2). No absolute resistance to any of the subtropical and tropical nematode species was observed and most genotypes showed moderate to high levels of nematode reproduction. In screening trials with *M. chitwoodi*, *M. fallax* and *M. hapla* an arbitrary level of five egg masses per plant has been used as the upper limit for selection as resistant (Janssen *et al.* 1996). Under such circumstances, only *S. stoloniferum* 93-STOL-2 would have been selected for resistance to *M. areneria* and to one isolate of *M. javanica*.

Discussion

The presence of resistance to root-knot nematodes in various wild tuber-bearing *Solanum* spp. as previously reported (Janssen *et al.* 1996), has been confirmed in this study. A high level of resistance to *M. chitwoodi* and *M. fallax* was observed in

genotypes of *S. bulbocastanum*, *S. hougasii*, *S. brachistotrichum*, *S. cardiophyllum* and *S. fendleri*. Since there were no genotypes of these *Solanum* species showing an infection level significantly different for *M. chitwoodi* and *M. fallax*, it is likely that the resistance to both nematode species is based on the same resistance gene(-s) occurring in these Central American *Solanum* species. This will be further investigated with crossing experiments. In the case of *S. chacoense* and *S. stoloniferum* significant differences between *M. chitwoodi* and *M. fallax* were observed for some genotypes, but resistance was not as effective as that observed in genotypes of the *Solanum* species mentioned above. *M. fallax* has only recently been recognised as a different species from *M. chitwoodi* (Karssen 1996), although morphologically, it is closely related to *M. chitwoodi*. Few differences are known with regard to host range (Van Meggelen *et al.* 1994; Karssen 1996) and the likely distribution of *M. fallax* seems comparable to that of *M. chitwoodi* (Karssen 1996). The similar behaviour of both nematode species on various Central American *Solanum* spp. is a further indication that *M. chitwoodi* and *M. fallax* should be considered as genetically closely related species, as compared to other *Meloidogyne* species like *M. hapla* and *M. incognita*.

Variability in virulence within *Meloidogyne* species was most noticeable with *M. hapla*. This species occurs world-wide as one of the four most common species of the genus (Sasser & Carter 1985) and large genetic variation within the species is expected. It is, however, surprising that large differences in virulence were found between *M. hapla* populations from the Netherlands on *Solanum* genotypes, which are native to Central- and South America (Hawkes 1990). The presence of virulent populations is not likely to be the result of a selection of virulent forms by these resistant *Solanum* spp., since resistance is not yet present in the currently used potato cultivars (Brown *et al.* 1994; Janssen *et al.* 1995). A comparable situation is found for tomato cultivars, bearing the Mi-gene from *Lycopersicon peruvianum*. Resistance breaking field populations of root-knot nematodes have been found that had not been previously exposed to resistant cultivars (Netscher 1977; Prot 1984; Fargette 1987; Roberts & Thomason 1989). Roberts and Thomason (1989) suggested that this virulence is genetically closely related to genes controlling other traits that improve the fitness of the population. Nevertheless the possibility of a recent introduction, in the last decades, of *Meloidogyne* populations from other areas and continents could also explain the occurrence of virulent *M. hapla* populations.

Little variation in virulence was noticed within *M. chitwoodi*. The population CHR of *M. chitwoodi* did not or hardly reproduced on *S. bulbocastanum* M94-98-1 and 93-57-5 respectively, which were susceptible to the other populations of *M. chitwoodi* and *M. fallax*. No tested population was virulent to the genotypes of *S. bulbocastanum*, but the existence of virulent *M. chitwoodi* populations on this *Solanum* species has been reported in the USA (Mojtahedi & Santo 1994).

The *Solanum* genotypes with resistance to *M. chitwoodi*, *M. fallax* and/or *M. hapla* were in general susceptible to *M. incognita*, *M. arenaria* or *M. javanica*. Only *S. stoloniferum* 93-STOL-2 was moderately resistant to some isolates of *M. arenaria* and *M. javanica*. Resistance to these subtropical and tropical nematode species has been observed in several *Solanum* species tested (Brücher 1967; Nirula *et al.* 1967, 1969; Jatala & Mendoza 1978) and efforts have been undertaken to transfer resistance into cultivated potatoes (Gomez *et al.* 1983*b*; Iwanaga *et al.* 1989). Although the nematode isolates used in this study do not represent the complete genetic variability of these high temperature adapted *Meloidogyne* species, it is not expected that the resistance to *M. chitwoodi*, *M. fallax* or *M. hapla* investigated will be effective against *M. incognita*, *M. javanica* and *M. arenaria*.

The occurrence of genetic variation within *Meloidogyne* spp. for characters like differences in morphology, biochemical and molecular markers, host range and (a-)virulence factors has been reported regularly, but has not lead to the distinction of well-defined groups within *Meloidogyne* spp., if ever possible. Variation in chromosome number, like the A- and B-race of *M. hapla* and aneuploid populations in various *Meloidogyne* spp., has been described (Triantaphyllou 1985), but merely complicates the characterisation of groups based on host ranges and (a-)virulence factors (Roberts 1995). A differential host test has been developed to differentiate (races of) *M. arenaria*, *M. incognita*, *M. javanica* and *M. hapla* (Sasser 1979; Sasser & Carter 1985), but the use of this test is restricted to practical purposes and meant for a preliminary identification. Host range differences within the species have also been described for *M. hapla* (Sasser 1972; Dalmasso & Bergé 1975) and *M. chitwoodi* (Santo & Pinkerton 1985), but only for the latter species it has lead to a differentiation into races. Race deviations have also been used to differentiate (a-)virulent populations on resistant host plants. The term 'B population' or later 'B-race' has been introduced for populations of *M. incognita*, *M. javanica* and *M. arenaria*, reproducing on resistant tomatoes carrying the Mi-gene (Riggs & Whitehead 1959; Netscher 1977) and a virulent population of *M. chitwoodi* on resistant *S. bulbocastanum* has been designated as race 3 (Mojtahedi & Santo 1994).

This study has shown that virulence exists within *M. hapla* and *M. chitwoodi* for several resistant *Solanum* spp. With *M. hapla*, the four populations used behaved differently on the set of selected genotypes of different *Solanum* species, indicating that more (a-)virulence factors are involved, which are present in different combinations. Assuming that (a-)virulence is genetically based, numerous combinations are possible depending on the number of resistance sources. Moreover, the (a-)virulence factors can inherit independently of other characters used for race differentiations. Like on root-knot nematode resistant cowpea both virulent and avirulent populations have been identified within the races 1 and 3 of *M. incognita* (Roberts *et al.* 1995). Therefore, we would prefer to base the identification of new

races on characters like host ranges and ploidy level, but not on the (a-)virulent behaviour of populations. For example, population HRO of *M. hapla*, virulent on *M. hapla* resistant *S. hougasii*, should not be described as a new 'race'. In line with this, race 3 of *M. chitwoodi* (Mojtahedi & Santo 1994) would be included in race 2 of *M. chitwoodi*, but designated as a population virulent on resistant *S. bulbocastanum*. Once resistance from wild *Solanum* species is incorporated into new cultivars and will be used in cropping systems, a biotype scheme as proposed by Roberts (1995) may be developed as a more convenient designation of populations.

In order to investigate virulence groups new procedures for multiplication, identification and maintenance of nematode populations are necessary. The current method of rearing populations on tomato plants aided by accurate species identification of females using isozyme patterns (Janssen *et al.* 1995) can not exclude the contamination of populations of the same *Meloidogyne* species differing in virulence factors, nor preserve the genetic variation of a population. To resolve the latter, the storage of nematode populations in liquid nitrogen is an effective and reliable method for long-term preservation of *Meloidogyne* germ plasm (Van der Beek *et al.* 1996). The multiplication on differential plants, *i.e.* resistant genotypes, and the use of molecular markers should aid to get an insight in the genetic background of virulence and other characters.

Resistance sources from various *Solanum* species are now available for the introduction of this trait into the cultivated potato. However, the existence of variation in virulence and interaction with different sources of resistance indicate that introgression of resistance into the cultivated potato should precede with caution. Moreover, no resistant genotype to all tested populations of *M. hapla*, *M. chitwoodi* and *M. fallax* was identified. To avoid selection of virulent populations or competitive nematode species, the introduction of resistance from more than one *Solanum* species into new potato cultivars is highly recommended. From our results it can be anticipated that this is time consuming but possible in the case of resistance to *M. chitwoodi*, *M. fallax* and/or *M. hapla*. Additionally, resistance to especially *M. hapla* needs to be evaluated against a large number of populations that would be representative for the pathogenicity of the species to ensure the effectiveness of the resistance before laborious efforts are undertaken to introduce this trait into new potato cultivars.

Chapter 4

Expression of resistance to the root-knot nematodes, *Meloidogyne hapla* and *M. fallax*, in wild *Solanum* spp. under field conditions.[1]

Summary

In 1995 two fields in the Netherlands, naturally infested with *Meloidogyne hapla* (Wageningen) and *M. fallax* (Baexem), were used to evaluate resistant and susceptible *Solanum* genotypes under natural conditions. In April, genotypes were planted in circular microplots. Soil samples were taken and analysed for the occurrence of second-stage juveniles every six weeks. From August onwards, large differences between resistant and susceptible genotypes in numbers of juveniles were found in the soil. For all resistant wild *Solanum* genotypes the level of infection in soil at the end of the growing season in October was equal to or lower than at the beginning. Glasshouse experiments were performed with the same genotypes and nematode populations (*i.e.* originally derived from these fields) and the results were comparable with the observations from the field. It is concluded that resistance, as selected in glasshouse trials, corresponds well with resistant behaviour in the field and that it is worthwhile to transfer the resistance from these *Solanum* sources to commercial potato cultivars for successful control of root-knot nematodes.

[1]) This chapter is based on: Janssen GJW, Janssen R, Van Norel A, Verkerk-Bakker J & Hoogendoorn J (1996) Expression of resistance to the root-knot nematodes *Meloidogyne hapla* and *M. fallax* in wild *Solanum* spp. under field conditions. Eur J Plant Pathol 102: 859-865

Introduction

In North Western Europe root-knot nematodes, *Meloidogyne* spp., are expected to become a serious pest in agriculture. For potato, *M. hapla* Chitwood and *M. chitwoodi* Golden *et al.* are the predominant *Meloidogyne* species and can cause severe economic losses in terms of yield reduction and quality damage of tubers. Both species cause necrotic spots inside the tubers and, especially *M. chitwoodi*, gall formations visible on the outside of the tubers. In the Netherlands, a deviant type of *M. chitwoodi* has been characterised by its isozyme pattern and preliminary differences in host range (Van Meggelen *et al.* 1994) and, very recently, this deviant type has been described as a new *Meloidogyne* species, *M. fallax* (Karssen 1996). However *M. fallax* and *M. chitwoodi* are thought to be genetically closely related (Janssen *et al.* 1996) and *M. fallax* causes similar symptoms of infection on potato as *M. chitwoodi*.

Resistance to root-knot nematodes would be very effective against these pests, but has been shown to be lacking in currently used cultivars (Brown *et al.* 1994; Janssen *et al.* 1995). In recent years, various *Solanum* species resistant to *Meloidogyne* spp. have been identified. In the USA, research has been focused on resistance to *M. chitwoodi* race 1 and 2 and *M. hapla* and highly resistant genotypes of *S. bulbocastanum* and *S. hougasii* were selected (Brown *et al.* 1989, 1991). Races 1 and 2 of *M. chitwoodi* are distinguished by means of their differential reproductivity on carrot and lucerne (Santo & Pinkerton 1985). In the Netherlands, screening trials in glasshouses have been performed with *M. chitwoodi*, *M. fallax* and *M. hapla* and have revealed numerous resistant *Solanum* species (Janssen *et al.* 1996). The populations of *M. chitwoodi* used for these screening trials resemble the originally described *M. chitwoodi* (race 1) (Golden *et al.* 1980). As a selection criterion for resistance an arbitrary chosen maximum number of 5 egg masses, representing 1 % successful reproduction of the total number of inoculated juveniles, was used (Janssen *et al.* 1996).

To validate whether such resistance is effective under natural circumstances it is necessary to evaluate susceptibility and resistance in the field. Additionally, a field experiment will not only indicate whether the resistance selected in the glasshouse is effective under field cropping conditions, but growing plants in naturally infested soil will allow prolonged exposure of plants to the nematodes and demonstrate the effect of possible multiple generations during a growing season. This report describes the evaluation of wild *Solanum* genotypes, resistant to *M. hapla* or *M. fallax*, in naturally infested fields and compare the results with those from glasshouse experiments.

Materials & Methods

Plant material

Extensive resistance screening trials in the glasshouse had revealed various *Solanum* species with resistance to *M. hapla*, *M. chitwoodi* and/or *M. fallax* (Janssen *et al.* 1996). For evaluation in the field *Solanum chacoense* 93-68-11, *S. gourlayi* 93-94-8, *S. hougasii* 93-71-5, *S. sparsipilum* 93-107-1 and the hybrid genotype *S. chacoense* x *S. tuberosum* 87-206-6 were selected for their resistance to *M. hapla*, while *S. sparsipilum* 93-107-8 was included as a susceptible wild genotype. For *M. fallax* the genotypes *S. fendleri* 93-114-12, *S. chacoense* 93-113-1, *S. bulbocastanum* 93-60-2, *S. stoloniferum* 93-STOL-4 were considered resistant and genotype *S. chacoense* 93-68-6 susceptible (Janssen *et al.* 1996). All tubers of selected and tested genotypes were obtained by growing plants under short day conditions in a glasshouse. Seed tubers of the cvs Nicola and Darwina, susceptible to *Meloidogyne* spp., were multiplied in a *Meloidogyne*-free field. All tubers were stored at 5 °C before use from March to September 1995.

Field experiments

Two fields in Wageningen and Baexem, both situated in the Netherlands and naturally infested with *M. hapla* and *M. fallax* respectively, were prepared for use in 1995. In Wageningen the first indication of infestation with *M. hapla* occurred in 1990 in a caraway crop. The following crops were wheat (1991), sugar beet (1992), potato (1993) and hemp (1994). In Baexem two successive years (1990 and 1991) of cultivation of evening primrose gave rise to a high infestation of *M. fallax*, although at that time it was still classified as *M. chitwoodi*. The following crops were scorzonera (1992), sugar beet or potato (1993) and sugar beet (1994). Both fields consisted of sandy soil with approximately 2.9 and 1.9 % organic matter, and a pH of 5.4 and 5.9 for Wageningen and Baexem respectively.

In April 1995 circular plastic containers (56 cm diameter x 35 cm deep) with no bottom were buried in the soil with a distance of 1.5 m between the centres of adjoining containers. The containers were used to prevent the growth of roots and stolons into neighbouring microplots. Genotypes were assigned to containers in four completely randomised complete blocks, in which each genotype was represented by three containers. Tubers were planted in pots in the glasshouse approximately three weeks before transplanting. Well-growing plants were selected and transplanted to the fields in Baexem and Wageningen on 21 and 24 April respectively. To avoid possible damage by night-frost, plants were twice covered with plastic for a few days during May. Weeds were regularly removed inside the containers and plants were protected

against late blight, *Phytophthora infestans*, with commonly used agrochemicals following the suppliers' recommendations. Tubers, if present, were harvested after the last soil sampling and both field experiments were terminated in October 1995.

Soil samples for nematode assays were taken approximately every 6 weeks by sampling randomly 5 soil cores (1 cm diameter x 30 cm deep) from each container, starting before planting on 4 April. Soil from the three containers with the same genotype within the block were treated as one sample for analysis. To analyse the number of active juveniles in the soil two sub-samples of 100 cm^3 soil were taken and juveniles were extracted from the soil using an Oostenbrink elutriator (s'Jacob & Van Bezooijen 1984). The nematodes were allowed to move from a cotton filter into a water layer overnight and the numbers of *Meloidogyne* juveniles in the suspension were counted. At the beginning and end of the experiment 20 additional cores per container were taken to perform a bio-assay with lettuce (Zondervan & Huiskamp 1987). For this assay, four-week-old lettuce plants (cv Norden) were transplanted in plastic pots, containing a sub-sample of 400 cm^3 soil. Each soil sample was represented in four replications and pots were randomly placed in a temperature controlled glasshouse (18 ± 2 °C). After five weeks the number of galls on the roots was counted and adjusted to numbers of infectious nematodes per 100 cm^3 soil.

For the statistical analysis square root transformation was used to obtain normal distribution of variance. The sampling dates and *Meloidogyne* spp. were separately analysed with ANOVA using Genstat (Payne *et al.* 1987).

Glasshouse tests

Second-stage juveniles of *M. fallax* and *M. hapla*, extracted from infested soil taken from the fields in Baexem and Wageningen respectively, were maintained on tomato plants (Janssen *et al.* 1995). To prepare inoculum, eggs were harvested from the roots by dissolving egg masses with 0.5 % NaOCl-solution (Hussey & Barker 1973). Juveniles were hatched in water and stored at 4 °C up to one month until use as inoculum.

Tubers were planted in clay pots of 350 cm^3, filled with moist silver sand and slow release NPK fertiliser. Pots were randomly placed in a temperature-controlled glasshouse (22 ± 2 °C). Inoculation with *M. fallax* or *M. hapla* followed two weeks after planting by supplying a water suspension of approximately 400 juveniles with an automatic syringe. Each genotype was tested in 16 replications. Eight weeks after inoculation egg masses on the roots were counted in eight of the replicates. The roots of the other eight were analysed for the total number of eggs produced using 1.0 % NaOCl-solution (Hussey & Barker 1973). Each *Meloidogyne* species was treated as a separate experiment for the analysis of variance after square root transformation of the data. The experiment was carried out from September to December 1995.

Results

Resistance to *M. hapla* and *M. fallax* in the various wild *Solanum* genotypes was clearly expressed in the field. The number of juveniles in the soil was found to have increased significantly in the soil around the susceptible genotypes from August onwards in both the field in Wageningen (Fig. 4.1) and in Baexem (Fig. 4.2). The actual start of a new generation of juveniles is likely to have occurred some weeks earlier, but could not be visualised due to the fairly wide intervals between the chosen sampling dates. The vigour and duration of growth varied between *Solanum* genotypes, but it did not have a major effect on the final level of infection, as is shown with the susceptible wild genotypes and commercial cultivars in both fields. Figures 4.1 and 4.2 show that the number of juveniles in the soil around resistant genotypes did not increase or even decreased over time for most selected resistant genotypes.

The reproductive factor (Rf = final population/initial population) (Oostenbrink 1966) was analysed and the results are in Tables 4.1 and 4.2 for *M. hapla* and *M. fallax* respectively. Of the resistant genotypes only *S. sparsipilum* 93-107-1 in Wageningen showed a slight but not significant increase in number of juveniles in October.

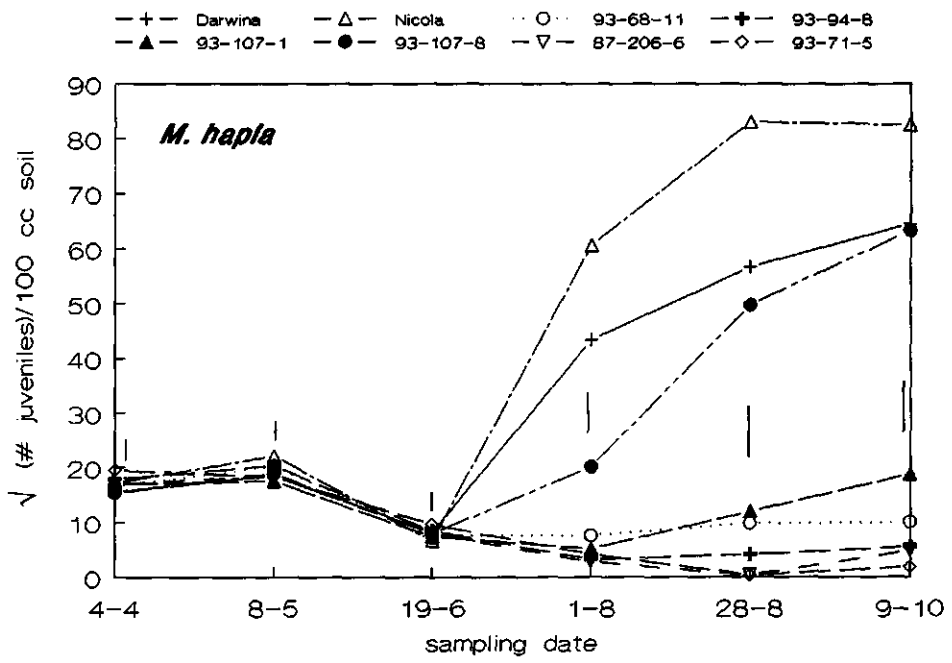


Figure 4.1. Square root number of juveniles of *M. hapla* per 100 cc soil in the field at Wageningen during growth of various *Solanum* genotypes. LSD-values per sampling date ($P<0.05$) are indicated by vertical strokes.

Table 4.1. Mean square root numbers of juveniles (√jv) and galls (√ga) in the lettuce bio-assay per 100 cc soil from the *M. hapla* infested field (Wageningen) before and after growing various *Solanum* genotypes and mean square root numbers of eggs (√eg) and egg masses (√em) in the glasshouse test. Reproductive factors (final population/initial population) were calculated using number of juveniles (Rf_{jv}) and galls (Rf_{ga}) in the field and number of eggs in the glasshouse (Rf_{eg}).

			Field experiment						Glasshouse test		
			April		October						
Solanum sp.	genotype	vigour[a]	√jv	√ga	√jv	√ga	Rf_{jv}	Rf_{ga}	√em	√eg	Rf_{eg}[b]
S. tuberosum	Nicola	3	17.21	7.50	82.54	5.45	17.04	0.54	5.56[c]	46.2[c]	6.2
S. tuberosum	Darwina	3	15.60	7.08	64.48	5.85	22.54	0.68	6.48[c]	39.7[c]	4.1
S. sparsipilum	93-107-8	2	17.91	7.78	63.28	5.51	17.36	0.50	4.35	70.1	13.1
S. sparsipilum	93-107-1	2	16.97	7.50	18.79	3.33	1.22	0.21	1.20	10.4	0.3
S. chacoense	93-68-11	4	16.56	7.40	10.12	1.99	0.37	0.09	0.43	11.8	0.5
S. gourlayi	93-94-8	2	15.38	7.87	5.56	1.45	0.12	0.04	1.19	8.7	0.2
S. chac.x S. tub.	87-206-6	4	16.99	7.81	4.95	1.25	0.11	0.04	0.13	1.3	0.0
S. hougasii	93-71-5	2	19.56	8.21	1.87	1.15	0.02	0.02	2.27	24.2	1.6
LSD (P<0.05)			3.12	0.98	8.77	0.90			1.27	14.3	

[a]) The general vigour and duration of growth of plants in the field during the season is expressed on a scale from 1 to 4 representing poor to excellent growth respectively.

[b]) As initial population the number of nematodes used for inoculation, 400 juveniles, is used.

[c]) Plants were in poor condition.

Table 4.2. Mean square root numbers of juveniles (√jv) and galls (√ga) in the lettuce bio-assay per 100 cc soil from the *M. fallax* infested field (Baexem) before and after growing various *Solanum* genotypes and mean square root numbers of eggs (√eg) and egg masses (√em) in the glasshouse test. Reproductive factors (final population/initial population) were calculated using number of juveniles (Rf_{jv}) and galls (Rf_{ga}) in the field and number of eggs in the glasshouse (Rf_{eg}).

			Field experiment						Glasshouse test		
			April		October						
Solanum sp.	genotype	vigour[a]	√jv	√ga	√jv	√ga	Rf_{jv}	Rf_{ga}	√em	√eg	Rf_{eg}[b]
S. tuberosum	Nicola	3	12.40	5.02	36.37	5.61	7.69	1.18	11.84	272.4	191.7
S. tuberosum	Darwina	3	12.16	5.27	37.68	6.45	8.92	1.49	8.40	161.4	69.3
S. chacoense	93-68-6	3	11.39	4.46	39.77	7.09	12.13	2.32	6.23	114.6	34.8
S. stoloniferum	93-STOL-4	3	10.34	4.88	8.07	2.68	0.60	0.27	0.14	5.3	0.2
S. chacoense	93-113-1	4	10.71	5.03	6.30	4.28	0.37	0.65	0.25	1.3	0.0
S. fendleri	93-114-12	2	11.75	4.91	7.19	4.93	0.41	0.95	0.18	1.7	0.0
S. bulbocastanum	93-60-2	3	11.65	5.22	1.98	1.59	0.04	0.13	0.13	1.1	0.0
LSD (P<0.05)			3.65	3.08	3.95	2.18			1.11	30.0	–

[a]) The general vigour and duration of growth of plants in the field during the season is expressed on a scale from 1 to 4 representing poor to excellent growth respectively.

[b]) As initial population the number of nematodes used for inoculation, 400 juveniles, is used.

For all other resistant genotypes the number of juveniles did not reach the infection level of the beginning of the season. It was noteworthy that *S. chacoense* 93-68-11 and to a lesser extent the hybrid 87-206-6 grew very vigorously in Wageningen during the season, resulting in enormous plants that were still green and flowering in October, but allowed hardly any reproduction of nematodes. The total of day °, measured at 10 cm depth with a base threshold temperature of 8.3 °C for *M. hapla* (Lahtinen *et al.* 1988) was 1530 day °, and this would have allowed at least 2 generations in Wageningen. With a total of 2100 day ° and a base threshold temperature - supposedly similar to *M. chitwoodi* - of 5.0 °C (Pinkerton *et al.* 1991) *M. fallax* could have produced up to 3 generations in Baexem.

With the bio-assay comparable rankings of genotypes were obtained (Table 4.1 and 4.2). However, the differences between high and low numbers of juveniles could not be expressed as well with the bio-assay due to the physical limitation to the maximum number of galls that a root system of lettuce can produce. Therefore, in the case of heavily infected soil, Rf-ratings estimated by the number of galls were much lower than Rf-ratings estimated by the number of juveniles.

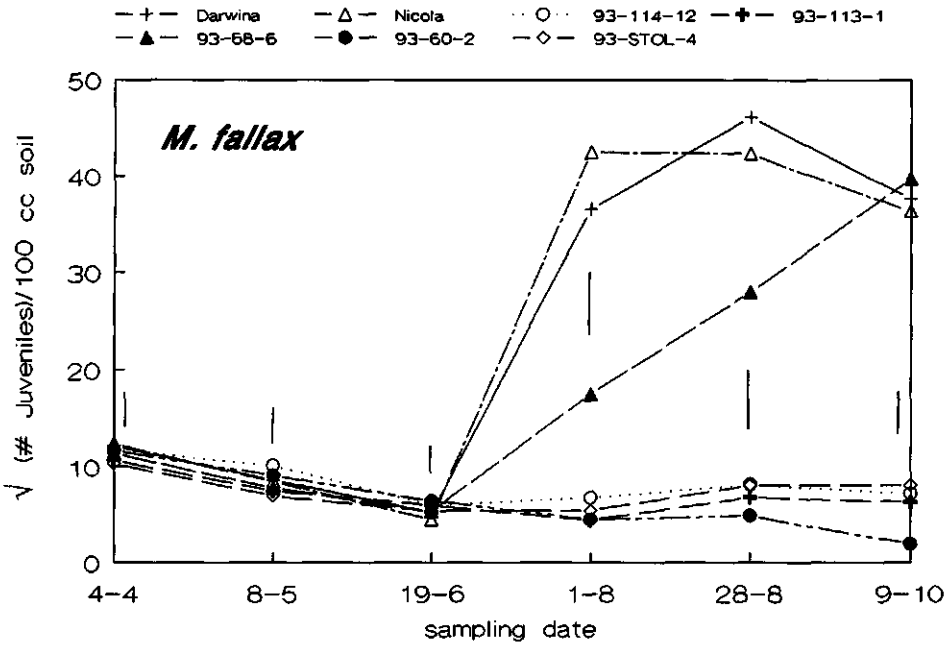


Figure 4.2. Square root number of juveniles of *M. fallax* per 100 cc soil in the field at Baexem during growth of various *Solanum* genotypes. LSD-values per sampling date ($P<0.05$) are indicated by vertical strokes.

The resistance observed in the field was very comparable to that observed in the glasshouse test (Tables 4.1 and 4.2). The results of the glasshouse tests are also in line with our earlier reported experiments with other nematode populations (Janssen *et al.* 1995, 1996). The only remarkable difference between the results of field and glasshouse tests was with *S. hougasii* 93-71-5. This genotype expressed a very high level of resistance to *M. hapla* in the field, while in the glasshouse test only a partial resistance was observed.

The various methods used to estimate differences in reproduction of root-knot nematodes in field and glasshouse experiments between resistant and susceptible genotypes gave mostly similar results. The coefficients of correlation are shown in Table 4.3. In the glasshouse test with *M. hapla* the plants of the cvs Nicola and Darwina were found to be in a rather poor condition and this is likely to have resulted in the low production of eggs and the rather low correlation between this value and the numbers of egg masses (r^2=0.50). In the bio-assay with *M. fallax* relatively large variation between replicates was found and the results were found to be less comparable with counts of juveniles and the results from the glasshouse than those of the bio-assay of *M. hapla*.

The cvs Nicola and Darwina had produced tubers of reasonable size and quantity at the end of the growing season in Baexem, but several tubers were severely malformed by the characteristic galls caused by *M. fallax*. Underneath the surface of the tubers an average infection of 1.25 females per cm^2 was observed after examination of 16 tubers. Most wild *Solanum* genotypes had formed enormous amounts of stolons, which were effectively limited by the containers, but no or few very small tubers were found. In Wageningen not only the cultivars but also the hybrid *S. chacoense* x *S. tuberosum* 87-206-6 had produced a large number of tubers of reasonable size. None of the tubers showed any visual symptoms of malformation. However inside the tubers of the cultivars an average infection of 0.25 females per cm^2 was observed after examination of 12 tubers. Inside the tubers of the hybrid no females or egg masses were observed.

Table 4.3. Coefficients of correlation between data for multiplication in the fields, measured by extraction of juveniles (√jv) and bio-assay (ga), and in the glasshouse, measured by counting eggs (√eg) and egg masses (em).

	M. hapla					*M. fallax*			
	√jv	ga	√eg	em		√jv	ga	√eg	em
√jv	1.00				√jv	1.00			
ga	0.94	1.00			ga	0.80	1.00		
√eg	0.71	0.70	1.00		√eg	0.75	0.45	1.00	
em	0.81	0.83	0.50	1.00	em	0.62	0.34	0.98	1.00

Discussion

The field experiments in Wageningen and Baexem demonstrated that resistance to *M. hapla* and *M. fallax* respectively, as selected in screening trials in the glasshouse, is also effective in suppressing the nematode populations under natural circumstances. Brown *et al.* (1994) also showed that resistance to *M. chitwoodi* derived from *S. bulbocastanum*, was stable after a prolonged exposure to infection in microplots in the field. Although some nematodes may have reproduced on the resistant *Solanum* genotypes, this was not at a high enough level to lead to an increase of root-knot nematodes after the growing season. Consequently, in combination with the expected natural decrease of the nematode population during winter time, a very low infection level in the next spring will be the result.

The differences in level of infestation in soil was better expressed by extracting and counting juveniles than by the lettuce bio-assay. The maximum number of galls on roots of lettuce is physically restricted by the size of the root system and therefore the bio-assay cannot demonstrate logarithmically higher infections. Even at the start of the growing period, the assessment of numbers of active juveniles in the soil by means of counting galls was approximately only 25 % of the numbers of juveniles extracted directly. Nevertheless, this bio-assay is frequently used in the Netherlands, since for this method staff need not be experienced in the identification of *Meloidogyne* juveniles amongst other nematodes in natural soil samples. The bio-assay method also allows juveniles to hatch from eggs, thus supposedly giving a better estimate of the level of infectious nematodes (Zondervan & Huiskamp 1987).

The field experiments were not suitable for detailed observations on tuber infection since the short day conditions necessary for tuberisation of most wild *Solanum* genotypes were lacking. Only tubers of the hybrid *S. chacoense* x *S. tuberosum* could be investigated and these appeared to be free of egg masses. It has been reported that genotypes of *S. bulbocastanum* with resistance in the roots also exhibited tuber resistance (Brown *et al.* 1994, 1995; Mojtahedi *et al.* 1995). However, the low incidence of juveniles in soil around the resistant genotype in Wageningen during the formation of tubers makes it possible that tubers may have escaped infection. The tubers of the common cultivars were more severely infected by *M. fallax* than by *M. hapla*, but this might be caused by the earlier penetration and reproduction of *M. fallax* (Santo & O'Bannon 1981).

The work reported here has shown that resistance from several different *Solanum* spp. is available to control root-knot nematodes in potato under field conditions. Although resistance to nematodes can remain effective after several consecutive years of exposure to infection (Roberts 1992), examples are also known where the introduction of a single nematode resistance gene into cultivated crops can lead to a selection pressure towards virulent populations or related nematode species. In the

Netherlands and UK, extensive use of cultivars with resistance to *Globodera rostochiensis*, based on the H_1 gene from *S. tuberosum ssp. andigena*, has lead to an increase of *Globodera pallida* populations (Evans 1993; Mulder 1994), whereas the use of the Mi gene in tomato, which can suppress populations of *M. incognita*, *M. arenaria* and *M. javanica*, can lead to a rapid selection of virulent populations in glasshouses (Jarquin-Barberena *et al.* 1991). In order to avoid high selection pressures, it is now necessary to work on the durable management of resistance to *Meloidogyne* spp. in potato by introducing not a single but different resistance genes from various *Solanum* sources, such as those reported here, into the potato gene pool.

Chapter 5

Dominant and additive resistance to the root-knot nematodes *Meloidogyne chitwoodi* and *M. fallax* in Central American *Solanum* species.[1]

Summary

The inheritance of resistance to *M. chitwoodi* and *M. fallax* in *S. fendleri*, *S. hougasii* and *S. stoloniferum* was studied assuming disomic behaviour of these polyploid *Solanum* species. Various populations were produced from crosses within the wild *Solanum* species; resistant x susceptible and reciprocal crosses (F1), self-pollinations (S1), testcrosses (TC) and self-pollinations (F2) of resistant hybrids, if possible. For the test crosses with *S. hougasii*, susceptible genotypes of *S. iopetalum* were used. In seedling tests, numbers of egg masses were counted after inoculation with *M. chitwoodi* or *M. fallax*. Almost all seedlings of F1 and S1 populations of *S. fendleri* appeared resistant, whereas TC and F2 populations of three different resistant hybrid genotypes segregated into resistant (having 1 or no egg mass) and susceptible plants (having more than 1 egg mass) of 1 : 1 and 3 : 1 ratios, respectively. The results clearly indicate the action of a single dominantly inherited gene and the symbol R_{mc2} is proposed for this gene. In the case of *S. hougasii*, F1 and S1 seedlings appeared mostly resistant. Difficulties were met in producing TC and F2 populations and only 4 TC populations were obtained, which segregated into a 1 : 1 ratio. These results also indicate the presence of a simple dominant factor. For both *S. fendleri* and *S. hougasii* no differences were observed between *M. chitwoodi* and *M. fallax*, indicating that resistance genes are the same for both nematode species. The F1, S1, and TC populations of *S. stoloniferum* segregated for the square root number of egg masses into normal-like distributions, which deviated between the *Meloidogyne* species used. The patterns indicate the presence of several additive genes and one or more genes effective to *M. fallax*, but not to *M. chitwoodi*. The relationship of resistance genes present in various Central American *Solanum* species is discussed.

[1]) This chapter is based on: Janssen GJW, Van Norel A, Janssen R & Hoogendoorn J (1997) Dominant and additive resistance to the root-knot nematodes *Meloidogyne chitwoodi* and *M. fallax* in Central American *Solanum* species. Theor Appl Genet (in press)

Introduction

The root-knot nematodes, *Meloidogyne* spp., are a potential threat for the cultivation of potato in North Western Europe and the Western states of USA. Especially *M. chitwoodi* Golden *et al.* and *M. fallax* Karssen can cause serious economic losses by means of yield reduction and quality damage of tubers. The recently described *M. fallax* (Karssen 1996) is thought to be genetically closely related to *M. chitwoodi* (Janssen *et al.* 1996). Resistance would be effective in controlling these pests, but appears to be absent in the currently used potato cultivars (Brown *et al.* 1994; Janssen *et al.* 1995).

Resistance has been identified in the wild tuber-bearing *Solanum* species *S. bulbocastanum* and *S. hougasii* (Brown *et al.* 1989, 1991; Janssen *et al.* 1996) and the introgression of the resistance into the potato gene pool is in progress (Brown *et al.* 1994). More recently, also other promising sources of resistance have been selected, like in diploid *S. brachistotrichum* and *S. cardiophyllum* and tetraploid *S. fendleri* and *S. stoloniferum* (Janssen *et al.* 1996). The former two species are genetically closely related to *S. bulbocastanum* and are considered as primitive species, very distantly related to *S. tuberosum*. The species *S. fendleri* and *S. stoloniferum* have been classified in the more advanced series *Longipedicellata* (Hawkes 1990) and are - in contrast to the former species - directly crossable with *S. tuberosum*. Also, the hexaploid species *S. hougasii* from the series *Demissa* is directly crossable with potato. Therefore, the introgression of resistance from these *Solanum* species into the cultivated potato can be achieved through sexual crosses, although difficulties can be expected due to genomic differences and differences in ploidy levels (Hermsen 1994). However, the success of introduction into the potato gene pool will largely depend on the inheritance of the resistance.

Although *S. fendleri*, *S. stoloniferum* and *S. hougasii* have a polyploid genome, these species are cytologically disomic, showing regularly 24 and 36 bivalent configurations of the tetraploid and hexaploid species respectively and only very low frequencies of multivalents during meiosis (Swaminathan & Hougas 1954; Marks 1965; Dvorák 1983; Matsubayashi 1991; Watanabe & Orrillo 1994). Moreover, a disomic inheritance of various traits in species from the series *Longipedicellata* and *Demissa* has been suggested and proven in genetic studies (McKee 1962; Cockerham 1970; Everhart & Rowe 1974; Malamud & O'Keefe 1976). Several researchers consider these so-called allopolyploid species to have arisen from natural hybridisations of two (prehistoric) *Solanum* species (*e.g.* Marks 1965; Hawkes 1990; Spooner *et al.* 1995). Subsequently, gradual changes in the originally similar chromosomes would have lead to diploidisation favouring balanced gamete formation (Matsubayashi 1991). Others suggest a genetic control of the suppression of homoeologous chromosome pairing (Lamm 1945; Dvorák 1983). In this study,

disomic inheritance will be considered for *S. fendleri*, *S. stoloniferum* as well as *S. hougasii*.

To investigate the genetic behaviour of traits like resistance the use of intraspecific populations is preferred to ensure normal meiotic reduction divisions leading to Mendelian segregation patterns. Use of hybrid populations of distantly related species can result in distorted segregations of simply inherited characters. As an example, a major locus, responsible for resistance to *M. chitwoodi* from *S. bulbocastanum* was found using RFLP markers, but the mapping population showed a distorted segregation as a result of irregular meiosis in the hybrid parent (Masuelli *et al.* 1995; Brown *et al.* 1996).

This study describes the inheritance of resistance to *M. chitwoodi* and *M. fallax* of wild *S. fendleri*, *S. stoloniferum* and *S. hougasii* by analysing cross populations within the *Solanum* species or between very related species.

Materials & Methods

Plant material

Genotypes of *S. fendleri*, *S. stoloniferum*, *S. hougasii* and *S. iopetalum*, resistant or susceptible to *M. chitwoodi* and *M. fallax*, had been selected from resistance screening trials (Janssen *et al.* 1996). Since no susceptible genotypes of *S. hougasii* had been found, susceptible genotypes of related *S. iopetalum* were used for crosses. Plants were crossed in a glasshouse during spring and summer of 1994 and 1995. Flowers were emasculated one or two days before anthesis (except for self-pollinations) and pollinated once flowers were open. Fruits were harvested six weeks after pollination. Resistant genotypes were crossed with susceptible ones and self-pollinated producing F1 and S1 populations respectively. Resistant F1 genotypes were selected from various hybrid populations and used for making testcross populations with susceptible genotypes (TC) and self-pollinations (F2). The level of resistance of parent- and hybrid genotypes was analysed in multiple glasshouse experiments using several nematode populations (Janssen *et al.* 1996; Janssen *et al.* 1997*b*). The characteristics of the genotypes used are presented in Table 5.1. The cross combinations and derived populations are described in Table 5.2, 5.3 and 5.4 for *S. fendleri*, *S. hougasii* with *S. iopetalum* and *S. stoloniferum* respectively.

Table 5.1. Origin of crossing parents and level of resistance to *M. chitwoodi* and *M. fallax*.

Solanum sp.	Genotype	source	*M. chitwoodi*	*M. fallax*
S. fendleri	93-89-6	BGRC 23568[a]	resistant[b]	resistant[b]
S. fendleri	93-89-21	BGRC 23568	resistant	resistant
S. fendleri	93-114-11	BGRC 8083	resistant	resistant
S. fendleri	93-114-12	BGRC 8083	resistant	resistant
S. fendleri	93-115-7	BGRC 8090	susceptible	susceptible
S. fendleri	93-115-14	BGRC 8090	susceptible	susceptible
S. fendleri	93-115-18	BGRC 8090	susceptible	susceptible
S. fendleri	M94-33-3	93-114-12 x 93-115-18	resistant	resistant
S. fendleri	M94-51-1	93-114-11 x 93-115-7	resistant	resistant
S. fendleri	M94-79-1	93-115-14 x 93-89-21	resistant	resistant
S. hougasii	93-71-3	BGRC 55203	resistant	resistant
S. hougasii	93-71-6	BGRC 55203	resistant	resistant
S. iopetalum	93-108-1	BGRC 8101	susceptible	susceptible
S. iopetalum	93-108-11	BGRC 8101	susceptible	susceptible
S. hou .x S. iop.	M94-11-3	93-71-3 x 93-108-1	resistant	resistant
S. hou .x S. iop.	M94-11-4	93-71-3 x 93-108-1	resistant	resistant
S. iop .x S. hou.	M94-32-2	93-108-1 x 93-71-6	resistant	resistant
S. iop .x S. hou.	M94-32-5	93-108-1 x 93-71-6	resistant	resistant
S. stoloniferum	93-STOL-1	BGRC 7229	mod. resistant	resistant
S. stoloniferum	93-STOL-3	BGRC 7229	mod. resistant	resistant
S. stoloniferum	93-STOL-4	BGRC 7229	mod. resistant	resistant
S. stoloniferum	93-STOZ-1	BGRC 7230	susceptible	susceptible
S. stoloniferum	93-STOZ-2	BGRC 7230	susceptible	susceptible
S. stoloniferum	M94-23-1	93-STOL-3 x 93-STOZ-2	mod. susceptible	resistant

[a]) BGRC-accessions are from the Dutch-German potato gene bank, Wageningen, The Netherlands
[b]) Level of resistance based on multiple resistance tests (*e.g.* Janssen et al. 1997*b*).

Nematode inoculum

The nematode populations 'CHE' of *M. chitwoodi* originating from Heide, the Netherlands, and 'FB' of *M. fallax* originating from Baexem, the Netherlands, were used in the resistance tests. These populations had also been used in previous resistance tests with the crossing parents included (Janssen *et al.* 1997*b*) and were maintained on tomato plants cv Nematex. The species identity was regularly verified by analysing single females on isozyme patterns of esterase and malate dehydrogenase (Esbenshade & Triantaphyllou 1990). To prepare inoculum, eggs were harvested from the roots by dissolving egg masses in 0.5 % NaOCl-solution (Hussey & Barker 1973). Juveniles were hatched in water and stored at 4 °C up to one month until use as inoculum.

Table 5.2. Populations from crosses within *S. fendleri*. Populations are derived from crosses of resistant parent genotypes (RP) and resistant F1 genotypes (RF1) with susceptible parent genotypes (SP), and from various self-pollinations.

		RP 93-89-6	RP 93-89-21	RP 93-114-12	RF1 M94-33-3	RF1 M94-51-1	RF1 M95-79-1	SP 93-115-7	SP 93-115-14	SP 93-115-18
RP	93-89-6	M94-34								M94-68
RP	93-89-21		M94-142						M94-146	
RP	93-114-11							M94-51		
RP	93-114-12			M94-29					M94-148	M94-33
RF1	M94-33-3				M95-241				M95-239	
RF1	M94-51-1					M95-238			M95-236	
RF1	M94-79-1						M95-244	M95-242		
SP	93-115-7						M95-243			
SP	93-115-14		M94-79	M94-122	M95-240	M95-237			M94-147	
SP	93-115-18	M94-120								

Table 5.3. Populations from crosses of *S. hougasii* and *S. iopetalum*. Populations are derived from crosses of resistant parent genotypes (RP) and resistant F1 genotypes (RF1) with susceptible parent genotypes (SP), and from various self-pollinations.

		RP	RP	SP	SP
		93-71-3	93-71-6	93-108-1	93-108-11
RP	93-71-3	M94-31		M94-11	
RP	93-71-6		M94-17		
RF1	M94-11-3				M95-232
RF1	M94-11-4				M95-233
RF1	M94-32-2				M95-234
RF1	M94-32-5				M95-235
SP	93-108-1	M94-13	M94-32	M94-108	

Table 5.4. Populations from crosses within *S. stoloniferum*. Populations are derived from crosses of resistant parent genotypes (RP) and resistant F1 genotypes (RF1) with susceptible parent genotypes (SP), and from various self-pollinations.

		RP	RP	RP	RF1	SP
		93-STOL-1	93-STOL-3	93-STOL-4	M94-23-1	93-STOZ-2
RP	93-STOL-1	M94-95				M94-131
RP	93-STOL-3		M94-24			M94-23
RP	93-STOL-4					M94-88
SP	93-STOZ-1				M95-229	
SP	93-STOZ-2	M94-132	M94-130	M94-96	M95-228	

Resistance tests

Resistance tests were performed to analyse the level of resistance to *M. chitwoodi* and *M. fallax* of seedlings during 1995 and 1996. For each *Solanum* species two resistance tests were carried out containing F1- and S1-populations in the first and F2- and TC-populations in the second test. Seeds were sown in a potting soil/silver sand (1 : 1) mixture and transplanted into square plastic tubes of 240 ml, filled with moist silver sand and NPK fertiliser. Tubes were put in trays and trays were placed randomly in a temperature-controlled glasshouse (22 ± 2 °C). Approximately three weeks after transplantation plants were inoculated with 400 juveniles of either *M. chitwoodi* or *M. fallax*. During the experiment, stolons were regularly cut to prevent ingrowth into neighbouring tubes. Plants were harvested eight weeks after inoculation. Roots were washed free from sand and for each seedling the number of egg masses was counted after staining with Phloxine B (Dickson & Strubble 1965).

Depending on the *Solanum* species and the number of seedlings available, populations were represented as randomly situated plots of 5 to 18 seedlings per tray and for each nematode species 4 to 8 trays were used. Each tray contained 4 plants of potato cv Nicola to serve as a susceptible control for possible miscellaneous nematode conditions.

In order to determine whether resistance to *M. chitwoodi* and *M. fallax* was the same or highly linked in *S. fendleri* and *S. hougasii*, 11 genotypes of TC population M95-236 and 15 of TC population M95-235 were tested in 4 replications to each nematode species. Seeds were sown in vitro on MS medium (Murashige & Skoog 1962) containing 30 g/l sucrose and shoots were cut, until enough clones were available. Two weeks after the last cutting, in vitro plantlets were transplanted in 350 ml stone pots, filled with moist silver sand and NPK fertiliser and the experiment was further carried out as described for the seedling tests. Least Significant Difference (LSD) was analysed with ANOVA using Genstat (Payne *et al.* 1987) after square root transformation of data.

Results

Resistance in S. fendleri

Most seedlings from the various F1 and S1 populations of *S. fendleri* appeared completely resistant to *Meloidogyne* spp. Only occasionally a seedling with more than one egg mass was observed. In Figure 5.1A the distribution pattern of the seedlings from the F1 population M94-122 and its reciprocal cross M94-148 is shown. For other cross combinations similar patterns were found. The population M94-147, derived from self-pollination of the susceptible genotype 93-115-14, showed a normal-like distribution ranging from 0 to 9 after square root transformation of the number of egg masses (Fig 5.1B). One seedling was found having no egg masses and was considered as an escape. The seedling populations which were obtained from testcrosses of resistant hybrids with a susceptible genotype, clearly segregated into groups of resistant and susceptible plants. An example of this segregation is shown with population M95-239 and its reciprocal cross M95-240 in Figure 5.1C. The F2 populations also segregated into distinct groups of resistant and susceptible plants, like M95-241 (Fig 5.1D).

Table 5.5. Mean square root number of egg masses of *M. chitwoodi* (CHE) and *M. fallax* (FB) on genotypes of *S. fendleri* M95-236 and (*S. hougasii* x *S. iopetalum*) x *S. iopetalum* M95-235. Means are based on 4 replications.

		genotype														
population	nem.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
M95-236	CHE	8.8	0.3	0.0	0.0	0.0	7.7	0.0	0.0	7.8	7.1	9.5				
	FB	8.6	0.0	0.0	0.0	0.0	7.0	0.0	0.0	8.0	7.4	8.9				
M95-235	CHE	7.1	0.0	8.3	6.6	7.3	7.3	0.3	0.3	5.3	6.6	0.0	0.0	0.0	2.4	0.0
	FB	7.3	0.0	7.0	7.1	8.4	6.9	0.0	0.0	5.9	7.4	0.0	0.3	0.0	5.0	0.0

LSD (P<0.05) = 1.2

Figure 5.1A-D. Distribution of levels of infection of seedling populations of *S. fendleri*, infected with *M. chitwoodi* (striped) or *M. fallax* (cross hatched). A: M94-122 + M94-148 (reciprocal cross), B: M94-147, C: M95-239 + M94-240 (reciprocal cross), D: M95-241. The infection level of parents is indicated with arrows.

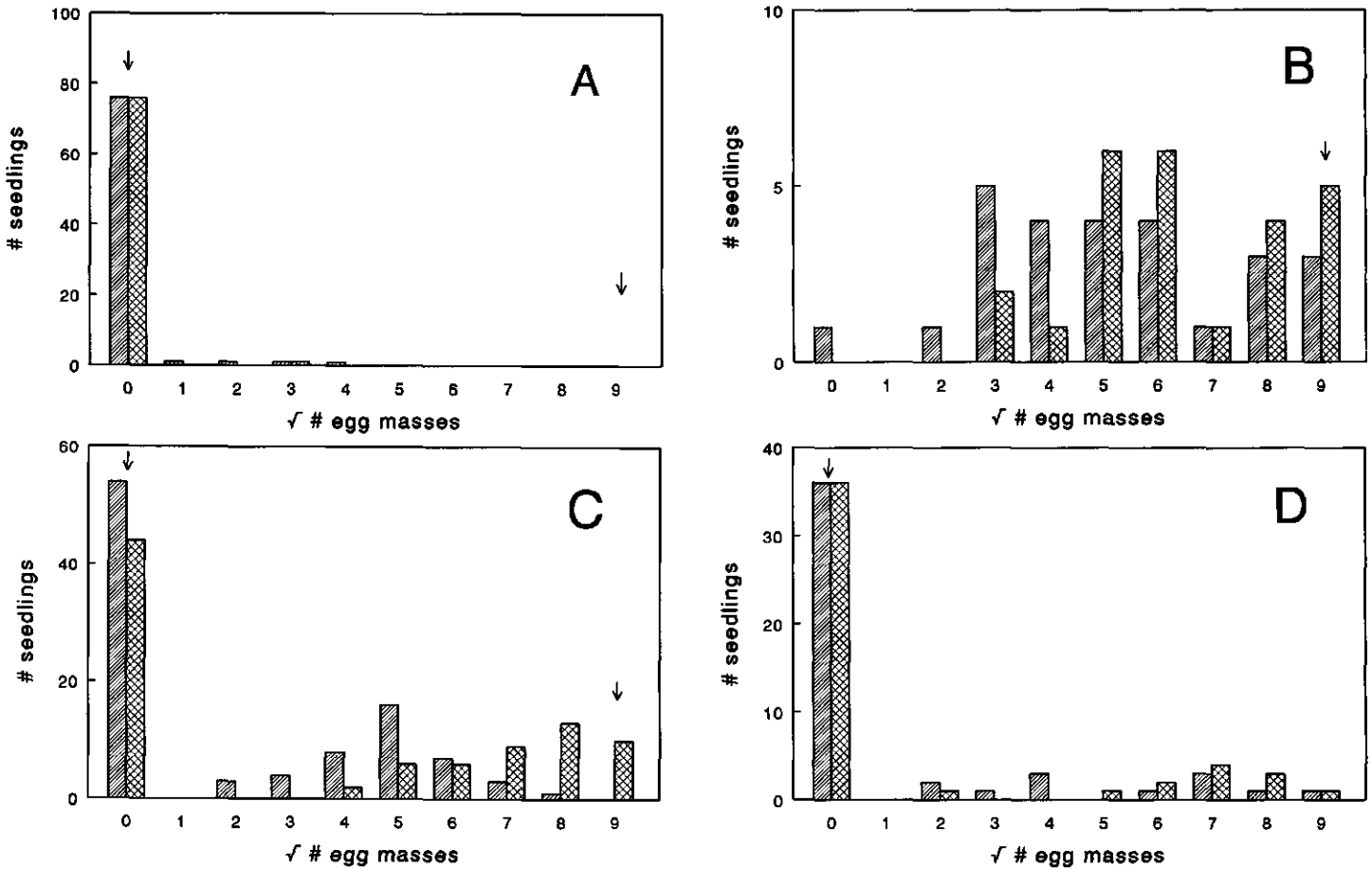


Figure 5.2A-D. Distribution of levels of infection of seedling populations of *S. hougasii* (x *S. iopetalum*), infected with *M. chitwoodi* (striped) or *M. fallax* (cross hatched). A: M94-31, B: M94-11 + M94-13 (reciprocal cross), C: M94-108, D: M95-234 + M95-235. The infection level of parents is indicated with arrows.

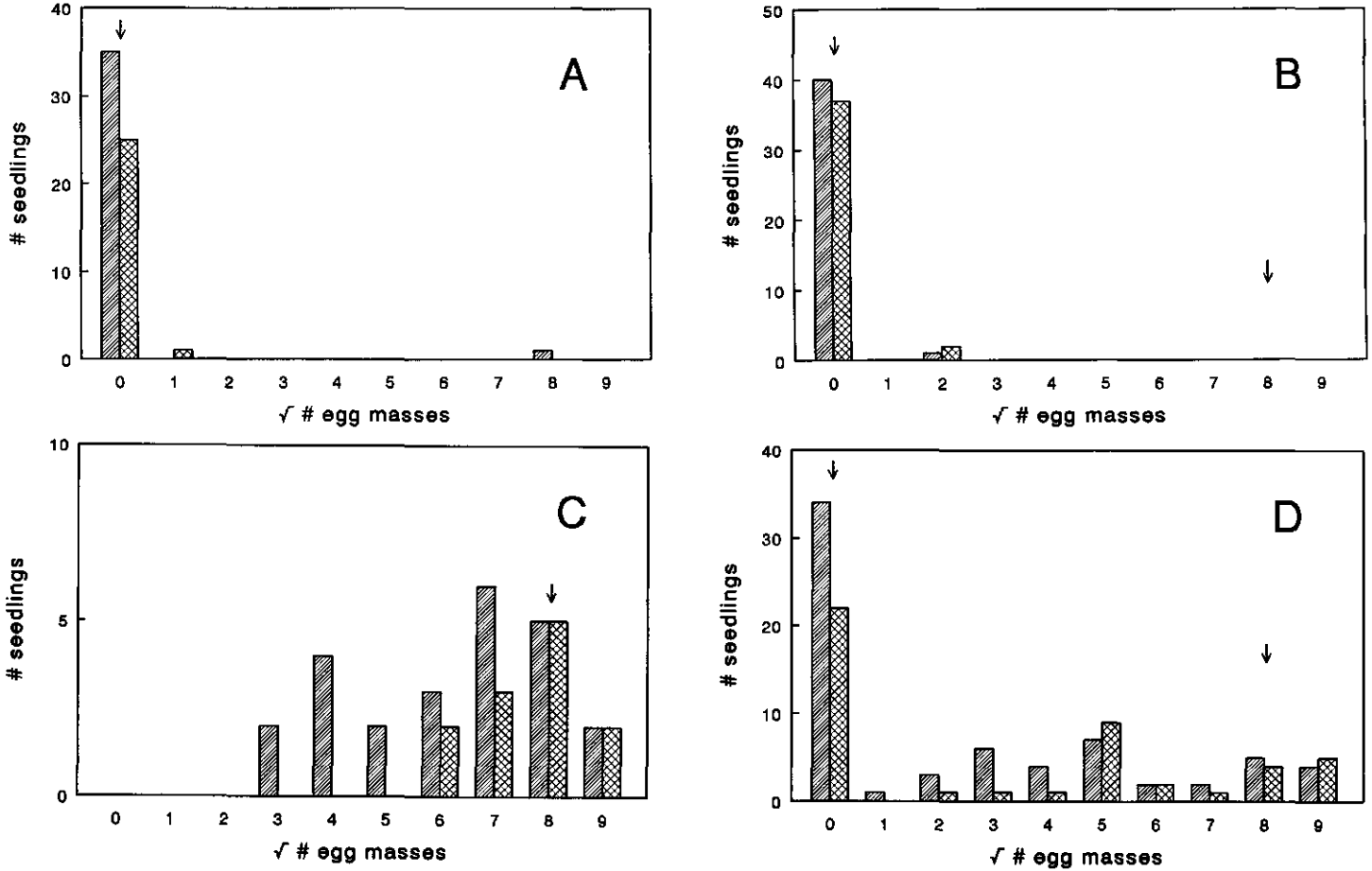
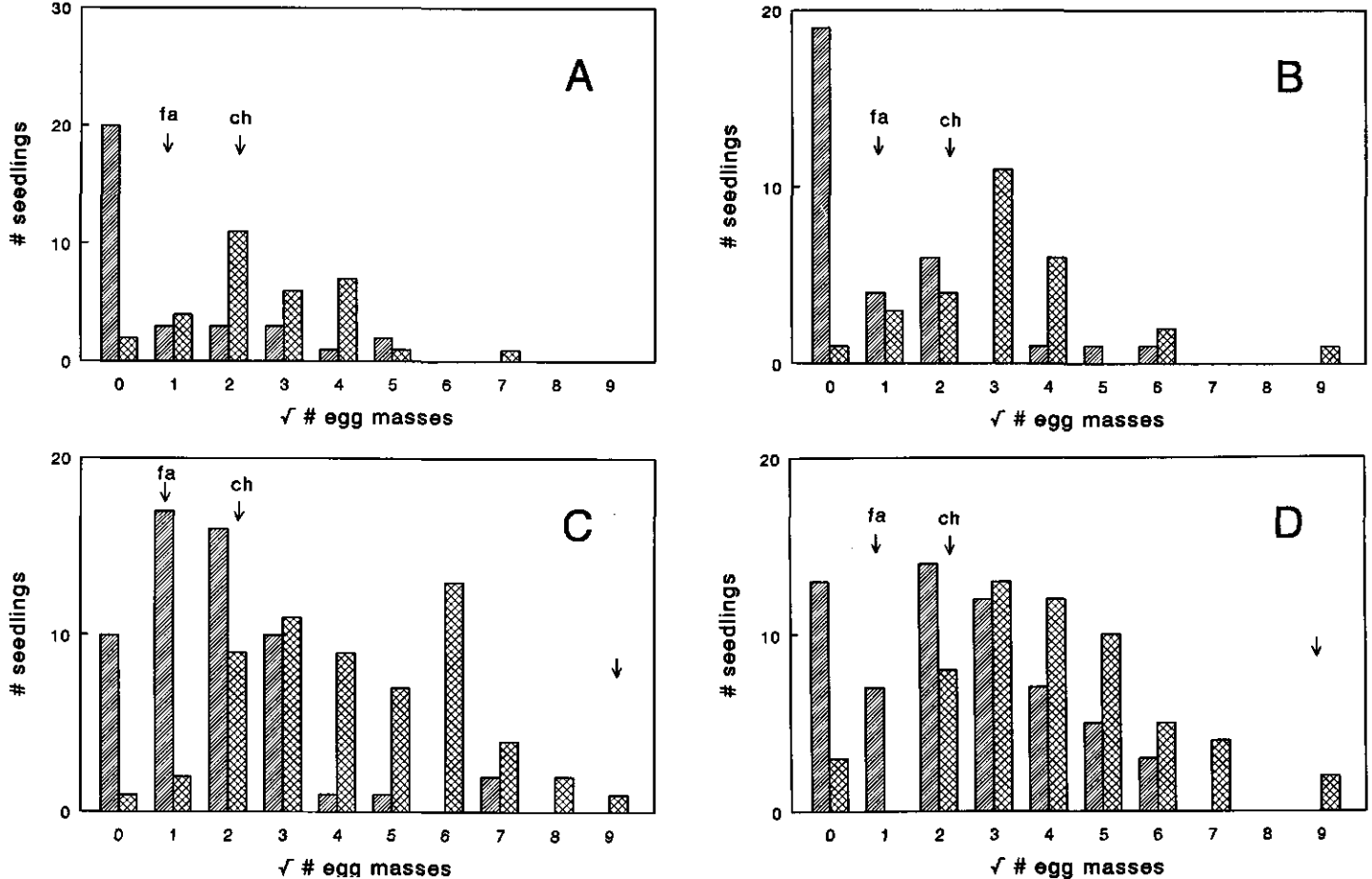
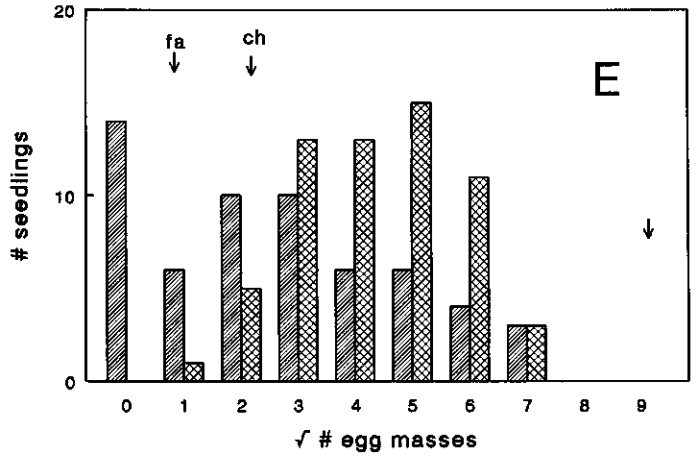


Figure 5.3A-F. Distribution of levels of infection of seedling populations of *S. stoloniferum*, infected with *M. chitwoodi* (striped) or *M. fallax* (cross hatched). A: M94-95, B: M94-24, C: M94-131 + M94-132 (reciprocal cross), D: M94-23 + M94-130 (reciprocal cross), E: M94-88 + M94-96 (reciprocal cross), F: M95-228 + M95-229. The infection level of parents is indicated with arrows.




20
10
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seedlings
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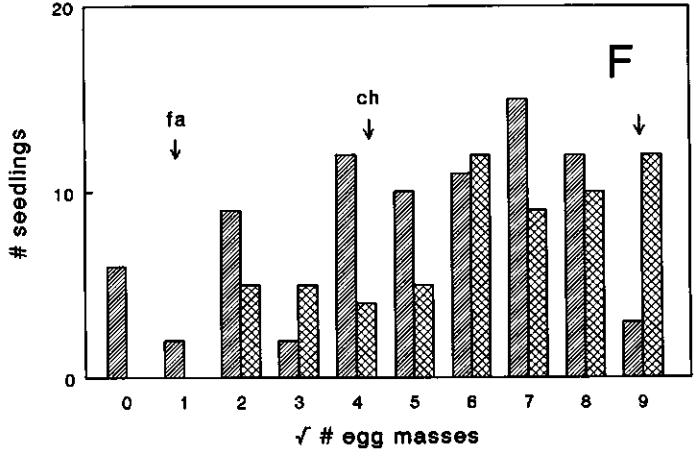
√ # egg masses


20
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seedlings
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0 1 2 3 4 5 6 7 8 9
√ # egg masses

In all tested seedling populations no clear deviant pattern was observed between the nematode species *M. chitwoodi* and *M. fallax*. Moreover, the 11 genotypes of M95-236, which were tested against both nematode species, were either resistant or completely susceptible to both (Table 5.5). In other experiments numerous genotypes of *S. fendleri* and interspecific hybrid- and backcrossed genotypes of *S. fendleri* with *S. tuberosum* have been screened and so far tests have not revealed any genotype with a distinct behaviour towards these nematode species (data not shown).

Based on the segregation patterns as shown in Fig 5.1A-D, in the genetic analysis plants having no or one egg mass were regarded resistant (R) and plants having more than one egg mass susceptible (S). The observed segregation R : S of tested populations of *S. fendleri* is shown in Table 5.6. Hardly any segregation was observed for all F1 and S1 populations. All TC populations showed a segregation pattern, which fitted a 1 : 1 distribution for R : S. The populations from the self-pollinations of the hybrid genotypes fitted a 3 : 1 segregation. In none of the cross combinations reciprocal differences were observed. Assuming disomic inheritance, the observed segregation patterns can be explained with a single dominantly inherited gene, which would be present in a homozygous form in the resistant parent genotypes.

Resistance in S. hougasii

Like *S. fendleri*, most seedlings from self-pollination of resistant genotypes of *S. hougasii* as well as from crosses with *S. iopetalum* appeared completely resistant. Figures 5.2A and 5.2B express the (lack of) segregation of populations from self-pollination of 93-71-3 and from crosses of this genotype with susceptible 93-108-1 respectively. The susceptible status of 93-108-1 is confirmed by the distribution of genotypes obtained from self-pollination (Fig 5.2C).

It was difficult to obtain seeds from testcrosses of resistant hybrids with susceptible genotypes of *S. iopetalum* and only crosses with the hybrid as female produced some seeds. All four TC populations segregated and M95-234 and M95-235 were pooled for the segregation pattern of Figure 5.2D. Since very few seeds were derived from self-pollinations, these were not tested. The 15 genotypes of TC population M95-235, which were tested against both *M. chitwoodi* and *M. fallax*, revealed no different behaviour between the species (Table 5.5). Other experiments testing several interspecific genotypes of *S. hougasii* with *S. tuberosum* confirmed that, like in *S. fendleri*, the resistance of *S. hougasii* to *M. chitwoodi* and *M. fallax* is the same or highly linked (data not shown).

Using the same criterion as for *S. fendleri* to distinguish between resistant and susceptible plants, hardly any susceptible plants were noticed in the F1 and S1 populations. The TC populations fitted a 1 : 1 segregation, but only for M95-234 and M95-235 numbers of seedlings were sufficient to validate this assumption (Table 5.7).

Assuming disomic behaviour of *S. hougasii*, monogenic dominantly inherited resistance is possible, but results are inconclusive and several alternative hypotheses can not be excluded.

Resistance in S. stoloniferum

In contrast to *S. fendleri* and *S. hougasii*, the level of resistance to *M. chitwoodi* and *M. fallax* in *S. stoloniferum* is not absolute and also different for the two nematode species (Table 5.1). The actual level of resistance was well represented by the mean level of resistance of the seedling populations from the self-pollination of 93-STOL-1 (Fig 5.3A) and 93-STOL-3 (Fig 5.3B), *i.e.* 1 and 2 for the square root number of egg masses of *M. fallax* and *M. chitwoodi* respectively. The seedlings from the crosses of the moderately resistant genotypes with susceptible 93-STOZ-2 showed a normal-like distribution for the square root number of egg masses from 0 to 7 with a mean of approx. 2 for *M. fallax* and a distribution from 0 to 9 with a mean of 3 to 4 for *M. chitwoodi* (Fig 5.3C-E).

Only one hybrid genotype, which had a reasonable level of resistance to *M. fallax* but moderately susceptible to *M. chitwoodi* was used to make testcrosses and the distribution of TC seedlings is shown in Fig 5.3F. The level of resistance to *M. fallax* was highly variable and the mean square root number of egg masses was approx. 5. Self-pollinations of the hybrid genotype were not successful, presumably due to the poor condition of the plant. The results indicate the action of possibly several additive genes responsible for resistance and at least one ore more resistance genes effective to *M. fallax*, but not to *M. chitwoodi*.

Discussion

The genetic analysis of *S. fendleri* revealed the likely presence of a monogenic and dominantly inherited factor responsible for resistance to both *M. chitwoodi* and *M. fallax*. Very recently, in *S. bulbocastanum* monogenic resistance to *M. chitwoodi* and *M. hapla* has been identified and designated as R_{mc1} (Brown *et al.* 1996) and this resistance appeared also effective towards *M. fallax* (Janssen *et al.* unpubl. results). The resistance in *S. fendleri* as described here does not suppress multiplication of *M. hapla* to a significant extent (Janssen *et al.* 1997*b*), indicating the existence of a different gene in *S. fendleri*. We propose the symbol R_{mc2} for the gene from *S. fendleri*. Although two different accessions of *S. fendleri* were used in this study, it is expected that the resistant genotypes from these accessions bear the same resistance gene. However, this needs to be confirmed with test crosses.

Table 5.6. Segregation patterns of various cross populations of *S. fendleri*.

Population	from cross		expected$_{R:S}$[a]	*M. chitwoodi* observed$_{R:S}$	χ^2-prob	*M. fallax* observed$_{R:S}$	χ^2-prob
M94-79	93-115-14 x 93-89-21	(SP x RP)	1 : 0	35 : 2	-	36 : 1	-
M94-146	93-89-21 x 93-115-14	(RP x SP)	1 : 0	30 : 2	-	31 : 4	-
M94-142	93-89-21 self-poll.	(RP ⊗)	1 : 0	33 : 3	-	39 : 0	-
M94-68	93-89-6 x 93-115-18	(RP x SP)	1 : 0	39 : 1	-	39 : 0	-
M94-120	93-115-18 x 93-89-6	(SP x RP)	1 : 0	30 : 1	-	30 : 2	-
M94-34	93-89-6 self-poll.	(RP ⊗)	1 : 0	32 : 1	-	37 : 0	-
M94-122	93-115-14 x 93-114-12	(SP x RP)	1 : 0	37 : 1	-	38 : 2	-
M94-148	93-114-12 x 93-115-14	(RP x SP)	1 : 0	39 : 0	-	39 : 1	-
M94-29	93-114-12 self-poll.	(RP ⊗)	1 : 0	37 : 2	-	37 : 1	-
M95-236	M94-51-1 x 93-115-14	(RF1 x SP)	1 : 1	25 : 19	0.37	20 : 27	0.31
M95-237	93-115-14 x M94-51-1	(SP x RF1)	1 : 1	22 : 24	0.77	23 : 25	0.77
M95-238	M94-51-1 self-poll.	(RF1 ⊗)	3 : 1	23 : 4	0.22	14 : 3	0.48
M95-239	M94-33-3 x 93-115-14	(RF1 x SP)	1 : 1	20 : 28	0.25	26 : 22	0.57
M95-240	93-115-14 x M94-33-3	(SP x RF1)	1 : 1	24 : 19	0.44	28 : 20	0.25
M95-241	M94-33-3 self-poll.	(RF1 ⊗)	3 : 1	36 : 12	1.0	36 : 12	1.0
M95-242	M94-79-1 x 93-115-7	(RF1 x SP)	1 : 1	24 : 24	1.0	24 : 24	1.0
M95-243	93-115-7 x M94-79-1	(SP x RF1)	1 : 1	23 : 23	1.0	24 : 22	0.76
M95-244	M94-79-1 self-poll.	(RF1 ⊗)	3 : 1	36 : 14	0.24	33 : 14	0.57

[a]) Expected segregation based on a monogenic dominant factor present in a homozygous form in the resistant parent genotype.

Table 5.7. Segregation of F1, S1 and TC populations of *S. hougasii*, crossed with *S. iopetalum*.

Population	from cross		expected$_{R:S}$[a]	*M. chitwoodi* observed$_{R:S}$	χ^2-prob	*M. fallax* observed$_{R:S}$	χ^2-prob
M94-11	93-71-3 x 93-108-1	(RP x SP)	1 : 0	20 : 0	-	20 : 0	-
M94-13	93-108-1 x 93-71-3	(SP x RP)	1 : 0	25 : 2	-	26 : 1	-
M94-31	93-71-3 self-poll.	(RP ⊗)	1 : 0	35 :1	-	26 : 0	-
M94-32	93-108-1 x 93-71-6	(SP x RP)	1 : 0	18 : 0	-	27 : 0	-
M94-17	93-71-6 self-poll.	(RP ⊗)	1 : 0	23 : 0	-	25 : 0	-
M95-232	M94-11-3 x 93-108-11	(RF1 x SP)	1 : 1	3 : 1	-	6 : 3	-
M95-233	M94-11-4 x 93-108-11	(RF1 x SP)	1 : 1	2 : 3	-	2 : 1	-
M95-234	M94-32-2 x 93-108-11	(RF1 x SP)	1 : 1	14 : 13	0.84	12 : 12	1.0
M95-235	M94-32-5 x 93-108-11	(RF1 x SP)	1 : 1	21 : 19	0.75	10 : 12	0.67

[a]) Expected segregation based on a monogenic dominant factor present in a homozygous form in the resistant parent genotype.

It has been considered that disomic inheritance is the most likely mode of fashion in *S. fendleri*. But when assuming tetrasomic inheritance, a single resistance gene present in a triplex form in the parent genotypes and in a simplex form in the F1 genotypes would also explain the segregation patterns observed. The chance of selecting three simplex genotypes randomly from a triplex x nulliplex cross would be 1/8. Taken into account the results described here, the occurrence of tetrasomic inheritance can not be excluded. Nevertheless, as already mentioned in the introduction, the disomic behaviour is thought to be most probable.

The results of the hexaploid *S. hougasii* also indicated the presence of simply inherited resistance to *M. chitwoodi* and *M. fallax*, but caution is needed because not enough genotypes and cross combinations were available for testing. Serious difficulties were encountered while making testcrosses and self-pollinations of the interspecific hybrid genotypes of *S. hougasii* and *S. iopetalum* and numerous attempts resulted in only few successful crosses. Also other researchers have reported successful hybridisation of several interspecific combinations of *Solanum* species within the series *Demissa* but high levels of sterility of the hybrids resulting in unsuccessful self-pollinations and backcrosses (Swaminathan & Hougas 1954; Hawkes 1955).

In previous studies the genotypes of *S. hougasii* expressed not only resistance to *M. chitwoodi* and *M. fallax*, but also moderate resistance to *M. hapla* (Janssen *et al.* 1997*b*), similar as expressed by resistant genotypes of backcrossed genotypes of *S. bulbocastanum* with *S. tuberosum* (Janssen *et al.* unpubl. results). The hexaploid species *S. hougasii* is only distinctly related to the primitive diploid species *S. bulbocastanum* and gene exchange through natural hybridisation is not likely to have occurred. Nevertheless, the similarity in working spectrum of resistance could indicate homology of the resistance genes. If the occurrence of resistance genes is not the result of recent introgression, resistance genes might have been conserved through the evolution of *Solanum* species. Combined with a continuous selection pressure towards resistance, this would explain the presence of resistance to *M. chitwoodi* and related *M. fallax* in various Central American *Solanum* species. All *Solanum* species investigated in this study as well as the resistant sources *S. bulbocastanum*, *S. brachistotrichum* and *S. cardiophyllum* have their natural habitat situated in Central America, primarily Mexico (Hawkes 1990) and resistance to *M. chitwoodi* and *M. fallax* seems rare in South American *Solanum* species (Janssen *et al.* 1996). Furthermore, *M. chitwoodi* has been found in different states of Mexico (Cuevas & Sosa Moss 1990) justifying this hypothesis.

Sometimes, most notably in the F1 and S1 populations of *S. fendleri* and *S. hougasii*, the cross population was found to be virtually completely resistant, but still occasionally a susceptible plant was observed. These positives are regarded as artefacts of the resistance tests due to ingrowth of susceptible plants into neighbouring

tubes. However, they could not have had any effect on the segregation of populations due to the large numbers of genotypes tested.

The resistance of *S. stoloniferum* could not be explained by a simple inheritance and polygenic inheritance seems likely. Furthermore, resistance factors to *M. chitwoodi* and *M. fallax* were not completely linked, in contradiction with the results observed with the other *Solanum* species, as indicated by the deviant segregation patterns and the difference in levels of resistance of the parents used. For the investigation of this type of polygenic and incomplete resistance, simple analysis of seedling populations is obviously not adequate and the use of replications of genotypes is necessary in order to decrease the experimental variation.

The introgression of resistance to root-knot nematodes from *S. fendleri*, *S. hougasii* and *S. stoloniferum* into cultivated potato has been initiated using the information obtained from these inheritance studies. In the case of the earlier found resistance from *S. bulbocastanum*, somatic hybridisations with *S. tuberosum* were necessary as a first step of gene transfer to *S. tuberosum* (Austin *et al.* 1993). With the *Solanum* species investigated direct sexual crosses with *S. tuberosum* were successful and currently resistant genotypes have been selected from first backcrosses. Future research will concentrate on the localisation of resistance genes to the root-knot nematodes in wild *Solanum* species using molecular markers, which will also enable marker-assisted selection to achieve rapid introduction of new resistance genes into new commercial potato cultivars.

Chapter 6

Introgression of resistance to root-knot nematodes from wild Central American *Solanum* species into *S. tuberosum* ssp. *tuberosum*.[1]

Summary

Crossing experiments were conducted to introduce resistance to the root-knot nematodes, *Meloidogyne chitwoodi* and *M. fallax*, from various wild Central American *Solanum* spp. into the cultivated potato, *S. tuberosum* ssp. *tuberosum*. No plants were obtained from the crosses of diploid *S. tuberosum* with diploid *S. bulbocastanum*, *S. brachistotrichum* and *S. cardiophyllum*. With regard to the interspecific crosses with polyploid *Solanum* species most efforts were put in producing tetraploid hybrids through inter-EBN (Endosperm Balance Number) crosses. From the crosses of tetraploid *S. tuberosum* (4 EBN) with tetraploid *S. stoloniferum* and *S. fendleri* (both 2 EBN), few seeds were derived leading to viable plants. In vitro culture of immature seeds also yielded several putative hybrid plants. From crosses of diploid *S. tuberosum* (2 EBN) with hexaploid *S. hougasii* (4 EBN) four hybrids were obtained through in vitro culture. The hybrid status was confirmed with RAPD markers and the ploidy level was analysed using flow cytometry. Backcrosses were made with selected hybrids and a variable number of seeds was produced depending on the hybrid genotype. The successful introgression of resistance into backcross populations is shown. A scheme is presented to introgress traits at a tetraploid level from allotetraploid *Solanum* species into autotetraploid *S. tuberosum* through sexual crosses. The relevance of EBN for potato breeding is discussed.

[1]) Parts of this chapter are based on: Janssen GJW, Van Norel A, Verkerk-Bakker B, Janssen R & Hoogendoorn J (1997) Introgression of resistance to root-knot nematodes from wild Central American *Solanum* species into *S. tuberosum* ssp. *tuberosum*. Theor Appl Genet (in press)

Introduction

Wild *Solanum* species are potential sources of valuable traits for potato breeding like resistances to various diseases and pests. However, not all *Solanum* species can be readily crossed with the cultivated potato, *S. tuberosum* ssp. *tuberosum*, due to the presence of pre- and/or postzygotic barriers, differences in ploidy levels and/or structural genomic differences (Ross 1986; Hawkes 1990; Hermsen 1994).

With regard to the postzygotic barriers, Johnston, den Nijs, Peloquin and Hanneman (1980) proposed the Endosperm Balance Number (EBN) hypothesis to explain the results of interspecific crosses between *Solanum* species. Each species with a certain ploidy level has been assigned a hypothetical value ranging from 1 to 4 (*e.g.* listed by Hawkes & Jackson 1992; Hanneman 1994) and only interspecific combinations with an equal EBN will have a normal endosperm development and give rise to viable seeds and hybrid plants. The EBN hypothesis bases the success of intra- and interspecific crosses on the female: male EBN ratio in the endosperm of 2 : 1 (Johnston *et al.* 1980) and seems to be genetically controlled by two or three unlinked loci (Ehlenfeldt & Hanneman 1988; Camadro & Masuelli 1995).

In order to circumvent incompatible reactions as a result of different EBN values several alternative strategies have been applied. In unreduced gametes, the so-called 2n gametes, the actual EBN number equals the somatic number and this can abolish the EBN difference in certain interspecific crosses. With the use of 2n gametes F1 hybrids or first backcrosses have been obtained from *S. stoloniferum* and *S. acaule* with *S. tuberosum* (Von Wangenheim 1955; Adiwilaga & Brown 1991). Also colchicine treatment of the lower EBN parent to double chromosome numbers has a similar effect and has made these interspecific (back-) crosses possible (Swaminathan 1951; Watanabe *et al.* 1992). The use of bridging species combined with 2n gametes or mitotic doubling has led to the development of complex hybrids bearing genetic material from diploid 1 EBN species like *S. commersonii*, *S. bulbocastanum*, *S. brevidens*, *S. etuberosum* and/or *S. pinnatisectum* (Dionne 1963; Chavez *et al.* 1988; Hermsen 1994; Bamberg *et al.* 1994). Somatic hybridisation is another approach to obtain interspecific genotypes as a first step of introgression. This technique has been applied for the establishment of hybrids of *S. tuberosum* with the diploid 1 EBN species *S. brevidens* (Barsby *et al.* 1984), *S. circaeifolium* (Mattheij *et al.* 1992), *S. bulbocastanum* (Austin *et al.* 1993), *S. commersonii* (Cardi *et al.* 1993) and *S. pinnatisectum* (Ward *et al.* 1994).

Still, the described alternatives can have certain disadvantages in the prolonged process of introgression. In the case of somatic hybrids, aneuploid chromosome numbers are common (Ramulu *et al.* 1989) and recombination is not automatically enhanced. The use of 2n gametes in crosses between tetraploid species or the treatment of triploid genotypes with colchicine will lead to hexaploid genotypes, which are

suitable for continued introgression. However, in these genotypes homoeologous pairing and recombination between chromosomes is not likely to occur due to preferential pairing of homologous chromosomes and disomic behaviour of the polyploid genome (Brown 1988; Watanabe *et al.* 1992). On the other hand, true tetraploid hybrids from sexual crosses should enhance homoeologous pairing and recombination and therefore facilitate introgression of desired traits (Iwanaga *et al.* 1991; Watanabe *et al.* 1992, 1994; Bamberg *et al.* 1994), but these interspecific hybrids have hardly been found due to the EBN differences between the wild *Solanum* species and *S. tuberosum*. Still, several successful inter-EBN combinations leading to viable hybrids have been reported (reviewed and discussed by Hermsen 1994).

Our interest concerns primarily the transfer of root-knot nematode resistance into the potato gene pool. High levels of resistance to *Meloidogyne chitwoodi* and *M. fallax* have been identified in several Central American *Solanum* species (Brown *et al.* 1989, 1991; Janssen *et al.* 1996). With regard to the resistant diploid 1 EBN species *S. bulbocastanum*, *S. brachistotrichum* and *S. cardiophyllum*, no successful crosses with *S. tuberosum* are known so far (Novy & Hanneman 1991). With the tetraploid 2 EBN species *S. stoloniferum* and *S. fendleri*, successful interspecific hybridisations with *S. tuberosum* have been reported, mostly as a result of 2n gametes and ploidy manipulations (Swaminathan 1951; Van Soest 1985; Adiwilaga & Brown 1991). However, on some rare occasions tetraploid hybrids of *S. stoloniferum* with *S. tuberosum* have been obtained (Von Wangenheim 1955), also in combination with embryo rescue and rescue pollination (Singsit & Hanneman 1991). The hexaploid 4 EBN species *S. hougasii* is sexually crossable with tetraploid *S. tuberosum*, but with diploid *S. tuberosum* a tetraploid level would be obtained giving better opportunities for introgression of genes (Ross 1986; Watanabe *et al.* 1992).

The present study describes attempts to cross root-knot nematode resistant *S. bulbocastanum*, *S. cardiophyllum*, *S. brachistotrichum*, *S. fendleri*, *S. stoloniferum* and *S. hougasii* genotypes with cultivated potato, *S. tuberosum*, and for some interspecific combinations with the use of in vitro culture of immature seeds. The first steps of introgression of resistance to *Meloidogyne* spp. from various Central American *Solanum* spp. through intra- and inter-EBN crosses will be described as well as the efforts to remain at a tetraploid level.

Materials and methods

Plant material

Genotypes were selected from trials for resistance to *Meloidogyne* spp. (Janssen *et al.* 1996). The characteristics of the genotypes used are presented in Table 6.1. The wild

Solanum genotypes have moderate to high levels of resistance to *M. chitwoodi* and *M. fallax*, whereas all *S. tuberosum* genotypes used are susceptible (Janssen *et al.* 1997*b*). All wild *Solanum* and *S. tuberosum* genotypes were good pollinators, according to the pollen production and pollen stainability with lactophenol-acid fuchsine. Wild *Solanum* genotypes were crossed with diploid and/or tetraploid *S. tuberosum* genotypes in a glasshouse from April to July 1994. Flowers were emasculated one or two days before anthesis and pollinated in the morning once flowers were open. Berries were harvested six weeks after inoculation. In the spring and summer of the following year selected hybrid genotypes were backcrossed with *S. tuberosum* genotypes, mostly differing from those used in the original cross.

Table 6.1. Some characteristics of *Solanum* species and the number of genotypes used.

Solanum species	accession[a]	ploidy	EBN	abbreviation	# genotypes	resistance[b]
S. bulbocastanum	8002, 8006	2x	1	blb	3	resistant
S. cardiophyllum	8024	2x	1	cph	3	resistant
S. brachistotrichum	7986	2x	1	bst	2	resistant
S. stoloniferum	7229	4x	2	sto	4	resistant
S. fendleri	8083, 23568	4x	2	fen	4	resistant
S. hougasii	55203	6x	4	hou	4	resistant
S. tuberosum	--	2x	2	tbr2	7	susceptible
S. tuberosum	--	4x	4	tbr4	5	susceptible

[a]) BGRC accessions from the Dutch-German potato gene bank, Wageningen, the Netherlands.
[b]) Resistance to the root-knot nematodes *Meloidogyne chitwoodi* and *M. fallax*.

In vitro seed culture

For some cross combinations, in vitro culture of immature seeds was applied to obtain hybrid plants. The procedure is based on dissecting berries and plating whole immature seeds in contrast to embryo-culture, where excised embryos are cultured. Young developing berries were harvested approximately 20 days after pollination, surface-sterilised by soaking for 10 sec in 70 % ethyl alcohol and for 10 min in 1 % NaOCl solution and then rinsed twice for 10 min in sterile water. Young seeds were carefully excised from the berries and placed in a sterile Petri dish with HLH 50 medium (Neal & Topoleski 1983). Dishes were put in the dark and after 14 days in the light in a growth chamber (23 ± 1 °C). Germinating seeds were transferred to sterile tubes containing MS 30 medium (Murashige & Skoog 1962). Rooted plants were transplanted in soil in the glasshouse and further treated as other genotypes.

Flow cytometry

Ploidy levels of *Solanum* genotypes were analysed using a flow cytometer (FCM). Young leaf tissue was chopped in 2 ml ice cold nucleus isolation buffer (1 mg/l 4,6-diamino-2-phenylindole (DAPI), 0.2 M mannitol, 10 mM 2(*N*-morpholino)ethane-sulphonic acid, 10 mM NaCl, 10 mM KCl, 10 mM spermine tetrahydrochloride, 2.5 mM ethylenediamine-tetra-acetic acid, 2.5 mM dithiothreitol, 0.05 % Triton X-100 and 0.05 % (w/v) sodium azide) (Bergervoet *et al.* 1996) and passed through an 88 µm mesh nylon sieve. The relative DNA content of isolated nuclei was measured using a Partec PAS II FCM. For calibrating the system a tetraploid genotype (potato cv Nicola) was used. The number of nuclei was plotted on a semi logarithmic scale and histograms were processed using software package FLOWS 1.0.

RAPD analysis

To verify the hybrid status of genotypes random amplified polymorphic DNA (RAPD) markers were used. Genomic DNA was extracted from deeply frozen leaf tissue following the procedure of Shure *et al.* (1983) with some minor modifications. One g of frozen leaf tissue was pulverised in a mortar in the presence of liquid nitrogen and taken up in a tube with 3 ml cold lysis buffer containing 5 M urea, 0.3 M NaCl, 0.05 M Tris-HCl (pH 7.5), 0.02 M EDTA, 2 % (w/v) sarcosyl and 0.05 % (w/v) sodium dodecyl sulphate. The mixture was gently stirred and 3 ml phenol/chloroform (1:1) was added. Tubes were shortly vortexed and phases were separated by low speed centrifugation. The upper phase was transferred into a clean tube, a 0.7 volume of ispropanol was added and after careful stirring the DNA cloud was taken out with a needle and rinsed with 70 % ethyl alcohol. DNA was then dissolved in 300 µl TE with 2 µl RNAse.

PCR amplification reactions were performed with a total volume of 23 µl containing 20 ng DNA, 25 ng primer (Operon), 100 µM each of dATP, dCTP, dGTP and dTTP, 2.5 µl 10 x Supertaq buffer (100 mM Tris-HCL, pH 9.0; 500 mM KCl; 0.1 % (w/v) gelatin; 15 mM MgCl; 1 % Triton X-100), 0.4 unit Supertaq Polymerase. Samples were overlaid with mineral oil to prevent evaporation. Several 10-mer primers were used and PCR amplification was performed in a Hybaid DNA thermal cycler in microtiter plates. Amplification products were separated by gel electrophoresis using 1.5 % agarose gel with TAE buffer and stained with ethidium bromide.

Resistance tests

The level of resistance to the root-knot nematodes *M. chitwoodi* and *M. fallax* was investigated for parent genotypes, putative hybrid- and backcross genotypes in various glasshouse experiments. Inheritance studies had revealed the likely presence of single dominant resistance genes in *S. fendleri* and *S. hougasii*, whereas the resistance of *S. stoloniferum* seemed to be based on several additive genes (Janssen *et al.* 1977*a*). The genotypes used of *S. fendleri* and *S. hougasii* appeared to be homozygous for the resistance. Genotypes were tested in 4 replications per nematode population as described by Janssen *et al.* (1997*b*). In this study, genotypes will be indicated as being resistant if the mean number of egg masses on roots was less than 5 % compared to the susceptible control, potato cv Nicola, and susceptible if roots of genotypes contained more.

Results

The results of the interspecific crosses are summarised in Table 6.2. The crosses of diploid *S. tuberosum* with the 1 EBN species *S. bulbocastanum*, *S. cardiophyllum* and *S. brachistotrichum* yielded in total only 2 berries, which were used for seed culture. Few seeds could be dissected from the berries and no plants emerged from these seeds during six months of culture (Table 6.3).

Most efforts were put in crossing 2 and 4 EBN *Solanum* species with *S. tuberosum*. Intra-EBN crosses were partially successful, but large differences were observed between reciprocal crosses indicating unilateral incompatibility (Table 6.2). The inter-EBN crosses often resulted in seedless berries, but some crosses produced few plump seeds in mature berries, which led to viable putative hybrid plants. There were indications that the number of seeds per cross and the success of a cross differed between the *S. tuberosum* genotypes used. In general, the best results were obtained with the cv Bildtstar. The use of the higher EBN parent as female seemed to give better results compared to the reciprocal cross, as indicated by Hermsen (1994). However, numbers of seeds were too low and climatological conditions too variable to analyse these observations statistically.

Also with immature seed culture, plants were derived from different inter-EBN crosses (Table 6.3). In contrast to the berries from the 2 x 1 EBN crosses, many developing seeds could be extracted from the berries of the 4 x 2 EBN and reciprocal crosses; the percentages of germinated seeds varied from 0 to 3.5 %. For comparison, seed culture was also applied to one intra-EBN combination, from which 13 % germinating seeds were obtained.

Table 6.2. Berry set and production of mature plump seeds of reciprocal crosses of wild *Solanum* species with *S. tuberosum*.

cross	EBN	ploidy	# pollinations	# berries	# mature seeds	# seeds/berry
blb x tbr2	1 x 2	2x x 2x	54	0	--	--
reciprocal	2 x 1	2x x 2x	25	0	--	--
cph x tbr2	1 x 2	2x x 2x	45	0	--	--
reciprocal	2 x 1	2x x 2x	41	1 (1[a])	--	--
bst x tbr2	1 x 2	2x x 2x	36	0	--	--
reciprocal	2 x 1	2x x 2x	31	1 (1)	--	--
sto x tbr2	2 x 2	4x x 2x	95	32 (1)	200	6.5
reciprocal	2 x 2	2x x 4x	39	7	0	0
sto x tbr4	2 x 4	4x x 4x	322	160 (8)	0	0
reciprocal	4 x 2	4x x 4x	76	36 (5)	30	1.0
fen x tbr2	2 x 2	4x x 2x	108	23	13	0.6
reciprocal	2 x 2	2x x 4x	47	15	620	41.3
fen x tbr4	2 x 4	4x x 4x	199	37 (8)	4	0.1
reciprocal	4 x 2	4x x 4x	116	4	1	0.3
hou x tbr2	4 x 2	6x x 2x	121	21 (3)	1	0.1
reciprocal	2 x 4	2x x 6x	65	2	0	0
hou x tbr4	4 x 4	6x x 4x	50	27	25	0.9
reciprocal	4 x 4	4x x 6x	49	0	--	--

[a]) Number of berries used for in vitro seed culture (see Table 6.3).

A number of putative hybrid genotypes was selected for verification of the hybrid nature and the presence of resistance (Table 6.4). The RAPD analyses showed the presence of unique bands from both parents in all genotypes except M94-135-1. This genotype showed only bands from the female parent (*S. stoloniferum*) after analysis of six primers and has probably arisen from accidental self-pollination or asexual hybridisation of maternal cells. An example of RAPD profiles of parents and their F_1 and BC_1-hybrids is shown in Figure 6.1. The ploidy analyses revealed that most hybrid genotypes had the expected ploidy level (examples in Figure 6.2A-B). The hybrids M94-126-1 and M94-126-2, obtained from crosses of *S. fendleri* with tetraploid *S. tuberosum*, were hexaploid, probably as a result of 2n eggs of *S. fendleri* which would have resolved the inter-EBN difference. The resistance tests showed that most hybrids were resistant to both *M. chitwoodi* and *M. fallax*. Only two of the hybrids with *S. stoloniferum* were susceptible.

Table 6.3. Germination and plant formation from immature seeds with in vitro culture.

From cross	EBN	# berries used	# imm. seeds plated	# plants obtained	% germination
tbr2 x cph	2 x 1	1	1	0	0
tbr2 x bst	2 x 1	1	3	0	0
sto x tbr2	2 x 2	1	68	9	13.2
sto x tbr4	2 x 4	8	329	1	0.3
tbr4 x sto	4 x 2	5	327	5	1.5
fen x tbr4	2 x 4	8	276	0	0
hou x tbr2	4 x 2	3	113	4	3.5

Backcrosses were made reciprocally between the true hybrids and *S. tuberosum*. Most seeds were obtained from the backcrosses of the hexaploid *S. fendleri*/ *S. tuberosum* hybrids with tetraploid *S. tuberosum* (Table 6.5). The backcrosses of the pentaploid hybrids from *S. hougasii* were successful only with the hybrid as female parent. Of the backcrosses with the inter-EBN hybrid genotypes, the crosses with the tetraploid hybrids from *S. fendleri* gave most seeds, but a few seeds were also produced in the backcrosses of the tetraploid hybrids from *S. stoloniferum* and *S. hougasii*. Again, best results were obtained using the hybrid as female. In the case of the tetraploid and pentaploid hybrids, pollen showed poor staining after treating with lactophenol-acid fuchsine indicating low male fertility.

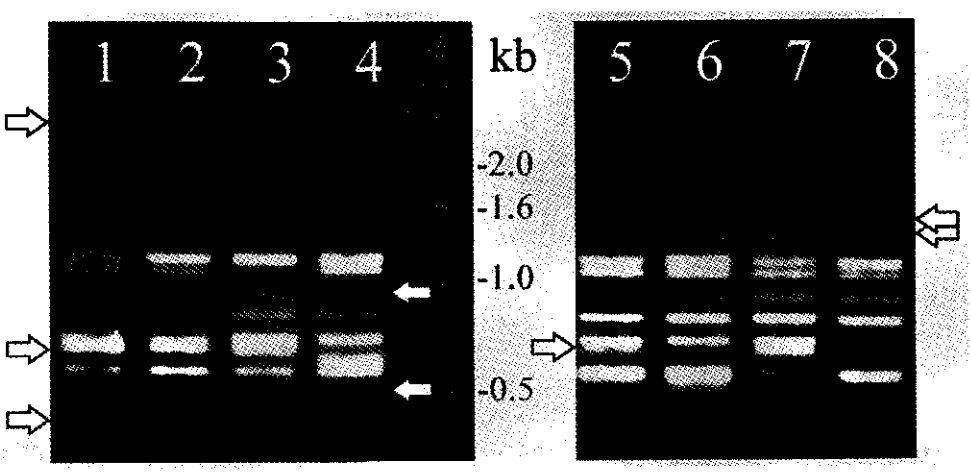


Figure 6.1. RAPD profiles of parents and interspecific hybrid- and backcrossed genotypes of *S. hougasii* and *S. tuberosum*, generated by Operon primer Y-13. Genotypes are 1. hou (female parent), 2. M94-111-3 (F_1), 3. M94-111-4 (F_1 and female parent of backcross), 4. tbr2 (male parent), 5. M95-203-2 (BC_1), 6. M95-203-3 (BC_1), 7. M95-203-4 (BC_1) and 8. tbr4 (male parent of backcross). Arrows indicate unique parental bands.

Table 6.4. Ploidy level and verification of hybrid nature of selected putative hybrid genotypes and the presence of root-knot nematode resistance.

	cross		ploidy [a]		hybrid?[b]	resistance
Genotype	parents	EBN	parents	F1-hybrid		
M94-133-1	tbr4 x sto	4 x 2	4x, 4x	4x	yes	resistant
M94-133-3	tbr4 x sto	4 x 2	4x, 4x	4x	yes	susceptible
M94-134-1	sto x tbr2	2 x 2	4x, 2x	3x	yes	susceptible
M94-135-1[c]	sto x tbr4	2 x 4	4x, 4x	4x	no	resistant
M94-124-1	tbr2 x fen	2 x 2	2x, 4x	3x	yes	resistant
M94-125-1	tbr4 x fen	4 x 2	4x, 4x	4x	yes	resistant
M94-126-1	fen x tbr4	2 x 4	4x, 4x	6x	yes	resistant
M94-126-2	fen x tbr4	2 x 4	4x,,4x	6x	yes	resistant
M94-127-1	fen x tbr2	2 x 4	4x, 2x	3x	yes	resistant
M94-144-1	fen x tbr4	2 x 4	4x, 4x	4x	yes	resistant
M94-109-1	hou x tbr4	4 x 4	6x, 4x	5x	yes	resistant
M94-110-2	hou x tbr4	4 x 4	6x, 4x	5x	yes	resistant
M94-111-3[c]	hou x tbr2	4 x 2	6x, 2x	4x	yes	resistant
M94-111-4[c]	hou x tbr2	4 x 2	6x, 2x	4x	yes	resistant

[a]) Analysed with a flow cytometer.
[b]) Hybrid nature was verified if unique RAPD bands from both parents were observed in the hybrid.
[c]) Obtained via in vitro seed culture.

Table 6.5. Berry set and production of mature plump seeds in backcrosses of interspecific hybrid genotypes with tetraploid *S. tuberosum*.

Original wild species used	hybrid used as parent	ploidy of backcross	# pollinat.	# berries	# seeds	# seeds/berry
S. stoloniferum	M94-133-1	4x x 4x	5	4	4	1.0
	reciprocal	4x x 4x	12	0	--	--
S. fendleri	M94-125-1	4x x 4x	38	9	63	7.0
	reciprocal	4x x 4x	9	0	--	--
S. fendleri	M94-126-1	6x x 4x	47	16	720	45.0
	reciprocal	4x x 6x	7	7	750	107.1
S. fendleri	M94-126-2	6x x 4x	26	11	500	45.5
	reciprocal	4x x 6x	10	9	450	50.0
S. fendleri	M94-144-1	4x x 4x	40	20	65	3.3
	reciprocal	4x x 4x	30	1	16	16.0
S. hougasii	M94-109-1	5x x 4x	35	6	46	7.7
	reciprocal	4x x 5x	4	0	--	--
S. hougasii	M94-110-2	5x x 4x	56	13	74	5.7
	reciprocal	4x x 5x	16	0	--	--
S. hougasii	M94-111-3	4x x 4x	33	19	9	0.5
	reciprocal	4x x 4x	40	0	--	--
S. hougasii	M94-111-4	4x x 4x	65	11	9	0.8
	reciprocal	4x x 4x	43	0	--	--

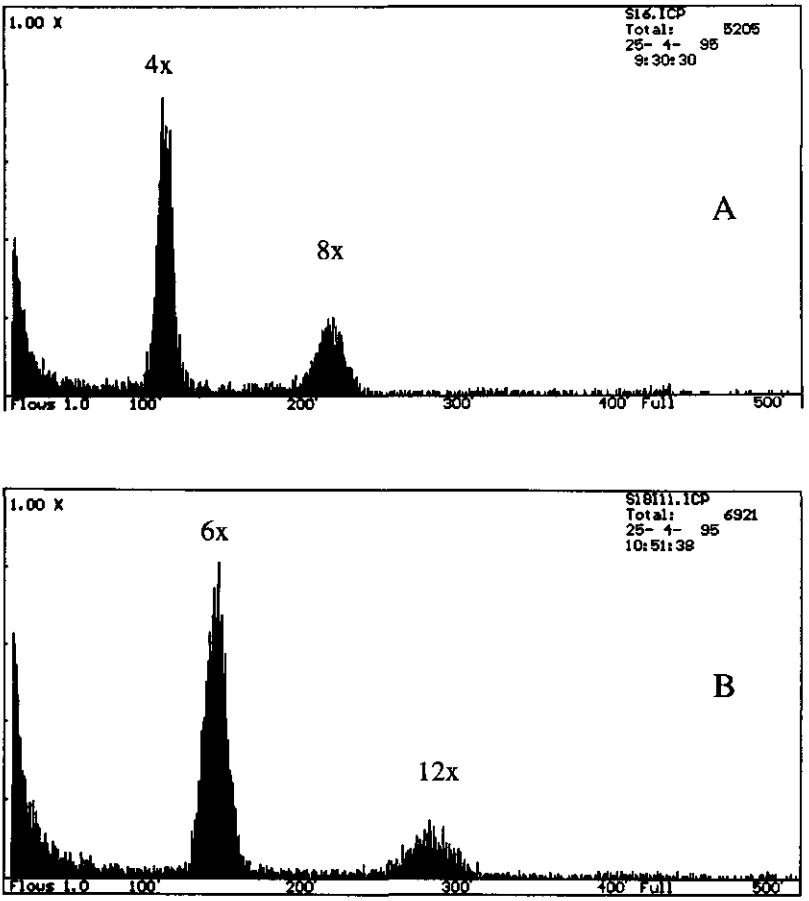


Figure 6.2A-B. Examples of profiles of DNA concentrations of hybrid genotypes, analysed with a flow cytometer. A. M94-111-3 (tetraploid) and B. M94-126-1 (hexaploid).

The hybrid status of two to three genotypes per backcross population was confirmed using RAPD's (Table 6.6). All genotypes of the two populations M95-206 and M95-207, which were tested for resistance, appeared to be resistant. This is in line with the expectation, that the F_1-hybrids M94-126-1 and M94-126-2 had arisen from a 2n gamete of *S. fendleri*. In that case one complete genome would still remain in all pentaploid genotypes leading to resistance. The other populations segregated into resistant and susceptible plants or showed no segregation (M95-203), the latter probably as a result of too few genotypes being available of this BC population.

Table 6.6. Ploidy level and verification of hybrid nature of some genotypes of various backcross populations and the presence of root-knot nematode resistance. (In brackets are the number of individuals tested).

Original wild species used	Population	cross parents	ploidy parents	ploidy BC hybrid	hybrid?	resistance
S. stoloniferum	M95-214	M94-133-1 x tbr4	4x, 4x	4x [3]	yes [2]	segregating [1 res., 2 susc.]
S. fendleri	M95-206	M94-126-1 x tbr4	6x, 4x	5x [2]	yes [2]	all resistant [9]
S. fendleri	M95-207	M94-126-2 x tbr4	6x, 4x	5x [2]	yes [2]	all resistant [10]
S. fendleri	M95-208	M94-125-1 x tbr4	4x, 4x	4x [5]	yes [2]	segregating [6 res., 7 susc.]
S. fendleri	M95-209	M94-144-1 x tbr4	4x, 4x	4x [4]	yes [2]	segregating [2 res., 6 susc.]
S. fendleri	M95-211	tbr4 x M94-144-1	4x, 4x	4x [2]	yes [2]	segregating [1 res., 2 susc.]
S. hougasii	M95-201	M94-110-2 x tbr4	5x, 4x	4x to 5x [3]	yes [2]	segregating [5 res., 3 susc.]
S. hougasii	M95-202	M94-109-1 x tbr4	5x, 4x	4x to 5x [5]	yes [2]	segregating [1 res., 5 susc.]
S. hougasii	M95-203	M94-111-4 x tbr4	4x, 4x	4x [4]	yes [3]	all susceptible [3]

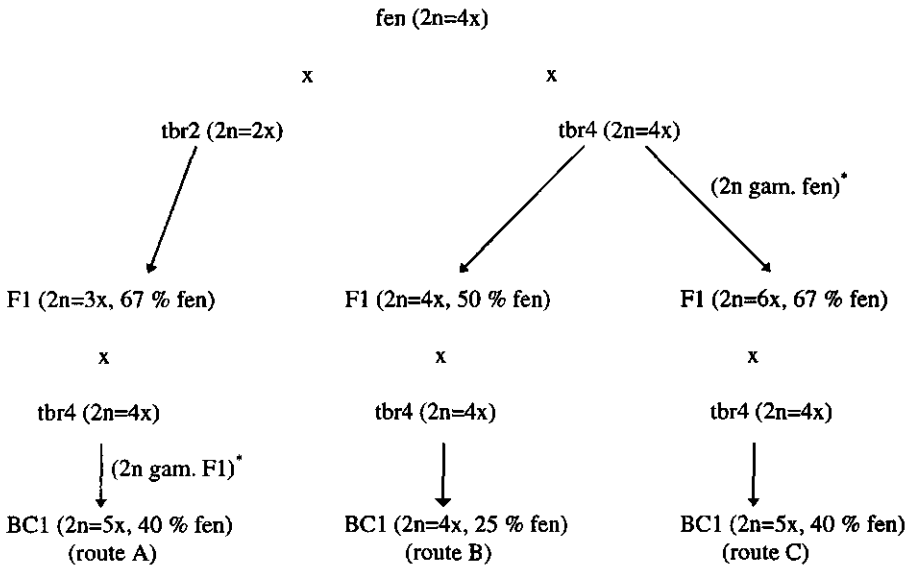
Discussion

The transfer of resistance to root-knot nematodes from *S. fendleri*, *S. stoloniferum* and *S. hougasii* into BC_1 genotypes through sexual crosses has been described. Most attention was paid to the production of true tetraploid hybrids to enhance recombination and promote rapid introgression (Iwanaga *et al.* 1991; Watanabe *et al.* 1992, 1994, Bamberg *et al.* 1994). With regard to the efficiency of introgression, a schematic representation is given in Figure 6.3 of possible routes to introgress traits from the allotetraploid species *S. fendleri* into *S. tuberosum*. The possibility of selecting true tetraploid hybrids and backcrosses (route B) enables a rapid introgression of traits, like resistance, without the dependence of 2n gametes or special treatments to double the genome to continue introgression. Similar schemes of the alternative routes A and C have been described earlier by Van Soest (1985), Adiwilaga & Brown (1991) and Watanabe *et al.* (1992). Route B, however, has distinct advantages. Besides an expected lower percentage of the wild genome in the F_1 and BC_1 than along the alternative routes, the desired tetraploid level is automatically reached regardless of negative selection of wild characteristics.

Whether recombination is truly enhanced depends on the pairing ability of the homoeologous chromosomes and formation of chiasmata leading to crossing-overs. A reduced homoeologous pairing of chromosomes in wide crosses can be expected due to structural chromosome differences, but this will depend on the relatedness *i.e.* genome constitutions of species (Matsubayashi 1991; Hermsen 1994). In general, recombination seems to occur in wide crosses between *Solanum* species. Cytological observations of tetraploid hybrids of hexaploid *S. demissum* with diploid *S. tuberosum* or *S. verrucosum* showed regular bivalent chromosome pairing between the homoeologous genomes (Ramanna & Hermsen 1979), whereas in tetraploid hybrids of *S. acaule* with *S. tuberosum* a high frequency of bivalents but also several univalents were observed (Watanabe *et al.* 1994). In diploid hybrids of *S. verrucosum* and *S. bulbocastanum* normal bivalent formation was found (Hermsen & Ramanna 1976), but a high univalent frequency in the diploid sexual hybrid of *S. circaeifolium* with *S. tuberosum* (Louwes *et al.* 1992). Also sexually obtained diploid hybrids of *S. brevidens* with *S. tuberosum* showed some meiotic aberrations, but chromosome associations suggested the likely occurrence of recombination between the genomes of non-tuberous and tuber-bearing species (Watanabe *et al.* 1995). It is expected that the chiasmata frequency will be lower in these wide interspecific crosses, but the advantage of at least some recombination at an early stage of introgression remains worthwhile.

Most successful inter-EBN crosses appear to have been the result of embryo-rescue or rescue-pollinations or a combination of these techniques (Iwanaga *et al.* 1991;

Figure 6.3. Efficiency of introgression of traits from *Solanum fendleri* into *S. tuberosum* via different routes.



*) In stead of 2n gametes, the doubling of the parent genotype through colchicine treatment would give a similar effect.

Singsit & Hanneman 1991; Watanabe *et al.* 1992, 1995). In the crossing experiments reported here, true tetraploid hybrids were obtained from crosses of *S. tuberosum* with *S. stoloniferum* and *S. fendleri* following normal crossing procedures. Tetraploid hybrids of *S. tuberosum* with *S. hougasii* were obtained after in vitro seed culture. One seed was derived from a mature berry but failed to germinate. Nevertheless, this might indicate that more crosses possibly would have yielded hybrid plants without having to resort immature seed culture. Such seed culture is a simplification of the embryo-culture technique, which has already proven to be powerful in overcoming crossing barriers (Hermsen 1994). By using embryo-rescue, hybrids have been obtained between the hexaploid weed black nightshade, *Solanum nigrum*, and potato cv Desiree (Eijlander & Stiekema 1994) and even sexual crosses between genera might be possible (Watanabe *et al.* 1995). In vitro seed culture is to be preferred in interspecific hybridisations, which are expected to be little successful but not impossible through sexual crosses, such as the cross combinations described in this study.

The EBN hypothesis is based on the (lack of) endosperm development in developing seeds (Johnston *et al.* 1980). However, there are more factors involved which determine the actual success of crosses and it is not an absolute rule. Since in some inter-EBN combinations 1 or 2 seeds per berry can be found, an arbitrary

criterion of 10 plump seeds per fruit has been used to determine the success of a cross and to assign an EBN value (Johnston & Hanneman 1980; Hanneman 1994). Without violating the theory on which the EBN hypothesis is based, it can be stated that EBN is not a major limiting factor for potato breeding, since a few hybrids are sufficient as a first step in gene transfer.

In the present study no hybrids were obtained from crosses between Central American 1EBN species and *S. tuberosum*. The low number of developing seeds present in the two berries, which could be extracted for in vitro culture, indicated that the incompatibility was the result of pre- rather than postzygotic barriers. Novy and Hanneman (1991) also reported lack of successful hybridisation of Central American 1 EBN species with *S. tuberosum*, despite the selection for 2n pollen or the use of genome doubled genotypes. The incompatibility was mainly based on a strong inhibition of pollen tube growth. In contrast, successful hybridisations have been reported with South American 1EBN species *S. commersonii, S. chancayense and S. circaeifolium* (Novy & Hanneman 1991; Louwes *et al.* 1992). It seems that the results of crosses with South American 1EBN species are well predicted with the EBN hypothesis. But the failure of interspecific crosses with Central American 1 EBN species is likely to be dominated by other processes than a genic imbalance of the endosperm, such as inhibition of pollen tube growth (Hermsen & Ramanna 1976; Fritz & Hanneman 1989; Novy & Hanneman 1991), somatoplastic and cytoplasmic-genic male sterility and structural genome differences (Hermsen & Ramanna 1976) and therefore can not be explained with the EBN hypothesis.

The hybrid status of genotypes and the process involved was analysed using the results from RAPD and ploidy analyses. With RAPD markers, one non-hybrid genotype was noticed and this actually demonstrated the value of this method. Based on morphological evaluation alone this genotype had not been excluded. Also in other research the RAPD technique has proven its potential ability as a tool to identify interspecific *Solanum* hybrids (Xu *et al.* 1993; Masuelli *et al.* 1995). With a flow cytometer different ploidy levels can be detected, but this technique can not reveal small deviations from the euploid chromosome number. Although the surplus or deficit of a single chromosome is not unlikely, the majority of tetraploid F_1 and BC genotypes are still presumed to have a balanced euploid number of 48 chromosomes.

The introgression of resistance to root-knot nematodes from *S. fendleri, S. stoloniferum* and *S. hougasii* has advanced to resistant BC_1 genotypes for further backcrosses. At this stage other agronomically important traits also need to be selected for at the cost of the majority of the traits from the wild species. The introgression strategy using true tetraploid hybrids (Figure 6.3, route B) is simple and efficient and should provide valuable breeding materials for a new generation of potato cultivars. In combination with marker-assisted selection, true tetraploid hybrids should even make a more efficient selection towards the wild *Solanum* genome possible.

Chapter 7

General discussion.

In this thesis, an evaluation of resistance to the root-knot nematodes *Meloidogyne chitwoodi*, *M. fallax* and *M. hapla* and the initial introgression into cultivated potato by interspecific hybridisation are described. This chapter will discuss the results in relation to durable nematode resistance and to future resistance breeding. The knowledge with regard to the durability and management of resistance to nematodes will be outlined, followed by an analysis of the resistance in wild *Solanum* spp. and the possibilities of co-evolution with root-knot nematodes. Lastly, some future perspectives of resistance research and -breeding will be discussed.

Durability of resistance

An important feature of resistance is the effectivity under natural cropping circumstances for numerous years, *i.e.* resistance needs to be durable. An example of durable resistance is the resistant *Prunus* rootstock cv Nemaguard, which bears simply inherited resistance to *M. incognita* and *M. javanica* (Sharpe *et al.* 1969). This rootstock has been used in commercial orchards, including peaches, almonds and apricots, and resistance has retained its effectivity despite an extensive use and hence continuous selection pressure on the nematodes for more than 35 years (Cook & Evans 1987; Robert 1992). Nevertheless, some virulent populations towards the resistance have been observed (*e.g.* Sharpe *et al.* 1969; Esmenjaud *et al.* 1996).

There are several processes, which can make nematode resistance ineffective: 1) the pathogen overcomes the resistance by mutations or other genetical adjustments, which influence the virulence pattern, 2) the nematode population is genetically diverse and already bears a small percentage of virulent genotypes, and 3) the resistance is not effective towards related pathogens, such as other species. All these processes lead to the ineffectivity of resistance as a direct result of selection pressure. Examples of each process are: 1) the artificial selection of virulent *Meloidogyne* genotypes, which reproduce through mitotic parthenogenesis, towards Mi-gene resistance (*e.g.* Riggs & Winstead 1959), 2) the gradual selection of virulent *Heterodera avenae* populations towards resistant cereals (Laserre *et al.* 1996) and 3) the present predominance in potato fields of *Globodera pallida* populations as a result of cultivating *G. rostochiensis* resistant potato cultivars (Evans 1993; Mulder 1994). The occurrence of and selection for virulence and management of resistance to prevent selection of virulence will be discussed.

Occurrence of and selection for virulence

Most information on virulence in *Meloidogyne* spp. is known with regard to the resistance in tomato based on the Mi gene, originally derived from *Lycopersicon peruvianum*. The resistance is based on a single dominant gene on chromosome 6 (Gilbert & McGuire 1956) and has been frequently bred into tomato cultivars since 1950 (Roberts & Thomason 1989). It is effective against *M. incognita*, *M. arenaria* and *M. javanica*, but not against *M. hapla* (*e.g.* Fassuliotis 1979). Since the end of 1950's, the occurrence of populations able to overcome the Mi-resistance was noticed (Sauer & Giles 1959) and these populations were hereafter designated as 'B-races' (Riggs & Whitehead 1959; Netscher 1977), not to be confused with the cytological polyploid race B of *M. hapla*. But besides the selection of virulence under cropping circumstances, naturally resistance-breaking *Meloidogyne* populations have been found, that were not previously exposed to resistant cultivars (Netscher 1977, 1978; Prot 1984; Fargette 1987; Roberts & Thomason 1989; Berthou *et al.* 1989; Fargette & Braaksma 1990).

Virulent populations of *M. incognita*, *M. arenaria* and *M. javanica* towards the Mi-resistance have also been obtained under laboratory conditions. Selection experiments showed a progressive increase in the proportion of virulent nematodes after each successive generation (Netscher 1977; Triantaphyllou 1987; Jarquin-Barberena *et al.* 1991; Castagnone-Sereno *et al.* 1994*b*), although the reproduction of naturally virulent populations on resistant cultivars remained superior over that of the laboratory selected populations (Jarquin-Barberena *et al.* 1991; Castagnone-Sereno *et al.* 1994*b*). The selected virulence remained present up to at least 18 generations under non-selective conditions indicating a stable genetic constitution (Netscher 1977; Jarquin-Barberena *et al.* 1991; Castagnone-Sereno *et al.* 1993, 1994*c*). Since *M. incognita*, *M. arenaria* and *M. javanica* are obligatory mitotically parthenogenetic species, other mechanisms than genetic recombination need to be responsible for the virulence. In theory, each step of increased virulence might have been the result of small-effect mutations with an unusual high frequency of 10^{-3} (Triantaphyllou 1987). Jarquin-Barberena *et al.* (1991) postulated a gene amplification system resulting in a highly progressive increase of virulence. Comparison of protein patterns of avirulent and selected virulent lines of *M. incognita* revealed a protein present in a smaller amount in the virulent lines, which might be involved in the recognition process of the plant-nematode interaction (Dalmasso *et al.* 1991).

Both the selected and naturally occurring Mi-virulent populations of *M. incognita* were unable to break through resistance in pepper (Castagnone-Sereno *et al.* 1992) and following experiments confirmed the specificity of Mi-virulent and Me3-virulent *M. incognita* populations on resistant tomato and pepper respectively (Castagnone-sereno *et al.* 1996). However, Roberts *et al.* (1990) demonstrated that naturally

virulent populations on Mi-resistance were able to break through other *M. incognita* resistance genes of *L. peruvianum*, whereas the laboratory selected Mi-resistance-breaking populations could not circumvent these other resistance genes.

It has been shown that the Mi-gene resistance breaks down at high temperatures (*e.g.* Dropkin 1969) and this phenomenon has been observed for other nematode resistance genes as well. Although it is considered as a threat to use this type of resistance, Cook and Evans (1987) postulated that in nature this phenomenon will help to preserve long-term stability of resistance by altering the selection pressure for virulence after the plant has been established and able to endure higher nematode densities.

In Chapter 3, the presence of virulent populations of *M. hapla* towards resistant genotypes of various wild *Solanum* species was shown. Differences in virulence between *M. hapla* populations have also been observed on resistant lucerne (Goplen *et al.* 1959; Griffin & McKenry 1989). The differences within *M. chitwoodi* were less explicit in the experiment described in Chapter 3, but virulent populations towards resistant *S. bulbocastanum* genotypes have been noticed (Mojtahedi & Santo 1994; Van der Beek, IPO-DLO Wageningen and GJW Janssen unpubl. results). Preliminary results indicated that in a population of *M. chitwoodi* a very low percentage of juveniles (0.02 %) was able to reproduce on resistant *S. fendleri* genotypes (unpublished results). It remains to be investigated in the near future whether this phenomenon is genetically based or not.

Management of nematode resistance

The observed presence of naturally virulent populations towards resistance genes emphasises the need for a cautious use of resistance in order to avoid a high selection pressure towards virulence and to preserve the effectivity of resistance. Besides virulence, also the selection of related nematode species, which are not suppressed by the resistance, should be avoided. But, in comparison with resistances to foliar fungi, nematode resistances will in general last longer because nematodes are dispersed more slowly than fungi and they have longer generation times (Cook & Evans 1987). In the absence of virulent genotypes or nematode species, which are not affected, resistance will remain effective for many consecutive years, as exemplified earlier with the *Prunus* rootstock cv Nemaguard. Consequently, the prevention of spread of root-knot nematodes will ultimately lead to lower selection pressure on resistance. A strict quarantine management on farms and plant material will not only be beneficial on short term, but also for the durability of resistance.

For a durable management of resistance, rotation of cultivars with resistance from different sources should be highly recommended to avoid a high selection pressure. In this perspective, resistance genes with a limited working spectrum or incomplete

resistance, as described in resistant *Solanum* sources to *M. hapla* populations in Chapter 3, can be useful and durable as well. When resistance genes with a restricted working spectrum have been implemented into new potato cultivars, it will be necessary to have a reliable diagnostic essay to recognise nematode species and virulence group(-s) for advisory work.

At present, resistance genes available are limited in most agricultural crops. At the end of the *Meloidogyne* resistance project, of which results are described in this thesis, potato progenitors with high levels of resistance from different *Solanum* species have been released to the Dutch potato breeding companies united in the 'Stichting Stimulering Aardappelonderzoek'. Still, it will probably take at least another 10 years before the first *Meloidogyne* resistant potato cultivars will become available for farmers. Therefore, other cultural practices, like crop rotation and fallow, will remain useful, also after the release of resistant cultivars. In intensive crop practises, even the use of nematicides might still be advised. In the case of infestation with the potato cyst nematode *G. pallida*, an integrated control system using resistant cultivars, nematicides and crop rotation is recommended (pers. comm. K. Evans, Rothamsted UK). Only with the availability of more resistant potato cultivars, which are based on different sources of resistance, and a diagnostic approach of the soil infestation, a cultivar rotation strategy as described above can be performed and totally abandon the use of nematicide treatments in the future.

Presence of resistance genes in *Solanum* spp.

The results from the screening trials, described in Chapter 2, clearly indicated that high levels of resistance to *M. chitwoodi* and *M. fallax* were merely detected in Central American *Solanum* spp., whereas resistance to *M. hapla* was found in species from both Central and South America. Interestingly, the resistance to *M. chitwoodi* and *M. fallax* was present in the primitive diploid as well as in the advanced polyploid Central American *Solanum* species. As already described in Chapter 1, the diploid Central American *Solanum* species from series *Bulbocastana* and *Pinnatisecta* are considered as primitive species, distinct from both the polyploid Central American species and all South American species. The Central American polyploid species from series *Demissa* and *Longipedicellata* are thought to have evolved millions of years later from amphidiploidisations of primitive Mexican ancestors with more advanced South American species (Hosaka *et al.* 1984; Hawkes 1990; Matsubayashi 1991).

The question arises why resistance to *M. chitwoodi* and *M. fallax* is present in both primitive and advanced Central American *Solanum* species, but lacking in the South American species. The occurrence of spontaneous interspecific hybridisations may have been responsible for gene flow between certain South American species (Hawkes

1962, 1990), but the primitive Central American species have strong sexual barriers to hybridise under natural circumstances with other *Solanum* species (Hawkes 1990; Matsubayashi 1991) making a recent introgression of resistance genes unlikely.

A more suitable hypothesis would be that resistance genes are genetically highly conserved and have been present in the wild ancestors of the current *Solanum* gene pool. In *S. stoloniferum* and *S. demissum* similar R-genes to *Phytophthora infestans* have been found and the occurrence of these genes in two species was also thought to be explained by a common ancestry (McKee 1962). More evidence for this hypothesis would be obtained if resistance genes of the different diploid and polyploid *Solanum* species would have large molecular homology in their genetic constitution. If resistance would have evolved more recently and independently in the primitive and advanced *Solanum* species this would lead to different genetic compositions and to different resistance mechanisms. This remains to be investigated.

But regardless which hypothesis approaches reality most, a continuous selection pressure towards resistance in Central America explains the lack of resistance in South American *Solanum* species and the necessity to maintain or develop resistance. In Mexico, *M. chitwoodi* appears to be a major economic pest on several crops including potato (Eisenback & Triantaphyllou 1991). An extensive survey in the state of Tlaxcala revealed the presence of 225 *Meloidogyne* infested lands, predominantly race 2 of *M. chitwoodi* (Cuevas 1995). Although further information about the spread and genetic diversity of *Meloidogyne* species in other states of Mexico is lacking, the possibility of *M. chitwoodi* being endemic in Mexico seems therefore likely.

Co-evolution of resistance and virulence?

If *M. chitwoodi* is an endemic pest in Mexico, the presence of resistance in Central American *Solanum* species may have been the result of co-evolution of plant and nematode species. Stone (1985) defined three conditions for co-evolution of resistance in plants and virulence in plant-parasitic nematodes: 1) resistance is confined to a highly specialised nematode species, 2) pathotypes, races or similar divisions within a species are demonstrated, and 3) the origin of wild hosts with resistance resembles the natural distribution of the pathogen. The only generally accepted case of co-evolution of plants with plant-parasitic nematodes is that of *Solanum* spp. with *Globodera* spp. (Stone 1979, 1985). Potato cyst nematodes are highly specialised to Solanaceous species, pathotypes are differentiated on their reproductive behaviour on a set of resistant genotypes (*e.g.* Kort *et al.* 1977) and both resistant *Solanum* spp. and *Globodera* spp. have an indigenous distribution in the highlands of Peru, Bolivia and Argentina. Another convincing evidence of co-evolution is the existence of a gene-for-gene relationship for the H_1 resistance gene from *S. tuberosum* ssp. *andigena* and the avirulence gene of *G. rostochiensis* Ro_1 (Janssen *et al.* 1991). A second gene-for-gene

interaction has been suggested for the H_2 resistance gene from *S. multidissectum* and the avirulence gene in *G. pallida* (Parrot 1981).

Taking into account the above mentioned conditions, *M. chitwoodi* does not completely fit the first condition. *Meloidogyne* spp. are considered as highly developed pathogens, able to modify host tissue to form complex feeding sites. However, *Meloidogyne* spp. - and *M. chitwoodi* in particular - have maintained an extensive host range. This would be in contradiction with the hypothesis that the evolutionary process of co-evolution of resistance and virulence is costly and leads to reciprocal adaptation and/or speciation of the pathogen (Thompson & Burdon 1992; Simms 1996). On the other hand, resistance of *S. bulbocastanum*, *S. hougasii*, *S. brachistotrichum*, *S. cardiophyllum* and *S. fendleri* to *M. chitwoodi* was found to be effective to the closely related *M. fallax*, but not to *M. incognita*, *M. arenaria* and *M. javanica* (Chapter 3), indicating a limited effectivity towards *Meloidogyne* spp.

With regard to the second condition, a virulent population of *M. chitwoodi* on resistant *S. bulbocastanum* has been found in California (Mojtahedi & Santo 1994). The occurrence of virulent genotypes of *M. chitwoodi* has also been noticed in the Netherlands (Van der Beek, IPO-DLO Wageningen and GJW Janssen unpubl. results). More differentiating results on *S. bulbocastanum* genotypes have been observed between *M. chitwoodi* populations, but these differences were considered to be specific for race 1 and 2 of *M. chitwoodi* (Brown *et al.* 1991), although only one population was used for each race.

To fulfil the third condition on the overlapping natural distributions of host and pathogen, it will be necessary to monitor the presence and genetic diversity of *M. chitwoodi* and related species in Central America, as has been performed in the state of Tlaxcala of Mexico (Cuevas 1995). Indirect evidence would be the presence of resistance to *M. chitwoodi* in other plant species, originating from Central America like *Zea mays* and *Phaseolus* spp. (Smartt & Simmonds 1995). Both gene pools might also provide better insight in the relationship of *M. chitwoodi* and *M. fallax*, since - accidentally or not - maize and beans are one of the few crop plants known to show differential responses towards these nematode species (Van Meggelen *et al.* 1994).

The best evidence of co-evolution would be revealed by the presence of a gene-for-gene interaction. The presence of single dominant resistance genes has been demonstrated in various *Solanum* spp. (Chapter 5; Brown *et al.* 1996). However, research on the genetic analysis of (a-)virulence is hindered by the mode of reproduction of *M. chitwoodi* through facultative meiotic parthenogenesis (Triantaphyllou 1985). The use of DNA fingerprint techniques, such as AFLP's, should enable to characterise individual genotypes and clarify the mode of reproduction from progeny tests, making a genetic analysis of (a-)virulence possible.

Future perspectives of resistance to nematodes

Genetically engineered resistance

Biotechnology offers alternative possibilities to introduce resistance than by introgression of resistance genes from genetic resources. One approach is to clone known resistance genes and to introduce them in other genotypes or other crop plants by transformation. Several resistance genes have been successfully cloned (reviewed by Bent 1996), including - very recently - the first nematode resistance gene to *Heterodera schachtii*, originating from the wild beet, *Beta procumbens* (Cai *et al.* 1997). Some research groups have tried to clone the Mi-gene in tomato (*e.g.* Aarts *et al.* 1991; Ho *et al.* 1992), but as far as known up till now without success (Williamson & Hussey 1996). Due to a highly reduced level of recombination in the introgressed region from wild *L. peruvianum* with the Mi gene (Messeguer *et al.* 1991; Ho *et al.* 1992), chromosome walking from nearby located markers is hampered. Besides problems related to cloning resistance genes and possible difficulties in the expression of genes inside alien plant genomes (Williamson & Hussey 1996), a major disadvantage of this strategy is that it will lead to an over-exploitation of the few known and cloned nematode resistance genes and probably to higher selection pressure on resistance-breaking nematode populations, as discussed before. Cloning of resistance genes is also being performed to gain better insight into the mechanism of the plant-pathogen interaction. In addition, new resistance genes may be identified by searching homologous sequences in plant genomes, since several plant disease resistance genes cloned so far appear to contain leucine-rich repeats and a nucleotide binding site. Eventually, new resistance genes may be constructed and cloned resistance genes improved once the structure and function of components of these genes have been studied intensively and fully understood (Bent 1996).

Another approach to induce resistance to nematodes is the interference with the initiation and/or development of giant cells. A basic requirement for this strategy is to identify genes which are induced solely in the invaded cells during the first hours after penetration of the nematode, and some highly specific genes have already been reported (Williamson *et al.* 1993; Opperman *et al.* 1994; Van der Eycken *et al.* 1996). The promotors of these genes are ideal for the construction of plant cell suicidal genes, *e.g.* genes with RNAse expression or genes which attenuate high metabolic activities after upregulation. Anti-nematode genes, such as those encoding for collagenases, proteinase-inhibitor proteins or toxins are also suitable as defence systems. Combined with a constitutive promotor, these anti-nematode genes may offer a broad defence mechanism against many nematode species (Sijmons *et al.* 1994; Atkinson *et al.* 1995). A related strategy is to generate plants that express a specific pathogen avirulence gene and a resistance gene under the control of a non-specific pathogen-

inducible promotor. In theory, this so-called 'two-component sensor system' will be effective against any pathogen - nematodes, fungi, viruses and bacteria -, which induces expression of the chosen promotor (De Wit 1992).

A third approach of novel resistance is by transforming plants with genes encoding monoclonal antibodies, so-called 'plantibodies', against nematode specific proteins (Schots *et al.* 1992). The binding of antibodies to these proteins can inhibit their function and thus disturb the host-pathogen interaction, as shown for artichoke mottled crincle virus in *Nicotiana benthamiana* (Tavladoraki *et al.* 1993). Monoclonal antibodies have been raised to secretions from oesophageal glands of *Meloidogyne* spp. (Hussey 1989*b*) and *Globodera* spp. (De Boer *et al.* 1996). Genes encoding (parts of) antibodies against the latter species have been transferred into potato and, at present, transgenic plants are tested under laboratory conditions to evaluate whether this strategy will result in useful levels of resistance or not (F. Gommers, Dept. Nematology WAU the Netherlands).

The described approaches of genetically engineered resistance show great potential in the near future, but their effectivity and stability still needs to be proven under practical cropping circumstances. Moreover, a successful introduction and establishment of these types of resistance in crop cultivars will depend on the acceptation of extra costs for seed material as a result of patents on techniques and genes. The benefits of genetically engineered resistance will therefore be highest, when the alternatives such as crop cultivars with natural resistance genes or alternative cultural practices are not available or economically interesting.

Improvement of resistance breeding using natural resources

Several wild *Solanum* spp. have already been used for the introgression of traits into cultivated potato, but the genetic base of potato cultivars is still rather limited and needs a permanent broadening (Ross 1986; Hawkes 1990). More knowledge from evaluation studies of wild species and the availability of new techniques to enhance the efficiency of potato breeding has led to changing concepts in breeding and probably better use of genetic resources. As an example, in the 1940's Ellenby found resistance to potato cyst nematodes in diploid wild *Solanum* species, but - according to him - it had a disadvantage in being diploid and wild (Ellenby 1952). Some years later, this wild species *S. vernei* has been used to introgress resistance genes to *G. pallida* and since 1980 cultivars with this resistance have become available in the Netherlands (Anonymous 1980). Moreover, potato breeding via the diploid level has gained better attention, since genes from wild diploid *Solanum* species can be transferred directly into (diploid) *S. tuberosum* and a disomic inheritance simplifies the genetic analysis of traits (Hougas & Peloquin 1958; Ross 1986). Dihaploids can be obtained from tetraploid plants after prickle pollination with haploid inducing *S. phureja* pollinators

and recovery of the tetraploid level by somatic chromosome doubling or sexually via 2n-gametes. The ease to perform these ploidy manipulations has made potato breeding via the diploid level a routine procedure in potato breeding programs, although different selection criteria should be considered at the different ploidy levels (Hutten *et al.* 1996)

The potential of natural genetic resources for potato is still expanding due to new methods to overcome natural hybridisation barriers. The use of embryo-rescue has resulted in hybrids of *S. tuberosum* with non-tuberous species *S. brevidens* (Watanabe *et al.* 1995) and *S. nigrum* (Eijlander & Stiekema 1994) and with somatic hybridisations inter-generic hybrids can be obtained, like hybrids of tomato and potato (Jacobsen *et al.* 1992). Still, the effort needed to introgress traits from donor species is directly correlated with the relatedness to the recipient *S. tuberosum*. Hence, also in the future it will be preferred to initiate the search for desired traits in existing cultivars, followed by primitive cultivated species, wild tuber-bearing *Solanum* spp. and then non-tuberous *Solanum* species and related genera.

In the research described in this thesis, root-knot nematode resistant progenitors were produced using *Solanum* sources, which have hardly been used in breeding programs (Ross 1986). These wild *Solanum* species also bear several other interesting traits like high levels of resistance to late blight, *Phytophthora infestans,* in *S. fendleri* and *S. hougasii* (unpubl. results) and it will be worthwhile to select for multiple traits during the process of introgression. Marker-assisted selection using molecular markers against the genetic background of the donor parent will improve the efficiency of introgression considerably. Additionally, selection of recombinants in the region of the desired traits and adjacent markers will ultimately limit the desired introgressed part (Melchinger 1990). In combination with these techniques and the earlier described breeding concepts, a new generation of potato cultivars with resistance to root-knot nematodes are within reach in the next decade.

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Summary

Root-knot nematodes, *Meloidogyne* spp., are world-wide one of the most damaging pests to arable farming. In North Western Europe, the species *M. chitwoodi*, *M. fallax* and *M. hapla* are becoming a serious problem in potato growing areas as a result of recent changes in crop rotation, that now include highly profitable host crops, and a reduced use of nematicides in potato. The root-knot nematodes can cause yield reduction and deteriorate the quality of the tuber to an unmarketable product. Since root-knot nematodes can multiply inside the tuber, infected potato tubers are a threat to infest *Meloidogyne*-free fields. To prevent this way of dispersal, quarantine measures will be in force for seed tubers in the countries of the European Community from August 1997.

Plant resistance is an effective, economical and environmentally safe alternative to control root-knot nematodes, but resistance is lacking in the presently used potato cultivars. The goal of the research described in this thesis was to identify and evaluate sources of resistance to *M. chitwoodi*, *M. fallax* and *M. hapla* and, if present, to initiate the transfer of resistance into cultivated potato.

A large screening of wild *Solanum* spp. was performed to identify sources of resistance to *M. chitwoodi*, *M. fallax* and/or *M. hapla* by selecting seedlings, which showed no to hardly any reproduction of the nematodes on the roots. High levels of resistance to both *M. chitwoodi* and *M. fallax* were observed in genotypes of *S. bulbocastanum*, *S. cardiophyllum*, *S. brachistotrichum*, *S. fendleri* and *S. hougasii*, whereas additionally moderate resistance to *M. fallax* was present in *S. stoloniferum* and *S. chacoense* and to *M. chitwoodi* in *S. gourlayi*. More *Solanum* species were selected with resistance to *M. hapla*, namely *S. bulbocastanum*, *S. brachistotrichum*, *S. cardiophyllum*, *S. arnezii*, *S. chacoense*, *S. tarijense*, *S. boliviense*, *S. gourlayi*, *S. microdontum*, *S. sparsipilum*, *S spegazzinii*, *S. sucrense*, *S. acaule* and *S. hougasii*. In general, resistance to *M. chitwoodi* and *M. fallax* was restricted to *Solanum* species originating from Central America, whereas resistance to *M. hapla* was present in numerous Central- and South American *Solanum* species. This might indicate that co-evolution has occurred between *M. chitwoodi* and related *M. fallax* and Central American *Solanum* species.

The following step was the evaluation of resistance with regard to the effectivity and working spectrum. In a glasshouse, resistant and susceptible *Solanum* genotypes were tested with two to four populations of *M. chitwoodi*, *M. fallax* and *M. hapla* to determine the level of resistance and to detect the presence of virulent populations within these *Meloidogyne* species. Resistant genotypes of *S. bulbocastanum*, *S. hougasii*, *S. cardiophyllum* and *S. fendleri* showed an almost absolute level of resistance and were able to suppress all populations of *M. chitwoodi* and *M. fallax*

tested. Some genotypes of *S. chacoense* and *S. stoloniferum* showed moderate resistance to *M. fallax*, but not or in a lesser extent to *M. chitwoodi*. In contrast, large differences in virulence were observed between the four tested populations of *M. hapla* on resistant genotypes of *S. bulbocastanum*, *S. hougasii*, *S. chacoense*, *S. gourlayi*, *S. sparsipilum* and *S. spegazzinii*. Some genotypes with resistance to *M. chitwoodi*, *M. fallax* and/or *M. hapla* were also tested against isolates of the tropical and subtropical *Meloidogyne* species *M. incognita*, *M. arenaria* and *M. javanica*, but resistance was not effective to these high temperature adapted species.

The effectivity of resistance of some selected wild *Solanum* species was also evaluated under natural field conditions. In two fields in the Netherlands, naturally infested with *M. hapla* or *M. fallax*, the level of infection of soil surrounding resistant and susceptible genotypes was followed during a growing season. From August onwards, large differences in number of second-stage juveniles were present between resistant and susceptible genotypes. At the end of the growing season, the level of infection in soil of resistant wild *Solanum* genotypes was equal or lower compared to the beginning, whereas soil surrounding susceptible wild and cultivated genotypes showed a 7- to 22-fold increase of nematode infection. The results were comparable with the resistance tests in glasshouse experiments.

An important feature for a rapid introgression of resistance is the inheritance and this has been investigated for the resistance to *M. chitwoodi* and *M. fallax* in *S. fendleri*, *S. hougasii* and *S. stoloniferum*. Although these *Solanum* species are polyploid, a disomic genetic behaviour can be expected as earlier indicated by cytogenetic and genetic studies. Various populations were produced from crosses between resistant and susceptible plants, self-pollinations and backcrosses within the wild *Solanum* species and segregation patterns of progenies in resistant and susceptible plants were analysed. The progeny tests of *S. fendleri* clearly indicated the action of a single dominantly inherited gene, effective against both *M. chitwoodi* and *M. fallax*, and the symbol R_{mc2} is proposed for this gene. In the case of *S. hougasii*, difficulties were met in producing backcross populations, but results also indicated the presence of a simple dominant factor for both nematode species. From the results of progeny tests of *S. stoloniferum*, it was concluded that several additive genes are involved.

The introgression of resistance from various wild Central American *Solanum* species into the cultivated potato has been initiated through interspecific hybridisation. Crosses were made between diploid *S. tuberosum* and diploid *S. bulbocastanum*, *S. brachistotrichum and S. cardiophyllum*, but no plants were obtained from these crosses. From crosses of tetraploid *S. tuberosum* with tetraploid *S. stoloniferum* and *S. fendleri*, and of diploid *S. tuberosum* with hexaploid *S. hougasii* few seeds leading to tetraploid hybrids were obtained, sometimes after in vitro culture of immature seeds. The hybrid status was confirmed with RAPD markers and the ploidy level was

analysed using flow cytometry. These cross combinations were thought not to be possible according to the Endosperm Balance Number hypothesis and the hybrids obtained are considered to be escapes. Backcrosses were made and a variable number of seeds leading to first backcrossed genotypes (BC_1's) was produced depending on the hybrid genotype. The introgression of resistance to root-knot nematodes from *S. fendleri*, *S. stoloniferum* and *S. hougasii* has now advanced to the evaluation of resistant BC_1's for other traits before continuation of further backcrosses.

In conclusion, resistance to the root-knot nematodes *M. chitwoodi*, *M. fallax* and *M. hapla* has been identified in various *Solanum* species and has the potential to become an effective tool to control these pathogens under field conditions after transfer into cultivated potato. The first steps of introgression of resistance into *S. tuberosum* has been made. The introduction of multiple sources of resistance in new potato cultivars will enable a resistance management based on durable exploitation of useful resistance genes from natural resources.

Samenvatting

Wortelknobbelaaltjes, *Meloidogyne* spp., zijn wereldwijd gezien één van de meest schadelijke pathogenen in de land- en tuinbouw. In noordwest Europa vormen de soorten *M. chitwoodi*, *M. fallax* en *M. hapla* in toenemende mate een probleem in de gebieden waar aardappels regelmatig geteeld worden. Dit is het gevolg van veranderingen in de gewasrotatie ten gunste van hoogopbrengende - maar voor de aaltjes vatbare - gewassen, zoals vollegrondsgroentes, en een gereduceerd gebruik van grondontsmettingsmiddelen bij de teelt van aardappel. De nematoden kunnen een aanzienlijke opbrengstreductie veroorzaken en bovendien de kwaliteit van de aardappelknollen zodanig aantasten, dat deze ongeschikt worden voor consumptie of industriële verwerking. Omdat wortelknobbelaaltjes zich in de knol kunnen vermeerderen, vormen geïnfecteerde knollen een besmettingsbron voor *Meloidogyne*-vrije akkers, wanneer deze gebruikt worden als pootgoed. Om deze manier van verspreiding te voorkomen zullen daarom vanaf medio 1997 quarantaine maatregelen gelden in de landen van de Europese gemeenschap om het gebruik van schoon aardappelpootgoed, d.w.z. vrij van besmetting met *M. chitwoodi* en *M. fallax*, na te streven.

Vruchtwisseling is vaak niet effectief genoeg of economisch rendabel om schade en populatie-opbouw van deze polyfage *Meloidogyne* soorten te vermijden en het gebruik van grondontsmettingsmiddelen dient zoveel mogelijk gereduceerd te worden. Daarentegen is resistentie van de plant een economisch aantrekkelijk en milieuvriendelijk alternatief, maar helaas blijkt deze afwezig te zijn in de huidig gebruikte aardappelrassen. Het doel van het onderzoek, dat is beschreven in dit proefschrift, was de identificatie en evaluatie van bronnen van resistentie tegen *M. chitwoodi*, *M. fallax* en *M. hapla* en, indien aanwezig, de initiatie van de introgressie van de resistentie naar de cultuuraardappel door middel van soortskruisingen.

Het genus *Solanum*, waar de gecultiveerde aardappel *Solanum tuberosum* ssp. *tuberosum* toe behoort, bestaat uit een grote groep van gecultiveerde en wilde *Solanum* soorten. De meeste van deze knoldragende *Solanum* soorten zijn getoetst als mogelijke bronnen van resistentie tegen *M. chitwoodi*, *M. fallax* en *M. hapla* door middel van het selecteren van zaailingen, die weinig tot geen reproductie van de nematoden op de wortels vertoonden. Een hoog niveau van resistentie tegen zowel *M. chitwoodi* als *M. fallax* werd gevonden in genotypen van *S. bulbocastanum*, *S. cardiophyllum*, *S. brachistotrichum*, *S. fendleri* en *S. hougasii*. Een middelmatig niveau van resistentie tegen *M. fallax* werd gevonden in *S. stoloniferum* en *S. chacoense* en tegen *M. chitwoodi* in *S. gourlayi*. Meer *Solanum* soorten bezaten resistentie tegen *M. hapla*, namelijk *S. bulbocastanum*, *S. brachistotrichum*, *S. cardiophyllum*, *S. arnezii*,

S. chacoense, *S. tarijense*, *S. boliviense*, *S. gourlayi*, *S. microdontum*, *S. sparsipilum*, *S. spegazzinii*, *S. sucrense*, *S. acaule* en *S. hougasii*. In het algemeen bleek de resistentie tegen *M. chitwoodi* en *M. fallax* alleen aanwezig te zijn in *Solanum* soorten, die van oorsprong in Midden Amerika groeien, terwijl de resistentie tegen *M. hapla* in zowel Midden- als Zuidamerikaanse *Solanum* soorten bleek voor te komen. Dit kan erop duiden, dat er co-evolutie heeft plaatsgevonden tussen *M. chitwoodi* en de gerelateerde soort *M. fallax* enerzijds en de genoemde Middenamerikaanse *Solanum* soorten anderzijds.

De volgende stap was de evaluatie van de effectiviteit van de resistentie. Onder kasomstandigheden zijn diverse - als resistent of vatbaar beoordeelde - *Solanum* genotypen getoetst met twee tot vier populaties van *M. chitwoodi*, *M. fallax* en *M. hapla* om het niveau van resistentie te bepalen voor elke *Meloidogyne* soort en de mogelijke aanwezigheid te detecteren van zogenaamde virulente nematode populaties, die door de resistentie heen breken. Genotypen van *S. bulbocastanum*, *S. hougasii*, *S. cardiophyllum* en *S. fendleri* bleken een vrijwel absoluut niveau van resistentie te vertonen en de resistentie was effectief tegen alle gebruikte nematode populaties van *M. chitwoodi* en *M. fallax*. Genotypen van *S. chacoense* en *S. stoloniferum* waren onvolledig resistent tegen *M. fallax* en waren matig tot volledig vatbaar voor *M. chitwoodi*. In tegenstelling tot de kleine verschillen, die aanwezig waren tussen populaties van *M. chitwoodi* en *M. fallax*, werden grote verschillen in virulentie waargenomen tussen de vier gebruikte populaties van *M. hapla* op de - als resistent geselecteerde - genotypen van *S. bulbocastanum*, *S. hougasii*, *S. chacoense*, *S. gourlayi*, *S. sparsipilum* en *S. spegazzinii*. Een aantal geselecteerde genotypen met resistentie tegen *M. chitwoodi*, *M. fallax* en/of *M. hapla* zijn eveneens getoetst met populaties van de tropische *Meloidogyne* soorten *M. incognita*, *M. arenaria* en *M. javanica*, maar de gevonden resistentie bleek niet of nauwelijks effectief te zijn tegen deze warmteminnende nematode soorten.

Van een beperkt aantal wilde *Solanum* soorten is de effectiviteit van de resistentie ook geëvalueerd onder natuurlijke veldomstandigheden. Op twee akkers in Nederland, die op natuurlijke wijze geïnfecteerd waren geraakt met *M. fallax* of *M. hapla*, zijn vatbare en resistente genotypen individueel opgekweekt in geïsoleerde microplots en is het infectie-niveau van de grond in de microplots gevolgd gedurende het groeiseizoen. Vanaf augustus waren er grote verschillen in aantallen *Meloidogyne* larven aanwezig tussen grondmonsters van vatbare en resistente genotypen. Aan het einde van het groeiseizoen was het niveau van infectie na groei van resistente genotypen gelijk of lager ten opzichte van de start van het groeiseizoen, terwijl het infectie-niveau na groei van vatbare genotypen 7 tot 22 maal was toegenomen. De gevonden resultaten bevestigden de waargenomen verschillen, die zijn verkregen in diverse toets-experimenten in de kas.

Voor een snelle introgressie van resistentie is het belangrijk om te weten wat de erfelijke basis is van de resistentie. Deze is onderzocht voor de resistentie tegen *M. chitwoodi* en *M. fallax* in *S. fendleri*, *S. hougasii* en *S. stoloniferum*. Alhoewel deze *Solanum* soorten polyploïd zijn, is voor de genetische analyse uitgegaan van een disome overerving aan de hand van - in de literatuur beschreven - cytogenetisch en genetisch onderzoek. Diverse populaties zijn geproduceerd door het maken van kruisingen en terugkruisingen van vatbare en resistente planten binnen de wilde *Solanum* soort. De populaties zijn als zaailingen op resistentie getoetst en de verkregen uitsplitsingen zijn nader geanalyseerd. De nakomelingschapstoetsen van *S. fendleri* wezen duidelijk op de aanwezigheid van een monogeen dominant overervende resistentie, die tegen zowel *M. chitwoodi* als *M. fallax* effectief is. Het symbool R_{mc2} is voorgesteld voor dit gen. In het geval van *S. hougasii* waren terugkruisingen minder succesvol, maar de resultaten wezen eveneens op eenvoudige dominante overerving van de resistentie tegen beide nematode soorten. Uit de resultaten van de nakomelingschapstoetsen van *S. stoloniferum* is geconcludeerd, dat er meerdere additief werkende genen betrokken zijn bij de resistentie tegen *M. fallax* en *M. chitwoodi*.

De introgressie van de resistentie vanuit verschillende wilde *Solanum* soorten naar de gecultiveerde aardappel is gestart met behulp van interspecifieke kruisingen. Er zijn kruisingen gemaakt tussen diploïde *S. tuberosum* en diploïde *S. bulbocastanum*, *S. brachistotrichum* en *S. cardiophyllum*, maar deze kruisingscombinaties bleken niet succesvol te zijn. Uit kruisingen van tetraploïde *S. tuberosum* met tetraploïde *S. stoloniferum* en *S. fendleri*, en van diploïde *S. tuberosum* met hexaploïde *S. hougasii* zijn enkele zaden ontstaan, waaruit tetraploïde hybride planten zijn verkregen. In sommige gevallen gebeurde dit na in vitro cultuur van onvolgroeide zaden. De hybride status van de planten werd bevestigd met behulp van RAPD merkers en het ploïdie niveau werd met behulp van een flowcytometer vastgesteld. Via terugkruisingen met tetraploïde aardappel werd - afhankelijk van de hybride - een variabel aantal zaden verkregen, waaruit de eerste terugkruisingsplanten (BC_1's) ontstonden. De introgressie van de resistentie tegen wortelknobbelaaltjes is momenteel gevorderd tot de evaluatie van resistente BC_1's op andere eigenschappen. Hierna zullen verdere terugkruisingen gemaakt worden.

Concluderend kan gesteld worden, dat er verschillende *Solanum* soorten zijn geïdentificeerd met resistentie tegen *M. chitwoodi*, *M. fallax* en/of *M. hapla*. Deze resistenties zullen na introgressie in nieuwe aardappelrassen een belangrijke bijdrage leveren om wortelknobbelaaltjes op een natuurlijke wijze effectief te bestrijden. De eerste stappen van het overbrengen van de resistentie naar de cultuuraardappel zijn gezet. Met de introductie van nieuwe aardappelrassen met resistenties, die afkomstig zijn van verschillende *Solanum* soorten, zal het mogelijk worden om een effectief en duurzaam resistentie management te ontwikkelen.

Account

The chapters 2 to 6 of this thesis are based on the following publications, respectively:

Janssen GJW, Verkerk-Bakker B, Van Norel A & Janssen R (1996) Resistance to *Meloidogyne hapla*, *M. fallax* and *M. chitwoodi* in wild tuber-bearing *Solanum* spp. Euphytica 92: 287-294

Janssen GJW, Van Norel A, Verkerk-Bakker B & Janssen R (1997) Intra- and inter-specific variation of root-knot nematodes, *Meloidogyne* spp., for resistance from wild tuber-bearing *Solanum* spp. Fund Appl Nematol 20 (in press)

Janssen GJW, Janssen R, Van Norel A, Verkerk-Bakker J & Hoogendoorn J (1996) Expression of resistance to the root-knot nematodes, *Meloidogyne hapla* and *M. fallax*, in wild *Solanum* spp. under field conditions. Eur J Plant Pathol 102: 859-865

Janssen GJW, Van Norel A, Janssen R & Hoogendoorn J (1997) Dominant and additive resistance to the root-knot nematodes *Meloidogyne chitwoodi* and *M. fallax* in Central American *Solanum* species. Theor Appl Genet (in press)

Janssen GJW, Van Norel A, Verkerk-Bakker B, Janssen R & Hoogendoorn J (1997) Introgression of resistance to root-knot nematodes from wild Central American *Solanum* species into *S. tuberosum* ssp. *tuberosum*. Theor Appl Genet (in press)

Other publications by the author:

Janssen GJW (1994) The relevance of races in *Ditylenchus dipsaci* (Kühn) Filipjev, the stem nematode. Fund Appl Nematol 17: 469-473

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Van Meggelen JC, Karssen G, Janssen GJW, Verkerk-Bakker B & Janssen R (1994) A new race of *Meloidogyne chitwoodi* Golden *et al.* 1980? Fund Appl Nematol 17: 93

Curriculum vitae

Gerardus Johannes Wilhelmus Janssen werd geboren op 23 april 1968 te Ottersum. Hij behaalde in 1986 het VWO-diploma aan het Elshof College te Nijmegen. In hetzelfde jaar begon hij aan de Landbouwuniversiteit te Wageningen (LUW) met de studie Plantenveredeling, die in juni 1992 met lof werd afgerond. In mei 1992 werd hij aangesteld als Assistent in Opleiding bij de vakgroep Plantenveredeling van de LUW en gedetacheerd bij het Centrum voor Plantenveredelings- en Reproductie Onderzoek (CPRO-DLO) te Wageningen. Van dit onderzoek zijn de resultaten in dit proefschrift beschreven. Van mei 1996 tot en met april 1997 werkt hij als toegevoegd onderzoeker bij CPRO-DLO aan een EG-project omtrent de resistentie tegen wortelknobbelaaltjes als onderdeel van de karakterisatie van deze nematoden.

Nawoord

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