Potato crop growth as influenced by potato cyst nematodes (*Globodera pallida*) and abiotic factors

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ABSTRACT

The objective of the research described in this thesis was to determine the major mechanisms by which potato cyst nematodes reduce potato crop growth and to explain interactions known to occur with cultivar and abiotic factors. Understanding of these interactions may lead to strategies that potato growers can use to minimise nematode damage.
The research concentrated on the interaction between nematodes and soil-related factors. Most experiments were carried out under field conditions. The effects of varying levels of nematode density, soil compaction, soil pH and phosphorus fertilisation on crop growth of potato cultivars were followed during crop development. Two methods for studying root growth in the field were compared and special attention was paid to the effects of nematodes on root growth.
It was found that potato cyst nematodes reduced crop growth at early stages of growth by inducing or aggravating phosphorus deficiency. Compensatory root growth alleviated phosphorus deficiency at later stages of growth. Application of phosphorus fertiliser reduced or prevented nematode-induced phosphorus deficiency, but could not fully prevent nematode damage. Obviously, penetration of nematodes into roots also impaired crop photosynthesis through mechanisms other than nutrient deficiency.
At late stages of growth, nematodes accelerated crop senescence. This was associated with reduced concentrations of nutrients in the foliage, though it seems likely that other mechanisms contribute to the effects. To resolve this, further study of the senescence of infested crops and options for its retardation is required.
It was found that tolerance of cultivars to potato cyst nematodes is associated with production of extra roots and large tops, a characteristic of late maturing potato cultivars. The influence of soil parameters on nematode damage was also found to depend on the amount of foliage produced. It is inferred that crops with large tops suffer little yield loss as they are able to maintain ground cover and light interception, even after infestation by potato cyst nematodes.
It is concluded that farmers can minimise yield loss by choosing tolerant cultivars and by cultural measures. It is suggested that initial nematode-induced phosphorus deficiency can be relieved by fertilisation methods that increase phosphorus in infested plants to adequate levels. Crop senescence may be delayed by increasing nitrogen in infested plants, e.g. by foliar application of a nitrogen fertiliser, but this requires further study.

Additional keywords:
biomass partitioning, cultivars, foliar nutrient concentrations, light interception, light use efficiency, nitrogen, root growth, soil compaction, soil pH, phosphorus, potassium

STELLINGEN

1. Minirhizotrons zijn ongeschikt voor bestudering van ruimtelijke verdeling van wortels in het veld.

   Dit proefschrift

2. Late rassen zijn toleranter voor aardappelcystenaaltjes dan vroege rassen doordat de knolgroei bij late rassen aanvankelijk achterblijft ten gunste van loof- en wortelgroei.

   Dit proefschrift

   Haverkort A J, De Ruijter F J, Boerma M & Van de Waart M 1996 Foliar calcium concentration of potato and its relation to genotype lateness and tolerance of cyst nematodes. Eur. J. Pl. Path. 102, 317-324

   Trudgill D L 1986 Yield losses caused by potato cyst nematodes: A review of the current position in Britain and prospects for improvement. Ann. Appl. Biol. 108, 181-198

3. Schade door aardappelcystenaaltjes is deels via gerichte toediening van fosfaat te voorkomen.

   Dit proefschrift

4. De in vitro waargenomen verlaging van wortelstrekking bij infectie door aardappelcystenaaltjes (Arntzen *et al.*, 1994) leidt onder veldomstandigheden niet tot afname van de totale wortellengte vanwege compensatiegroei van wortels.

   Dit proefschrift

   Arntzen F K, Visser J H M en Hoogendoorn J 1994 The effect of the potato cyst nematode Globodera pallida on in vitro root growth of potato genotypes, differing in tolerance. Ann. Appl. Biol. 124, 59-64

5. De afschaffing in 1994 van de verplichte AM-bemonstering voor de consumptie-aardappelteelt maakt *Globodera pallida* tot een voorlopig weer ongezien probleem.

6. De voorgenomen herstructurering van de varkenssector naar aanleiding van de varkenspest leidt tot verlaging van de mestoverschotten maar draagt weinig bij aan pestpreventie.

7. De 'handen' in het spreekwoord "Vele handen maken licht werk" dienen letterlijk opgevat te worden want "vele hoofden leiden tot tragere besluitvorming".

8. De alom bekende 'Hollandse zuinigheid' is betrekkelijk, gezien de langzame groei van het gebruik van de deelauto.

   Stichting voor Gedeeld Autogebruik

9. Volgens de wet 'gelijke behandeling' zou op ijsbanen ook rechtsom geschaatst moeten kunnen worden.

10. Waarschuwingen tegen RSI kunnen niet vaak genoeg herhaald worden.

11. Voetbal kan zonder scheidsrechter gespeeld worden.

12. Uit oogpunt van diversiteit in het landschap moeten naast verschraalde zandverstuivingen en heidevelden ook rijk bemeste akkers in stand worden gehouden.

Stellingen behorende bij het proefschrift van Frank de Ruijter:

"Potato crop growth as affected by potato cyst nematodes (*Globodera pallida*) and abiotic factors".

Wageningen, 13 maart 1998.

VOORWOORD

Nu het proefschrift is afgerond en de promotiedatum nadert, is het een goed moment om stil te staan bij al diegenen die mij stimuleerden en ondersteunden bij de totstandkoming hiervan.

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Na de proeven en de analyses komt het schrijven: opzet bedenken, bespreken, bijstellen, schrappen, toevoegen, oppoetsen, ... Kortom: een hele klus. Handig is het daarbij om ervaringen te delen met anderen die in hetzelfde schuitje zitten. Ik heb met plezier deelgenomen aan het promovendi-overleg 'Nutrient dynamics and soil structure' van de

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Frank de Ruijter
Wageningen, januari 1998

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CHAPTER 1

GENERAL INTRODUCTION

Potato (*Solanum tuberosum* L.) is the second most widely cultivated arable crop in the Netherlands, the first is silage maize. In 1996, of the total arable area of 807,000 hectares, about 39,000 ha were under seed potatoes, 84,000 ha under ware potatoes and 63,000 ha under starch potatoes (Anon., 1996). In the Netherlands, potatoes are generally grown in high cropping frequencies of once in four years and in the starch potato growing areas even as often as once in two years. In the latter areas in particular, potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) are major pests of potato and cause severe yield losses. About 90 percent of the infested sites are infested by *G. pallida* (Van der Burgt *et al.*, 1995).

Potato cyst nematodes

Potato cyst nematodes parasitise various solanaceous species (Stelter & Engel, 1975) and are of economic importance as a pest of potato, which is the only host species grown as an arable crop in the Netherlands. The nematodes survive in the soil as eggs within a cyst and may persist in this stage for many years, withstanding drought and frost (Oostenbrink, 1966). When a host crop is grown, exudates from the roots stimulate the eggs to hatch (Triffitt, 1930). Subsequently, the juveniles invade the roots, thereby destroying root cells, and establish a feeding site (Jones, 1981). In the roots, the nematodes remain sessile and after three moults, the females swell and develop into a cyst, containing a large number of eggs. In temperate conditions, potato cyst nematodes have one major generation a year (Evans, 1969).

Control of potato cyst nematodes

The damage suffered by a potato crop largely depends on the population density of potato cyst nematodes at planting (Brown, 1969; Elston *et al.*, 1991; Oostenbrink, 1966; Seinhorst, 1965). Yield loss can therefore be prevented by reducing population densities to low levels. The potato cyst nematodes can be controlled by a wide crop rotation or application of nematicides. The option of lengthening crop rotation is often not attractive to the arable farmer, as there are too few other crops that are profitable. The other option, the use of nematicides, is expensive: soil fumigation costs about Dfl 1000 per hectare (Roeterdink and Brantjes, 1992), and is increasingly being restricted in the Netherlands by legislation to protect the environment. From 2000 onwards, frequency of soil fumigation will be restricted to a maximum of once per five years (Anon., 1991).

A third way of reducing nematode population densities is to grow cultivars resistant to potato cyst nematodes. Such cultivars restrict or prevent nematode multiplication

(Trudgill, 1991). However, juveniles do penetrate the roots of resistant cultivars and these cultivars do vary in their tolerance of potato cyst nematodes (Dale *et al.*, 1988; Evans, 1982; Trudgill and Cotes, 1983a; Trudgill *et al.*, 1983). They may be intolerant, meaning that they cannot withstand or recover from the damaging effects of nematodes. Resistant intolerant cultivars will control potato cyst nematodes in severely infested fields, but will also suffer a relatively large reduction in yield. Therefore, these cultivars should preferably also be tolerant of potato cyst nematodes.

Environmental interactions

There are many environmental interactions which affect the level of tolerance expressed by a cultivar (Dale *et al.*, 1988; Evans and Haydock, 1990; Trudgill, 1986). Factors such as soil type, crop husbandry, weather or interaction with secondary organisms can affect the degree of yield loss.

In general, nematodes reduce yield more in sandy soils than in peat soils (Trudgill, 1986). Evans and Haydock (1990) have suggested that the better crop performance in peat soils could result from the better moisture retention characteristics of these soils. In experiments, however, irrigation has often increased the tuber yield of both the control treatment and the nematode treatment, therefore having little effect on the losses caused by nematodes (Haverkort *et al.*, 1992; Whitehead *et al.*, 1984). It has also been found that fertiliser application improves cultivar tolerance of nematode attack. Trudgill (1980, 1987) found that it increased growth of untreated plants more than that of nematicide-treated plants and Dale *et al.* (1988) confirmed this finding, reporting the smallest yield losses on fields with the highest fertiliser inputs. Soil pH has also been demonstrated to influence the effect of soil type on tolerance (Mulder, 1990, 1994). In a container experiment, Haverkort *et al.* (1993) found that at similar infestation levels, nematodes reduced tuber yield by 19 percent at pH_{KCl} 4.5 but by 44 percent at pH_{KCl} 6.5.

It is not completely clear in what ways these soil factors affect yield loss. In this thesis, I attempt to understand the mechanisms by which nematodes affect crop growth that hold the key to explanation of the various interactions.

Potato crop growth and damage mechanisms

Potato cyst nematodes affect many crop growth characteristics, both above-ground and below-ground (summarised by Evans and Haydock, 1990; Haverkort and Trudgill, 1995; Trudgill, 1986). Their effects on crop growth originate when the juveniles invade the roots. However, effects of nematode infestation are not limited to the roots, as the above-

ground and below-ground plant parts interact. Fig. 1.1 is a schematic representation of a potato crop. Above-ground, the leaves intercept light and take up CO_2 to produce biomass. Below-ground, the roots absorb nutrients and water. Above and below-ground growth or functioning are interdependent, as nutrients and water are necessary for photosynthesis and dry matter accumulation, whereas root growth depends on the amount of intercepted light and the resulting overall growth. The relationship between shoot and root can be seen as a functional equilibrium. When growth is limited by an essential substance that must be absorbed by the roots, root growth is relatively favoured; when the limiting factor has to be absorbed by the shoot, growth of above-ground parts is relatively favoured (De Willigen and Van Noordwijk, 1987; Brouwer, 1983). Therefore, disturbance of growth or functioning in either the above-ground or the below-ground part will have an effect in the other part.

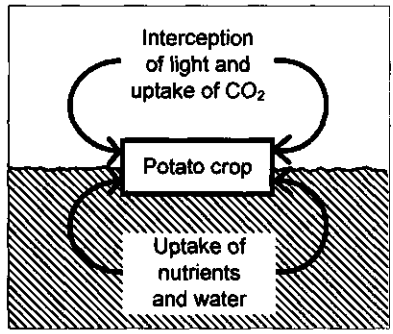

Figure 1.1. Schematic representation of resource capture of a potato crop.

To elucidate the nematode damage mechanisms, it is necessary to study growth over time, as this enables the direct effects caused by the nematodes to be distinguished from the secondary effects that are caused by an altered growth pattern. To do this, the various hypotheses that have been put up so far for the physiological mechanisms by which nematodes reduce crop growth are evaluated. Primary responses originate in the root system when juveniles penetrate the roots. It seems likely that extraction of assimilates for growth of the nematodes themselves is of minor importance, as total nematode biomass is only about 100 kg per hectare[1]. Yield loss is therefore mainly caused by the effects of nematodes on the crop. This is supported by the different effects that nematodes have on

[1] Estimated from the relationship between number of eggs (n) and cyst weight (w, in µg):
n= 8*w (Arntzen, 1993), and a population density of 200 eggs per gram of soil in the top 30 centimeter.

tolerant and intolerant cultivars. In the literature, hypotheses on nematode damage mechanisms refer to disturbed plant hormone systems, nutrient deficiency, or water stress. Schans (1991) and Schans and Arntzen (1991) asserted that cyst nematodes affect the hormonal balance of the plant, impairing crop photosynthesis. Three days after inoculation of second stage juveniles of *G. pallida* at a density of 65 juveniles per gram of soil, leaf photosynthesis was reduced by 35 percent and transpiration rates by 38 percent (Schans, 1991). Thirty days after planting four potato cultivars in pots of soil with 100 eggs of *G. pallida* per gram of soil, photosynthetic rates per unit leaf area were 70% lower than in uninfested controls (Schans and Arntzen, 1991). The photosynthetic rates increased again in the following weeks.

Nematodes can also slow down crop growth by inducing nutrient deficiency. Crops infested by potato cyst nematodes generally have reduced concentrations of nitrogen, phosphorus and potassium in the foliage (Trudgill *et al.*, 1975a, 1975b). Moreover, the shoot to root ratio is reduced (Trudgill and Cotes, 1983b; Evans, 1982), indicating that the roots are not functioning properly. Further evidence for nematode-induced nutrient deficiency was produced by Trudgill (1980), who showed in a pot test that nematode damage interacted strongly with the availability of N and of P. Doubling the amount of N and P more than doubled top weight in the absence of nematicide. At seven weeks after planting, the additional fertilisation increased leaf nutrient concentrations and reduced nematode damage. These results were corroborated in field experiments on nematode-infested soil, where application of compound fertiliser increased tuber yield more in the absence of a nematicide than with a nematicide (Trudgill, 1987).

Potato cyst nematodes affect plant water relations in a similar way as drought. However, at early stages of growth, Haverkort *et al.* (1991a) found that nematodes reduced plant dry matter production more than was to be expected on the basis of impaired water relations alone. They concluded that there must be a direct effect of nematode infestation on the dry matter production which corroborates the hormonal effect on photosynthesis reported by Schans (1991) and Schans and Arntzen (1991). In field experiments, Haverkort *et al.* (1992) found only additive effects on crop growth following a combination of potato cyst nematodes and drought. This did not confirm the expectation that nematode-infested crops would be more susceptible to drought. Evans *et al.* (1975) concluded that nematodes, together with secondary pathogens, lead to earlier root senescence and thereby reduce uptake of water, even when there is sufficient water in the profile. The subsequent drought stress may increase leaf senescence and cause the crop to senesce earlier (Evans *et al.*, 1977).

Finally, it is supposed that nematodes may increase senescence at the end of the season because they alter crop growth. Reduced initial growth may give further yield reductions when e.g. roots colonise the soil insufficiently and nutrients or water in the root zone become depleted at the end of the season.

Outline and objectives of this thesis

The research in this thesis aimed to determine the major mechanisms at the crop level by which potato cyst nematodes reduce potato growth and to explain the possible interactions with cultivar and soil type. Only the soil-related factors were examined in this research, which investigated the interaction with soil compaction, soil pH and phosphorus fertilisation. Special attention was paid to the effects on root growth and nutrient uptake, as damage originates from nematodes invading the roots. Trudgill (1986) suggests that towards the end of the season, restricted root extension may lead to insufficient soil colonisation and exhaustion of nutrients within reach, leading to nutrient deficiency. Clearly, it is inappropriate to study these indirect effects in the artificial conditions of a pot test: field experiments are required.

In the field, root systems are difficult to quantify and the information obtained varies for different root measurement methods (Mackie-Dawson and Atkinson, 1991). Therefore, two methods for studying root growth in the field were compared: minirhizotron observations and core sampling. The aim was to identify the most suitable method to obtain information on temporal and spatial development of potato roots. This work is described in Chapter 2.

To elucidate nematode damage mechanisms and the interaction with soil type, growth of the potato crop was studied over time. The experiments, conducted at various degrees of soil compaction, and the findings relating to the effects of nematodes on growth of different cultivars are described in Chapter 3. The effects on interception of solar radiation, photosynthesis, dry matter production and partitioning, root growth and functioning, and crop nutrient status were determined, to explain the variation between cultivars and soils in yield loss caused by cyst nematodes.

The interaction of nematodes with different levels of soil pH and P fertilisation was studied in another field experiment and under controlled conditions. The aim was to examine the effects of potato cyst nematodes and soil pH on potato growth and in particular the role of phosphorus in nematode damage. Special attention was paid to effects on root growth and distribution and to crop nutrient status at early stages of growth. These are discussed in Chapter 4. The treatment effects on leaf nutrient concentrations and on subsequent crop growth are described in Chapter 5. The thesis concludes with a General Discussion in which the results are summarised and the implications for farming are discussed.

CHAPTER 2

A COMPARISON OF SOIL CORE SAMPLING AND MINIRHIZOTRONS TO QUANTIFY ROOT DEVELOPMENT OF FIELD-GROWN POTATOES

ABSTRACT

Root growth of potato (*Solanum tuberosum* L.) is sensitive to soil conditions. A reduced root system size can result in reduced uptake of water and/or nutrients, leading to impaired crop growth. To understand the mechanisms by which soil conditions affect crop growth, study of temporal and spatial development of roots is required.

In field experiments, effects of soil temperature, soil compaction and potato cyst nematodes (*Globodera pallida*) on root growth of potato cultivars were studied using two methods: core sampling and vertically oriented minirhizotrons.

Minirhizotrons showed relatively more roots in deeper soil layers than core sampling, probably because of preferential root growth along the tube. Spatial distribution of roots should therefore be analysed by core sampling.

To eliminate differences in spatial distribution, total root systems as measured by both methods were compared. Nematodes, cultivars and time did not affect the relationship between both methods. Soil compaction, however, affected it because of a strong respons of root length in bulk soil and small differences in root number against the minirhizotron, suggesting that soil coring has to be used to study effects of different bulk densities.

With both methods, sequential measurements of roots give the net effect of root growth and decay. Data on root turnover can only be obtained with minirhizotrons by comparing video recordings of different dates. Other information obtained with minirhizotrons is the average orientation of roots. Moreover, the minirhizotron method has the advantage of demanding less labour.

INTRODUCTION

Root growth of potato is sensitive to soil conditions. Root system size can be reduced by both abiotic factors such as soil compaction (Boone *et al.*, 1978), and biotic factors such as cyst nematodes (Trudgill, 1986). This often results in reduced uptake of water and/or nutrients followed by impaired shoot growth and low tuber yields (Evans and Haydock, 1990; Trudgill, 1986). To understand the mechanisms by which soil conditions affect crop growth, analysis of root growth and root damage is required. However, root systems are difficult to quantify and information obtained varies with the method used (Mackie-Dawson and Atkinson, 1991). An ideal method to study root systems would yield accurate information about temporal and spatial development of the roots, without demanding too much time or labour.

Mackie-Dawson and Atkinson (1991) described a range of methods that are currently available to study plant-root systems in the field. Of these methods we have chosen core sampling and minirhizotrons for comparison of effects of biotic and abiotic factors on root development of field-grown potatoes. Core samples are taken by auger and the roots are separated from the soil by washing on sieves. Root length is then measured by counting the number of intersections between roots and a regular pattern of lines. Soil core sampling is therefore a destructive method which requires much time and labour. Minirhizotrons are glass tubes, which are inserted in the soil. Roots growing against the minirhizotron are observed through an endoscope or recorded with a video camera (Smit *et al.*, 1994; Vos and Groenwold, 1983). With minirhizotrons, root growth can be analysed non-destructively and sequential observations can be made of the same soil location. Besides root number, root turnover and senescence rate also can be measured with minirhizotrons.

Both methods of root measurement have been compared before (Böhm, 1974; Bragg *et al.*, 1983; Heeraman and Juma, 1993; Majdi *et al.*, 1992; Vos and Groenwold, 1987). A general problem with the correlation between these methods is that minirhizotrons underestimate root growth in the top layer and overestimate it in deeper soil layers (Heeraman and Juma, 1993; Parker *et al.*, 1991). This may be caused by preferential root growth along the tube (Gregory, 1979). Bragg *et al.* (1983) compared vertical and 45° angled minirhizotrons with soil core sampling and concluded that tracking of roots along minirhizotrons depends on the insertion angle of the tube. However, they drew their conclusions after omitting data of the top 30 cm. Recalculating data of their Table 1 shows that when more soil layers are included in the regression, the vertical minirhizotrons correlated equally well or even better with root length than angled minirhizotrons (Table 2.1). Omitting less soil layers improved the correlation between data from core samples and those of vertical minirhizotrons, while it reduced the correlation between data from core samples and angled minirhizotrons.

Table 2.1. Correlation coefficients (r) between soil coring and 45° angled or vertically oriented minirhizotrons (recalculations after Table 1 of Bragg *et al.*, 1983).

Excluded soil layer (cm)	July 8		August 12	
	Angled	Vertical	Angled	Vertical
0-5	0.19	0.90	0.48	0.84
0-10	0.65	0.92	0.85	0.94
0-15	0.71	0.91	0.87	0.92
0-20	0.71	0.86	0.88	0.90
0-25	0.69	0.79	0.87	0.85
0-30	0.82	0.69	0.89	0.79
0-35	0.78	0.57	0.87	0.77
0-40	0.72	0.59	0.85	0.77
0-45	0.30	0.74	0.77	0.68

Angular insertion thus does not seem to improve minirhizotron performance significantly. Additional evidence for this point of view stems from Mackie-Dawson *et al.* (1989), who found that tracking did not differ between vertical and 45° angled tubes. Therefore, we used vertically oriented minirhizotrons for comparison with soil core sampling.

The accuracy of different methods to detect relative effects of different soil factors on root development has not received much attention. Parker *et al.* (1991) found that installation of minirhizotrons may change soil bulk density and may therefore be less useful than soil core sampling to study the effect of tillage methods on potato root growth. The aim of this study was to identify the most suitable method to obtain information on effects of biotic and abiotic soil factors on temporal and spatial development of potato roots, while minimising required labour. Therefore, we compared the results of both methods for different soil temperatures, nematode infestation levels and soil bulk densities.

MATERIAL AND METHODS

Five field experiments were carried out to study root growth of potatoes (*Solanum tuberosum*, Table 2.2). In all experiments, roots were observed with minirhizotrons, in Exp. 2, 3 and 4 soil core samples were taken as well.

Soil core sampling was carried out between two plants within the ridge (Exp. 2) or between four plants in flat soil (Exp. 3 and 4, Fig. 2.1). Roots sampled with soil cores were washed free from soil on sieves (Exp. 2) or by hydropneumatic elutriation (Smucker

et al., 1982; Exp. 3 and 4) and root length was determined by the line intersect counting method (Tennant, 1975).

The minirhizotrons consisted of glass tubes of 1.2-1.5 m length and 0.06 m outside diameter which were placed vertically in the soil directly after planting. First a hole was made by an auger with a diameter 2 mm smaller than the minirhizotron, and then the minirhizotron was forced manually into the soil leaving about 0.4 m above the soil surface. The protruding part was covered with plastic sheet (black inside and white outside) and plugged with foam rubber to prevent light from entering, reduce solar warming, and to prevent condensation of water vapour inside the glass tube. The position of the minirhizotrons relative to the plants is shown in Figure 2.1. Roots were observed several times during the growing season by means of an endoscope (Table 2.2).

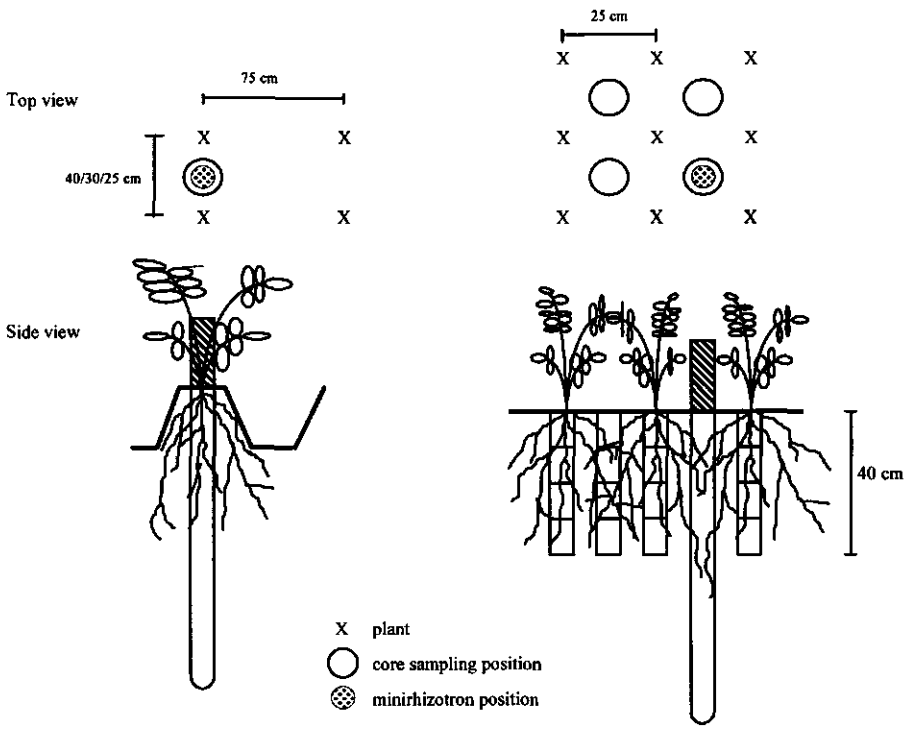

Figure 2.1. Diagram of locations of minirhizotron tube and core sampling positions relative to the plants. Left: Exp. 1, 2 and 5; right: Exp. 3 and 4.

Table 2.2. **Details of the experiments.**

	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5
Year	1982	1984	1991	1992	1980
Site	Lelystad	Lelystad	Assen	Assen	Wageningen
Treatments	soil temp.	soil temp.	soil compaction nematodes cultivar	soil compaction nematodes cultivar	cultivar
Cultivars	Eersteling	Eersteling	Darwina Elles	Darwina Elles	Bintje Saturna
Planting date	April 1	March 19	May 16	May 13	April 21
Planting pattern (m x m)	0.75 x 0.30	0.75 x 0.25	0.25 x 0.25	0.25 x 0.25	0.75 x 0.40
Number of replicates	1	1	4	4	1
Number of minirhizotrons per plot	4	4	1	1	3
Max. depth of minirhizotron (m)	1.1	1.1	0.8	0.8	0.8
Soil type	calcareous clay	calcareous clay	sand	sand	sand
Soil % organic matter	2.2	2.2	4.8	6.2	-
Soil pH-KCl	7.4	7.4	4.9	4.9	-
Dates of minirhizotron observation	May 26 June 4, 17 July 2	May 3, 8, 24 June 6, 20 July 3	June 20 July 8, 16 August 1, 15 October 25	June 15 July 11 August 11 September 1 October 10	June 5, 12, 26 July 15, 29 September 1, 15
Dates of soil core sampling		June 6	June 10 July 2 July 24 August 19	July 6 August 24	

Every two cm, the aluminium rod of the endoscope contained registration holes. At each depth the endoscope was rotated 360° and roots intersecting a mark on the ocular were counted. Additionally, the rod was lowered vertically at eight wind directions and roots were counted.

From the ratio between horizontal and vertical countings the average orientation of roots, in degrees angle to the vertical, was calculated. To correct for unequal increments, the horizontal countings were divided by 0.375 π. Figure 2.2 shows the method for roots

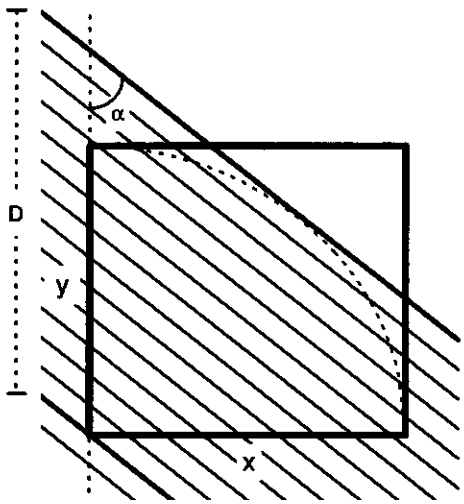

Figure 2.2. Schematic representation of parallel roots with root density D (cm cm^{-2}), growing at an angle α (in degrees to the vertical). X and y represent the horizontal and vertical line along which intersects with roots are counted. From these countings the orientation of the roots is calcultated (see text).

growing parallel at one angle to the vertical with root density D (cm cm^{-2}). The angle is calculated by:

$$\alpha = \arctan\left(\frac{\text{vertical countings}}{\text{horizontal countings}}\right) = \arctan\left(\frac{\sin\alpha \cdot D}{\cos\alpha \cdot D}\right) = \arctan\left(\frac{\sin\alpha}{\cos\alpha}\right)$$

Because we only count total numbers of intersections with horizontal and vertical lines, combinations of different growing directions are averaged. When for example three different growing directions occur, the average orientation is calculated as:

$$\alpha = \arctan\left(\frac{\sin\alpha_1 + \sin\alpha_2 + \sin\alpha_3}{\cos\alpha_1 + \cos\alpha_2 + \cos\alpha_3}\right)$$

This method exactly gives the average orientation of roots when growing directions are distributed symmetrically around one angle. Otherwise, a small error is made. The

maximum error is 4.1° and is found with two angles, 0° and 90°, occurring at a frequency of 0.75 and 0.25.
Calculation of the average angle of the roots gives no information about the root system form. However, the average angle of the newly formed roots can be calculated by comparing the increase of horizontal and vertical intersects between different observation dates.

Experiments 1 and 2

The effect of soil temperature on root and crop growth was studied. The soil was heated with water of 30°C in polyethylene tubes placed 60 cm deep at a spacing of 75 cm. Tubers were planted in ridges, between two heating tubes. Heating started on May 4 in Exp. 1 and March 17 in Exp. 2.
Minirhizotrons were placed within a row halfway between two plants and between two heating tubes (Fig. 2.1). In Exp. 2 on June 6, four core samples per treatment were taken at two depths (0-0.18 m, 0.18-0.36 m) at positions equivalent to those of the minirhizotrons.

Experiments 3 and 4

The effect of nematodes and soil compaction on root and crop growth was studied. Both fields were heavily infested with *Globodera pallida* (12 living juveniles per cm^3 soil in Exp. 3 and 22 in Exp. 4). In both experiments half the area was fumigated with Monam (400 l ha^{-1} in Exp. 3, 600 l ha^{-1} in Exp. 4) to reduce nematode densities to 1 living juvenile per cm^3 soil in Exp. 3 and 3 in Exp. 4. Two levels of soil compaction were created by either disrupting the soil with a rotary cultivator ('loose') or compacting it three times with a roller ('compacted'). The bulk soil density between depths of 0.02 and 0.27 m was 1.29 g cm^{-3} of the loose soil and 1.45 g cm^{-3} of the compacted soil. Single-stem plants were planted on flat soil without ridges. Further details of soil preparation and plant material are given in Chapter 3.
To analyse root death, roots growing against the minirhizotrons were recorded by video, twice during each experiment. In Exp. 3, video recordings were made on July 16 and 30, in Exp. 4 on July 14 and August 12. For each experiment, the recordings of both dates were compared by counting the number of living roots observed on the first date that had died or disappeared by the time of the second recording. Roots were considered to be dead when their diameter had decreased, obviously by loss of turgor. Senescence did not

always lead to changes in root colour so this could not be used as alternative criterion for root death.

In Exp. 3, soil core samples of 7 cm diameter were taken at four positions in the plot, diagonally in between plants, at four depths (0-0.1, 0.1-0.2, 0.2-0.3, 0.3-0.4 m, Fig. 2.1). Pre-planting sampling in both trial fields revealed poorly penetrable soil, with little organic matter, below 0.4 m depth, so during the experiments sampling was largely restricted to the upper soil layers. For each depth the samples were mixed and sub samples of 0.5 kg were taken. The same sampling procedure was applied in Exp. 4 except that only two sampling depths were used (0-0.2 and 0.2-0.4 m).

Experiment 5

Root growth was studied using two potato cultivars, Bintje and Saturna, respectively tolerant and intolerant of drought according to the Netherlands Recommended List of Varieties (Ebskamp *et al.*, 1994). Three minirhizotrons were placed in the row between two plants for each cultivar (Fig. 2.1).

RESULTS

Experiments 1 and 2

Figure 2.3 shows the number of intersects of roots with a horizontal line at different depths along the minirhizotron on June 6. Roots grew deeper into the soil of the heated treatment and the total number of observed roots was higher than that of the unheated treatment.

From the horizontal and vertical countings the average orientation of roots was calculated (Table 2.3). The orientation of roots was not influenced by soil heating but the angle with the vertical increased over time. This was due to an increase of more horizontally oriented roots early in the season and decrease of more vertically oriented roots late in the season.

Table 2.4 shows the results from core sampling and the horizontal countings of the minirhizotrons. Soil heating had no significant effect on root growth. Vertical root distribution, however, was significantly different between both methods. With core sampling there were relatively few roots in the subsoil (18-36 cm) compared to the topsoil (0-18 cm) and the ratio (topsoil/subsoil) was higher with core sampling than with minirhizotrons (Table 2.4).

Table 2.3. Average orientation of roots (in degrees deviation from the vertical) as calculated from minirhizotron observations in Experiments 1, 2 and 5. Between brackets the average angle of newly formed roots (+) or disappeared roots (-) since the previous observation date.

Experiment 1			Experiment 2			Experiment 5		
Date	Heated	Not heated	Date	Heated	Not heated	Date	Bintje	Saturna
			May 3	26	--			
			May 8	36 (+51)	26			
May 26	49	45	May 24	42 (+53)	36 (+36)			
June 4	47 (+45)	47 (+49)	June 6	43 (*[1])	43 (+62)	June 5	49	47
June 17	55 (*[1])	49 (+52)				June 12	49 (+49)	50 (+53)
			June 20	45 (-27)	48 (+63)	June 25	53 (+61)	54 (+71)
July 2	57 (-55)	53 (-37)	July 3	44 (-47)	52 (+71)	July 15	54 (-50)	58 (*[1])
						Sept. 1	55 (-50)	55 (-65)
						Sept. 15	58 (*[1])	57 (*[1])

[1] No angle calculated because of simultaneously decrease of horizontal and increase of vertical countings or vv.

Table 2.4. Root length density (cm cm^{-3}) and minirhizotron countings (number of horizontal countings) for heated and non-heated soil. LSD (0.05) for comparison of average ratio is 3.26.

Method	Treatment	Soil layer		Average ratio
		0-18	18-36	0-18/18-36
Core sampling	Heated	4.9	0.9	
	Not heated	3.9	1.3	4.16
Minirhizotrons	Heated	56	106	
	Not heated	77	62	0.89

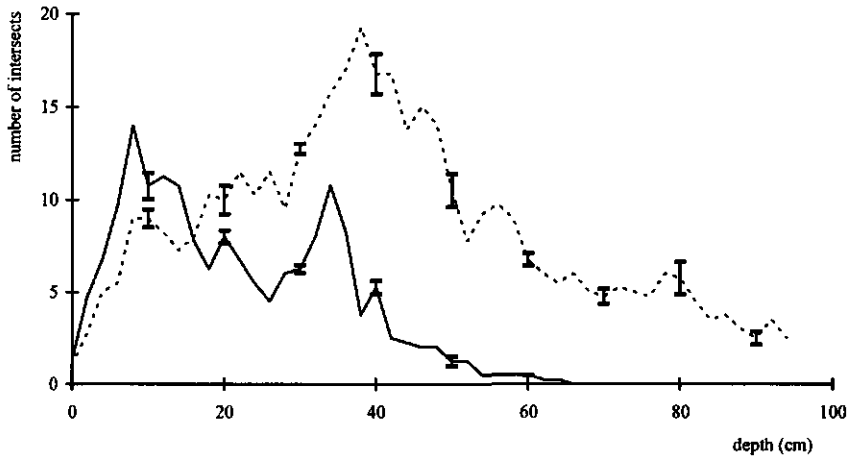

Figure 2.3. Number of intersects of roots with a horizontal line around a minirhizotron vs depth (cm) for heated (- - - -) and unheated (——) soil on June 6, (Exp. 2). Error bars indicate standard error of the mean (n=4).

Experiments 3 and 4

Figure 2.4A shows the rooting profile as measured with a minirhizotron of cultivar Darwina, growing in loose, fumigated soil on different observation days. The number of intersects with horizontal lines and the rooting depth increased during the season. Figure 2.4B shows the results of core sampling for the same treatment at about the same sampling dates as in Figure 2.4A. Root length density hardly decreased with depth for this treatment. We had not expected any root growth deeper than 40 cm because of poorly penetrable soil. This was confirmed by testing with soil coring, which revealed only a few roots deeper than 40 cm. Root growth along minirhizotrons, however, clearly went deeper than 40 cm. In the fumigated loose treatments in Exp. 3 on August 19, 52 percent of the roots of cv Darwina and 44 percent of the roots of cv Elles grew deeper than 40 cm along the minirhizotron. In Exp. 4 on August 24 these values were 54 percent for cv Darwina and 71 percent for cv Elles.

The ratio between horizontal and vertical countings of root number against the minirhizotrons in Exp. 4 revealed only small differences in average orientation of the roots for the different treatments. Differences between cultivars and treatments were small and not

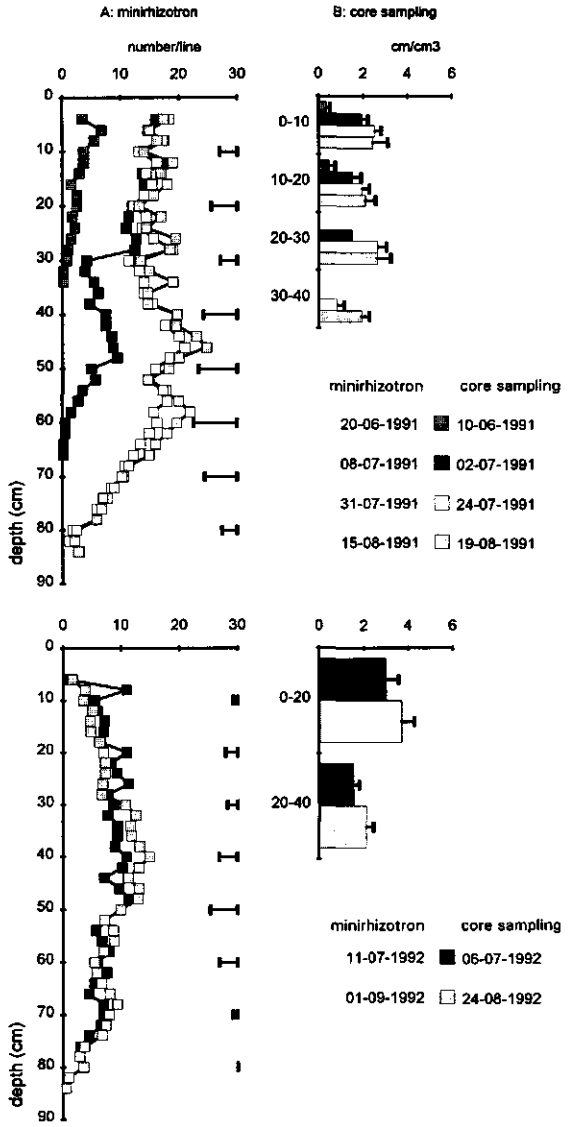

Figure 2.4. Rooting profiles of cultivar Darwina, growing in loose, fumigated soil. A: minirhizotron observations (number of intersects per horizontal line), B: soil coring between four plants ($cm\ cm^{-3}$). Top: Exp. 3, bottom: Exp. 4. Error bars indicate standard error of the mean (s.e.m.; n=3 for core sampling in Exp. 3 and n=4 for core sampling in Exp. 4 and for minirhizotrons). For the minirhizotron observations only the highest standard error among the observation dates is given.

Table 2.5. Fractions of roots that died between day 197 and 211 (Exp. 3) and day 193 and 224 (Exp. 4). For Exp. 3 different letters mean statistically significant differences (P=0.05, comparison of all means), for Exp. 4 only the main effect of compaction was statistically significant (P=0.05).

Year	Cultivar	Loose		Compacted	
		Fumigated	Not fumigated	Fumigated	Not fumigated
Exp. 3	Darwina	0.085a	0.434b	0.567b	0.426b
	Elles	0.106a	0.161a	0.074a	0.452b
Exp. 4	Darwina	0.229	0.413	0.211	0.267
	Elles	0.269	0.303	0.153	0.219

significant. The average angle to the vertical was 45° on July 11, 45° on August 11 and 48° on September 1. Between these observation dates the number of intersects hardly varied so no average of newly formed roots between observation dates could be calculated.

Root death was analysed by comparing the video recordings of roots visible at the mini-rhizotrons at two observation dates (Table 2.5). In Exp. 3, effects of treatments differed between cultivars. In Exp. 4 only the mean effect of compaction was significant.

The relation between number of horizontal intersects (minirhizotrons) and root length density (soil cores) was not affected by cultivar and soil fumigation so data were averaged

Table 2.6. Comparison of results of minirhizotron observations and soil core samples, expressed as the ratio of root number/root length density (sum of horizontal intersects/cm cm^{-3}). Between brackets the number of observations. S.e.d. is standard error of difference for comparison of all averages.

		soil layer				
		0-10	10-20	20-30	30-40	s.e.d.
Exp. 3	loose	58.4 (16)	49.6 (16)	35.8 (12)	101.4 (8)	
	compacted	65.8 (16)	58.8 (16)	82.6 (12)	148.4 (8)	42.3
		0-20		20-40		
Exp. 4	loose	14.3 (16)		61.7 (16)		
	compacted	85.5 (16)		110.2 (16)		17.3

for these treatments. The relation between both methods differed with soil layer and was affected by soil compaction, especially in Exp. 4 (Table 2.6). Soil compaction increased the number of intersects relative to root length. In deep soil layers the number of intersects relative to root length was higher than in the upper layer.

Because of this interaction between depth and method, observations of roots at all depths were summed to compare total root systems as measured by both methods. The total number of horizontal intersects between 0 and 0.8 m was compared to total root length between 0 and 0.4 m (Fig. 2.5). As total root systems were compared, data were analysed by linear regression forced through the origin. The slope of the regression (number of intersects relative to total root length) was influenced by treatment, but not consistently. Nematodes had no significant effect on the slope of the regression. Compaction, however, significantly increased the slope of the regression line in Exp. 4 (Fig. 2.5C and D) and in Exp. 3 with cultivar Elles (Fig. 2.5B), mainly because the number of intersects was not influenced by compaction whereas root length was strongly reduced.

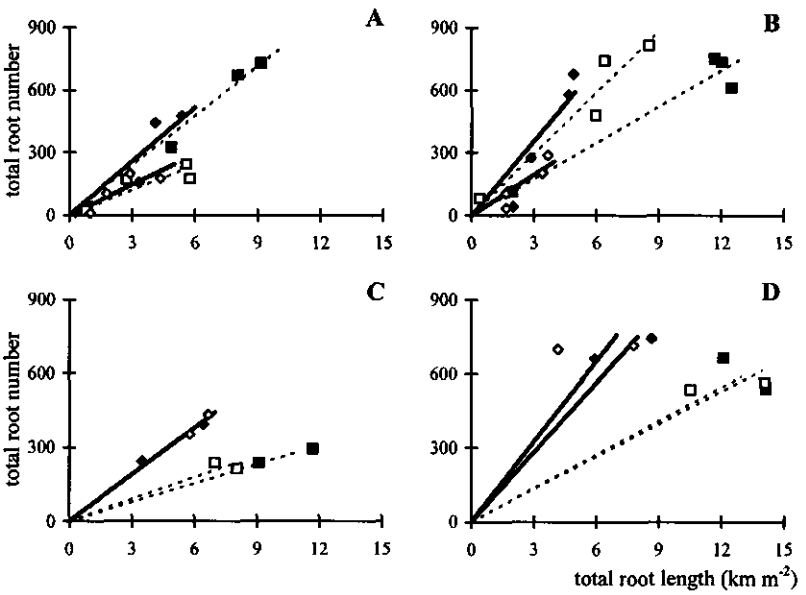

Figure 2.5. Comparison of total number of intersects of roots with horizontal lines per minirhizotron and total root length (km m^{-2}) measured by core sampling for loose, fumigated (■); loose, not fumigated (□); compacted, fumigated (◆) and compacted, not fumigated (◊). A: Darwina, Exp. 3; B: Elles, Exp. 3; C: Darwina, Exp. 4; D: Elles, Exp. 4. Regressions through the origin for compacted soil differed significantly from loose soil in Exp. 4 ($P \leq 0.02$) and in Exp. 3 for cv Elles fumigated only ($P=0.01$).

The time required for measurements with both methods is shown in Table 2.7. In our experiments, root measurements with core sampling took 1.5x more time than direct minirhizotron observations, and took 2x more time than video recordings of root growth in minirhizotrons.

Experiment 5

Root growth observed with minirhizotrons of both cultivars is given in Figure 2.6. Cultivar Bintje had more roots in deeper layers than cultivar Saturna, whereas Saturna showed a more abundant root development in the upper layers. The average orientation of roots of both cultivars increased during the season (Table 2.3).

DISCUSSION

Spatial development

We found an interaction between method and depth (Table 2.4 and 2.6). In deeper soil layers minirhizotrons showed relatively more roots than were found with core sampling. Problems with the correlation between these methods in different soil layers have been reported before. Minirhizotrons underestimated root growth in the top layer and overestimated it in the subsoil (Heeraman and Juma, 1993; Parker *et al.*, 1991). However, generally good correlations between core sampling and minirhizotrons have been reported. On closer study, however, these good correlations have only been found when the top soil layer was not used in the regression. The omitted layer ranged from the top 10 to 30 cm (Bragg *et al.*, 1983; Parker *et al.*, 1991; Vos and Groenwold, 1987). Explanations for poor root growth against the minirhizotron in the top layer are effects of drought (Vos and Groenwold, 1987), compacted soil (McMichael and Taylor, 1987) or poor soil-tube contact in tilled soil (Parker *et al.*, 1991). Still, regression with only data from deeper soil layers seems dubious. In deep soil layers root growth tends to be overestimated by the minirhizotron method because of preferential root growth along the tube (Heeraman and Juma, 1993; Parker *et al.*, 1991). When the top soil layers are omitted, the extent of tracking will affect the correlation between soil coring and minirhizotrons. Moreover, in the case of potatoes most of the roots usually are located in the top 30 cm (Parker *et al.*, 1991), so correlations between both methods for only deeper layers are of limited interest.

Table 2.7. Time (h) required per method for one object (i.e. one minirhizotron or 1 core divided in four depths) late in the growing season when many roots are present (Exp. 3 and 4).

	minirhizotron		core sampling
total current root density			
installation (only once)	(0.5)	sampling	0.25
counting horizontal intersections	0.5	washing	0.25
counting vertical intersections	0.5	root length measurements	1.0
total	1.0	total	1.5
root dynamics			
video recording	0.2		
analysing video recording	0.5		

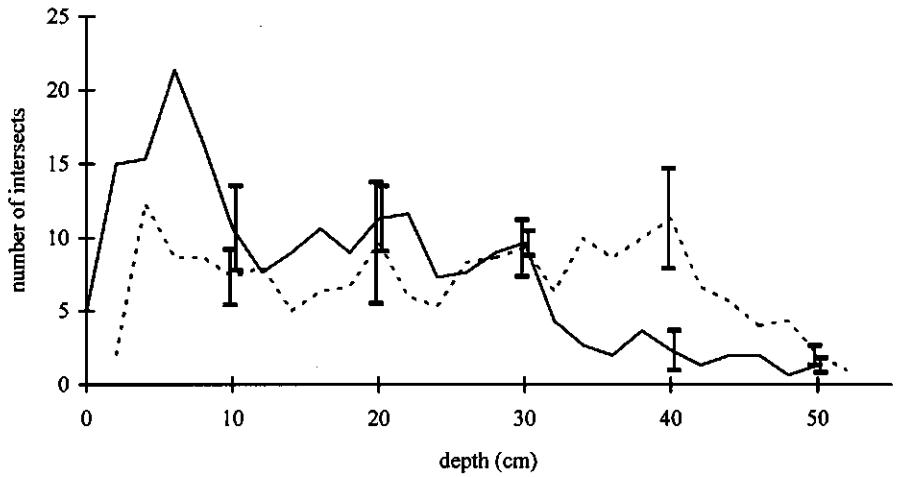

Figure 2.6. Number of intersects of roots with horizontal lines around a minirhizotron placed in the ridge vs depth (cm) for cultivars Bintje (- - - -) and Saturna (——) on June 26, (Exp. 5). Error bars indicate standard error of the mean (n=3).

The possible occurrence of tracking limits the use of minirhizotrons to study the spatial distribution of roots. To minimise bias due to tracking, Buckland *et al.* (1993) proposed to count only first and last points of contact of roots with the minirhizotron. In this manner, roots that track the tube are counted only once which prevents overestimation of total root growth. Because root tips of roots tracking the tube are found in relatively deep soil layers, this method does still not prevent the observed rooting profile bias towards deeper soil layers. Using this method, Buckland *et al.* (1993) had good results with sparsely distributed tree roots, but not with pasture roots. We conclude that when spatial distribution is an important part of the study root growth should be analysed by core sampling (Mackie-Dawson and Atkinson, 1991). For study of relative effects of non-soil factors, such as cultivar differences in our Exp. 5, the use of minirhizotrons in the field is useful. For example, we found more roots in deeper soil layers with the drought tolerant cultivar Bintje than with the intolerant cultivar Saturna, indicating that differences in drought tolerance may be based on differences in rooting.

Total root systems

As root distribution was influenced by the presence of the minirhizotrons, our observations of different soil layers in Exp. 3 and 4 were summed to compare total root systems as measured by both methods. We found an effect of soil compaction on the relation between total number of intersects and total root length, especially in Exp. 4 (Fig. 2.5). Compaction increased the number of intersects relative to root length. This change in slope was mainly caused by a decrease in total root length while the number of intersects hardly differed. Apparently, when soil is compacted roots can grow more easily along the minirhizotron-soil interface than in the bulk soil, indicating that installation of the tubes has reduced treatment differences in bulk density. Effects of installation of minirhizotrons on bulk density of the surrounding soil have been reported before (Brown and Upchurch, 1987; Taylor *et al.*, 1990).

There were no clear effects of nematodes and soil temperature on the relation between the two methods and total root systems can be measured by both. However, some bias may occur because potato cyst nematodes are found mainly in the top 40 cm of the soil (Whitehead, 1977). When root growth is shifted downwards by the minirhizotrons, observed roots in the subsoil may be less infected by nematodes. However, we did not measure this.

We conclude that total root systems can be measured by both soil coring and minirhizotrons but when effects of differences in soil bulk density on root growth are to be studied, roots observed with minirhizotrons may not be representative of roots growing in bulk soil and the method of soil coring is preferred.

Root dynamics

When total living roots are counted at different dates, both methods give net root growth or death. With minirhizotrons, one root system is observed during time and differences are caused by growth or decay of that root system. Soil coring will show more variation because different plots have to be used for the different samples. We found no effect of time on the relation between total roots systems as observed by both methods.
Root dynamics can be studied in more detail with minirhizotrons because particular roots can be recognised at later observations and root death can be observed. For this, video recordings have to be taken. With the video recordings we found no clear effects of treatments on root death (Table 2.5). Root death at the minirhizotron surface may differ from that in the bulk soil because root density at the glass surface may be higher. When all roots that normally would grow in the space occupied by the tube are present at the glass surface, the concentration of roots near the tube is higher than in the bulk soil. This may lead to earlier local depletion of nutrients or lower numbers of nematodes per root and thereby affect death rate of the observed roots. However, we did not measure this.

Orientation of roots

An advantage of minirhizotrons over core sampling is the possibility to study the orientation of roots in the soil. We found that the average orientation of roots increased during the season. The average angle of roots gives no information about the root system form. Analysis of the increase or decrease of horizontal and vertical countings may give information about the average orientation of newly formed roots or recently disappeared roots. In our experiments, however, the additional information was limited because angles calculated from the differences between two observation dates differed not much from the average angle of total roots. We calculated the average orientation for all roots against the minirhizotron surface. Dividing the countings along vertical lines into several soil layers might give more information about orientation of first arrived and later formed roots.
At an average orientation of roots of 45°, counting only along horizontal lines would be sufficient and the required observation time for the minirhizotron method would be halved. However, we found that the average orientation of roots is not always 45°. With an average angle of 50° to the vertical, root growth is underestimated by 10% when only roots intersecting horizontal lines are counted.

CONCLUSIONS

We have concluded that for study of spatial distribution soil coring is the best method. For studying effects of biotic and abiotic factors on total root systems, both soil coring and minirhizotrons can be used. However, when these factors interfere with bulk density, minirhizotrons cannot be used and soil coring is recommended.

With minirhizotrons more detailed information can be obtained on root dynamics than with core sampling. When information about effects on root turnover is important, the minirhizotron method is preferred. Additional advantages of the minirhizotron method compared to core sampling are the lower labour requirement and the opportunity to measure the average orientation of roots.

CHAPTER 3

ANALYSES OF THE EFFECTS OF POTATO CYST NEMATODES (*GLOBODERA PALLIDA*) ON GROWTH, PHYSIOLOGY AND YIELD OF POTATO CULTIVARS IN FIELD PLOTS AT THREE LEVELS OF SOIL COMPACTION

ABSTRACT

Field experiments were carried out in 1991 and 1992 on sandy soil highly infested with the potato cyst nematode *Globodera pallida*. Half the trial area was fumigated with a nematicide to establish two levels of nematode density. Three levels of soil compaction were made by different combinations of artificial compaction and rotary cultivation. Two potato cultivars were used in 1991 and four in 1992.

Both high nematode density and soil compaction caused severe yield losses, of all cultivars except cv. Elles which was tolerant of nematode attack. The effects of the two stress factors were generally additive. Analysis of the yield loss showed that nematodes mainly reduced cumulative interception of light while compaction mainly reduced the efficiency with which intercepted light was used to produce biomass. This indicates that nematodes and compaction affect growth via different damage mechanisms.

Nematodes reduced light interception by accelerating leaf senescence, by decreasing the specific leaf area and indirectly by reducing overall crop growth rate. Partitioning of biomass between leaves, stems and tubers was not affected by nematode infestation but compaction decreased partitioning to leaves early in the growing season while increasing it during later growth stages.

The effects of nematodes and compaction on root length dynamics and nutrient uptake were also additive. This suggests that the commonly observed variation in yield loss caused by nematodes on different soil types is not related to differences in root system expansion between soils of various strength.

Cv. Elles, which showed tolerance of nematodes by relatively low yield losses in both experiments, was characterized by high root length density and thick roots. These characteristics did not confer tolerance of soil compaction, since compaction affected root lengths and tuber yields equally in all cultivars.

In the first experiment only, high nematode density led to decreased root lengths and lower plant nutrient concentrations. The yield loss which occured in the second experiment was attributed to the effects of nematodes on other aspects of plant physiology.

INTRODUCTION

Cyst nematodes (*Globodera* spp.) may cause severe yield losses in potato. The amount of loss depends on the potato cultivar, the genotype and density of the nematode, and the soil type (Trudgill, 1986; Evans and Haydock, 1990). How these factors affect yield loss is not completely clear. The ranking of potato genotypes for tolerance of cyst nematodes varies between years and sites (Dale *et al.*, 1988; Hancock and Holliday, 1992). Whitehead and Nichols (1992) showed that soil density may be involved in the site effect. They found that the mean yield loss of two cultivars in two years increased from 40 to 56% when compacted soil, severely infested with *Globodera rostochiensis* Woll., was loosened. Soil compaction strongly retards the extension of potato root systems (Boone *et al.*, 1978), and may indirectly arrest nematode population development. To clarify such interactions, closer examination of the mechanisms by which nematodes cause yield loss seems warranted.

Yield loss is often associated with reduced light interception by the crop due to reduced crop leaf area (Trudgill *et al.*, 1990; Haverkort *et al.*, 1992). Whether infection by nematodes reduces leaf area by decreasing leaf growth, accelerating leaf senescence, lowering the specific leaf area, or by a combination of these mechanisms, is unclear.

Yield loss may further be associated with effects of the nematodes on photosynthesis. Thirty days after planting four potato cultivars in pots of soil with 100 eggs g^{-1} of *G. pallida* Stone, photosynthetic rates per unit leaf area were 70% lower than in uninfested controls (Schans and Arntzen, 1991). The photosynthetic rates increased again in the following weeks. Since this research was carried out under artificial conditions, with little rooting space in the pots, limited nutrient availability, and very high nematode densities, it is still unclear whether nematodes also impair photosynthesis under field conditions, and to what degree the effect may account for observed yield losses.

Two hypotheses have been put forward for the physiological mechanisms underlying the nematode effect. Trudgill (1980; Trudgill *et al.*, 1975a; Trudgill and Cotes, 1983a, 1983b) proposed that nematodes reduce size and efficiency of the root system, leading to chronic nutrient deficiency and early crop senescence. Schans (1991; Schans and Arntzen, 1991), on the other hand, asserted that cyst nematodes primarily affect the hormonal balance of the plant, leading to stomatal closure and impaired crop photosynthesis. According to the latter hypothesis, disturbance of carbon metabolism, and not nutrient metabolism, impairs crop growth. Evidence from field experiments is scarce. Schans' hypothesis has so far not been tested in the field, while Trudgill found stronger reductions of plant nutrient concentrations by nematodes in pot experiments than in the field.

The objective of the research presented in this paper was to identify the major ways in which potato cyst nematodes affect crop growth in the field. The effects of nematodes on

leaf growth, senescence and photosynthesis of field-grown nematode-tolerant and intolerant potato cultivars were assessed. The effects were measured at various levels of soil compaction, to examine how soil type affects the magnitude of yield loss. We also studied the effects of treatments on root system size and activity and crop nutrient status to examine possible mechanisms of damage and to explain the variation between cultivars and soils in yield loss caused by cyst nematodes.

MATERIALS AND METHODS

Experimental factors

Two field experiments were carried out, in 1991 and 1992, on different farmers' fields on sandy soil (4.8-6.2% organic matter; pH 4.9) infested with *Globodera pallida*, near Assen, the Netherlands. Three experimental factors were included: nematode density, level of soil compaction, and cultivar.

In both experiments half the area was fumigated about six weeks before planting with sodium methyldithiocarbamate (Monam 510 g l^{-1} a.i.; 400 and 600 l ha^{-1} in 1991 and 1992, respectively) to establish a lower level of nematode population density. Two to four weeks after fumigation, initial nematode density was estimated by soil sampling. In each plot (1991: n = 48; 1992: n = 96), about five dm^3 soil was taken, cysts were filtered out and put in potato root diffusate to count the number of emerging second stage juveniles (Van Haren, 1995).

Three levels of soil compaction were established, one week before planting in both experiments, by driving three (severely compacted), one (lightly compacted) or zero (non-compacted) times over the soil with a 1 m wide motorized roller of 2700 kg. The density of the non-compacted soil was decreased further by means of rotary cultivation.

The effects of compaction treatments on soil strength were measured in all plots once during each growing season by means of penetrometry (Bengough, 1991). In 1991, the effects of compaction on soil pore size distribution and total pore volume between depths of 0.02 and 0.27 m were assessed in each plot by measuring water loss from intact wet soil samples subjected to a range of suction tensions (Glinski and Lipiec, 1990).

In 1991, two late maturing, *G. pallida*-resistant potato cultivars were used, Darwina and Elles, in 1992 the early maturing, susceptible cvs Bintje and Mentor were added (Anon., 1992a). Farming practice suggests that only cv. Elles has a high tolerance of nematodes (M. Boerma, HLB Assen, pers. comm. 1990). In April of both years, seed tubers of these cultivars were planted in containers under an open-air rain shelter (1991) or in a greenhouse (1992). Four weeks after planting the tubers were taken out of the soil, and

ongrowing shoots with roots were carefully detached. These single-stem plantlets, with an average length of 15 cm, were transplanted to the trial fields, on May 16, 1991 and May 13, 1992. The plantlets were set out at a spacing of 0.25 m x 0.25 m, on flat soil, no ridges were made, to promote homogeneous rooting. Plot size was 4.5 m x 3.0 m, giving a total of 216 plants per plot.

In both years fertilizer was applied on the basis of soil samples taken to 0.4 m deep, to give pre-planting levels of N, P and K of 0.023, 0.030 and 0.050 kg m^{-2}, respectively. The fungicide maneb/fentin acetate was sprayed several times, according to normal farming practice, for protection against *Phytophthora infestans*. In 1991 natural rainfall was sufficient to keep the crops amply provided with water, but in 1992 a total of 150 kg water m^{-2} was supplied between planting and the end of June.

In both years a split-plot design was used with four randomized blocks. Each block contained six main plots, for the six combinations of fumigation and soil compaction treatments. The subplots within the main plots were randomly allocated to the two (1991) or four (1992) cultivars used.

Measurements

Ground cover by green foliage was measured weekly. In 1991, a metal frame 0.75 x 0.75 m, divided in 100 equal squares, was used to estimate percentage ground cover visually as the number of squares more than half filled with green leaves (Haverkort *et al.*, 1991b). In 1992 ground cover was assessed using portable equipment for measuring red and green reflectance of the crop. The two methods give comparable data (Haverkort *et al.*, 1991b).

Shoot and tuber biomass were measured at four harvest dates (1991: June 10, July 2 and 24, August 19; 1992: June 15, July 6, August 3 and 24). At each harvest, patches of nine neighbouring plants per plot were harvested for separate determination of dry mass of tubers, stems, green, yellow and dead leaves. At least 0.5 m (i.e. two border plants) separated patches harvested at different dates. The area of a sample of green leaves was measured for calculation of the leaf area index. Fresh and dry weight, nitrogen, phosphorus and potassium content of leaves, stems and tubers and, in 1991 only, nitrate content of the leaves were also determined at each harvest. The nutrient concentrations were determined in only two of the four blocks. After foliage death, tuber yield was assessed in a final harvest of 24 plants per plot, taken from the central plot area. In 1992, measurements on plants growing on lightly compacted soil were restricted to ground cover and final tuber yield.

Diurnal courses of crop photosynthetic rate of cv. Darwina, in non- and severely compacted soil at both levels of nematode density, were measured at ambient light intensity with mobile field equipment (Louwerse and Eikhoudt, 1975). In 1991,

photosynthesis was measured between July 1 and 5, and between July 29 and August 2. In 1992, measurements were carried out between July 13 and 17, and between August 10 and 14. Additional measurements of leaf photosynthetic rate at light saturation were taken concurrently in all cultivars. These measurements were done using a portable leaf chamber analyzer (LCA; Analytical Development Co. (ADC), UK), while holding an incandescent lamp, providing 300 W m^{-2} of photosynthetically active radiation, over the enclosed leaf for at least one minute before taking readings.

Root weight and length were assessed at each harvest in 1991, and at the second and fourth harvests in 1992. In 1991, 36 soil core samples, each 200 cm^3, were taken at nine positions in the plot, five directly below plants and four diagonally in between, at four depths (0-0.1, 0.1-0.2, 0.2-0.3, 0.3-0.4 m). Pre-planting sampling in both trial fields had revealed poorly penetrable soil, with few nutrients and organic matter, below 0.4 m depth, so during the experiments sampling was largely restricted to the upper soil layers. For each depth the five samples taken below plants were mixed, as were the four samples taken between plants, and subsamples of 0.5 kg were derived. The same procedure was applied in 1992 except that only two depths were sampled (0-0.2 and 0.2-0.4 m). The 0.5 kg subsamples were taken to the laboratory, roots were washed free from soil by hydropneumatic elutriation (Smucker *et al.*, 1982) and root length was determined by the line intersect counting method (Tennant, 1975). After counting, the roots were collected to determine their fresh and dry weight. Total root length (km m^{-2}) was calculated as the average root length density (m m^{-3}) for the various sampling positions, multiplied by 0.4 x 10^{-3} to account for the 0.4 m rooting depth, and for conversion from m to km.

Root length was also assessed non-destructively in the field, by the use of minirhizotrons placed in all plots, except those with the intermediate level of soil compaction. The mini-rhizotrons were glass tubes 1.20 x 0.06 m placed vertically in the soil to a depth of 0.85 m, exactly in between plants, so their outer circumference was 0.15 m away from the four nearest planting positions. Twice each year (July 16 and 30, 1991; July 14 and August 12, 1992) video recordings were made of the rooting profile along the tubes (Upchurch and Ritchie, 1983). The tapes were later examined to estimate the rate of root senescence, by counting the fraction of roots present at the first recording that had disappeared or visibly deteriorated by the time of the second recording.

Air temperature and incident solar radiation data throughout the growing season were obtained from Eelde weather station about 17 km north of the trial fields. Soil temperature data for both years were taken from Nieuw-Beerta weather station 51 km east. These data compared well to soil temperatures measured at five positions in the trial fields from July to October 1992, by thermocouples placed 0.10 m deep. Average soil temperatures were lower in 1991 than in 1992 from planting until the end of June (13.7°C compared to 17.3°C), but were similar thereafter (about 18.7°C on average in July and August of both years).

Calculations and statistical analysis

The time courses of ground cover were used to calculate cumulatively intercepted photosynthetically active radiation (PARCUM: MJ m^{-2}) by the crops. Daily light interception was estimated from ground cover percentage multiplied by the amount of incoming radiation. PARCUM was then calculated by summing all daily interception values over the growing season. The efficiency with which the crops used the intercepted light to produce biomass (LUE: g MJ^{-1}) was estimated by linear regression of total biomass at the various intermediate harvest dates on corresponding PARCUM-values (Monteith, 1977).

The rate of leaf senescence was quantified as the average percentage of leaves that died daily between the second and fourth harvest. This was calculated as minus the natural logarithm of the proportion of leaves that survived from harvest 2 till 4, divided by the number of days between harvests.

Root senescence was quantified similarly as leaf senescence, using the video recordings from minirhizotrons of living and dead roots.

Statistical analysis was carried out separately for the 1991 and 1992 experiments. All measurements were analysed using analysis of variance (ANOVA) corresponding to the split-plot designs used. Standard errors of difference between treatments at given degrees of freedom were derived from the ANOVA's.

RESULTS

Nematicide and compaction treatments

Cyst nematode populations in non-fumigated soil were 12 ± 2.0 (S.E.M.; n = 24) and 22 ± 2.6 (S.E.M.; n = 48) living juveniles cm^{-3} soil in 1991 and 1992, respectively. In fumigated soils there were 1 ± 0.7 (S.E.M.; n = 24) and 3 ± 0.5 (S.E.M.; n = 48) living juveniles cm^{-3} soil in 1991 and 1992, respectively.

In 1991, soil bulk density between depths of 0.02 and 0.27 m, was 1450, 1390 and 1280 kg m^{-3}, respectively, for severely, lightly and non-compacted soils, respectively. In 1992, soil densities were 1460, 1390 and 1300 kg m^{-3}, respectively.

The effect of the treatments on soil penetration resistance was most pronounced above a depth of 0.3 m (Fig. 3.1). Although the established ranges of soil bulk density were similar in the two years, soil rolling increased penetration resistance more in 1991 than in 1992 (Fig. 3.1). Moderate and severe compaction reduced total pore volume from 51% to

47 and 45%, respectively. This reduction was mostly accounted for by a decreased frequency of pores wider than 0.0003 m, in the soil layer above a depth of 0.10 m. Since total pore volume was reduced only slightly, the compaction treatments will not have interfered with oxygen supply to the roots. Soil water content was also little affected by compaction. The water content between depths of 0.02 and 0.27 m in severely compacted, moderately compacted and loose soil was 270, 270 and 250 kg m^{-3}, respectively, on June 27, 1991, and 190, 180 and 170 kg m^{-3} on August 11, 1992.

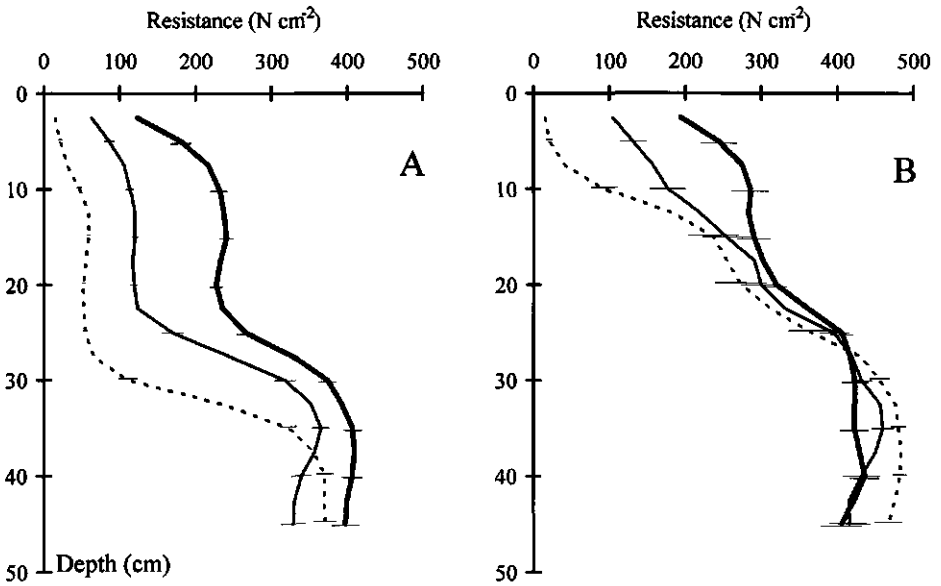

Figure 3.1. Soil penetration resistance (N cm^{-2}) between depths of 0 and 45 cm, at three levels of soil compaction. Resistance was assessed by means of a 1 cm^2 cone penetrometer. A: 3 October 1991 (n=48); B: 8 August 1992 (n=16). Horizontal bars indicate standard errors of means. - - - Non-compacted, —— lightly compacted, ▬▬ severely compacted.

Table 3.1. Final tuber yield (g dry matter m^{-2}), cumulative interception of photosynthetically active radiation (PARCUM: MJ m^{-2}) and crop light use efficiency (LUE: g MJ^{-1}). SED = standard error of difference (value in parentheses is for the comparison within the same level of nematode density or compaction).

	Year	Cultivar	Nematode density		Compaction level		
			Low	High	Loose	Light	Severe
Yield	1991	Darwina	1223	489	1013	863	692
		Elles	1363	1103	1475	1349	875
		SED (d.f. = 18)	55.9 (37.3)		68.5 (45.7)		
	1992	Darwina	1177	890	1131	1032	938
		Elles	1386	1388	1510	1373	1279
		Bintje	757	612	906	654	492
		Mentor	1117	814	1012	937	948
		SED (d.f. = 54)	72.9 (71.4)		89.3 (87.4)		
PARCUM	1991	Darwina	709	263	526	506	426
		Elles	777	625	791	737	576
		SED (d.f. = 18)	26.5 (16.9)		32.4 (20.7)		
	1992	Darwina	772	649	726	720	684
		Elles	915	882	917	915	864
		Bintje	614	514	630	575	487
		Mentor	670	571	635	614	613
		SED (d.f. = 54)	18.4 (18.1)		22.6 (22.2)		
LUE	1991	Darwina	2.31	1.76	2.32	2.06	1.72
		Elles	2.33	1.94	2.58	2.11	1.72
		SED (d.f. = 18)	0.108 (0.106)		0.132 (0.130)		
	1992	Darwina	2.07	2.06	2.25	-	1.88
		Elles	2.16	1.95	2.09	-	2.02
		Bintje	1.82	1.69	1.85	-	1.66
		Mentor	2.03	1.82	2.04	-	1.81
		SED (d.f. = 36)	0.135 (0.138)		0.135 (0.138)		

-, no data collected

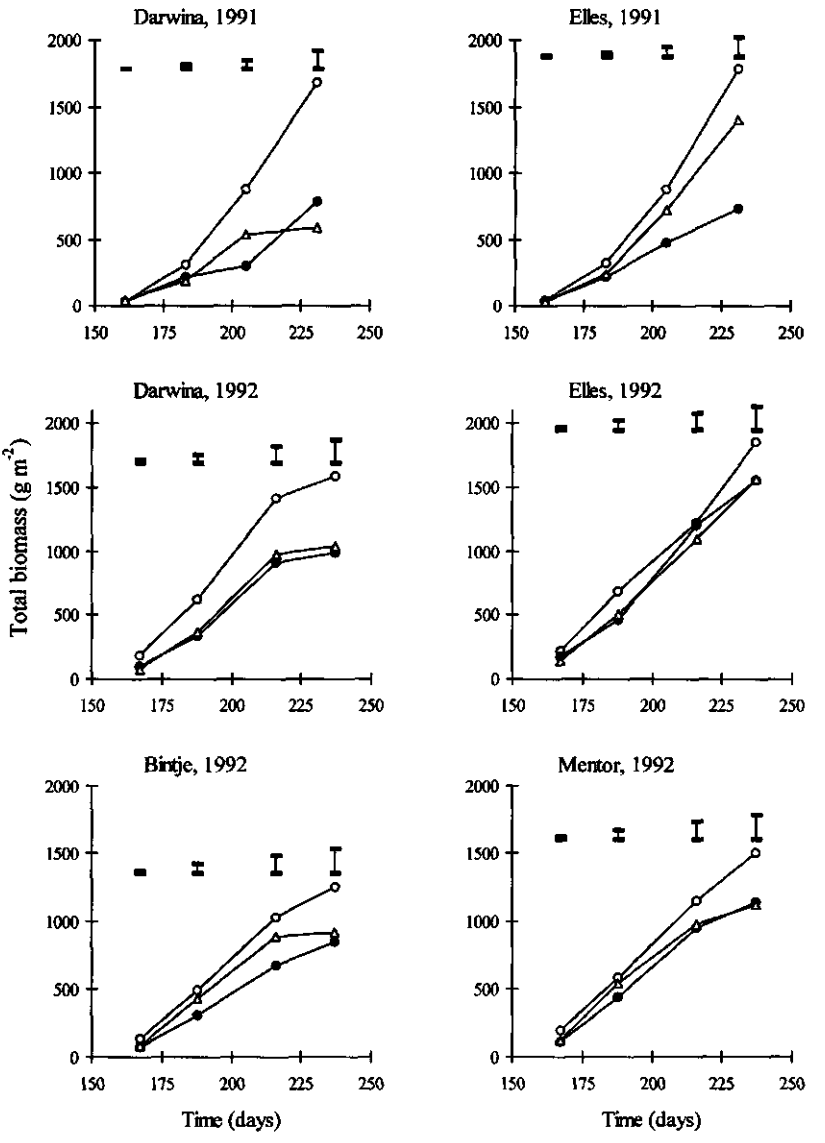

Figure 3.2. Time courses of total dry biomass (g m^{-2}), excluding roots, for various treatments. The final harvest date, when only tubers were weighed, is excluded. Vertical bars above harvest data indicate standard errors of difference (1991: d.f. = 18; 1992: d.f. = 36). —O— Non-compacted, fumigated; —●— compacted, fumigated; —Δ— non-compacted, not fumigated.

Crop growth and yield

High nematode density and soil compaction reduced crop growth rates more strongly in 1991 than in 1992. Cultivars were equally affected by soil compaction but nematodes affected cv. Elles less than the other cultivars (Fig. 3.2). Combining nematodes and compaction reduced crop growth additively (not shown).

In both years, the reduced crop growth rates resulted in statistically significantly lower final tuber yields (Table 3.1). In 1991 the average yield loss was 40% with nematodes, and 37% with severe soil compaction. In 1992 the losses were only 12% and 16%, respectively, for cvs Darwina and Elles, with similar values for cvs Bintje and Mentor (Table 3.1). The cultivars responded differently to the nematodes. Cv. Elles was not affected by high nematode density except in the compaction treatments of 1991. The other cultivars always suffered yield loss by nematodes. No such differences between cultivars were found for the effect of soil compaction.

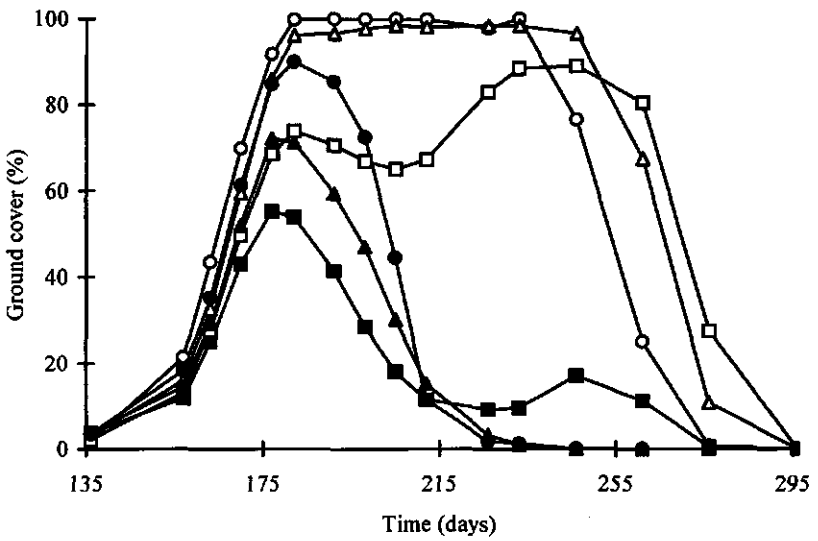

Figure 3.3. Time courses of percentage ground covered by green leaf area for cv. Darwina, 1991. Fumigated: —O— loose, —Δ— light compacted, —□— severely compacted. Not fumigated: —●— loose, —▲— light compacted, —■— severely compacted.

Ground cover

The variation in yield largely reflected the differences in time courses of ground cover. Figure 3.3 shows this for the example of cv. Darwina in 1991. The other cultivars reacted similarly, apart from cv. Elles which suffered negligible effects from nematodes. Nematodes accelerated foliage death, especially in 1991, thus reducing ground cover mainly during the second half of the growing season. Compaction retarded or prevented canopy closure, but delayed crop senescence by one or two weeks.

PARCUM and LUE

The coefficients of determination (r^2) for regressions of biomass on cumulatively intercepted photosynthetically active radiation (PARCUM) averaged 0.96 and 0.93 for 1991 and 1992, respectively. High nematode density and soil compaction reduced both PARCUM and crop light use efficiency (LUE) in both years (Table 3.1). Nematodes reduced PARCUM of cvs Darwina and Elles on average by 41% in 1991 and by 10% in 1992. The corresponding effects on LUE were 20% and 5% (Table 3.1). Severe compaction reduced LUE, by 30% and 11% in 1991 and 1992, respectively, whereas the corresponding reductions of PARCUM were 24% and 9%. For LUE, no significant statistical interactions between cultivar and level of nematode density or compaction were found, but for PARCUM these interactions were significant in both years.

Partitioning

Data of dry weights for leaves, stems and tubers, as determined at the consecutive harvests, were used to calculate the changes during the growing season of partitioning of growth between plant organs (Fig. 3.4). In each case the fraction of growth occurring in tubers increased with time, but somewhat slower in cv. Elles than in the other cultivars. High nematode density did not markedly affect partitioning in any of the cultivars. Compaction on the other hand, slightly decreased partitioning to leaves early in the growing season, but in most cases increased it thereafter. The partitioning patterns for the combination of severe soil compaction and high nematode density (not shown) were similar to those for soil compaction alone. Partitioning to stems was not affected by any of the treatments.

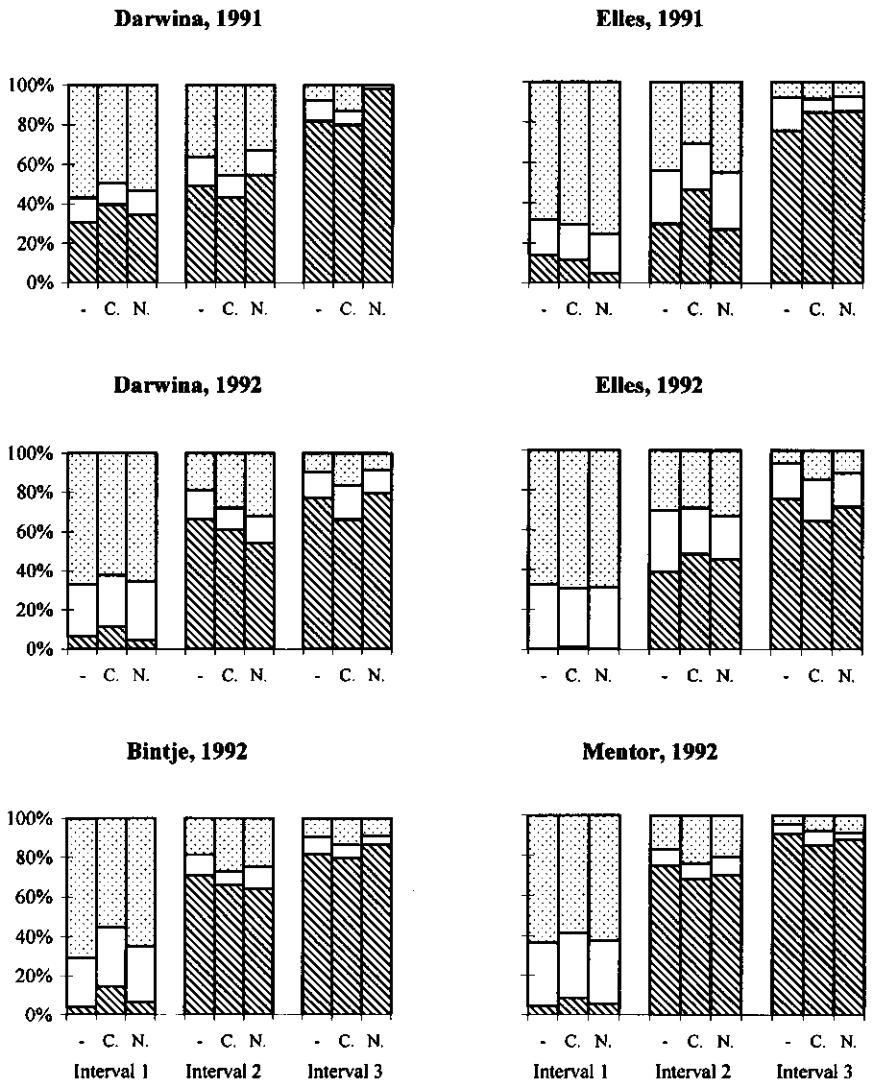

Figure 3.4. Partitioning of growth of dry biomass between leaves ⊠, stems □ and tubers ▨, for non-compacted, fumigated soil (-), severely compacted, fumigated soil (C) and non-compacted, not fumigated soil with high nematode density (N). Partitioning was calculated for three consecutive time intervals: between planting and first harvest, between first and second harvest, and between second and fourth harvest. The three intervals are represented by the three groups of columns shown for each cultivar in both experimental years.

Specific leaf area

Specific leaf area (SLA) was determined at the first four harvest dates as the area-dry weight ratio of green leaves. In 1991 the average SLA was about 22 m^2 kg^{-1} at all dates, but in 1992 SLA increased from 12.2 m^2 g^{-1} at the first harvest to 18.6 and 17.5 m^2 kg^{-1} at the third and fourth harvest, respectively.
In both years, SLA was reduced significantly and additively by nematodes and soil compaction. The effects were strongest in 1991, when severe compaction reduced SLA by 19% and nematodes reduced it by 9%. In 1992 the effects were 7% and 4%, respectively. SLA only differed significantly among cultivars in 1992, when SLA was about 16% higher in cv. Elles than in the other cultivars.

Leaf senescence

The late maturing cultivar Elles consistently showed the lowest rates of leaf senescence between the second and fourth harvests (Table 3.2). In 1991, both nematodes and soil compaction accelerated leaf senescence, but in 1992 nematodes had no effect, while compaction slightly delayed leaf senescence.

Table 3.2. Average daily rate of leaf senescence between harvests 2 and 4 (% d^{-1}). SED = standard error of difference (value in parentheses is for the comparison within the same level of nematode density or compaction).

Year	Cultivar	Nematode density		Compaction level		
		Low	High	Loose	Light	Severe
1991	Darwina	2.6	8.7	4.8	5.5	6.5
	Elles	0.7	1.0	0.5	0.8	1.1
	SED (d.f. = 18)	1.59 (1.51)		1.94 (1.85)		
1992	Darwina	3.3	2.6	3.2	-	2.7
	Elles	2.0	2.5	2.6	-	1.8
	Bintje	12.9	15.2	13.0	-	15.1
	Mentor	8.3	11.2	13.4	-	6.1
	SED (d.f. = 36)	1.73 (1.73)		1.73 (1.73)		

-, no data collected

Photosynthesis

In each of the four periods that crop photosynthesis was measured, crop photosynthetic rate of cv. Darwina varied strongly among treatments, as illustrated in Fig. 3.5A for the first five-day period. The variation was partly caused by differences in ground cover and, therefore, differences in light interception. To account for this, crop photosynthetic rate was analysed in relation to the amount of intercepted light, calculated as before (Fig. 3.5B). Photosynthetic Light Use Efficiency (PLUE) in non-light saturating conditions was determined as the initial slope of the linear regression discarding all data taken at ambient light intensities above 75 W m^{-2} photosynthetically active radiation (Table 3.3; r^2 averaged 0.91, n>272). PLUE was reduced by both nematodes and soil compaction. In both years, the effect of nematodes was strongest during the second period of measurement.

Measurements of leaf photosynthetic rate at light saturation (PMAX) showed similar effects as on PLUE (Table 3.3), apart from the measurements on August 13, 1992 where compaction increased PMAX.

Root length dynamics

In both years root length increased mainly in the number of days between planting and early July (Fig. 3.6). On non-compacted soil with few nematodes, all cultivars reached root lengths between 9 and 16 km m^{-2}. Root length was largest in cv. Elles, with only small differences among the other cultivars. In 1991, high nematode density and soil compaction reduced root length strongly, especially in cv. Elles. In 1992, root length was greater than in 1991 but the treatments had no statistically significant effect (Fig. 3.6). The large root length reduction of cv. Elles in 1991 was more than offset by its high inherent root length, so for each treatment and harvest time cv. Elles still had a larger root system than cv. Darwina.

Spatial distribution of roots

Root length data for the various sampling positions were used to calculate the relative vertical and horizontal extension of roots. Vertical extension was quantified as the root length density below 0.2 m depth divided by the density above 0.2 m. Horizontal extension was quantified similarly as the ratio of root length densities in between planting

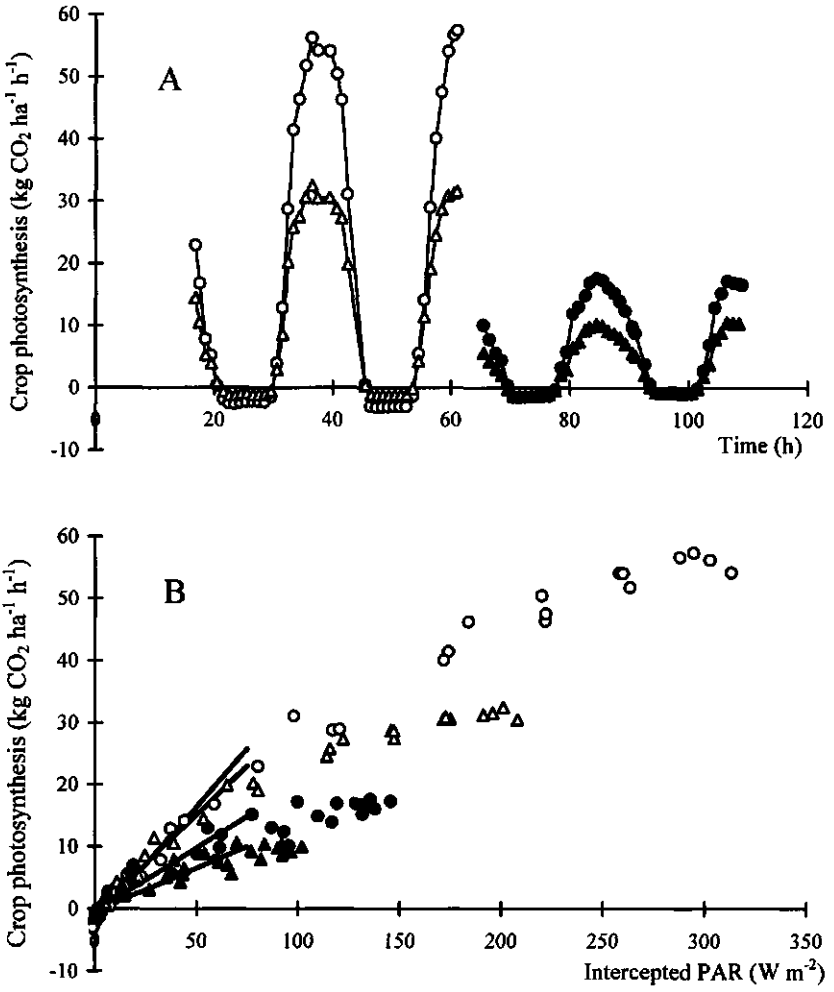

Figure 3.5. Measurements with crop enclosures, cv. Darwina, 1-5 July 1991. A: Diurnal courses of crop photosynthetic rate (kg CO_2 ha^{-1} ground area h^{-1}), for various treatments; B: Same data on photosynthetic rate as in A, but relative to concurrent rates of interception of photosynthetically active radiation (W m^{-2} ground area). The graph also shows the linear regression lines for photosynthetic rate as a function of intercepted radiation under non-light saturating conditions (below 75 W m^{-2}), the slopes of which indicate crop photosynthetic light use efficiency (PLUE, see Table 3.3). —O— Non-compacted, fumigated; —●— compacted, fumigated; —Δ— non-compacted, not fumigated; —▲— compacted, not fumigated.

Table 3.3. Crop Photosynthetic Light Use Efficiency (PLUE; g CO_2 MJ^{-1}) and leaf photosynthetic rate at light saturation (PMAX; mg CO_2 m^{-2} s^{-1}). SED = standard error of difference.

	Year	Period	Cultivar	Nematode density	Compaction level	
					Loose	Severe
PLUE	1991	July 1-5	Darwina	Low	10.4	5.7
				High	8.8	3.8
		July 29-Aug. 2	Darwina	Low	12.2	8.2
				High	3.0	5.4
	1992	July 13-17	Darwina	Low	9.1	7.7
				High	9.1	7.5
		Aug. 10-14	Darwina	Low	7.4	7.3
				High	6.0	5.9
PMAX	1991	July 5	Darwina + Elles	Low	0.52	0.49
				High	0.57	0.40
			SED (d.f. = 3)		0.060	
		July 16	Darwina + Elles	Low	0.69	0.70
				High	0.58	0.55
			SED (d.f. = 5)		0.089	
	1992	Aug. 13	Darwina + Elles	Low	0.36	0.45
				High	0.27	0.43
			Bintje + Mentor	Low	0.33	0.32
				High	0.29	0.35
			SED (d.f = 4)		0.041	

positions and directly below plants. Even at the latest harvest, a fully homogeneous root distribution, with both ratios equal to one, was not found (Table 3.4). High nematode density reduced root spatial extension only in 1991, with average reductions of 30 and 13% for vertical and horizontal extension, respectively (Table 3.4). Severe soil compaction affected vertical and horizontal extension in 1991 by 32 and 55%, respectively, and in 1992 by 10 and 43%, respectively (Table 3.4). There were no statistically significant interactions between high nematode density and soil compaction for horizontal or vertical root extension. The spatial distribution of roots did not differ significantly among the cultivars.

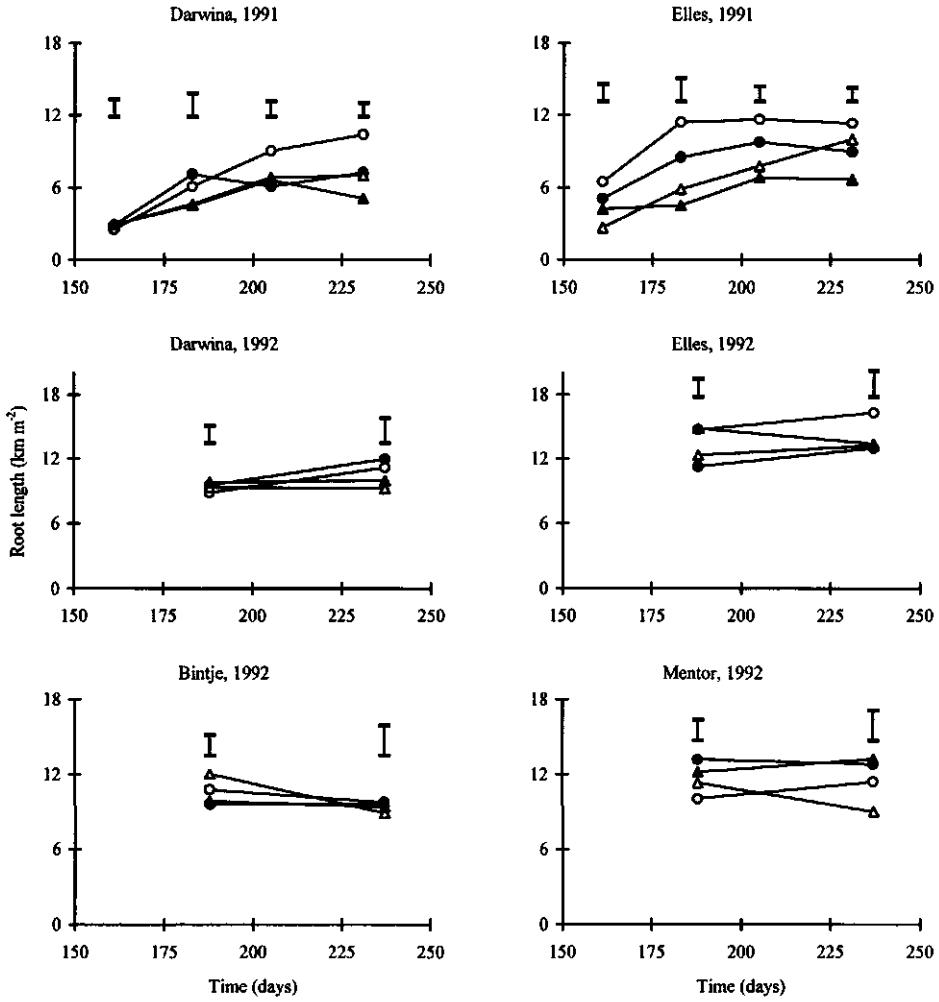

Figure 3.6. Time courses of total root length (km m^{-2}), for various treatments. Vertical bars above harvest data indicate standard errors of difference (1991: d.f. = 12; 1992: d.f. = 36). —O— Non-compacted, fumigated; —●— compacted, fumigated; —Δ— non-compacted, not fumigated; —▲— compacted, not fumigated.

Table 3.4. Spatial distribution of roots at harvest 4 (August 19, 1991; August 24, 1992). Vertical extension is calculated as the ratio of root length density (RLD, m root cm^{-3} soil) at depth 0.2-0.4 m relative to RLD at depth 0.0-0.2 m. Horizontal extension is calculated as the ratio of RLD in soil columns (0.0-0.4m) between plants relative to RLD in soil columns below plants. Differences between cultivars were not significant, so only average data for all cultivars are given. SED = standard error of difference.

	Year		Nematode density		Compaction level		
			Low	High	Loose	Light	Severe
Vertical extension	1991	mean	0.61	0.43	0.63	0.50	0.43
		SED (d.f. = 18)	0.066			0.081	
	1992	mean	0.51	0.68	0.63		0.56
		SED (d.f. = 36)	0.080			0.080	
Horizontal extension	1991	mean	0.70	0.61	0.96	0.57	0.44
		SED (d.f. = 18)	0.119			0.146	
	1992	mean	0.73	0.71	0.92		0.52
		SED (d.f. = 36)	0.092			0.092	

Specific root length and root-shoot ratio

Soil compaction reduced root weight to a similar extent as root length. High nematode density primarily affected root weight and thus increased the specific root length (SRL: m root g^{-1} root weight), especially in 1991 (Table 3.5). The cultivars differed strongly for SRL, with cv. Elles having the lowest SRL at both nematode densities in both years.
Root-shoot ratios (Table 3.6) decreased strongly during the season, but treatment effects were consistent, so only data for the second harvest are given. Soil compaction increased root-shoot ratio in both years (Table 3.6), but high nematode density did not cause any statistically significant effects. Cultivar differences in root-shoot ratio resembled those in SRL, but in the opposite direction: cv. Elles had the highest root-shoot ratio for most treatments in both years.

Table 3.5. Specific root length (m g^{-1} root fresh weight), average of four harvest dates. Effects of soil compaction were not statistically significant and are not shown in the table. SED = standard error of difference (value in parentheses is for the comparison within the same level of nematode density).

Year	Cultivar	Nematode density	
		Low	High
1991	Darwina	79	113
	Elles	56	62
	SED (d.f. = 12)	13.2 (9.3)	
1992	Darwina	73	54
	Elles	32	41
	Bintje	65	98
	Mentor	59	57
	SED (d.f. = 36)	17.3 (16.6)	

Table 3.6. Root-shoot ratio at the second harvest (July 2, 1991; July 6, 1992), calculated as the dry weight ratio of the roots and the sum total of stems, leaves and tubers. Root dry weight is estimated as 0.08 times root fresh weight. SED = standard error of difference (value in parentheses is for the comparison within the same level of soil compaction).

Year	Cultivar	Compaction level		
		Loose	Light	Severe
1991	Darwina	0.028	0.040	0.049
	Elles	0.054	0.071	0.111
	SED (d.f. = 12)		0.0241 (0.0187)	
1992	Darwina	0.050		0.079
	Elles	0.071		0.106
	Bintje	0.040		0.066
	Mentor	0.062		0.101
	SED (d.f. = 36)		0.0208 (0.0205)	

Root senescence

In both years root senescence rate was about 1 % d^{-1} for all cultivars growing under optimal conditions, i.e. fumigated, non-compacted soil. Statistically significant acceleration of root senescence due to nematodes or compaction was only observed in 1991. In that year either high nematode density or soil compaction or both accelerated root senescence of cv. Darwina, up to 4-6 % d^{-1}, but only the combination of both stress-factors caused a similar acceleration in cv. Elles.

Nutrient concentrations

At all harvest dates, concentrations of nitrogen, phosphorus and potassium were measured in leaves, stems and tubers. The concentrations decreased during the growing season, especially in leaves and stems. An exception to this pattern was found in plants on compacted soil in 1991, where the initial reduction in leaf and stem N-concentration was reversed at the later harvests (Fig. 3.7). In most other cases treatment effects on nutrient concentrations remained consistent during the season, so only seasonal averages were subjected to analysis of variance (Table 3.7).

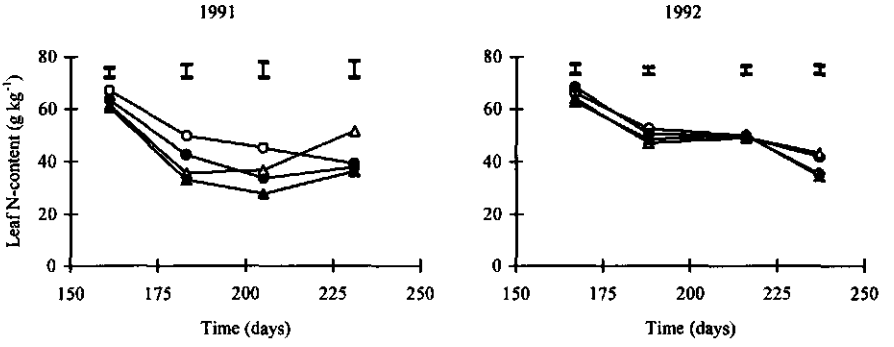

Figure 3.7. Time courses of leaf nitrogen concentration (g N kg^{-1} dry matter) in cultivar Darwina. Treatments and symbols as in Fig. 3.6. Vertical bars above harvest data indicate standard errors of difference (1991: d.f. = 6; 1992: d.f. = 12).
—O— Non-compacted, fumigated; —●— compacted, fumigated;
—Δ— non-compacted, not fumigated; —▲—compacted, not fumigated.

Table 3.7. Nutrient concentrations (g kg^{-1} dry matter), for non-compacted, fumigated soil (Control), non-fumigated soil (Nematodes), severely compacted soil (Compaction) and non-fumigated, severely compacted soil (Nematodes + Compaction). Data are averages for all harvest dates. For each of the 19 combinations of year (1991/1992), organ (leaves, stems, tubers) and nutrient (total nitrogen, nitrogen in nitrate, phosphorus, potassium) a separate analysis of variance was carried out and standard errors of difference (SED) calculated.

Year	Treatment	Leaves				Stems			Tubers		
		N	NO_3^-	P	K	N	P	K	N	P	K
1991	Control	52.2	4.4	4.2	50.3	29.0	3.6	66.5	15.2	2.5	21.6
	Nematodes	46.7	2.7	3.7	40.2	22.1	2.5	42.2	12.3	1.9	16.8
	Compaction	47.1	1.7	3.4	40.3	21.6	2.4	40.3	14.5	1.9	18.8
	Nematodes + Compaction	41.4	1.5	3.0	31.0	18.9	2.2	28.4	13.4	2.1	18.0
	SED (d.f. = 6)	1.53	0.43	0.22	2.22	1.60	0.16	2.53	1.24	0.06	0.75
	Darwina	45.6	2.3	3.5	41.0	22.6	2.5	43.2	14.0	2.1	19.0
	Elles	47.4	2.5	3.7	38.9	21.9	2.8	44.2	13.1	2.2	18.6
	SED (d.f. = 6)	0.47	0.10	0.05	0.99	0.79	0.08	1.68	0.71	0.06	0.72
1992	Control	51.6	-	3.3	47.0	35.3	2.8	58.9	15.8	2.3	21.9
	Nematodes	50.0	-	2.9	41.6	33.3	2.1	52.1	15.5	1.9	20.8
	Compaction	51.0	-	2.9	43.4	34.9	2.2	56.2	16.4	1.9	22.3
	Nematodes + Compaction	48.5	-	2.7	40.5	33.6	2.0	51.2	17.0	1.8	22.5
	SED (d.f. = 12)	0.26		0.10	1.26	0.95	0.11	3.15	0.35	0.07	0.63
	Darwina	50.7	-	3.0	39.9	35.1	2.3	54.3	15.3	1.9	21.9
	Elles	53.3	-	3.3	43.3	33.0	2.8	55.9	14.2	2.1	21.8
	Bintje	48.6	-	2.9	44.1	34.9	2.3	53.8	18.6	2.1	22.2
	Mentor	48.5	-	2.7	45.2	34.1	1.8	54.5	16.6	1.8	21.5
	SED (d.f. = 12)	0.90		0.05	0.97	0.97	0.06	1.34	0.42	0.07	0.36

-, no data collected

Both high nematode density and soil compaction significantly decreased all nutrient concentrations in 1991. In 1992 the effects were much smaller, albeit still statistically significant except for N and K in stems and leaves (Fig. 3.7, Table 3.7). Percentage reductions in nutrient concentration were lowest for nitrogen and highest for potassium. However, leaf nitrate-N, only measured in 1991, responded most strongly to the treatments. The reductions were largest in stem tissue and lowest in the tubers.

Compared to the effects of nematodes and soil compaction, differences among cultivars in nutrient concentration were small (Table 3.7). Moreover, only in three cases (all in 1992:

leaf P and K, stem P) significant interactions of cultivar with soil treatment were found. These interactions explained less than 3.2% of measured variation, so the nutrient concentrations of the cultivars differed as little in their response to treatments as in their average values. Table 3.7 only shows the average values.

Nutrient inflow rates

The data of nutrient concentrations were combined with data of total biomass and root length, to calculate nutrient inflow rates, i.e. rates of nutrient uptake per unit root length per day (Fig. 3.8). High nematode density and soil compaction reduced inflow rates for nitrogen, phosphorus and potassium. No consistent differences between years or cultivars were found, except for a higher nutrient inflow rate in cv. Darwina in the absence of stress factors.

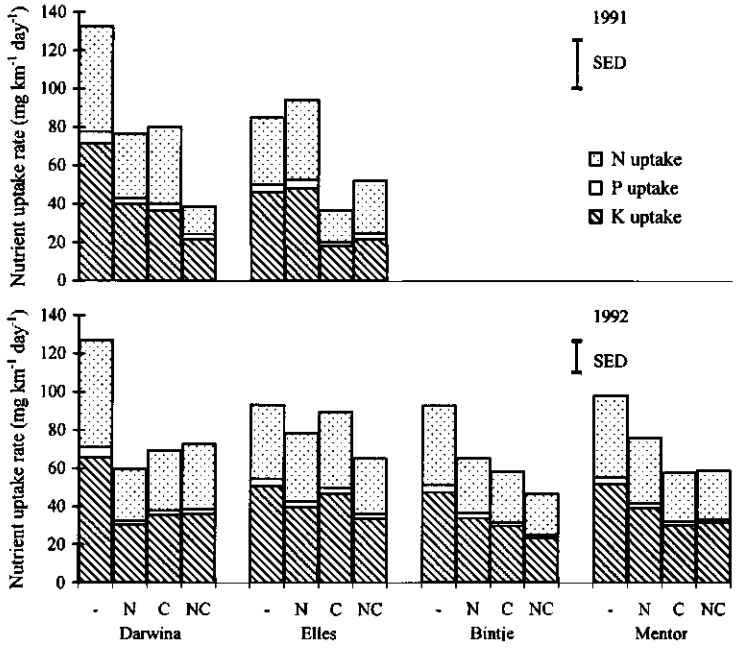

Figure 3.8. Average inflow rates (mg nutrient km^{-1} root d^{-1}), between first and fourth harvest, of nitrogen-N, phosphorus-P and potassium-K, for non-compacted, fumigated soil (-), non-compacted, non-fumigated soil (N), severily compacted, fumigated soil (C) and severily compacted, non-fumigated soil (NC). Error bars indicate standard errors of difference for total nutrient inflow rate (N + P + K).

DISCUSSION

Effectivity of nematicide and compaction treatments

Nematicide application reduced nematode density by 91% in 1991, and by 85% in 1992. It is not clear what caused the greater nematicide effectivity, at lower dose rate, in 1991. The nematicide applied may have killed other soil organisms interacting with the crops, but since cyst nematode density was high in both years, we assume that the established differences in nematode density dominated the effects of fumigation on crop growth.
In 1991, the compaction procedure was more effective in increasing soil penetration resistance at depths greater than 15 cm, than it was in 1992 (Fig. 3.1), possibly because of the low soil water content in the latter year. Compaction probably did not affect the nematodes themselves, because unlike migratory plant-parasitic nematodes, which lack a protective cyst, cyst nematodes are not sensitive to mechanical damage (Boag, 1988).

Interaction of nematodes and soil type

Both high nematode density and soil compaction reduced final tuber yields (Table 3.1). The effects were mostly additive with but one exception: cv. Elles suffered yield loss due to nematodes only on compacted soil in 1991. Our experiments therefore did not confirm the results of Whitehead and Nichols (1992), who found greatest losses on loose soil. Variation among soil types in yield loss by similar densities of nematodes (Trudgill, 1986; Evans and Haydock, 1990) therefore is probably caused by differences in soil characteristics other than penetration resistance to root growth.

Differences between nematodes and soil compaction

The effects of soil compaction were similar for all cultivars in our experiments, so no cultivar with tolerance of compaction was identified. The cultivars did respond differently to nematodes, with cv. Elles the most tolerant. Nematodes and compaction also differed in that only the nematode effect varied strongly between the two experimental years. We conclude that nematodes and compaction affect potato growth by different damage mechanisms, which may explain their largely additive effect on yield.
In the absence of interactions, the main effects are summarized in Table 3.8 for cvs. Darwina and Elles, grown in both experiments. Yield loss due to nematodes averaged 26% for these cultivars over both years. This was mainly accounted for by reduction in

cumulative light interception (PARCUM) with less reduction of the light use efficiency (LUE) (Table 3.8). Compaction caused similar losses as nematodes (27% on average) but mainly affected LUE. Part of the reductions of PARCUM were caused by reduced LUE-values, because a lower LUE will lead to a reduced crop growth rate resulting in less light intercepting leaf area. However, both nematodes and compaction also reduced PARCUM more directly than via LUE, namely by reducing SLA and accelerating leaf senescence (Table 3.8). Compaction further reduced PARCUM by decreasing the fraction of biomass allocated to leaves early in the growing season (Fig. 3.4). Nematodes did not affect partitioning between leaves, stem and tubers, and any change of root-shoot partitioning was probably small (Table 3.6).

This analysis of the component factors determining PARCUM reveals further differences between the damage mechanisms of nematodes and soil compaction. Nematodes mainly reduce PARCUM by accelerating leaf senescence, while compaction mainly reduces PARCUM by reducing LUE and SLA and initially also by decreasing partitioning to leaves (Table 3.8; Fig. 3.4). Therefore compaction mainly delays crop closure early in the growing season, whereas nematodes primarily accelerate foliage death during later growth stages (compare Fig. 3.3).

The measurements of crop and leaf photosynthesis (PLUE, PMAX) tend to confirm the observed reductions of LUE (Table 3.8), except for the increase in PMAX by compaction at the end of the growing season. The late-season stimulative effect of compaction, which was also observed for ground cover (Fig. 3.3) may be explained by slower depletion of soil nutrients and water by the slow growing plants on compacted soil.

Differences among cultivars for tolerance to nematodes

The same analysis of PARCUM and LUE can be applied to explain the relatively strong tolerance of cv. Elles to nematodes. Cv. Elles suffers equal reductions of LUE as the other cultivars and its tolerance is therefore based on a smaller reduction of PARCUM (Table 3.8). Surprisingly, nematodes affected SLA and leaf senescence of cv. Elles to the same extent as in the other cultivars, while partitioning was unaffected in all cultivars, as indicated above. We conclude that the tolerance of cv. Elles is not caused by a better response to nematode infestation, but rather by a more favourable growth habit, in which much leaf area is produced because of a prolonged partitioning of biomass to leaves (Fig. 3.4) and a high SLA. This confirms the findings of Trudgill (1986), who found relatively low nematode-induced yield loss in cultivars with large tops. Since prolonged partitioning to leaves is a common characteristic of late maturing potato cultivars, we expect yield loss to be negatively correlated with cultivar lateness.

Table 3.8. Average effects (% change compared with plants from fumigated, non-compacted plots) of high nematode density and severe soil compaction on crop characteristics of cvs Darwina and Elles in both experiments.

Crop characteristic			Effect of nematodes		Effect of
	Darwina	Elles	1991	1992	compaction
Yield [1]	-42	-9	-40	-12	-27
PARCUM [1]	-39	-12	-41	-10	-15
LUE [1]	-12	-13	-20	-5	-20
SLA	-7	-6	-9	-3	-11
leaf senescence [2]	+107	+34	+139	+4	+7
PLUE [3]	-25		-40	-9	-23
PMAX [3]			-13	-15	+7

[1] Data derived from Table 3.1.

[2] Data derived from Table 3.2.

[3] Data derived from Table 3.3.

Root growth

Root length growth can be considered as the product of three factors: overall crop growth rate, the fraction of growth that appears as root biomass, and the specific root length. Net root length increase is the difference of this product and root senescence rate. Both net root length increase and its four constituents have been quantified so the main determinants of treatment effects can now be identified.

Soil compaction reduced net root length growth (Fig. 3.6) less than overall crop growth (Fig. 3.2, Table 3.1), although root senescence was accelerated and specific root length unchanged. Compaction thus must have stimulated allocation of biomass to roots, as also indicated by increased root-shoot ratios in both years (Table 3.6).

Nematodes also accelerated root senescence in 1991 but seemed to increase specific root length in both years (Table 3.5). Nematodes may have increased root branching near infected root tips, thereby increasing the frequency of thin roots and increasing average specific root length, but we did not collect morphological data to test this assumption. Although root-shoot ratios were not statistically significantly increased at high nematode density (Table 3.6), nematodes may have stimulated allocation of biomass to roots if an effect on root-shoot ratio was masked by increased root senescence.

Cultivar Elles differed greatly from the other cultivars in root characteristics. Although the specific root length of cv. Elles was lowest (Table 3.5), its total root length was highest (Fig. 3.6) because of the large fraction of biomass allocated to roots (Table 3.6). Nematodes and soil compaction increased root senescence in cv. Elles less than in cv. Darwina, especially in 1991. Possibly, the relatively thick roots of cv. Elles afforded some protection against damage.

In summary, whenever root length was reduced, the main cause was accelerated root senescence rather than impaired root growth. Plants tended to minimize the reduction of root length density by increasing allocation to roots and, with nematodes, by increasing the specific root length.

Nutrient concentrations

Nutrient concentrations were positively correlated with total root length. The large reductions of root length in 1991 (Fig. 3.6) coincided with large reductions of nutrient concentrations (Table 3.7), while both variables were relatively unaffected by treatments in 1992. Both root length and leaf concentrations of nitrogen and phosphorus were significantly higher in cv. Elles than in the other cultivars. However, concentration-reducing treaments generally increased root-shoot ratios (Table 3.6), leaving less plant weight to be supplied with nutrients per unit root weight and length. We therefore conclude that the correlation between concentrations and root length has no causal basis, and variation in concentrations must therefore be explained by factors affecting uptake per unit root length (Fig. 3.8) rather than root system size.

High nematode density and soil compaction may have reduced the capacity for nutrient uptake by restricting vertical and horizontal extension of roots (Table 3.4), leading to insufficient soil colonization and exhaustion of nutrients within reach. However, a direct effect of nematode infection on uptake capacity of roots cannot be excluded.

Mechanisms of yield loss

Both reduction of light interception and reduction of light use efficiency have been shown to be involved in the response of potato cultivars to nematodes and soil compaction. However, these reductions are but the end-result of primary responses originating in the root system. The reported data on root length dynamics and nutrient uptake allow us to elucidate the link between root and shoot responses. In 1992, high nematode density and soil compaction had little effect on nutrient concentrations (Table 3.7), nor did they affect leaf senescence (Table 3.2). However, high nematode density did decrease yields in 1992,

by 23% on average for the intolerant cultivars Darwina, Bintje and Mentor, while compaction reduced yields by 20% (Table 3.1). We conclude that nutrient deficiency was not the likely cause of damage in 1992. Nematodes and compaction probably did not induce water stress either, since the plants were repeatedly irrigated, and soil water contents remained equally high for all treatments. Passioura (1988) and Masle (1990) have shown for drought and soil compaction, respectively, that soil-related stress factors may trigger hormonal root signals which impair shoot functioning. The same mechanism has been proposed, on the basis of pot experiments, for potato cyst nematodes (Schans, 1991), following earlier findings that abscisic acid concentrations were increased after potato cyst nematode infection (Fatemy *et al.*, 1985). Possibly, the present results for 1992, i.e. nematode damage without nutrient deficiency, may be explained in a similar manner.

In 1991, yield losses were much higher than in 1992. Moreover, the losses were accompanied by strong reductions of nutrient concentrations. Nutrient deficiency may therefore have caused the extra high losses. Occurrence of nutrient deficiency in 1991 may also explain why only in that year leaf senescence rates were accelerated (Table 3.2). However, it seems probable that even in 1991 nutrient deficiency only occurred in the second half of the growing season, since differences between nutrient concentrations only became prominent from the time of the second harvest (Fig. 3.7A). Foliage development was by then already strongly reduced by nematodes so root signalling rather than nutrient deficiency may have caused the initial delay of crop growth.

The results do not allow conclusions as to which of the nutrients became deficient, since concentrations of N, P and K were all reduced. Lorenz and Tyler (1983; cited by Walworth and Muniz, 1993) reported that mid-season concentrations in potato leaf blades of 5% N, 0.4% P and 3.5% K are sufficient for maximum growth. If these data apply to our cultivars and conditions as well, only phosphorus levels may have become deficient (Table 3.7). Nutrient concentrations were reduced to similar extent in all cultivars, including the tolerant cultivar Elles, which suffered relatively little yield loss. However, the absolute uptake rate of nutrients (g m^{-2} soil d^{-1}) was always highest in cv. Elles, and dry matter increase was least impaired. Although this does not prove a causal relationship, it does suggest that uptake rates are better suited than concentrations as indicators of nutrient deficiency. The high level of tolerance of cultivar Elles may thus be explained by its producing the largest root system and thereby maintaining the highest nutrient uptake rates. A positive correlation of root length of potato cultivars with tolerance of cyst nematodes has earlier been indicated by Evans and Haydock (1990).

It is not completely clear why high nematode density led to strong root length reduction and nutrient deficiency only in 1991, given that the initial density of nematodes was higher in the following year. The higher soil temperatures in 1992 may have provided better conditions for root growth (Fig. 3.6), but that does not explain the smaller effect of

nematodes on root length. Possibly, movement and penetration efficiency of the nematodes was hampered by the somewhat drier conditions in 1992, even though the irrigation was sufficient to prevent any visible drought symptoms on the plants themselves and control yields were similar in both years.

The results of this paper do not explain the common observation that nematode damage varies with soil type (Trudgill, 1986). Nematodes and compaction mostly showed additive effects, both at the level of overall crop growth and at the level of the underlying processes of root length dynamics and nutrient uptake. Variation in soil density or penetration resistance thus does not seem to affect the plant-pest relationship.

The field experiments reported here lead to the following general scheme of nematode-related damage to crops. The primary response to nematodes, possibly initiated by hormonal root signals, involves impairment of photosynthesis, a decrease in specific leaf area, and possibly also a decreased allocation of biomass to tubers and shoot in favour of root growth. A secondary response may follow if nematode infection is so severe that root lengths are strongly decreased, leading to chronic nutrient deficiency (Trudgill, 1980) in the second half of the growing season, and, finally, acceleration of crop senescence.

In this paper we have analysed nematode-related damage mechanisms in some detail, and identified various ways in which crop growth was affected. This will provide data for the further development of a computer model which simulates dynamically the interaction between the growing crop and the nematode population in soil and roots (Van Oijen *et al.*, 1995). The model is intended to define the characteristics that make a cultivar tolerant.

CHAPTER 4

POTATO GROWTH AS AFFECTED BY POTATO CYST NEMATODES (*GLOBODERA PALLIDA*) AND SOIL PH. 1. ROOT GROWTH AND NUTRIENT UPTAKE

ABSTRACT

The effects of potato cyst nematodes (*Globodera pallida*) and soil pH on potato crop growth were studied in the field and in the Wageningen Rhizolab. The experimental field was infested with potato cyst nematodes and two levels of nematode density were established by fumigation with a nematicide. Prior applications of calcium carbonate resulted in pH_{KCl} levels of 4.8 and 6.1. Two levels of phosphorus fertiliser were applied: either 0 or 225 kg P ha^{-1}.

In the Wageningen Rhizolab, soil of both pH levels from the field was used after treatment with 1 MRad gamma irradiation to kill the nematodes. Subsequently, half of the soil was inoculated with cysts to give a nematode density of 30 viable juveniles per gram of soil.

At 61 days after planting, crop growth in the field experiment at pH 6.1 was limited by P deficiency and the ratio root length to leaf area (RL/LA) was increased. Phosphorus fertilisation relieved P deficiency and decreased the RL/LA from 3.27 to 1.88 km m^{-2}. Nematodes induced or aggravated P deficiency, but had a small effect on RL/LA. Both nematodes and high soil pH reduced nutrient uptake per unit root length.

In the Wageningen Rhizolab, directly after planting, the number of roots visible against minirhizotrons was reduced by nematodes and lagged behind the control. However, the increase in root number of the nematode treatment continued longer until root number was higher than that of the control. The increased root number of the nematode treatment was restricted to the top 30 centimetres and nematodes reduced rooting depth. The absence of an effect of nematodes on the RL/LA in the field experiment where P deficiency was induced was explained by a delayed compensatory root growth.

INTRODUCTION

Potato cyst nematodes (*Globodera* spp.) are major pests of potato in the Netherlands and cause severe yield losses. Potato cyst nematodes survive in the soil as eggs within a cyst. When a host crop is grown, exudates from the roots stimulate the eggs to hatch (Triffitt, 1930). Subsequently, the juveniles invade the roots, thereby destroying root cells, and induce a feeding site (Jones, 1981). These processes have a direct effect on root elongation as root length is reduced within a few days (Arntzen *et al.*, 1994; Rawsthorne and Hague, 1986). When measured in field experiments, crops infested by potato cyst nematodes generally have a reduced root weight several weeks after planting. However, nematodes reduce top growth more than root growth, resulting in a reduced ratio of top weight to root weight (Evans, 1982; Trudgill and Cotes, 1983b). This indicates that nematodes not only reduce root elongation but also impair physiological functioning of the roots.

Nematodes reduce nutrient uptake, as concentrations of nitrogen, phosphorus and potassium in the foliage of infested crops are reduced (Evans and Franco, 1979; Trudgill *et al.*, 1975a, 1975b). Reduced growth of infested plants may therefore be caused by nutrient deficiency induced by the nematodes. Trudgill (1980) showed in a pot test that nematode damage interacted with the availability of N and of P. Doubling the amount of N and P more than doubled total biomass of infested plants and reduced nematode damage from 71 percent to 25 and 29 percent. These results were corroborated in field experiments where application of compound fertiliser increased percentage ground cover and yield more at high nematode densities than at low densities (Trudgill, 1987).

Another indication of decreased nutrient uptake caused by potato cyst nematodes is the interaction between nematode damage and soil pH (Haverkort *et al.*, 1993). In a container experiment, nematodes reduced tuber yield by 19% at pH_{KCl} 4.5, but by 44% at pH_{KCl} 6.5. Soil pH affects the solubility of ions in the soil and thereby the availability of phosphorus. On acid soils, below a pH_{KCl} of 4.5, phosphorus availability is reduced by increases in the availability of aluminium. At high soil pH, increased concentrations of calcium result in increased phosphorus fixation. The increased yield loss caused by potato cyst nematodes at pH_{KCl} 6.5 compared with pH_{KCl} 4.5 may be explained by an effect of both soil pH and nematodes on the uptake of phosphorus.

The aim of our study was to examine the effects of potato cyst nematodes and soil pH on potato growth and in particular the role of phosphorus in nematode damage. Therefore, we carried out experiments in which we varied nematode density, soil pH and phosphorus availability. This paper describes the effects of potato cyst nematodes and soil pH on root growth and nutrient uptake. The effects on foliar nutrient concentrations and crop growth are discussed in Chapter 5.

MATERIALS AND METHODS

Two experiments in 1995 studied the effects of potato cyst nematodes (*Globodera pallida*) and soil pH on above and below-ground growth of potato cv. Mentor. Mentor is a mid-late variety (Anon., 1992a) and is susceptible to and intolerant of potato cyst nematodes (Velema and Boerma, 1988). Both experiments, one in the field and one in the Wageningen Rhizolab, were carried out with soil of the same origin, a sandy soil with about 6% organic matter.

Experiment 1, field experiment

The field experiment was in Vlagtwedde, the Netherlands on a site that was naturally infested with potato cyst nematodes. The pH of the soil, measured as pH_{KCl}, was 5.0 in 1984 and by applying various amounts of calcium carbonate over four years pH values of 5.2 and 6.9 were obtained. Since 1987 no calcium carbonate was applied and pH values decreased to 4.8 and 6.1.

There were six blocks, each of which was split for the two levels of soil pH. Perpendicular to this, each block was split and one half was fumigated with sodium methyl-dithiocarbamate (Monam, 51% a.i.; 600 litre ha^{-1}) on March 24. The four subblocks were further split into two plots that received either zero or 225 kg P per hectare, applied as triple super phosphate. The phosphorus fertilizer and a basal dressing of 230 kg N and 125 kg K were applied prior to the soil fumigation. Plot size was 8 m x 4.5 m.

Nematode densities prior to planting were determined by sampling about 400 g of soil of each plot to a depth of 25 cm. After rinsing of each sample all cysts were counted and 300 cysts were soaked for one week in tap water. The cysts were crushed and the eggs were exposed to fresh potato root diffusate. Ten days later, total emerged juveniles were counted. With this procedure, more than 95 percent of the viable eggs are detected (Regeer, 1997).

The availability of phosphorus was determined 11 weeks after fertilisation and after ridging by measuring the amount of water-extractable soil phosphate (Pw) (Sissingh, 1971) in soil samples taken in the centre of the ridge to a depth of 30 cm.

Seed potatoes, class A, grade 35-55 mm, were planted on April 21 at a spacing of 30 cm within rows and 75 cm between rows. Before canopy closure, 61 days after planting, a group of twelve plants per plot was harvested. After foliage death, 146 days after planting, tubers of 24 plants per plot were harvested. At the first harvest, total fresh weight of the above-ground plant parts was determined and a sample of ten stems was taken and divided into stems and leaves. Leaf area was determined with a LiCor 3100 Area Meter

(Li-Cor Inc., Lincoln, Nebraska, USA). Tubers and the underground part of the stem with stolons and thick roots were dug up and washed. Dry weight of the different plant parts was determined after drying for 24 hours at 105 °C and N, P and K concentrations in the dry matter were determined in samples of leaves, stems and tubers. Total nitrogen concentration was determined using the Dumas-method (Macro N, Foss Heraeus), phosphorus concentration was assessed colorimetrically (Starrcol) after destruction with H_2SO_4/HNO_3 and potassium concentration was measured by atomic sorption (AS Varian AA10).

Root length at 61 days after planting was assessed by taking soil core samples of 4.77 cm diameter in the ridge between plants at three depths (0-15, 15-30 and 30-45 cm). In each plot two replicate samples were taken. The soil samples were stored at -18°C until processing. After thawing, the roots were washed free from soil by hydropneumatic elutriation (Smucker *et al.*, 1982) and root length was determined by the line intersect counting method (Tennant, 1975). Total root length (km m^{-2}) was calculated by multiplying root length density (cm cm^{-3}) with the volume of soil per square metre of surface area for each soil layer of 15 cm. For this, a homogeneous horizontal distribution of roots was assumed, and a triangular shape of the ridges, being 20 cm high and 75 cm wide.

Uptake of N, P and K per unit root length was calculated by dividing total nutrient uptake at 61 days after planting by the total root length at that time.

Experiment 2, Rhizolab experiment

This experiment was carried out in the Wageningen Rhizolab. For a detailed description of research methodology and main functions of this facility we refer to Van de Geijn *et al.* (1994) and Smit *et al.* (1994). The following is a brief description.

The experiment was carried out in four compartments of 1.25 x 1.25 m and 2 m deep. The bottom 1 m of each compartment was filled with coarse sand without organic matter. On top of this a 70 cm layer of a sandy soil with 4% organic matter was placed. Soil for the top 30 cm was taken from the field of Exp. 1 of both levels of soil pH and before fertilisation and soil fumigation. The Pw of the collected soil was 62 for pH 4.6 and 58 for pH 6.1. The soil of both pH levels was irradiated with 1 Mrad gamma radiation that killed all nematodes. Fungi and bacteria are less affected by this level of radiation (Becking, 1971). The soil of both pH levels was split and one half was inoculated with cysts of *Globodera pallida* to a density of 30 viable juveniles per gram of soil. Before inoculation, the content of the cysts was determined by exposing duplicate samples of 500 cysts to potato root diffusate and counting the number of emerged juveniles.

The soil for the top 30 cm was fertilised with amounts equivalent to 100 kg N, 44 kg P and 166 kg K per hectare. For an equal distribution of nematodes and fertiliser, the soil was thoroughly mixed in a concrete mixer.

During filling of the compartments, glass minirhizotrons of 6 cm diameter were installed horizontally at various depths (5, 10, 15, 20, 30, 45, 60, 85 cm). By horizontal placement, effects of the minirhizotrons on spatial distribution of roots through tracking along the glass surface (Chapter 2) were avoided. To measure soil moisture and temperature, ceramic cups and hydrophilic microporous tubes were installed at several depths. Periodically, soil solution was extracted and mineral nitrogen concentrations were determined with a TRAACS 800 continuous flow analysis system.

Single-stem plantlets were used rather than seed tubers to diminish plant variation. Sixteen days before transplanting, tubers were placed 5 cm deep in trays filled with potting compost. On May 12, the plants were carefully detached from the mother tuber and shoots of equal size were selected. The planting pattern was 20 x 25 cm, 30 plants per compartment. The same planting pattern was applied to the guard rows surrounding the compartment.

During growth, soil moisture content was measured regularly by measuring the dielectric constant of the soil and water supply was kept optimal by drip irrigation. No rain water reached the compartments as the Rhizolab was equipped with a rain shelter that unfolds when rain is detected. The crop was protected against late blight (*Phytophthora infestans*) by spraying regularly with a fungicide, according to standard farming practice.

Roots showing at the minirhizotron surface were recorded every 14 days with a video camera (Smit *et al.*, 1994). The video tapes were processed by counting the number of roots visible in each image of 14 mm x 18 mm, 36 images per minirhizotron. Branches were counted as individual roots. For each minirhizotron, the 36 images were averaged and a single number of roots per cm^2 minirhizotron surface was calculated.

Development of the crop was followed by measuring regularly height of the crop. Concentrations of N, P and K were determined in the second leaflet from the top of the topmost fully expanded leaf at several dates. Before foliage death, 101 days after planting, the entire crop was harvested and total crop biomass excluding fibrous roots and foliar nutrient concentrations were determined.

The limited availability of the Rhizolab space did not allow for replication of treatments. To minimise coincidental differences between the compartments, uniform planting material and homogenised soil were used. Potato cyst nematodes and soil pH were applied such as to create clear differences between the treatments.

RESULTS

Experiment 1, field experiment

Prior to planting, nematode population densities at pH 4.8 were almost twice as high as at pH 6.1 (Table 4.1). Soil fumigation reduced initial nematode population densities to low levels of 5 and 6 juveniles per gram of soil for pH 4.8 and 6.1 respectively. The availability of phosphorus, expressed as Pw, was 34 percent higher at pH 4.8 than at pH 6.1 (Table 4.1). Phosphorus fertilisation increased the Pw value by 34 percent at both pH levels, giving a larger absolute increase of Pw value at pH 4.8 than at pH 6.1. Fumigation did not affect the Pw value.

The different treatments significantly affected total biomass at 61 days after planting (Table 4.1). Final tuber yield was affected similarly and the effects on crop growth are described in more detail in Chapter 5. Root length density (RLD) in soil cores between

Table 4.1. Effects of treatments on initial nematode density (juveniles per gram of soil) and phosphorus availability index (Pw) at planting, and their effects on total dry biomass (Ytot, g m^{-2}) and root length density (cm cm-3) at three depths between plants in the center of the ridge at 61 days after planting. Experiment 1. P- = 0, P+ = 225 kg P ha^{-1}.

Treatment			Nematode density	Pw	Ytot (g m^{-2})	Root length density 0-15 cm	15-30 cm	30-45 cm
pH 4.8	P-	Fumigated	5	58	284	2.16	2.24	1.66
		Nematodes	47	64	194	1.25	1.77	0.98
	P+	Fumigated	5	84	289	2.01	1.86	1.34
		Nematodes	47	80	219	1.40	1.57	0.62
pH 6.1	P-	Fumigated	6	44	178	2.11	2.39	1.13
		Nematodes	25	47	107	1.51	1.93	0.66
	P+	Fumigated	6	63	249	1.87	2.08	0.81
		Nematodes	25	59	160	1.08	1.50	0.52
LSD (0.05)			6	10	34	0.64	0.75	0.48

plants in the ridge was decreased by nematodes (Table 4.1). Phosphorus fertilisation also reduced RLD but these reductions were smaller than those caused by nematodes. The effect of soil pH on RLD varied and interacted significantly with depth, mainly because average RLD at 30-45 cm depth was about 30 percent smaller at pH 6.1 than at pH 4.8. At 0-15 and 15-30 cm depth, differences between both pH levels were small. Nematodes and phosphorus fertilisation did not affect vertical distribution of the roots, as there were no significant interactions with depth. There were no significant interactions between the treatments on RLD. Neither were significant interactions found when the initial nematode population density was taken into account by multiple regression analysis.

Nematodes did not significantly affect the ratio between total root length and total leaf area (RL/LA) per plant (Table 4.2). RL/LA was affected by both soil pH and phosphorus fertilisation. RL/LA varied between 4.12 km m^{-2} at pH 6.1 without phosphorus fertiliser and 1.67 km m^{-2} at pH 4.8 with phosphorus fertiliser.

Uptake of N, P and K per unit root length was significantly increased by phosphorus fertilisation (Table 4.2). Soil pH significantly affected uptake of P per unit root length, e.g., without P fertilisation uptake of P was 0.202 g km^{-1} at pH 4.8 and 0.092 g km^{-1} at pH 6.1. Nematodes significantly reduced uptake of P and K per unit root length. There were no interactions between treatments on nutrient uptake per unit root length, nor when the differences in initial nematode population density were taken into account.

Table 4.2. Effects of treatments on the ratio between root length and leaf area (RL/LA, km m^{-2}) and nutrient uptake per unit root length (g km^{-1}), Experiment 1, 61 d.a.p. P- = 0, P+ = 225 kg P ha^{-1}.

Treatment			RL/LA (km m^{-2})	Nutrient uptake of the roots (g km^{-1})		
				N	P	K
pH 4.8	P-	Fumigated	2.08	2.09	0.202	2.73
		Nematodes	2.41	1.79	0.136	2.31
	P+	Fumigated	1.75	2.50	0.271	3.41
		Nematodes	1.67	2.43	0.232	2.94
pH 6.1	P-	Fumigated	3.27	1.43	0.092	1.95
		Nematodes	4.12	1.19	0.064	1.49
	P+	Fumigated	1.88	2.42	0.200	3.25
		Nematodes	2.06	2.32	0.167	2.95
LSD (0.05)			0.90	0.67	0.053	0.79

Experiment 2, Rhizolab experiment

Differences in growth at an early stage in the season are shown in Table 4.3 where crop height at 46 days after planting of the plantlets is shown. Nematodes reduced crop height similarly at both levels of soil pH. At the end of the experiment, 101 d.a.p., total biomass was strongly reduced by nematodes at both pH levels. Soil pH did not affect crop height nor total biomass.

The average number of roots per cm^2 minirhizotron surface increased rapidly in the top 30 centimetres during the first half of June (Fig. 4.1). The number of roots of both control treatments in the topsoil increased until a maximum on June 15 and declined thereafter. From this date onwards, roots were found in the subsoil.

Directly after planting, root growth was reduced by both high soil pH and nematodes (Fig. 4.1). Until July, the number of roots per cm^2 minirhizotron surface in the topsoil was higher at pH 4.6 than at pH 6.1. Soil pH did not affect the number of roots in the subsoil.

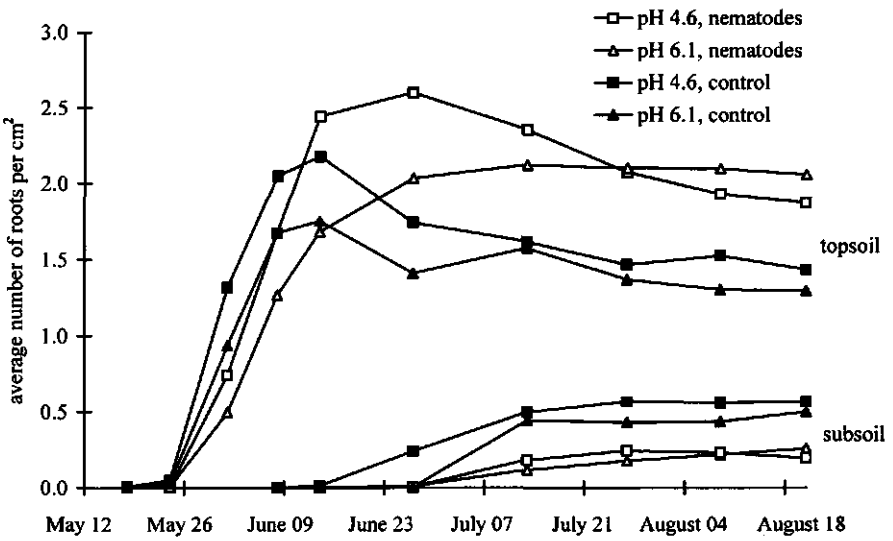

Figure 4. 1. Average number of roots per cm^2 of minirhizotron surface in respectively topsoil and subsoil with time, Experiment 2. For topsoil the observations with the minirhizotrons at 5, 10, 15, 20 and 30 cm depth were averaged, for subsoil the minirhizotrons at 45, 60 and 85 cm depth.

Nematodes initially reduced the number of roots in the topsoil but prolonged root formation, and from mid June onwards, the nematode infested crops had more roots in the top 30 cm than the uninfested crops. Nematodes decreased the number of roots per cm^2 of minirhizotron surface in the subsoil, below 30 cm. Despite this, nematodes increased total root number (topsoil + subsoil) in the second half of the growing season. The maximum depth at which roots were found was 85 cm for the control treatments and 60 cm for the nematode treatments.

Effects of nematodes on foliar nutrient concentrations changed with time (Table 4.4). At the first sampling date, 31 d.a.p., concentrations of N_{total}, NO_3 and P in leaflets of the topmost fully expanded leaf were reduced by nematodes, whereas foliar K concentrations were increased or not affected. During growth, foliar nitrogen concentrations of the controls decreased faster than those of the nematode treatment and in July total nitrogen and NO_3 concentrations of the nematode treatment were higher than those of the control. Foliar P concentrations decreased over time and concentrations of the nematode treatment were lower than those of the control. On the first two sampling dates, it was found that nematodes had strongly reduced foliar P concentrations. At later stages of growth, the effects were smaller. The effect of nematodes on foliar K concentration varied. On June 26 and on August 21, foliar K concentrations were reduced by nematodes. On the other sampling dates, no differences were found.

The effect of soil pH on foliar nutrient concentrations varied. On the first sampling date, it was found that high soil pH did not affect N_{total} concentration but increased the concentration of NO_3. P concentration in the foliage was not affected, K concentration was reduced. In the course of time the effects of high soil pH varied but at the end of the experiment on August 21, foliar N and K concentrations were increased whereas foliar P concentration was decreased.

Table 4.3. Crop height (cm) on June 27 (46 d.a.p.) and total crop biomass (g m^{-2}, dry weight) on August 21 (101 d.a.p.) of four combinations of soil pH and nematode infestation level (juveniles per gram of soil), Experiment 2.

Treatment		Crop height (cm),	Total crop biomass (g m^{-2}),
pH_{KCl}	Nematodes	June 27	August 21
4.6	0	100	2762
	30	72	1693
6.1	0	100	2596
	30	74	1846

Table 4.4. Nutrient concentrations (g kg^{-1}) in dry matter of the second leaflet from the top of the topmost fully expanded leaf, Experiment 2

Treatment		Date of sampling				
		June 12	June 26	July 10	July 24	August 21[1]
	N_{total} (g kg^{-1})					
pH 4.6	control	74.0	64.3	55.1	51.3	42.9
	nematodes	66.8	60.3	59.0	54.6	45.2
pH 6.1	control	74.2	65.1	58.0	49.5	43.4
	nematodes	67.2	63.6	56.9	52.5	48.0
	NO_3 (g kg^{-1})					
pH 4.6	control	4.36	1.28	0.51	0.19	-
	nematodes	4.11	1.28	1.87	0.57	-
pH 6.1	control	6.13	1.99	0.73	0.10	-
	nematodes	4.50	2.39	1.18	0.60	-
	P (g kg^{-1})					
pH 4.6	control	7.41	7.09	4.24	4.62	2.55
	nematodes	4.40	5.18	3.70	3.82	2.37
pH 6.1	control	7.05	6.91	3.85	4.56	2.33
	nematodes	4.52	4.59	3.60	4.00	2.02
	K (g kg^{-1})					
pH 4.6	control	45.8	40.0	29.5	26.2	45.2
	nematodes	45.2	29.3	32.9	25.8	36.1
pH 6.1	control	40.5	39.7	31.3	27.8	49.2
	nematodes	43.5	33.5	26.2	27.7	41.4

[1] concentrations in entire green leaves

Fig. 4.2 shows the cumulative amounts of soluble mineral nitrogen at various soil depths with time. Nitrogen in soil solution of the top soil layers was soon depleted and by the end of June no soluble mineral nitrogen was found in the top 30 cm. With nematodes, soluble mineral nitrogen decreased at a lower rate and nitrogen in the layer 50-70 cm became not depleted, whereas of the control nitrogen in this layer was depleted at July 10, 59 d.a.p. Soil mineral nitrogen in the 70-100 cm layer was lowest with the control treatment at pH 4.6. There were no clear effects of the soil pH treatments on the amount of soluble mineral nitrogen in the different soil layers.

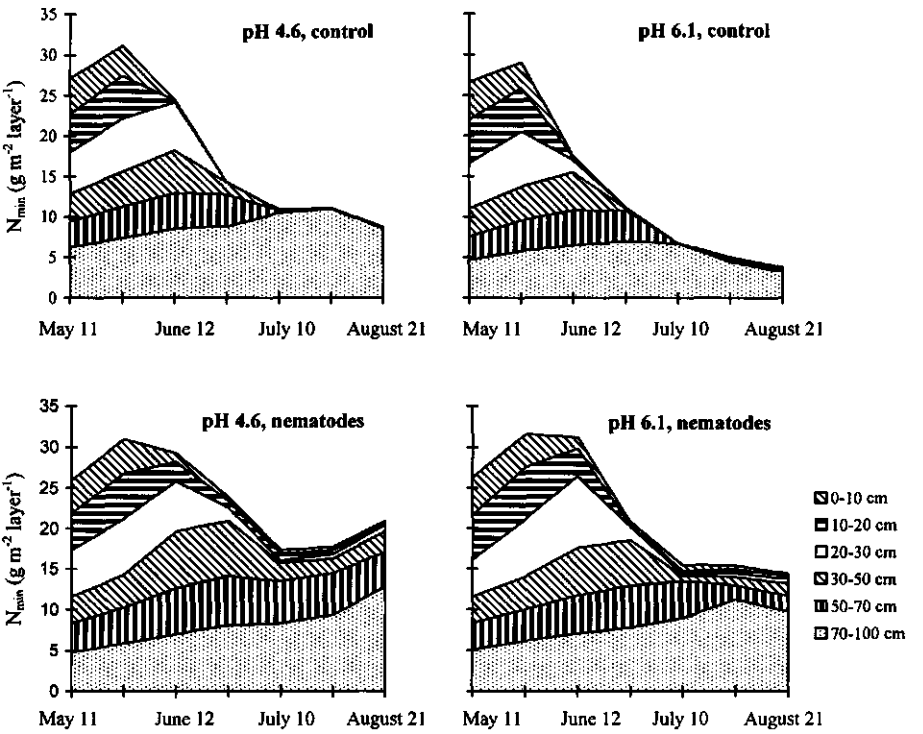

Figure 4.2. Amounts of soluble mineral nitrogen in various soil layers with time in Experiment 2.

DISCUSSION

Effects of soil pH

In Exp. 1, the initial nematode density differed between levels of soil pH and was almost twice as high at pH 4.8 than at pH 6.1 (Table 4.1). The effects of soil pH are therefore best studied within the fumigated treatment, where nematode densities were equally low for both levels of soil pH.

Without nematodes, high soil pH reduced total biomass in Exp. 1, also found previously by Mulder (1994). This was likely caused by phosphorus deficiency, as total biomass was strongly increased by phosphorus fertilisation. Phosphorus deficiency may have resulted from low phosphorus availability, as the Pw value was lowest at pH 6.1.

In Exp. 2, high pH had no effect on crop height nor total biomass, but it reduced root number from planting onwards. The reduced root number may result from a reduced root elongation at high soil pH (Tang *et al.*, 1996), but apparently the root system was still able to sustain plant growth. In Exp. 1, effects of soil pH on root length density varied, but in the deepest sampled soil layer it was lower at pH 6.1 than at pH 4.8. This may not be caused by a reduced root elongation, but rather result from reduced overall crop growth and subsequent smaller root system, as the ratio of root length to leaf area (RL/LA) was increased.

The ratio of root length to leaf area is a comparison of the degree of above and below-ground resource capture and gives information on growth limiting factors (Brouwer, 1983; Körner and Renhardt, 1987). The increased ratio at pH 6.1 indicates that growth was restricted by insufficient below-ground resource capture. Evidently, phosphorus uptake was restricted, as phosphorus fertilisation reduced the RL/LA from 3.27 to 1.88 km m^{-2}, a level similar to the 2.08 km m^{-2} at pH 4.8 (Table 4.2).

We conclude that in our experiments the effect of soil pH was mainly caused by differences in the availability of phosphorus. The Pw values ranged from 44 to 64 and were increased by P fertilisation to 63 and 84. These values are above the target value of 30 in the Netherlands recommendation scheme (Anon., 1992b). However, potato crops require a relatively high P status of the soil and, based on model calculations, Van Noordwijk *et al.* (1990) estimated the Pw requirement for potato on sandy soils to be between 44 and 87. Our experiments support this as phosphorus fertilisation increased biomass production.

Effects of potato cyst nematodes

Nematodes reduced total biomass and in Chapter 5 it is concluded from foliar concentrations of nitrogen, phosphorus and potassium that nematodes induced or aggravated phosphorus deficiency at early stages of growth. Uptake of phosphorus strongly depends on total root length (De Willigen and Van Noordwijk, 1987) and the damage mechanism of the nematodes could be a reduction of total root length. However, above-ground growth was equally reduced, reducing the demand for nutrients. Based on fresh weights, root to shoot ratio's are often increased (Evans, 1982; Trudgill and Cotes, 1983b). Our results give evidence that nematodes affect the functioning of the roots and reduce uptake per unit root length (Table 4.2).

The negative effect of nematodes on root functioning and subsequent phosphorus deficiency was expected to give compensatory root growth and an increased root length to leaf area ratio, as was found at pH 6.1. Nematodes infestation led to some increase of RL/LA but not significantly and much smaller than the effects of soil pH and phosphorus

fertilisation (Table 4.2). However, we sampled at only one occasion in Exp. 1 and the observed differences were the result of processes from the previous nine weeks. The "time course" study in the Wageningen Rhizolab (Exp. 2) showed that nematodes reduced the number of roots against the minirhizotrons directly after planting and delayed the increase of root number (Fig. 4.1). This corresponds to studies of the effects of nematodes on root growth *in vitro*, where nematodes reduced root elongation within a few days (Arntzen *et al.*, 1994; Rawsthorne and Hague, 1986). However, root number of the nematode infested crops increased over a longer period and at about one month after planting there were more roots with nematodes than with the control. This compensatory root growth was likely a response to phosphorus deficiency, as nematodes strongly reduced foliar phosphorus concentrations (Table 4.4). By the time that the nematode treatments had more roots than the controls, the differences in foliar phosphorus concentrations were small. Then, the nematode treatments had an increased number of roots, sustaining a decreased foliage.

A prolonged exploration of new parts of the soil by the roots will cause nematodes to hatch from the cysts that penetrate the roots and reduce elongation. Until most nematodes are hatched and have penetrated the roots, compensatory root growth and reduced root elongation will occur simultaneously. This may explain the absence of compensatory root growth of the nematode infested crop at the first harvest in the field, whereas it was present at pH 6.1. Phosphorus deficiency will therefore be overcome after depletion of the nematode pool when compensatory root growth indeed will increase root length. Because of a wider plant spacing in the field experiment, complete rooting of the soil, depletion of the unhatched nematode pool and subsequent recovery of P deficiency will be slower than in the Wageningen Rhizolab.

Nematodes affect spatial root distribution and often reduce root growth in deeper soil layers, thereby diminishing access to water and nutrients (Evans and Haydock, 1990). In a Rhizolab experiment similar to Exp. 2, Haverkort *et al.* (1994) found that nematodes increased root growth in the topsoil and strongly reduced root formation in the subsoil. As nitrogen in soil solution of the top soil was soon depleted, Haverkort *et al.* (1994) concluded that reduced growth and early senescence of an infested crop may be caused by nitrogen deficiency at the end of the season. In our experiments, nematodes slightly reduced root growth in the subsoil (Fig. 4.1). We also found that nitrogen in soil solution of the top soil was soon depleted (Fig. 4.2). However, crop growth was also reduced and thereby the demand for nitrogen. At the end of Exp. 2, we found an increase of the foliar nitrogen concentrations with nematodes and therefore no lack of nitrogen.

Interaction between soil pH and potato cyst nematodes

The greater nematode density in Exp. 1 at pH 4.8 than at pH 6.1 (Table 4.1) complicates providing a quantitative description of nematode damage in relation to soil pH. There were no significant interactions between the effects of soil pH and either the soil fumigation treatment or the initial nematode population density on root length density or nutrient uptake per unit root length. In Chapter 5, however, a significant interaction was found between the effects of soil pH and initial nematode population density on total biomass. The absence of interaction effects on root length density and nutrient uptake per unit root length may be explained by the single measurement date in Exp. 1. The observed differences on this date were the result of cumulated effects on root elongation, nutrient uptake per unit root length and compensatory root growth.

CHAPTER 5

POTATO GROWTH AS AFFECTED BY POTATO CYST NEMATODES (*GLOBODERA PALLIDA*) AND SOIL PH. 2. FOLIAR NUTRIENT CONCENTRATIONS AND CROP GROWTH

ABSTRACT

Potato cyst nematodes (*Globodera pallida*) cause severe yield losses in potato. Crops infested by potato cyst nematodes generally have reduced concentrations of nitrogen, phosphorus and potassium in the foliage. This study aimed to investigate whether slow initial growth of nematode-infested crops is caused by nutrient limitation. In field and container experiments we studied the effects of potato cyst nematodes on foliar nutrient concentrations and crop growth. Phosphorus concentration appeared to correlate best with total crop biomass at early stages of growth, and the role of phosphorus in nematode damage was further investigated in a field experiment with different levels of nematodes, soil pH and phosphorus fertilisation.

Nine weeks after planting, total crop biomass ranged from 107 g m^{-2} for the treatment with nematodes at pH_{KCl} 6.1 without phosphorus fertiliser to 289 g m^{-2} for the fumigated treatment at pH_{KCl} 4.8 with phosphorus fertiliser. The differences in total biomass for the various treatments were explained by differences in foliar phosphorus concentration. Nematodes reduced total biomass by inducing or aggravating P deficiency. Additional P fertilisation increased phosphorus concentration but did not overcome nematode damage. At high, non-limiting levels of foliar phosphorus concentration, nematodes still reduced total biomass. High soil pH reduced growth, mainly by reducing the availability of phosphate. Results suggested interaction between nematodes and soil pH, nematode damage being higher at pH_{KCl} 6.1 than at pH_{KCl} 4.8.

INTRODUCTION

The losses due to potato cyst nematodes (*Globodera* spp.) depend on the population density of the nematode at planting, the potato cultivar, the weather and the soil type (Evans and Haydock, 1990; Trudgill, 1986). Damage is more severe on light than on heavy soils (Evans and Trudgill, 1992). This apparently does not depend on soil density, as in Chapter 3 the effects of nematodes and soil compaction on yield were generally cumulative. In the effect of soil type, soil pH may play a role. In a container experiment, Haverkort *et al.* (1993) found that nematodes reduced tuber yield with 19% at pH_{KCl} 4.5, but with 44% at pH_{KCl} 6.5.

Yield loss following nematode infestation is often associated with reduced light interception by the crop due to reduced crop leaf area. Infested crops show delayed canopy closure and senesce earlier than uninfested crops (Haverkort *et al.*, 1992; Mulder, 1994; Trudgill *et al.*, 1990). The effects at the end of the growing season are but the end-result of primary responses originating in the root system when juveniles penetrate the roots. At the beginning of the season, growth of nematode infested crops can be reduced due to a reduced photosynthetic rate and due to a dry matter distribution unfavourable for the formation of leaf area. Thirty days after planting of four potato cultivars in pots of soil with 100 eggs of *G. pallida* per gram of soil, photosynthetic rates per unit leaf area were 70% lower than in uninfested controls (Schans and Arntzen, 1991). The photosynthetic rates increased again in the following weeks. Schans (1991) and Schans and Arntzen (1991) asserted that cyst nematodes primarily affect the hormonal balance of the plant, leading to impaired crop photosynthesis. Conversely, nutrient deficiency can affect photosynthetic rates (Nátr, 1992) and crops infested by potato cyst nematodes generally have reduced foliar concentrations of nitrogen, phosphorus and potassium (Trudgill *et al.*, 1975a, 1975b). Trudgill (1980) showed in a pot test that nematode damage interacted strongly with the availability of N and of P. Doubling the amount of N and P more than doubled top weight when the nematodes were not controlled, but had little effect when the nematodes had been controlled with a nematicide. The effects of increased availability of N and P were apparent at both 7 and 14 weeks after planting, when additional fertilisation increased foliar nutrient concentrations and reduced nematode damage. These results were corroborated in field experiments at sites infested by potato cyst nematodes, where application of compound fertiliser increased growth more in the absence of a nematicide than with a nematicide (Trudgill, 1987).

The effect of fertilisation on growth and on the reduction of nematode damage can be through increased initial growth and earlier canopy closure, or by prolongation of the growing season. To understand more about the nematode damage, we focused on the early stages of crop growth, particularly on the role of nutrients in nematode damage during

these stages. To do this we sought to answer the following questions: (1) do nematodes induce nutrient limitation during early crop growth? If so: (2) which nutrients are involved, (3) which nutrient is most limiting, (4) is nematode induced nutrient deficiency remediable, and (5) how does soil pH influence the effects of nematodes on nutrients? This paper describes the effects of potato cyst nematodes and soil pH on foliar nutrient concentrations and on crop growth. In Chapter 4, the effects on root growth and nutrient uptake are described.

MATERIALS AND METHODS

Three field and two container experiments studied the effects of potato cyst nematodes on crop growth and nutrient uptake (details are in Table 5.1). The experimental fields were naturally infested with potato cyst nematodes. Two levels of infestation were obtained by soil fumigation of half the area with the nematicide Monam (active ingredient sodium methyldithiocarbamate, 51%), applied by rotary spading injector. For the container experiments, soil was taken from infested fields and half of it was irradiated with 1 MRad gamma radiation to kill the nematodes for the control treatment. Nematode densities prior to planting were determined in soil samples. Cysts were extracted by using the Schuiling centrifuge and after removing adhering organic matter by acetone, the number of cysts was counted. The cysts were crushed to expose eggs and juveniles and mixed with water. In duplicate samples of the solution the number of viable eggs and juveniles, with a distinct border between the oesophagus and intestinal region (LaMondia *et al.*, 1986), were then counted. In Exp. 5, 300 cysts were soaked for one week in tap water and crushed. The eggs were exposed to fresh potato root diffusate for 10 days and after that, the total number of emerged juveniles was counted. With this procedure, more than 95 percent of the viable eggs are detected (Regeer, 1997).

Total crop biomass excluding fibrous roots was measured between 43 and 116 days after planting and nutrient concentrations in the leaves were determined. Dry weight was determined by drying samples of the plants for 24 hours at 105°C. Total nitrogen concentration in the dry matter was determined using the Dumas-method (Macro N, Foss Heraeus), phosphorus concentration was assessed colorimetrically (Starrcol) after destruction with H_2SO_4/HNO_3 and potassium concentration was measured by atomic sorption (AS Varian AA10).

The crops were adequately protected against *Phytophthora infestans* by spraying the fungicide maneb/fentin acetate several times, according to current farming practice.

Table 5.1. Details of the experiments.

Year	Experiment 1 1989	Experiment 2 1990	Experiment 3 1990	Experiment 4 1991	Experiment 5 1995
Site	Eeserveen field	Eeserveen field	Wageningen containers	Wageningen containers	Vlagtwedde field
Soil type	sandy	sandy	peaty	peaty	sandy
Organic matter	6.5%	6.5%	10%	22%	6%
Soil pH_{KCl}	5.2	5.2	5.5	4.5, 6.5	4.8, 6.1
Soil treatment	Monam[1)], 500 l·ha^{-1}	Monam[1)], 500 l·ha^{-1}	irradiation, 1 MRad	irradiation, 1 MRad	Monam[1)], 500 l·ha^{-1}
Nematode density (viable eggs/g dry soil)	6 and 26	6 and 58	0 and 19	0 and 27	between 2 and 57
Other treatments	irrigation: with, without	irrigation: with, without	drought: none, early	soil pH: 4.5, 6.5	P-fertilisation: with, without
Cultivars	Astarte Darwina Desiree Elles Mentor	Darwina Desiree Elles Mentor	Mentor	Mentor	Mentor
Planting date	April 20	April 17	April 24	May 2	April 21
Planting pattern	0.3 x 0.75 m	0.3 x 0.75 m	6 plants/container	6 plants/container	0.3 x 0.75 m
Harvest date	July 19	July 18	June 6	June 24	June 21, Aug. 15, Sept. 14
Number of replicates	2	2	3	3	6

1) active ingredient: sodium methyldithiocarbamate 51%

Experiments 1 and 2

The effects of nematodes and drought on growth of different cultivars were studied in two field experiments in Eeserveen, the Netherlands (Table 5.1). Nematode densities prior to planting were determined by taking soil samples (100 g) of each plot to a depth of 30 cm. Differences in soil water availability were obtained by irrigating one half of the area at regular intervals by overhead sprinkler irrigation. Irrigation was applied mainly from mid-July onwards.

The trials were laid out in a split-split-plot design with two replicates, irrigation was in the main plots, fumigation was in the subplots and cultivars were in the sub-subplots. At the harvest, eight plants per plot were harvested.
Prior to planting and following soil analysis and recommendations, the field was fertilised with 180 kg N, 52 kg P and 125 kg K per hectare. By the end of June an additional amount of 30 kg N per hectare was applied. More details about these experiments are given by Haverkort *et al.* (1992).

Experiment 3

The effects of nematodes and early drought were studied in a container experiment under a rain shelter in Wageningen (Table 5.1). Polyester containers, measuring 0.6 x 0.9 m by 0.4 m high were filled with sandy soil from an experimental field near Assen, the Netherlands. Nematode densities prior to planting were determined in a bulked soil sample of 350 g.
At planting, the total mineral nitrogen content of the soil was 11.3 g per container. The P and K status of the soil were not determined. Additional fertilisation consisted of 4.1 g P and 10.5 g K per container. Six tubers were planted per container. At planting, the soil was at field capacity and the control treatments were watered twice a week to maintain its soil moisture content. For the early drought treatment, containers were not watered until 43 days after planting (d.a.p.).
The experiment was carried out in three blocks within which were randomised containers with or without nematodes and watered or not watered. Containers were placed in three rows with one guard container at both ends of each row. Space between the containers was about one meter. More details on this experiment are given by Fasan and Haverkort (1991).

Experiment 4

Effects of nematodes and soil pH were studied in containers under a rainshelter in a similar set-up as Exp. 3. Soil with a pH_{KCl} of 4.5 and 6.5 was taken from an experimental field in Tweede Exloërmond, the Netherlands. On this field, different levels of soil pH were obtained by application of various amounts of calcium carbonate over several years. Nematode densities prior to planting were determined in a bulked soil sample of 350 g.
At planting, the total mineral nitrogen content of the soil was 11.2 g per container. The P and K status of the soil were not determined. Additional fertilisation consisted of 5.6 g N, 4.1 g P, 7.1 g K and 1.6 g Mg per container. More details on this experiment are given by Haverkort *et al.* (1993).

Experiment 5

The effects of nematodes, soil pH and phosphate fertilisation on crop growth and foliar nutrient concentrations were studied in a field experiment in Vlagtwedde, the Netherlands (Table 5.1). The design of this experiment is described in detail in Chapter 4. Therefore, a brief description is given here.

There were six blocks, each of which was split for two levels of soil pH (pH_{KCl} 4.8 and 6.1). Perpendicular to this, each block was split for the soil fumigation treatment. The 24 subblocks were further split into two plots that received either no fertiliser or 225 kg P per hectare, applied as triple super phosphate. All plots were equally fertilised with 230 kg nitrogen and 125 kg potassium per hectare. Plot size was 8 m x 4.5 m. On 61 and 116 days after planting, twelve plants per plot were harvested. After foliage death, 146 days after planting, tubers of 24 plants per plot were harvested.

RESULTS

Experiment 1 to 4

Fig. 5.1 shows the relationship between total crop biomass (excluding fibrous roots) and foliar nutrient concentrations at the periodic harvests in Exp. 1 to 4. Nematodes reduced total biomass and often also reduced foliar nutrient concentrations (open symbols vs. closed symbols). In general, total biomass was positively correlated with concentrations of P and K. Relationships between total biomass and N concentration were less clear and varied between experiments. In Exp. 3 and 4, foliar concentrations of nitrogen did not differ between fumigated and non-fumigated treatments, whereas total biomass and P and K concentrations were decreased in the non-fumigated treatment (Fig. 5.1C and D).

The relationship between foliar K concentration and total biomass was not always positive. In Exp. 2 (Fig. 5.1B) high levels of nematodes reduced total biomass. With nematodes (non-fumigated) a significant negative relationship was found ($P<0.001$, R^2_{adj} 0.65) and the highest dry matter yields were found at the lowest K concentrations.

In Fig. 5.1, deficiency (left arrow) or sufficiency (right arrow) levels are indicated on the abscissa. These levels were calculated with data on concentrations in leaf blade and in petiole from Lorenz and Tyler (1983; cited by Walworth and Muniz, 1993) and with a leaf composition of 75 percent blade and 25 percent petiole (pers. comm. J. Vos). As harvest dates varied, levels for midseason are indicated for Exp. 1 and 2 and levels for early season were taken for Exp. 3 and 4.

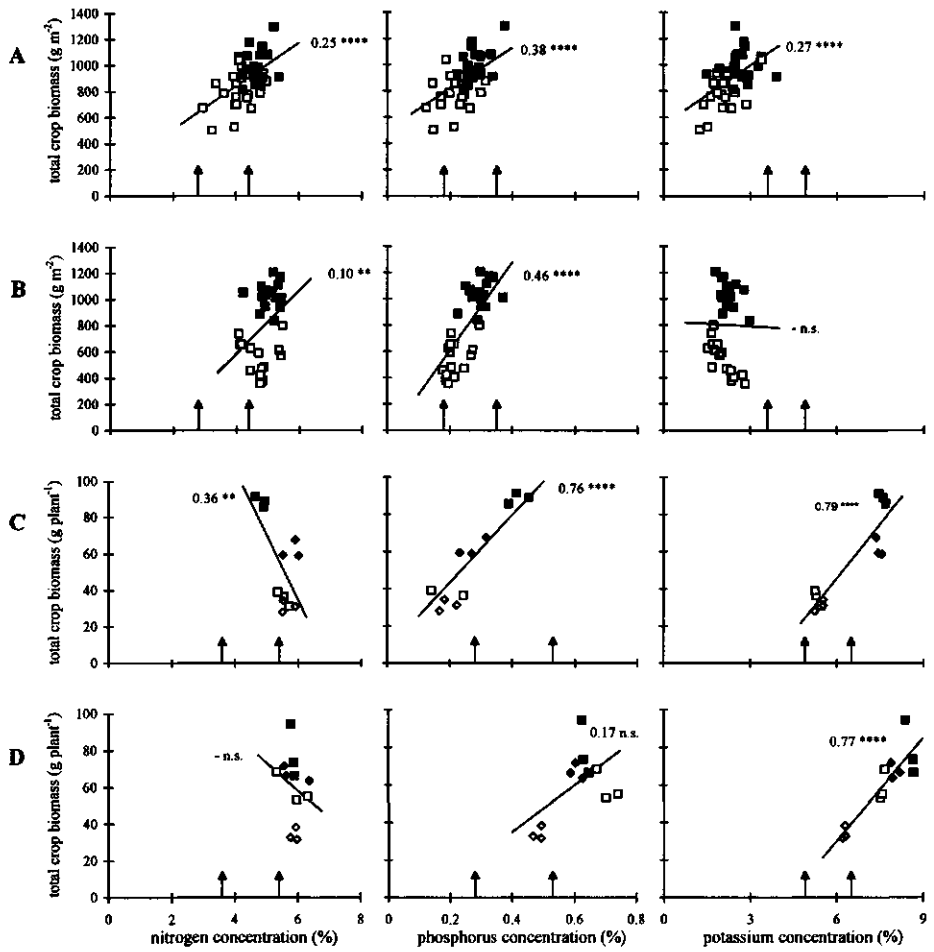

Figure 5.1. Total biomass (g m^{-2} and g $plant^{-1}$) vs. nutrient concentrations of the green leaves (%). Closed symbols: fumigated soil, open symbols: non-fumigated soil. Numbers in the graph indicate the R^2_{adj} of the regression, asterisks indicate significancy by which the slope of the regression differs from zero: **** P<0.001, *** 0.001<P<0.01, ** 0.01<P<0.05 and * 0.05<P<0.10. A: Exp. 1, 90 d.a.p.; B: Exp. 2, 92 d.a.p.; C: Exp. 3, 43 d.a.p., squares: control, diamonds: drought; D: Exp. 4, 53 d.a.p., squares: pH_{KCl} 4.5, diamonds: pH_{KCl} 6.5. The arrows on the abscissa indicate deficiency (left arrow) or sufficiency (right arrow) levels according to Walworth and Muniz (1993).

As most irrigation in Exp. 1 and 2 was applied from mid-July onwards, after the time of the periodic harvest, effects of irrigation were small and not significant. In Exp. 3, early drought reduced total biomass and foliar P concentration (Fig. 5.1C). Drought hardly affected foliar K concentration and increased N concentration. With nematodes, drought did not affect total biomass nor foliar nutrient concentrations.
The effect of nematodes on total biomass and on foliar P and K concentrations interacted with the soil pH treatment. Yield loss by nematodes was greater at pH 6.5 than at pH 4.5 and at pH 6.5 yield loss was associated with decreased concentrations of P and K.

Experiment 5

The role of phosphorus in the mechanism of nematode damage was studied further in Exp. 5. Table 5.2 shows the effects of soil pH, phosphate fertilisation and soil fumigation on water-extractable soil phosphate (Pw) and initial nematode population density. Phosphate fertilisation clearly increased the Pw but the Pw was decreased by high soil pH. The Pw value of the non-fertilised treatment at pH 4.8 was 61, equal to the Pw value of fertilised soil at pH 6.1. The nematode population density differed between the pH levels and was at pH 6.1 almost half that of pH 4.8.
The different treatments significantly affected total biomass at 61 days after planting (d.a.p.) and final tuber yield (Table 5.3). High levels of nematodes in non-fumigated soil reduced total biomass by 24 to 40 percent at 61 d.a.p. The relative effects on final tuber yield were similar. There was a significant interaction between effects of soil pH and phosphorus fertilisation on total biomass at 61 d.a.p. Total biomass was higher at pH 4.8 than at pH 6.1 and was increased by phosphate fertilisation, but the increase was greater at pH 6.1 than at pH 4.8. Interactions between soil fumigation and pH or phosphorus

Table 5.2. Average values for the different treatments in Exp. 5

	pH_{KCl}		LSD (0.05)
	4.8	6.1	0.2
Phosphate availability index (Pw)			
Non-fertilised	61	45	
Fertilised	82	61	7
Nematode density (living juveniles/g soil)			
Fumigated	5	6	
Nematodes	47	25	6

fertilisation were not significant. However, the initial nematode density at pH 6.1 was half that at pH 4.8 (Table 5.2) but gave similar yield reductions as at pH 4.8 (Table 5.3). When the initial nematode density was taken into account in a multiple regression analysis, significant effects on total biomass were found for the initial nematode density, soil pH and for the interaction between the nematode density and soil pH.

Table 5.3. Average values of total crop biomass (g m^{-2}) and concentrations of nitrogen, phosphorus and potassium in the green leaves (%) in Exp. 5. LSD = least significant difference. P+ = 225 kg P ha^{-1}, P- = no phosphorus fertiliser, Fum = fumigated, Nem = nematodes.

	Total crop biomass		Damage (%)	Foliar nutrient concentrations					
				Nitrogen		Phosphorus		Potassium	
	Fum.	Nem.		Fum.	Nem.	Fum.	Nem.	Fum.	Nem.
61 days after planting									
pH 4.8 P+	289	219	24	5.81	5.32	0.622	0.506	5.13	4.06
pH 4.8 P-	284	194	32	5.92	4.94	0.561	0.380	4.75	4.03
pH 6.1 P+	249	160	36	5.64	5.00	0.456	0.388	5.47	4.44
pH 6.1 P-	178	107	40	5.45	4.96	0.370	0.302	5.14	4.66
LSD (0.05)	34		21	0.35		0.055		0.59	
116 days after planting.									
pH 4.8 P+	1699	1224	28	4.17	3.42	0.151	0.112	2.70	2.49
pH 4.8 P-	1596	1134	29	3.93	3.47	0.132	0.117	3.27	2.53
pH 6.1 P+	1544	981	36	3.85	3.60	0.116	0.113	4.48	3.85
pH 6.1 P-	1210	766	37	3.70	3.75	0.112	0.125	4.23	4.02
LSD (0.05)	208		22	0.41		0.021		0.76	
146 days after planting[1]									
pH 4.8 P+	1439	1028	29	–	–	–	–	–	–
pH 4.8 P-	1374	963	30	–	–	–	–	–	–
pH 6.1 P+	1206	822	32	–	–	–	–	–	–
pH 6.1 P-	1004	598	40	–	–	–	–	–	–
LSD (0.05)	117		18						

[1] only tubers harvested

In Fig. 5.2A-C, total crop biomass at 61 days after planting is plotted against foliar concentrations of nitrogen, phosphorus and potassium. There was a positive relationship between total biomass and foliar nutrient concentrations. Foliar N concentrations were reduced by nematodes (Table 5.3) but the reduced dry matter production could not be attributed to nitrogen limitation, as P fertilisation led to large differences in total biomass at equal N concentrations (Fig. 5.2A). The variation in total biomass was associated with a relatively small variation in N concentration (Fig. 5.2A) and relatively large variation in P concentration (Fig. 5.2B). K concentrations were around or below the indicated deficiency level. A positive trend between total biomass and K concentration was visible but the relationship was weak (Fig. 5.2C). As in Exp. 2, at high levels of nematodes a significant negative relationship was found ($P=0.028$, R^2_{adj} 0.13) between total biomass and foliar K concentration.

On fumigated soil, the reduced total biomass at pH 6.1 without P fertilisation (Fig. 5.2B, closed circles) was associated with low values of foliar P concentration, close to the deficiency level (left arrow on the abscissa in Fig. 5.2B). P fertilisation increased P concentration and total biomass (Fig. 5.2B, closed triangles). Nematodes reduced yield and reduced foliar nutrient concentrations within each combination of soil pH and P fertilisation (Table 5.3 and Fig. 5.2, open vs. closed symbols). At low P concentrations (± 0.4%), total biomass was similar for the fumigated and nematode treatment. At high P concentrations (≥ 0.5%), close to the sufficiency level, yields were higher on fumigated soil than with nematodes (Fig. 5.2B).

Multiple regression analysis showed that variation in total biomass was significantly ($P\leq0.006$) explained by a quadratic effect of P concentration, by the initial population density of the nematodes and by the interaction between P concentration and nematode density. In Fig. 5.2B, regression lines for the effects of P concentration are drawn for the average nematode densities on fumigated and non-fumigated soil, respectively 5 and 36 living juveniles per gram of soil.

Figure 5.2D-F show the relationship between total biomass and foliar nutrient concentrations at 116 days after planting. By then, about half of the leaves had turned yellow or were dead. Nutrient concentrations in the green leaves had decreased compared to the levels at 61 days after planting (Table 5.3). Contrary to the first harvest date, at 116 days after planting there was no clear positive relationship between total biomass and foliar P concentration (Fig. 5.2E). Between total biomass and K concentration (Fig. 5.2F) there was a negative relationship. When fumigated or non-fumigated plots were observed separately, this negative relationship was more clear. Despite the absence of a positive relationship between total biomass and foliar nutrient concentrations, the relative effects of the different treatments on total biomass were similar to those at 61 d.a.p. (Table 5.3).

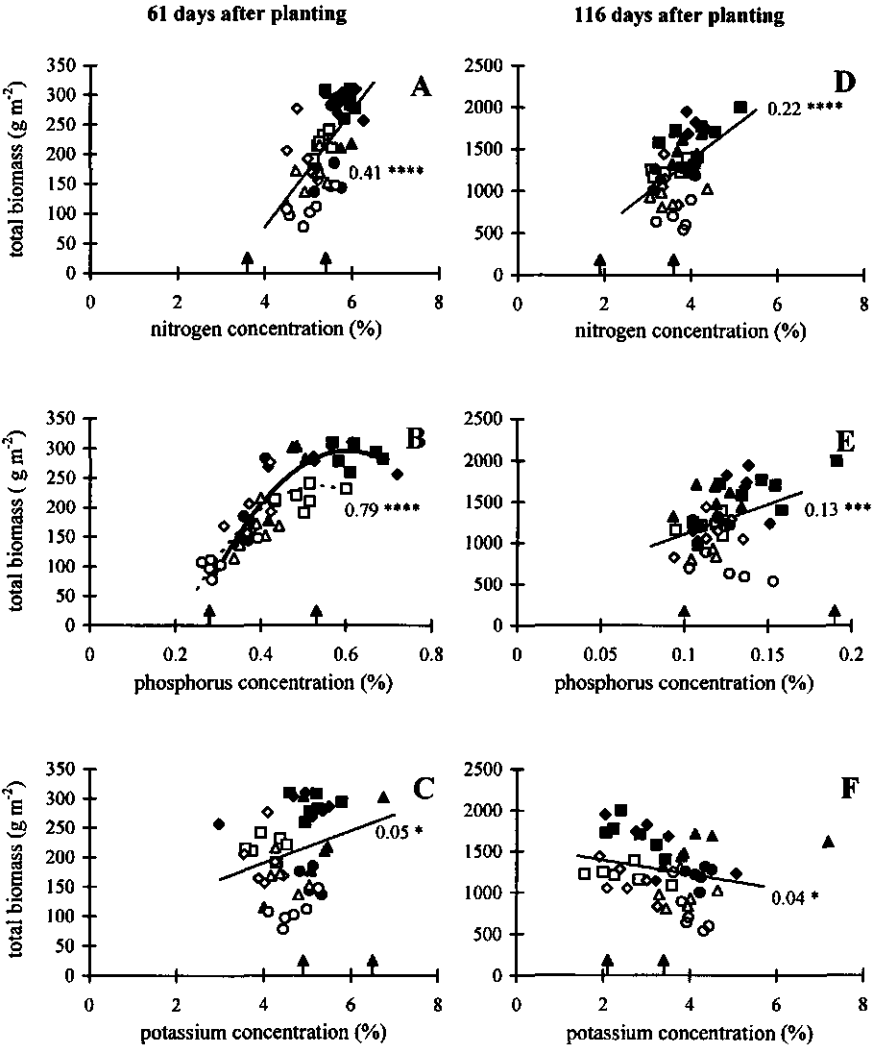

Figure 5.2. Total crop biomass (g m^{-2}) vs. nutrient concentrations of the green leaves (%), Exp. 5A, B, C: 61 d.a.p.; D, E, F: 116 d.a.p. Closed symbols: fumigated soil, open symbols: non-fumigated soil. □ pH_{KCl} 4.8, P+; ◊ pH_{KCl} 4.8, P-; Δ pH_{KCl} 6.1, P+; O pH_{KCl} 6.1, P-. Numbers in the graph indicate the R^2_{adj} of the regression, asterisks indicate significancy of the slope of the regression: **** P<0.001, *** 0.001<P<0.01, ** 0.01<P<0.05 and * 0.05<P<0.10. The arrows on the abscissa indicate deficiency (left arrow) or sufficiency (right arrow) levels according to Walworth and Muniz (1993). Note the different horizontal axes in Fig. 5B and 5E.

DISCUSSION

Biomass production and nutrient concentrations

Chemical nematicides reduced population densities in the field experiments with on average 83 percent and gamma radiation completely killed the nematodes in the container experiments (Table 5.1 and 2). Both treatments may have killed other soil organisms interacting with the crops, but since nematode densities were high we attributed the effects of fumigation and gamma radiation on crop growth to differences in nematode density.

We found in most cases positive relationships between total biomass and foliar concentrations of nitrogen, phosphorus and potassium (Fig. 5.1 and Fig. 5.2). This is generally found after infestation with nematodes (Trudgill *et al.*, 1975a, 1975b; Trudgill, 1980, 1987), indicating that nematodes reduce both yield and nutrient uptake per unit biomass. However, the relationships were not always positive, sometimes absent (Fig. 5.1B for K or 5.1D for N) or negative (Fig. 5.1C for N). A negative relationship between total biomass and foliar concentration of a nutrient is expected when growth is reduced by factors other than that nutrient and the reduced growth gives less dilution of the absorbed amount of nutrient. For non-limiting nutrients, however, healthy plants are able to maintain relatively constant nutrient concentrations by physiological control of nutrient uptake (De Willigen and Van Noordwijk, 1987). Then, no relationship between total biomass and foliar nutrient concentration will be found. When growth is limited by nutrient deficiency, physiological control cannot counteract a reduction in uptake as roots are taking up at maximum rate. Then, the nutrient will be maximally diluted giving low nutrient concentrations.

To determine which deficiencies contributed to the reduction of total biomass induced by nematodes in our experiments, three different approaches have been used:

1. comparison of the relationship between foliar nutrient concentrations and total biomass (Exp. 1-5),
2. comparison of the foliar nutrient concentrations with the deficiency-sufficiency ranges known from the literature (Exp. 1-5),
3. the effect of changed availability of a supposed limiting nutrient on nematode damage (Exp. 5).

In Exp. 1 to 4 we found that in most cases total biomass was positively related with foliar P and K concentrations. In Exp. 1 and 2 also a positive relationship between total biomass and foliar N concentration was found, but in Exp. 3 and 4, N concentrations were hardly

affected by nematodes and sometimes increased, whereas total biomass was reduced by nematodes. Therefore, in these experiments the growth reduction by nematodes was not caused by nitrogen limitation.

Since we did not vary fertilisation in Exp. 1 to 4, we compared concentrations of N, P and K with deficiency levels from the literature to investigate whether these might have been limiting. The use of critical nutrient concentrations is awkward, as these depend on the sampled plant part, the age of tissue and environmental variables as moisture supply, temperature and light intensity (Bates, 1971). Therefore, the deficiency and sufficiency levels can only be used to get an indication of which element may have been limiting. Between the different experiments, the range of concentration levels varied and the lowest values of P and K were sometimes below deficiency levels from the literature. This may be a result of including old leaves with low nutrient levels in the bulked sample. The K concentrations in Exp. 1 and 2 were all lower than the deficiency level. However, it is not plausible that K was limiting growth in Exp. 2, as there was no positive relationship between total biomass and K concentration. In this experiment, differences in total biomass may have been caused by differences in P uptake. As P concentration of the treatments with the lowest total biomass was in most cases near the deficiency levels, and in most cases a positive relationship between total biomass and P concentration was found, the role of P in nematode damage was studied in more detail in Exp. 5 by varying soil pH and phosphate fertilisation.

In Experiment 5, we found that total biomass was strongly correlated with P concentration at 61 d.a.p. On fumigated soil at pH 6.1 without P fertilisation, yield was reduced by P limitation as fertilisation increased P concentrations and yield. The Pw value of this treatment was 44, indicated as relatively high by the Netherlands fertiliser recommendation scheme (Anon., 1992b). However, potato is responsive to P and Van Noordwijk *et al.* (1990) calculated that for non-P-limited growth on four sandy soils Pw values were required from 44 to 87.

Effects of potato cyst nematodes

Nematodes reduced P concentrations at 61 d.a.p. and the effect of nematodes on growth could largely be attributed to P limitation, as P concentrations similar to that of the limiting concentration of the treatment 'pH 6.1 without P fertilisation on fumigated soil' gave similar yields (Fig. 5.2B). Lower P concentrations showed a further yield decrease. However, not all damage by nematodes could be attributed to P limitation. At high P concentrations of the fumigated treatment, variation in P concentration did not lead to variation in total biomass, indicating that at this time P concentrations higher than 0.5% were not limiting. At these non-limiting concentrations, nematodes reduced total biomass.

Apparently potato cyst nematodes reduced crop growth by different mechanisms. At low levels of phosphate fertilisation, phosphorus was limiting and 'other' mechanisms acted at high phosphate fertilisation levels. Reduced crop growth after nematode infestation without nutrient limitation was also found in Chapter 3.

At 116 d.a.p., there was no relationship between total biomass and foliar P concentration. In Chapter 4 is was concluded that the initial P deficiency induced by nematodes was counteracted by compensatory root growth. This, however, could not compensate for the initial growth reduction by the nematodes. At the end of the growing period, 146 d.a.p., nematodes reduced tuber yields by 29 to 40 percent.

Trudgill (1987) found that application of additional NPK fertiliser increased growth of nematode-infested plants more than that of nematicide-treated plants. Nematode damage was therefore reduced by additional fertilisation. We found increased total biomass at the non-fumigated treatment after application of additional P fertiliser but this also occurred with the fumigated treatment (Table 5.3). The percentage damage by nematodes tended to be reduced by application of additional P fertiliser, but this effect was small and not significant. At low P-fertility levels, nematodes reduced growth by P limitation and fertilisation reduced P limitation and increased growth. However, fertilisation reduced P limitation also at the fumigated treatment and percentage damage was hardly reduced. At high P-fertility levels, nematodes induced some P limitation but reduced growth also by other mechanisms, indicated by the difference between the solid and the dotted line in Fig. 5.2B. Application of P fertiliser relieved the P limitation but not the other mechanisms and percentage damage was hardly reduced. This limits the possibilities to reduce nematode damage by additional fertilisation for cultivar Mentor in the studied environment.

Effects of soil pH

High soil pH reduced total biomass and final tuber yield (Table 5.3). The effects of soil pH on growth were largely reflected in foliar P concentration (Fig. 5.2B) and together with the observed increased ratio of root length to leaf area (Chapter 4), it is concluded that in our experiments growth at high soil pH was reduced by phosphorus limitation. This was mainly caused by a reduced phosphorus availability, as the Pw value was lowest at pH 6.1. A reduced Pw value was not the only effect of high soil pH, as at pH 6.1 with phosphorus fertiliser, the Pw value was similar to that at pH 4.8 without phosphorus fertiliser, but total biomass at pH 6.1 was still reduced (Table 5.1). This may also have been caused by phosphorus deficiency as the average foliar P concentration was reduced from 0.56 percent at pH 4.8 to 0.46 percent at pH 6.1 (Table 5.3). However, the root

length to leaf area ratio did not differ between these treatments, suggesting that the below-ground resource capture was not limiting (Chapter 4).

Interaction between soil pH and potato cyst nematodes

Providing a quantitative description of nematode damage in relation to soil pH is complicated by the greater nematode density at pH 4.8 than at pH 6.1 (Table 5.2). The effect of soil fumigation on total biomass and final tuber yield was similar at both pH levels (Table 5.3). However, when the initial nematode density was taken into account in a multiple regression analysis, a significant interaction between initial nematode density and soil pH was found. This corroborates the previously found interactions by Mulder (1994) and Haverkort *et al.* (1993). In addition to the results described by Haverkort *et al.* (1993), the effects of the treatments of this experiment on foliar nutrient concentrations are shown in Fig. 5.1D. The low yields of the combined effect of soil pH and nematodes coincide with reduced foliar concentrations of P and K. Though these concentrations are relatively high compared to the indicated deficiency level, nutrient uptake may be responsible for this interaction effect. This is supported by Trudgill (1987), who found that nematode damage was highest at low fertilisation levels. The interaction in Exp. 5 may therefore result from the low phosphorus availability at pH 6.1.

CHAPTER 6

GENERAL DISCUSSION

POTATO CROP GROWTH

In this thesis, various crop processes that are affected by potato cyst nematodes have been investigated to ascertain the mechanisms by which potato cyst nematodes reduce tuber yield and to explain the interaction between nematodes and abiotic factors. The processes studied are shown schematically in Fig. 6.1. The diagram in the centre describes the processes that determine crop growth and indicates the interactions between them. The top part describes the above-ground plant parts, the shaded area the below-ground parts.

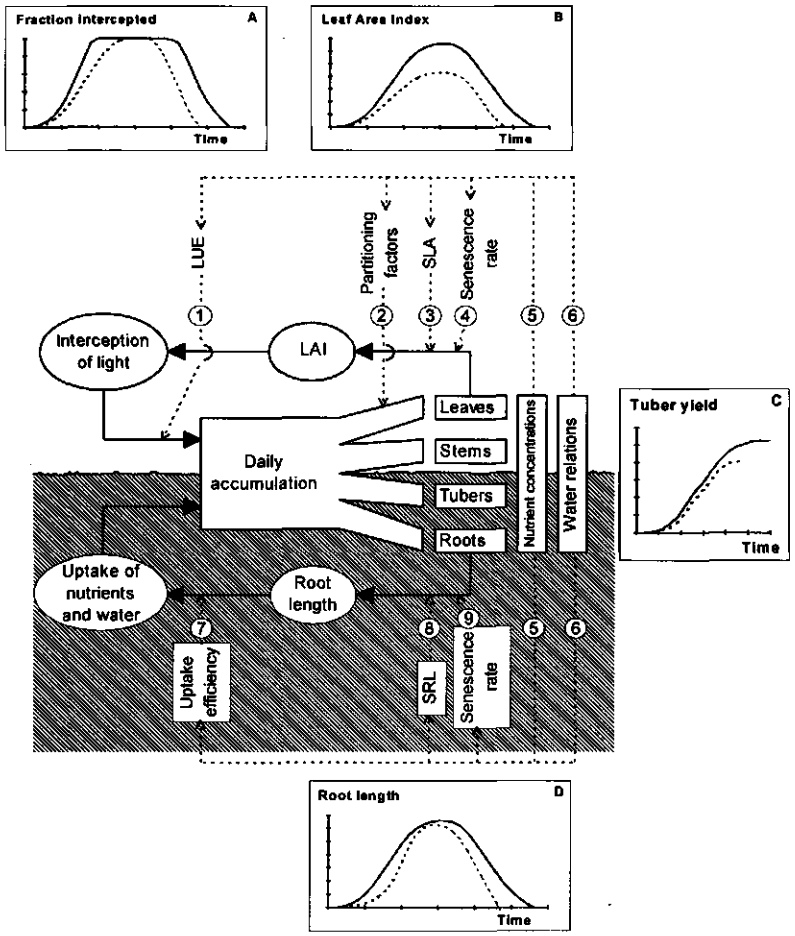

Figure 6.1. Schematic representation of crop growth of potato. For explanation: see text.

The processes described result in time courses of crop characteristics and weights of plant parts shown in the graphs A to D of Fig. 6.1. In these graphs, examples are shown for a nematode-free (solid line) and a nematode-infected crop (broken line). The discussion on crop growth of potato that follows is based on the diagram in Fig. 6.1.

Crop growth strongly depends on the interception of light. After emergence, the leaves intercept light that is used to convert CO_2 and H_2O into biomass. The newly formed dry matter is allocated to leaves, stems, roots and, after tuber initiation, to tubers. New leaves contribute to the light-intercepting leaf area and after emergence growth is exponential as new leaves contribute to the increased light interception, they increase growth and lead to the formation of more new leaves. Because of shading, the effect of added leaf layers diminishes and at full ground cover, additional leaf growth does not lead to increased interception of light.

Below-ground, the roots absorb water and nutrients. Water is mostly transpired by the leaves. The resulting water flow within the plant distributes the nutrients to the various plant parts. Above and below-ground growth and functioning of organs are interdependent as nutrients and water are necessary for photosynthesis and dry matter accumulation, whereas root growth depends on assimilates from the shoot. This interdependence is strictly regulated: when growth is limited by an essential substance that has to be absorbed by the roots, root growth is relatively favoured; when the limiting factor has to be absorbed by the shoot, growth of the above-ground parts is relatively favoured (Brouwer, 1983).

NEMATODE EFFECTS ON CROP GROWTH

At a high level of integration, crop growth can be analysed as the product of light interception by the crop and the efficiency with which intercepted light is used to produce biomass (Monteith, 1977). Nematode damage can largely be explained by reduced interception of light (Chapter 3; Mulder, 1994; Haverkort and Trudgill, 1995). This is shown in graph A of Fig. 6.1 by the reduced ground cover at the beginning and at the end of the season. The reduced ground cover is the result of decreased leaf area expansion at early stages of growth and/or an increased leaf senescence rate at the end of the growing season (graph B). As depicted in Fig. 6.1, various processes affect light interception and crop growth and nematodes may retard leaf area expansion by changing the partitioning factors (2), or by reducing specific leaf area (3) or light use efficiency (1). These parameters are affected by nutrient concentrations (5) and leaf-water relations (6). Nematodes can alter uptake of nutrients and water by influencing root formation (2 and 8) and uptake efficiency (7). Light use efficiency can also be reduced by hormonal

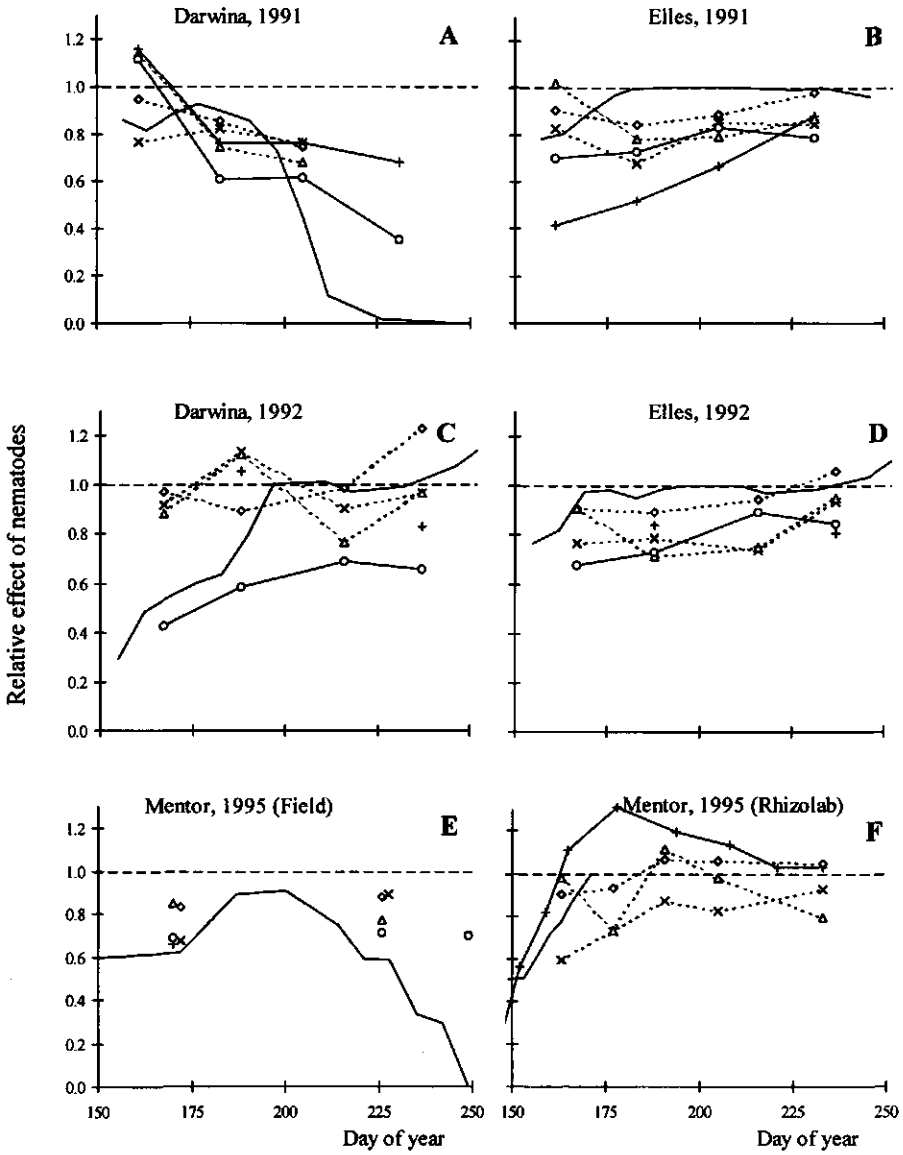

Figure 6.2. Time courses of the effects of potato cyst nematodes relative to the control for root length (+—+), percentage ground cover (—), total dry biomass (O — O) and the concentrations of nitrogen (◇---◇), phosphorus (x---x) and potassium (Δ---Δ) in the green leaves (based on dry matter).

signalling. On the basis of pot experiments, Schans (1991) and Schans and Arntzen (1991) speculated that when nematodes invade the roots they trigger hormonal root signals that are transmitted to the above-ground plant parts and impair photosynthesis.
At the end of the season, the rate of leaf senescence (4) can be increased through the effects of nematodes on nutrient concentrations (5) and leaf water relations (6). These effects can be indirect as the reduced overall crop growth or changed allocation patterns may reduce the number of roots that are able to penetrate the subsoil (Haverkort *et al.*, 1994). This does not immediately lead to insufficient water or nutrient uptake because early in the growing season sufficient water and nutrients are available in the topsoil. Late in the season, however, the limited rooting depth leads to exhaustion of nutrients or water within reach, causing nutrient deficiency and water stress (Evans *et al.*, 1977). As lack of water and nutrients late in the season results from growth disturbing effects in the first part of the growing season, it is necessary to know the chronology of nematode effects on crop growth and which effects are initial and which are secondary. This entails studying nematode effects in the order in which they become apparent during the growing season, though multiple mechanisms often operate simultaneously.
Results of the experiments described in Chapters 3, 4 and 5 are summarised in Fig. 6.2. The graphs show the effects of potato cyst nematodes over time, relative to the control for root length, ground cover, total biomass excluding fibrous roots, and foliar concentrations of nitrogen, phosphorus and potassium. In the following assessment of the findings, I will first discuss nematode damage mechanisms at the crop level at early and at later stages of growth. Subsequently, differences between cultivars and the relationship between nematode damage and abiotic factors will be discussed. This chapter concludes with a discussion of the agronomic implications of the results of the research.

Nematode effects at early stages of growth

On the first measurement date, it was found that nematodes had reduced ground cover and, in most cases, the total biomass (Fig. 6.2). As total biomass at these early stages of growth consists mainly of ground covering stems and leaves, a concurrent effect on both was to be expected. The various underlying mechanisms that may explain the reduced growth will now be discussed (see Fig. 6.1).
The cause of the reduced early growth of nematode infected crops may have been nutrient deficiency, as foliar nutrient concentrations were also found to be depressed on the first measurement date (Fig. 6.2). Phosphorus was the foliar nutrient reduced most and in Chapter 5 it was shown that nematodes induced or aggravated phosphorus deficiency at early stages of growth by influencing uptake efficiency. The effect on uptake efficiency was inferred from the finding that root length was reduced equally to or less than total

biomass, whereas total nutrient uptake was decreased (Fig. 6.2E; Chapter 4). Other researchers have found a reduced shoot to root ratio in nematode-infected crops (Evans, 1982; Trudgill and Cotes, 1983b), leaving less plant weight to be supplied with nutrients per unit root weight or length. From this it can be inferred that growth is limited by below-ground processes (Brouwer, 1983; Körner and Renhardt, 1987). However, when the effects of nematodes on root growth are studied *in vitro*, nematodes reduce root elongation within a few days (Arntzen *et al.*, 1994; Rawsthorne and Hague, 1986). These direct effects of nematodes on roots would result in an increased shoot to root ratio but in my experiments it seems that these effects were quickly overruled by compensatory root growth. Compensatory root growth was clearly found in the Wageningen Rhizolab, where some weeks after planting the infested crop was smaller but had more roots than the control (Fig. 6.2F, Chapter 4).

The nematode damage to the crop cannot fully be ascribed to an induced nutrient deficiency. In the experiment described in Chapter 5 it was found that a high application rate of phosphorus fertiliser prevented nematode-induced phosphorus deficiency but nematodes still reduced growth. And in an earlier Chapter (3, see also Fig. 6.2C), it was found that nematodes reduced total biomass and ground cover but did not always affect foliar nutrient concentrations. Evans and Haydock (1990) suggested that nematodes reduce growth by inducing drought stress. Haverkort *et al.* (1991a) compared the effects of drought and nematodes, finding that drought led to a higher water use efficiency whereas nematodes reduced water use efficiency at early stages of growth and increased it at later stages. They suggested that at early stages of growth nematodes not only affect stomatal processes but also photochemical or biochemical processes. This corresponds with the hypothesis of Schans (1991) and Schans and Arntzen (1991), who speculated that when nematodes invade the roots, they trigger hormonal root signals that are transmitted to the above-ground plant parts and impair photosynthesis. The effects of nematodes on leaf photosynthesis in the pot experiment reported by Schans and Arntzen (1991) decreased in the course of time, which they suggested to be a result of depletion of inoculum or a dilution of the signal as the amount of foliage increased. Finally, it seems unlikely that nematodes will affect water uptake causing drought stress at early stages of growth, as at that time crop water demand is low and the shoot to root ratio of young plants is low. At later stages, however, drought stress is more likely to occur.

At early stages of growth, leaves do not senesce and growth is determined by radiation, and also by the light use efficiency, dry matter partitioning and specific leaf area (Fig. 6.1). In my experiments, nematodes reduced both light use efficiency and specific leaf area but had no effect on dry matter partitioning at the early stages of growth (Chapter 3). The effects I found on light use efficiency and specific leaf area cannot be attributed unequivocally to underlying mechanisms, as both can be reduced by phosphorus deficiency (Nátr, 1992), and by hormonal signalling or drought stress (Davies *et al.*, 1990;

Jones and Corlett, 1992; Munns and Sharp, 1993; Passioura and Gardner, 1990). Therefore, the only conclusion warranted, given my findings and the literature, is that both hormonal signalling and phosphorus deficiency are involved in the initial growth reduction after nematode infestation.

Nematode effects at late stages of growth

A nematode-infested crop generally senesces earlier at the end of the growing season. Evidence for this was presented in Chapter 3 (and see also Fig 6.2A and E), but has also been presented by other researchers. Mulder (1994) compared the effects of potato cyst nematodes on ground cover and light interception for a number of cultivars, before and after canopy closure (see graph A in Fig. 6.1). He found that about one third of the reduction in light interception was before canopy closure, the remaining two thirds was attributable to earlier senescence of the crop. From this it can be concluded that differences in crop senescence greatly influence differences in nematode damage.

Senescence is known to be regulated by many environmental and internal factors, the major environmental factors being limited water and nutrient (especially nitrogen) availability (Gan and Amasino, 1997). In many annual species the number of senescing leaves increases during seed development, allowing the assimilated nutrients in the foliage to be transported to and stored in the reproductive organ. There are two major models to explain this phenomenon: in one, strong nitrogen demand from the reproductive tissues causes leaf senescence, in the other, reproductive tissues produce a senescence hormone that is transported to leaves to activate the senescence programme (Gan and Amasino, 1997).

In Chapter 3, the differences in damage between the two experiments resulted from differences in crop senescence, where high yield losses coincided with strong reductions of nutrient concentrations and increased leaf senescence (compare Fig 6.2A and C). This corresponds to the first model, as the reduced concentrations lead to a reduced pool of foliar nitrogen, which may hasten senescence at the end of the season when tubers withdraw nitrogen from the leaves (Dyson and Watson, 1971). In addition, nematodes hardly affect the timing of tuber initiation, resulting in a reduced amount of foliage throughout the season as growth of foliage stops some time after the start of tuber growth. At the end of the season, the nitrogen pool in the foliage will be exhausted faster in a crop with a small amount of foliage than in a crop with a large amount of foliage.

Withdrawal of nitrogen from the foliage by the tubers increases when nutrient uptake stops early. This can be caused by depletion of the rooted volume of soil as nematodes reduce root system expansion (Haverkort *et al.*, 1994; Trudgill, 1986). Whether nitrogen in the rooted volume of soil becomes depleted is uncertain as crop size is also reduced and

thereby the demand for nitrogen. Moreover, in the experiments described in this thesis, nematodes only slightly reduced spatial distribution of the roots (Chapters 3 and 4) and all nitrogen in the rooted soil can be absorbed with root length densities down to 1 cm cm^{-3} (De Willigen and Van Noordwijk, 1987). Another way in which nutrient uptake ceases is when the roots senesce. In Chapter 3, it was shown that earlier crop senescence of nematode-infested crops coincided with increased root senescence. Not only nutrient uptake, but also cytokinin production stops when roots senesce. Leaf senescence is initiated when cytokinin levels in the leaves fall below a threshold (Buchanan-Wollaston, 1997). In this thesis, root vitality was not specifically studied but it requires more attention, as it may influence the duration of crop growth.

As suggested above, drought stress may occur at later stages of crop growth and cause increased leaf ageing and senescence. During growth, the shoot to root ratio increases (Brouwer, 1983), as does the crop demand for water. The roots therefore absorb water at a higher rate. Nematodes apparently reduce the water uptake capacity or water conductivity of the roots; this can be inferred from the fact that on days with a high evaporative demand infested crops wilt, even when soil water was available abundantly (see Evans et al. (1977) and also own observations in the Wageningen Rhizolab). The increased senescence of nematode-infested crops may therefore result from drought stress.

Finally, interaction of potato cyst nematodes with other pathogens may also be responsible for increased crop senescence, as described in the review of Evans and Haydock (1990). Potato cyst nematodes may interact with *Rhizoctonia solani*, *Verticillium dahliae* and *V. albo-atrum*.

In conclusion, several mechanisms may be responsible for the increased senescence of nematode-infested crops. In part, they originate from the reduced crop growth at early stages of growth. Root vitality and longevity also seem to be important in crop senescence. This should be studied in more detail to elucidate whether crop senescence is indeed induced by root senescence and to determine the role of nutrients, water or the hormonal balance in crop senescence.

TOLERANCE OF POTATO CYST NEMATODES

Cultivar differences

Cultivars differ in tolerance of potato cyst nematodes (Dale *et al.*, 1988; Evans, 1982; Evans and Haydock, 1990; Trudgill and Cotes, 1983a; Trudgill *et al.*, 1983; Trudgill, 1986). Schans and Arntzen (1991), studied cultivars differing in tolerance and did not observe differences in the effects of nematode infection on photosynthetic rates. The

mechanism of hormonal signalling, therefore, will generally reduce the initial growth of crops infested by potato cyst nematodes. This was confirmed in Chapter 3, where it was shown that the tolerant cultivar Elles suffered equal reductions of the light use efficiency as the other cultivars. Given that Elles showed only a small reduction of total intercepted light, it seems likely that the tolerance of this cultivar is based on a more favourable growth habit, A prolonged partitioning of biomass to leaves assures that, even with some growth retardation, full ground cover is reached. This confirms the findings of Trudgill (1986), who found relatively low nematode-induced yield loss in cultivars with large tops. Since prolonged partitioning to leaves is a common characteristic of late-maturing potato cultivars, yield loss is negatively correlated with cultivar lateness (Haverkort *et al.*, 1996).
In addition to the prolonged partitioning of biomass to leaves, cultivar Elles was characterised by a high root length density and thick roots (Chapter 3). In the experiments, nematodes reduced root length in general (Fig. 6.2). Root length of the tolerant cultivar Elles, however, recovered from nematode infestation (Fig. 6.2B), whereas the root length of the infested and intolerant cultivar Darwina decreased further during time (Fig. 6.2A). Evans (1982) found that the tolerant cultivar Cara produced extra roots in the presence of nematodes, and Evans and Haydock (1990) concluded that this ability is clearly a mechanism that will lead to tolerance. In Chapter 4, compensatory root growth was found to compensate for reduced root functionality.
Production of extra roots is a mechanism to restore a reduced functionality. Therefore, it can be inferred that as well as being associated with the production of large tops, tolerance will be associated with the ability to produce extra roots.

Influence of soil parameters

Damage by potato cyst nematodes interacts with soil type (Trudgill, 1986). In this thesis, the effects of soil density, soil-pH and phosphorus fertilisation on nematode damage were studied. The effects of nematodes and soil compaction on yield were generally cumulative (Chapter 3). Soil pH, however, affected nematode damage, and at similar nematode densities the damage was greater when soil pH was higher (Chapters 4 and 5). Interaction between soil type and nematode damage may arise from an effect of soil type on the nematodes themselves. Cyst nematodes, however, are not sensitive to mechanical damage because of their protective cyst (Boag, 1988) and were not affected by soil compaction.
The evidence for a possible effect of soil pH on nematodes is conflicting. The hatching of larvae of potato cyst nematodes is reported to be optimal at pH 2.5 (Robinson and Neel, 1956), at pH 6 (Ellenby, 1946) or to be constant between pH 3 and 8 (Fenwick, 1951). As reported in Chapters 4 and 5, natural infestation levels in the field were found to be higher at pH_{KCl} 4.8 than at pH_{KCl} 6.1 (respectively 47 and 25 viable juveniles per gram of soil).

This is similar to findings of Haverkort *et al.* (1993) who reported an initial nematode density of 17.5 at pH_{KCl} 4.5 and 8.8 at pH_{KCl} 6.5. The inverse relation between soil pH and nematode number is probably attributable to better crop growth at low pH, which allowed higher multiplication rates. It indicates that the lower nematode damage at pH_{KCl} 4.8 was not caused by a decreased nematode population density.

The cumulative effects of nematodes and soil compaction on yield suggest that variation in yield loss caused by nematodes on different soil types is not related to differences in root system expansion between soils of various strength (Chapter 3). However, in one of the two years of experimentation, there was an exception on the cumulative effects: the nematode-tolerant cultivar Elles showed no nematode damage on loose soil but nematodes reduced final tuber yield by 45 percent on compacted soil. The combined effects of soil compaction and nematodes reduced the foliage to such an extent that full ground cover was not reached. Apparently, the effects of soil compaction on growth forestalled the advantageous effect of large tops, thus removing the tolerance property of cultivar Elles. This corresponds to the earlier described nematode damage mechanism of reduced initial growth, a smaller foliage at the time of tuber initiation and a subsequent earlier senescence.

Given that soil pH *per se* is not expected to affect nematode population density, it can be postulated that the interaction between the effects of nematodes and soil-pH on growth and tuber yield arises from their effects on crop growth. Both nematodes and high soil pH reduced foliar phosphorus concentrations (Chapter 5). If only one factor is operative damage may be limited, as compensatory root growth can offset part of the reduced phosphorus uptake, and a small reduction of foliar P concentrations to values just below optimal does not give large yield reductions. However, for the combined effect, phosphorus uptake is further reduced and compensatory root growth cannot prevent phosphorus deficiency. Soil pH mainly affected crop growth through the effect on phosphorus avaoilability (Chapter 4 and 5) This is similar to varying levels of P fertilisation and the results found in my experiments correspond to the observation of Trudgill (1987) who reported that nematode damage was highest at low fertilisation levels.

The interaction between soil type and nematode damage depends on the yield potential of the site and differs between cultivars as it is strongly related to ground cover and the amount of foliage. On favourable sites and under favourable conditions, there is substantial crop growth, with ample foliage that maintains full ground cover for some time, even after infestation by potato cyst nematodes. When the site is unfavourable for growth (compacted soil or low fertility) the detrimental effects of nematodes further reduce growth so that full ground cover cannot be attained. This will reduce light interception and substantially depress yields.

As mentioned above, potato cyst nematodes may interact with secondary pathogens, increasing senescence and yield loss. Differences in nematode damage between fields might therefore be caused by differences in the presence of other pathogens. This possibility was not investigated in this thesis.

AGRONOMIC IMPLICATIONS

Potato cultivars

Tolerance is associated with large tops and with the ability to produce extra roots after nematode infection. These characteristics are related to late maturing cultivars, so growing such cultivars will restrict nematode damage. The cultivars should preferably also be resistant, to reduce or prevent nematode multiplication. The resulting low nematode population densities prevent future problems with potato cyst nematodes. For *G. pallida*, however, the choice of resistant cultivars is limited, and resistant cultivars do not always have the desired commercial qualities. There remains a role for breeders to combine tolerance and resistance in cultivars with the desired commercial qualities.

Improving tolerance by cultural measures

In Chapter 5 it was shown that nematode-induced phosphorus deficiency could be relieved by phosphorus application. However, in the experiment an excessive amount of phosphate was applied which is not suitable for current practice. Methods such as foliar application of phosphorus or in-row fertiliser placement might boost phosphorus in infested plants to adequate levels, thereby preventing nematode-induced phosphorus deficiency and limit damage. The benefits of these methods should be investigated. Potato leaves can absorb foliar applied phosphorus (Lewis and Kettlewell, 1993) and positive effects of foliar applications of nutrients on yield have been found (Albregts and Howard, 1986; Clarkson and Scattergood, 1982). To prevent leaf damage, nutrient concentrations in the spray solution have to be low, limiting the amount of nutrients which can be supplied by one foliar spray (Marschner, 1986). As nematode-induced phosphorus deficiency occurs in small plants with a small leaf area, multiple sprayings will be necessary for a sufficient uptake of foliar-applied phosphorus. In-row fertiliser placement increases the availability of nutrients near the plant and enhances the growth of young plants (Knittel, 1988). Thus, a similar phosphorus availability near the plants as in the experiment of Chapter 5 may be obtained and prevent nematode-induced phosphorus deficiency. Some damage, however,

will remain because, as shown in this thesis, nematode damage cannot be fully ascribed to nutrient deficiency.

At the end of the growing season, a nematode-infested crop senesces earlier and the resulting reduced amount of intercepted light contributes strongly to the yield reduction after nematode infestation. The cause of increased senescence of nematode-infested crops was not specifically studied in this thesis, but discussed earlier in this Chapter in the paragraph on nematode effects at late stages of growth. The reduced initial growth of nematode-infested crops gave a reduced amount of foliage throughout the season resulting in earlier foliage death. Therefore, increasing the initial growth of nematode-infested crops, e.g. by preventing phosphorus deficiency, may delay foliage death and reduce nematode damage.

Leaf senescence is largely regulated by limited water and nitrogen availability (Gan and Amasino, 1997) and options to remedy early senescence of nematode-infested crops by irrigation or fertilisation requires further study. Nematode-induced drought stress seems difficult to remedy, given that even when abundant soil water was available, the infested crops wilted on days with a high evaporative demand. Nitrogen deficiency towards the end of growth of infested crops may be relieved by a supplemental nitrogen dressing. Millard and Robinson (1990) found positive effects on tuber yield of late applications of N as foliar sprays. The effects of foliar application of nitrogen on leaf area duration and yield of nematode-infested crops merits further study.

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SUMMARY

Potato cyst nematodes (*Globodera* spp.) are one of the major pests of potato (*Solanum tuberosum* L.) and cause severe yield losses. Yield loss can be prevented by reducing population densities to low levels. This can be achieved by crop rotation, application of nematicides or growing resistant cultivars. A wide crop rotation and the use of nematicides are economically or environmentally less attractive. The most attractive option is to grow cultivars that are resistant to potato cyst nematodes, as these cultivars restrict or prevent nematode multiplication. However, these cultivars may be intolerant, meaning that they cannot withstand or recover from the damaging effects of nematodes. Tolerance to potato cyst nematodes varies between cultivars and is not related to the level of resistance. Furthermore, yield loss is affected not only by the cultivar and initial nematode population density, but also by environmental factors such as soil type, crop husbandry, weather and secondary pathogens. Understanding of these interactions is important for potato growers in order to be able to take appropriate measures to minimise nematode damage.

The objective of the research described in this thesis was to determine the major mechanisms by which potato cyst nematodes reduce potato crop growth and to explain the interactions with cultivar and abiotic soil factors observed by other researchers. Nematodes disrupt crop growth processes from the beginning of the season onwards, so it can be postulated that altered growth patterns such as restricted root extension may increase damage at later stages of growth when nutrients or water are exhausted in the rooted volume of soil. Therefore, the experiments were largely carried out under field conditions and potato crop growth characteristics were studied over time.

The effects of potato cyst nematodes on the crop start when the juveniles invade the roots. Therefore, special attention was paid to the effects of nematodes on root growth. In Chapter 2, two methods for studying root growth in the field, core sampling and observations with vertically oriented minirhizotrons, are compared to identify the most suitable method to obtain information on development of potato roots in the field. It is concluded that spatial distribution of roots should be analysed with core sampling, as the minirhizotrons affected vertical distribution of the roots, probably because of preferential root growth along the tube. Both methods proved suitable for studying temporal development of roots; sequential measurements of roots give the net effect of root growth and decay. Data on root turnover can only be obtained with minirhizotrons by comparing video recordings of different dates.

Chapter 3 describes two field experiments in which the effects of nematodes on growth of four different cultivars were studied at various levels of soil compaction, to examine the role of soil strength in nematode damage. Both high nematode density and soil compaction caused severe yield losses of all cultivars except Elles, which was tolerant of nematode attack. Cultivar Elles was characterised by thick roots, a high root length density and it showed a prolonged period of partitioning of biomass to the leaves. The effects of nematodes and soil compaction were generally cumulative, suggesting that variation in yield loss caused by nematodes on different soil types is not related to differences in root system expansion between soils of various strengths. However, in one of the two years of experimentation anomaly was found in the additive effects: the nematode-tolerant cultivar Elles showed no nematode damage on loose soil but on compacted soil nematodes reduced final tuber yield by 45 percent. The combined effects of soil compaction and nematodes reduced the foliage to such an extent that full ground cover was not reached. It is surmised that the effects of soil compaction on growth forestalled the advantageous effect of large tops, thus removing the tolerance property of cultivar Elles. In one experiment, high nematode density led to decreased root lengths, lower plant nutrient concentrations and increased senescence. The yield loss which occurred in the second experiment was attributed to the effects of nematodes on other aspects of plant physiology.

Analysis of field and container experiments showed that potato cyst nematodes reduced leaf nutrient concentrations and crop growth, and that leaf phosphorus concentration correlated best with total crop biomass at early stages of growth. As soil pH affects phosphorus availability and interacts with nematode damage, the effects of potato cyst nematodes, soil pH and phosphorus fertilisation on potato crop growth were studied in the field and in the Wageningen Rhizolab (Chapters 4 and 5). At 61 days after planting, crop growth in the field experiment at pH_{KCl} 6.1 was limited by P deficiency and the ratio of root length to leaf area (RL/LA) was increased. Application of 225 kg P ha^{-1} relieved P deficiency and decreased the RL/LA from 3.3 to 1.9 km m^{-2}. Nematodes induced or aggravated P deficiency, but had a small effect on RL/LA. Additional P fertilisation increased leaf phosphorus concentration and crop growth, but did not overcome nematode damage. At high, non-limiting levels of leaf phosphorus concentration, nematodes still reduced total biomass.

In the Wageningen Rhizolab, directly after planting, the number of roots visible against minirhizotrons was reduced by nematodes and lagged behind that of the control. However, the increase in root number of the nematode treatment continued longer until root number was higher than that of the control. The increased root number of the nematode treatment was restricted to the top 30 centimetres and nematodes reduced rooting depth.

It is concluded that potato cyst nematodes induce phosphorus deficiency at early stages of growth, which is overcome later in the season by compensatory root growth. High soil pH mainly reduced growth by reducing the availability of phosphorus. It is suggested that the

effect of both nematodes and soil pH on phosphorus uptake explains the earlier observed interaction between soil pH and potato cyst nematodes on potato growth.

In Chapter 6, the damage mechanisms of potato cyst nematodes are discussed in the context of the results of the previous chapters and the literature. From studies of potato crop growth over time, conducted to elucidate nematode damage mechanisms and their interactions with abiotic factors, it is concluded that both hormonal signalling and phosphorus deficiency are involved in the initial growth reduction after nematode infestation. At the end of the growing season, senescence of nematode-infested crops is increased. It is postulated that this is caused by drought stress, resulting from reduced root conductivity, or by nitrogen deficiency. Leaves senesce when tubers withdraw nitrogen from the foliage. This may be hastened by nematodes, as they reduce nitrogen concentrations in the foliage and reduce the nitrogen pool by decreasing the amount of foliage. No clear-cut conclusions can be drawn, however, and it is noted that the causes of senescence require further study.

The experiments and literature reports suggested that a high tolerance of cultivars to potato cyst nematodes is associated with production of extra roots and large tops, a characteristic of late-maturing potato cultivars. The influence of soil parameters on nematode damage also depended on the amount of foliage produced. It is argued that crops with large tops suffer little yield loss as they are able to maintain ground cover and light interception, even after infestation by potato cyst nematodes. In addition to using late cultivars, it is advantageous to have a site with a high yield potential, as this will reduce yield loss.

The thesis concludes with a discussion of the practical implications of the results. Given that a wide crop rotation and the use of nematicides are economically or environmentally less acceptable, growing resistant and tolerant cultivars is the most attractive option to reduce nematode densities. Tolerance of potato cyst nematodes is not only related to the cultivar but can be improved by cultural measures. Initial nematode-induced phosphorus deficiency can be alleviated by fertilisation methods that increase phosphorus in infested plants to adequate levels. Crop senescence may be delayed by increasing nitrogen in infested plants, e.g. by foliar application of a nitrogen fertiliser, but this requires further study.

SAMENVATTING

Aardappelcysteaaltjes (*Globodera* spp.) vormen een belangrijke plaag in de aardappelteelt (*Solanum tuberosum* L.) en kunnen veel schade veroorzaken. Schade kan voorkomen worden door populatiedichtheden van de aaltjes te verlagen, hetgeen bereikt kan worden via gewasrotatie, het gebruik van nematiciden of door de teelt van resistente rassen. Een ruime gewasrotatie en het gebruik van nematiciden zijn vanuit economisch of milieukundig oogpunt minder aantrekkelijk. De aantrekkelijkste optie is de teelt van rassen met resistentie tegen aardappelcysteaaltjes omdat deze de vermeerdering van de aaltjes verhinderen of beperken. Resistente rassen kunnen echter intolerant zijn, wat betekent dat ze niet bestand zijn tegen aaltjesaantasting of niet kunnen herstellen na infectie door de aaltjes. Tolerantie voor aardappelcysteaaltjes staat los van de graad van resistentie en verschilt tussen rassen.

Naast afhankelijkheid van ras en initiële aaltjesdichtheid wordt schade ook beïnvloed door omgevingsfactoren zoals grondsoort, teeltwijze, klimaat en aantasting met secundaire pathogenen. Een beter begrip van deze interacties is belangrijk voor aardappeltelers zodat geschikte maatregelen genomen kunnen worden om aaltjesschade te minimaliseren.

Het doel van het in dit proefschrift beschreven onderzoek was het vaststellen van de belangrijkste mechanismen waarmee aardappelcysteaaltjes de groei van aardappel reduceren en een verklaring te geven voor de interacties tussen aardappelcysteaaltjes en abiotische bodemfactoren zoals die gevonden zijn door andere onderzoekers. Vanaf het begin van het seizoen verstoren aaltjes gewasgroeiprocessen. In de loop van het seizoen kunnen deze initiële effecten via veranderde groeipatronen de schade vergroten, bijvoorbeeld via een verminderde worteldiepte en uitputting van voedingsstoffen of water in de bewortelde zone tegen het eind van het seizoen. Om deze effecten goed te kunnen bestuderen zijn de proeven grotendeels uitgevoerd onder veldomstandigheden en is de groei van het gewas in de tijd gevolgd.

De eerste effecten van aaltjes op gewasgroei ontstaan wanneer juvenielen de wortels binnendringen. Speciale aandacht is daarom besteed aan de effecten van aaltjes op wortelgroei. In Hoofdstuk 2 worden twee methoden vergeleken om wortelgroei in het veld te bestuderen: boorbemonstering en waarneming met verticaal geplaatste glazen buizen (minirhizotrons). Er werd gezocht naar de meest geschikte methode om informatie te krijgen over de ontwikkeling van aardappelwortels in het veld. De conclusie was dat de ruimtelijke verdeling van wortels het beste waargenomen kan worden via boorbemonstering. Dit omdat de minirhizotrons de verticale verdeling van de wortels beïnvloedden, waarschijnlijk vanwege voorkeursgroei van wortels langs de buis. Voor

bestudering van de wortelontwikkeling over de tijd bleken beide methoden geschikt: opeenvolgende metingen geven het netto effect van groei en afbraak van wortels. Meer specifieke informatie over wortelturnover kan echter alleen via minirhizotrons verkregen worden door video-opnamen van verschillende waarnemingsdata te vergelijken.
Hoofdstuk 3 beschrijft twee veldproeven waarin de effecten van aardappelcysteaaltjes op de groei van vier verschillende aardappelrassen bekeken werden. Dit gebeurde bij drie niveaus van bodemdichtheid om het effect van verschil in indringingsweerstand in de bodem op aaltjesschade te bekijken. Zowel aaltjes als bodemverdichting veroorzaakten grote schade bij alle cultivars, behalve bij Elles die tolerant bleek tegen aaltjesaantasting. Het ras Elles had dikke wortels, een hoge worteldichtheid en een lange periode waarin bladgroei plaatsvond. De effecten van aaltjes en bodemverdichting waren in het algemeen additief. Dit geeft aan dat variatie in schade door aardappelcysteaaltjes op verschillende grondsoorten niet gekoppeld is aan verschillen in worteluitbreiding tussen bodems met verschillende indringingsweerstand. In één van de twee proefjaren werd echter een uitzondering op de additieve effecten gevonden: de tolerante cultivar Elles leed geen aaltjesschade op losse grond, maar op verdichte bodem reduceerden de aaltjes de uiteindelijke knolopbrengst met 45 procent. De combinatie van verdichting en aaltjes reduceerde de hoeveelheid loof dusdanig dat een volledige bodembedekking niet bereikt werd. Verondersteld wordt dat de bodemverdichting een dusdanige groeireductie gaf, dat het voordeel van een grote loofhoeveelheid niet bereikt werd en de tolerantie-eigenschap van Elles verdween. In één proef veroorzaakte aaltjesaantasting een afname in de wortellengte, lagere nutriëntengehaltes in het gewas en vervroegde gewasafsterving. Schade in de tweede proef werd toegeschreven aan effecten van aaltjes op andere plantenfysiologische aspecten.
Uit bestudering van veld- en bakkenproeven bleek dat aardappelcysteaaltjes zowel nutriëntengehaltes in het blad als de gewasgroei reduceerden. De totaal geproduceerde hoeveelheid biomassa correleerde het beste met het fosfaatgehalte in het blad van een jong gewas. De rol van fosfaat in het schademechanisme werd bekeken in proeven in het veld en in het Wageningen Rhizolab (Hoofdstukken 4 en 5). Omdat de fosfaatbeschikbaarheid in de bodem wordt beïnvloed door de zuurgraad van de bodem en omdat bodem-pH invloed heeft op de schade door aaltjes, werd de invloed van aardappelcysteaaltjes, bodem-pH en fosfaatbemesting op gewasgroei onderzocht. Bij een pH_{KCl} van 6.1 was op 61 dagen na poten de gewasgroei in het veld gereduceerd door fosfaatgebrek en was de verhouding wortellengte - bladoppervlak (RL/LA) verhoogd. Bemesting met 225 kg P ha^{-1} hief het fosfaatgebrek op en verlaagde de RL/LA van 3.3 naar 1.9 km m^{-2}. Aantasting door aardappelcysteaaltjes induceerde of versterkte het fosfaatgebrek maar had weinig effect op de RL/LA. Fosfaatbemesting verhoogde zowel het fosfaatgehalte in het blad als de gewasgroei maar kon aaltjesschade niet voorkomen omdat bij hoge, niet-beperkende fosfaatgehaltes de biomassaproductie nog steeds gereduceerd was.

In het Wageningen Rhizolab werd het aantal zichtbare wortels tegen minirhizotrons direct na poten verminderd door aaltjes en bleef achter bij de controle. Het wortelaantal nam bij aaltjes echter toe over een langere periode, zodat er uiteindelijk meer wortels waren dan bij de controle. De verhoging van het wortelaantal door aaltjes bleef beperkt tot de bovenste 30 centimeter van de grond, beneden de bouwvoor van 30 cm verminderden aaltjes het aantal wortels en de bewortelingsdiepte.
Het onderzoek leidde tot de conclusie dat aardappelcysteaaltjes vroeg in het seizoen fosfaatgebrek induceren, hetgeen later in het seizoen ongedaan gemaakt wordt door compensatiegroei van wortels. Een hoge bodem-pH reduceerde de groei voornamelijk via verlaging van de fosfaatbeschikbaarheid. Het effect van zowel aaltjes als bodem-pH op de fosfaatopname kan de eerder waargenomen interactie verklaren tussen effecten van aardappelcysteaaltjes en bodem-pH op aardappelgroei.
In Hoofdstuk 6 worden de schademechanismen van aardappelcysteaaltjes besproken in relatie tot de resultaten uit de diverse hoofdstukken en de literatuur. De effecten op gewasgroei van aardappel werden bestudeerd gedurende het seizoen om inzicht te krijgen in schademechanismen en de wijze waarop interactie met omgevingsfactoren tot stand komt. De conclusie is dat zowel hormonale signalen als fosfaatgebrek betrokken zijn bij de initiële groeireductie na aaltjesaantasting. Aan het einde van het groeiseizoen sterven door aaltjes aangetaste gewassen eerder af. De stelling is dat dit veroorzaakt kan worden door droogte, hetgeen voortvloeit uit een verlaagd wortelgeleidingsvermogen, of door stikstofgebrek. Bladeren sterven af wanneer knollen er stikstof uit onttrekken. Aaltjes kunnen dit versnellen omdat deze de stikstofvoorraad in het blad verkleinen door verlaging van de stikstofgehalten en verlaging van de hoeveelheid blad. Duidelijke conclusies omtrent de vervroegde afsterving van door aaltjes aangetaste gewassen kunnen echter niet getrokken worden en verder onderzoek naar de oorzaken van afsterving is nodig.
Uit de proeven en literatuur komt naar voren dat hoge tolerantie van rassen voor aardappelcysteaaltjes samengaat met productie van extra wortels en veel loof, een eigenschap van late rassen. Ook het effect van bodemfactoren op aaltjesschade hangt af van de geproduceerde hoeveelheid loof. Gewassen met veel loof ondervinden minder schade omdat deze in staat zijn de bodem volledig bedekt te houden, zelfs na aantasting door aardappelcysteaaltjes, en zodoende maximaal zonlicht onderscheppen. Naast het gebruik van late rassen is het dus voordelig om te telen op een veld met een hoog opbrengstpotentieel waardoor de schade beperkt blijft.
Het proefschrift sluit af met een discussie over de consequenties van de resultaten voor de praktijk. Aangezien een ruime gewasrotatie en het gebruik van nematiciden economisch of milieukundig minder gewenst zijn, is de teelt van resistente rassen de aantrekkelijkste wijze om populatiedichtheden van aaltjes te reduceren. Om schade te beperken dienen deze rassen tolerant te zijn. Tolerantie hangt af van het ras maar kan verhoogd worden via

teeltmaatregelen. Door aaltjes geïnduceerd fosfaatgebrek kan opgeheven worden via bemestingsmethoden die het fosfaat in aangetaste planten in voldoende mate verhogen. Gewasafsterving kan uitgesteld worden door de hoeveelheid stikstof in aangetaste planten te verhogen, bijvoorbeeld via bladtoediening van een stikstofmeststof. Deze methoden dienen voor praktische toepassing nog onderzocht te worden.

ACCOUNT

The chapters 2 - 5 in this thesis have been published or are submitted to the following journals:

2. De Ruijter F J, Veen B W and Van Oijen M 1996 A comparison of soil core sampling and minirhizotrons to quantify root development of field-grown potatoes. Plant and Soil 182: 301-312.

3. Van Oijen M, De Ruijter F J and Van Haren R J F 1995 Analyses of potato cyst nematode-related effects on growth, physiology and yield of potato cultivars in field plots at three levels of soil compaction. Annals of Applied Biology 127: 499-520

4. De Ruijter F J and Haverkort A J Potato growth as affected by potato cyst nematodes (*Globodera pallida*) and soil pH. 1. Root growth and nutrient uptake. European Journal of Plant Pathology. Submitted.

5. De Ruijter F J and Haverkort A J Potato growth as affected by potato cyst nematodes (*Globodera pallida*) and soil pH. 2. Foliar nutrient concentrations and crop growth. European Journal of Plant Pathology. Submitted.

OTHER PUBLICATIONS OF THE AUTHOR

- De Ruijter F J and Haverkort A J 1996 Effect of potato cyst nematodes on nutrient uptake of potato. *In* Abstracts 13th Triennial Conference of the European Association for Potato Research, Veldhoven, the Netherlands, p 371-372.
- De Ruijter F J and Haverkort A J 1996 Potato crop growth and nutrient concentration as influenced by soil-pH and potato cyst nematodes. *In* Book of Abstracts, 4th Congress of the European Society for Agronomy, Veldhoven, the Netherlands, p. 534-535.
- De Ruijter F J and Jansma J E 1994 De bol in getal. Modelmatige beschrijving van produktie- en milieuvariabelen van bloembolgewassen met behulp van het rekenmodel TCG_CROP. Rapport 17, AB-DLO, Wageningen, 36 pp. + 13 pp. bijl.
- De Ruijter F J and Van Oijen M 1994 The effect of potato cyst nematodes and soil compaction on growth of some potato cultivars. Rapport 6, AB-DLO, Wageningen, 40 p. + 35 p. App.
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- Van Oijen M, De Ruijter F J and Van Haren R J F 1995 Modelling the interaction between potato crops and cyst nematodes. *In* Haverkort A J and MacKerron D K L (Eds), Potato ecology and modelling of crops under conditions limiting growth. Kluwer Academic Publishers, p 185-195.
- Van Oijen M, De Ruijter F J and Ammerlaan F H M 1993 Simulation of root growth reduction following compaction of nematode infested soil. *In* Abstracts 12th Triennial Conference of the European Association for Potato Research, Paris, France, p. 135-136.

CURRICULUM VITAE

Frank de Ruijter werd geboren op 1 november 1966 te Terneuzen en bracht zijn jeugd door in de Flevopolder waar hij opgroeide op een akkerbouwbedrijf te Biddinghuizen. Na het behalen van het VWO-diploma aan het Christelijk College Nassau Veluwe te Harderwijk begon hij in 1984 met de studie Landbouwplantenteelt aan de toenmalige Landbouwhogeschool, nu Landbouwuniversiteit (LUW), te Wageningen. In Augustus 1990 studeerde hij af met als doctoraalvakken Landbouwplantenteelt, Nematologie en Theoretische Productie Ecologie. Tijdens zijn studie heeft hij zijn praktijk doorgebracht bij het Seed Potato Production Centre in het toenmalige Noord Jemen.
Van september 1990 tot januari 1992 werkte hij bij het toenmalige Centrum voor Agrobiologisch Onderzoek (CABO-DLO), het huidige DLO-Instituut voor Agrobiologisch en Bodemvruchtbaarheidsonderzoek (AB-DLO), in onderzoek naar effecten van aardappelcysteaaltjes op gewasgroei van aardappel. Vanaf september 1992 is hij in dienst bij het AB-DLO en heeft hij het onderzoek naar de effecten van aardappelcysteaaltjes en abiotische factoren op gewasgroei van aardappel voortgezet en beschreven in dit proefschrift. Tevens heeft hij gewerkt aan modelmatige beschrijving van productie- en milieuvariabelen van bloembolgewassen voor verkenning van milieuvriendelijke bloembolproductiesystemen met behulp van interactieve meervoudige doelprogrammering. Dit wordt voortgezet in een studie naar ecologisering van landbouwproductiesystemen, waarin de modellen uitgebreid worden met meer gewassen en met dierlijke systemen. Sinds eind 1997 werkt hij aan modellering van nutriëntenstromen op bedrijfsniveau en binnen de Nederlandse landbouw.