Solanum sisymbriifolium (Lam.): A trap crop for potato cyst nematodes

Abstract

Timmermans, B.G.H., 2005. *Solanum sisymbriifolium* (Lam.): A trap crop for potato cyst nematodes. Potato cyst nematodes (PCN), *Globodera pallida* (Stone) and *G. rostochiensis* (Woll.) continue to be a major pest in potato growing areas, in spite of existing control measures. Therefore, *Solanum sisymbriifolium* (Lam.) was introduced as a trap crop for PCN in Western Europe. The current study was performed to collect quantitative information on the ecology, agronomy and potential nematode reduction of *S. sisymbriifolium* crops in The Netherlands.

The emergence rate of *S. sisymbriifolium* was almost zero at temperatures below 8 °C. The relation between germination and temperature at sub-optimal temperatures is adequately described with expolinear and quadratic equations. These are relatively simple, and applicable in modelling germination of other plant species grown in conditions near their base temperature. Plantings between May and the end of July led to a crop with full ground cover. Initial growth was slow, but indeterminate and accumulated amounts of above-ground biomass were high (more than 10 tons ha^{-1} dry matter after 100 days). Root length density could be reasonably well assessed from above-ground crop characteristics, and was linked to theoretically possible nematode reductions. In a greenhouse study in containers, nematode population reduction was found to be related to root length density and length of the growth period of the crop. After 150 days of crop growth the PCN population density was reduced with 75% on average; in the soil layer with maximum root length density of 5.8 cm cm^{-3} the PCN population reduction was 86%. A general formula was derived to calculate the time gained to reduce the PCN population density below threshold levels, comparing a scenario with cultivation of one crop of *S. sisymbriifolium*, followed by non-hosts or fallow, with a scenario with natural decay of the PCN population (non-hosts or fallow throughout). Calculations indicated that 75% reduction of PCN amounts to 4 years time gain to sanitation. *S. sisymbriifolium* is highly tolerant to PCN, enabling successful growth in PCN infested soil. Furthermore, it is highly resistant against several isolates of *Phytophthora infestans* (Mont.) de Bary, with low infection efficiency and lesion growth rates. Therefore, *S. sisymbriifolium* is an interesting PCN trap crop, deserving further development.

Keywords: *Solanum sisymbriifolium*, *Globodera pallida*, *Globodera rostochiensis*, germination, light use efficiency, crop growth, crop management, root length density, hatching, trap crop, tolerance, *Phytophthora infestans*, resistance.

Nao dees veer gooj jaore
Is het den toch gedaon
Alles is biejein gezoch en aafgewirktj
Noe is het tied om wiejer te gaon

Mer wo de waeg auch haer veure moog
En waat wiejer alles passere zal
Ei klein stökske van mich zal neet verangere
Det is waal te huere aan miene Ingelse kal

Ich bun oppe Echterhei opgegreujd
En kin se noch ummer waal wete
Es se kieks nao allemaol klein dinger
Die zeen der gans deep ingeslete

Mien gewuentes vinje ze hiej nog altied vreamdj
Sjroeap onger de kiës, dao hoofse neet mèt aan te kómme
Nae, zoea loeas zeen ze hiej noch neet gewore
Auch al höbbe ze waal de vlaai äövergenómme

Eder waek woord hiej hel gewirktj
Mer es het vriedes ‘s naomiddes woor
Zoea teange vief oer, den woord dea wage gepaktj
Womèt ich den nao het zuije voor

Ein aantal van uch höbbe noeajt begrepe
Womèt ich mich al dea tied bezig höb gehaoje
Of geer noe oet het zuje of noorde kómptj, stuit zich geliek
Ich zal uch neet langer in spanning haoje

Kiek mer ins waat het gewore is

Contents

CHAPTER 1

General introduction

Potato cyst nematodes (PCN), *Globodera rostochiensis* (Woll.) and *G. pallida* (Stone) are a permanent threat in potato production areas. On a world scale, PCN were estimated to account for more than 12% losses in crop yield (Urwin *et al.*, 2001). Also in Europe the problem is present: a first structured survey taken in England and Wales showed that PCN were present in 64% of all sampled sites (Minnis *et al.*, 2002). Annual yield losses in the UK were estimated at 30 million Euro (Haydock and Evans, 1998). At least since 1948 (Mulder, 1994) nematodes have been present in Dutch potato production areas and ever since prevention and control measures have been taken.

History of potato growing and PCN in Europe

Potatoes became an important food crop in Ireland at the end of the seventeenth century and in Western England, Scotland, Germany and The Netherlands by the end of the eighteenth century. Many peasant farmers became so dependent on the crop, that the appearance of potato late blight (*Phytophthora infestans*) in 1845 and 1846, suddenly causing huge losses, brought famine to Ireland (Evans *et al.,* 1975). Following this disaster, new plant material was imported from South America to breed for late blight resistance and early maturity, and it is suspected that with this breeding material also the first PCN were brought to Europe (Evans *et al.,* 1975). Other theories on possible means of transport suggest PCN to have been present in Peruvian Guano, once used widely throughout Europe as a fertilizer (Inagaki and Kegasawa, 1973). The first infections were noticed near Kühn in Germany in 1881 and the species was called *Heterodera rostochiensis* (Wollenweber, 1923). Later a second species of PCN, *Heterodera pallida*, was identified and described (Stone, 1973). These names were changed to *Globodera rostochiensis*, also called the yellow or golden potato cyst nematode, and *G. pallida*, called the pale or white potato cyst nematode.

Brief biology of PCN

PCN are named after their soil-borne survival structure, the cyst, which consists of the toughened integument of the dead female body, filled with embryonated eggs (Mulder and Van der Wal, 1997). After the host potato crop is harvested, these cysts remain in

the soil and each year part of them hatches spontaneously, causing an annual decline of their population size (Devine *et al.*, 1999). However, part of the cysts survives for many years without a host crop (some report more than 20 years, Jones *et al.*, 1998). If PCN are stimulated by potato root exudate or tomato root exudate the eggs can hatch within a few days (e.g., Fenwick, 1952; Ellenby and Gilbert, 1958). Research has shown that a small group of chemical compounds (at least nine, Devine and Jones, 2000), called hatching factors, is causing this reaction. Some were purified, e.g. solanoeclepin A (Mulder *et al.*, 1996) and their structure was characterized, which was of quite complex nature (Schenk *et al.*, 1999; Benningshof *et al.*, 2002). Furthermore, not only hatching stimulators were present in root exudates, but also hatching inhibitors (Byrne *et al.*, 1998). The hatching agents are active at extremely low concentrations (*in vitro* at less than 2.1×10^{-8} M) (Devine and Jones, 2000b). Some reports state that nematodes show seasonal variation in the degree of hatching when exposed to hatching factors due to diapause (Fenwick and Reid, 1953; Muhammad, 1994), although Scholte and Vos (2000) found no variation in hatchability during the growing season (May – September).

If stimulated to hatch by hatching factors of a host crop, the nematodes invade its roots (Jones, 1981). Inside these roots, the nematodes migrate through the cortex to the region of cell differentiation and to the periphery of the vascular tissue, passing from cell to cell, cutting through cell walls with their stylets and rupturing or destroying cells on their way (Mulder and Van der Wal, 1997). Once arrived near the stele, the nematode starts to feed by piercing cells. Injection of saliva in these cells induces the formation of a feeding site or syncytium: cell walls of adjacent cells partly dissolve and a large, multi-nucleate structure with a dense granular cytoplasm is formed. This has the characteristics of a transfer cell and expands gradually (Jones and Northcote, 1972; Mulder and Van der Wal, 1997). In the root, differentiation into male and female nematodes takes place and the nematodes sexually reproduce. During the course of the season, the new cysts are formed, each containing up to 300 or 400 eggs. Possible population multiplication factors of the potato cyst population in one season are quite variable, ranging from 2.5 to 150 (!) for low initial infestation levels (Seinhorst, 1982a). The multiplication factor is density dependent (Trudgill *et al.*, 1996), meaning that small populations may increase enormously, whereas larger populations have lower multiplication factors due to damage to the root system of the host crop and increased competition between the nematodes.

The invasion of the nematodes in the roots has a strong influence on potato plants: leaf nutrient concentrations are lower, leaf senescence is accelerated, root growth is slower but continues longer during the crop cycle and overall growth is strongly reduced compared to the controls (De Ruijter and Haverkort, 1999; Van Oijen *et al.*,

1995). With higher infection density, the decrease in growth and dry matter production is higher (Mulder *et al.*, 1997), and the damage to the tuber production is higher as well. Furthermore, infestation results in an economic damage for farmers that extends beyond the direct loss in potato production. It is forbidden to produce propagation material on infested fields of those plant species with soil adhering to the product. An important example of the consequences for the Dutch Flevo Polders is the prohibition of cultivation of flower bulbs on PCN-infested fields. From a legislative viewpoint PCN are a quarantine disease.

Control options

Several measures are available to control PCN: wide crop rotations, cultivation of resistant cultivars or the use of nematicides. Each of these has its own advantage and set-backs as discussed below and additional control measures are therefore welcomed.

The use of crop rotations (Urwin *et al.*, 2001) to control the nematodes implies a very low frequency (once every 6 – 7 years) of potato in the rotation. This prevents the PCN from reaching population densities high enough to substantially reduce yield. As potato is an economically profitable crop, at least in Dutch farming conditions, such a rotation is often undesirable, and other control measures need to be taken (Schomaker and Been, 1999).

The use of resistant cultivars is often limited by their availability. In some market outlets resistant cultivars are present, e.g., the ware potato cv. ‘Santé’, that have been reported to reduce multiplication of some populations of *G. pallida* to a large extent (Whitehead, 1991), but still there is a lack of commerciall-attractive cultivars with *G. pallida* resistance (Urwin *et al.*, 2001).

The introduction of (partially) resistant potato cultivars derived from *Solanum vernei* and *S. tuberosum* subsp. *andigena* started a process of selection for pathotypes within the nematode population that were capable of reproducing on the resistant cultivars. In the late 1960s and early 1970s, resistances derived from *S. tuberosum* subsp. *andigena* were broken. Furthermore, results of field trials and data from growers showed that cultivars with resistances derived from *S. vernei* had high yield losses under moderate or high infestation levels of incompatible nematode populations (Mulder, 1994). This demonstrated that resistant cultivars derived from *S. tuberosum* subsp. *andigena* also had a relatively high level of tolerance, which was absent in resistant cultivars derived from *S. vernei* (Mulder, 1994). At first, development of tolerance was discouraged since it was not in line with the policy aimed at keeping low nematode levels. However, a reasonably degree of tolerance appeared necessary, and the current view is that resistant cultivars also need to show tolerance. Use of

(partially) resistant cultivars led to an increase in virulence in PCN populations (Turner and Fleming, 2002). As a consequence at least five different pathotypes of *G. rostochiensis* and seven pathotypes of *G. pallida* appeared over time (Fleming and Powers, 1998). Moreover, gradually *G. pallida* became the dominant species (Trudgill *et al.*, 2003). Breeding for resistances has been successful, but did a not bring a permanent solution of the PCN problems. The latter is especially true for the ware potato and seed potato sector, as it has been found to be difficult to combine PCN resistance with all other desired traits. The number of required traits of cultivars are less in starch potato production and here the use of (partially) resistant cultivars has been and still is a major method to control PCN. However, also in this sector, the battle between breeder and nematode continues as there are indications that *G. rostochiensis* reappears on the scene; this might indicate that the latest generations of resistant cultivars insufficiently control *G. rostochiensis*.

Since 1968, the use of nematicides (mostly 1,3-dichloropropene and methyl isothiocyanate) was promoted in The Netherlands as the major solution to problems with PCN (Schomaker and Been, 1999). Soil fumigations under optimum circumstances reduce population densities of plant-parasitic nematodes by 80%, enabling short rotations (at least one potato crop every three years). However, effects achieved in practice were often not that high and percentages of reduction reached were insufficient to prevent the population from increasing. Moreover, also the cost/ benefit ratio of the soil fumigants was poor (Schomaker and Been, 1999). In soils, the breakdown of frequently used soil fumigants (such as 1,3-dichloropropene) accelerated due to adapted microflora, reducing its effect on nematodes (Lebbink *et al.*, 1989).

In conclusion, therefore, there is scope for other control measures, e.g., the development of new (biological) nematicides (Twomey *et al.*, 2002) or the use of trap crops (Halford *et al.*, 1999; Scholte, 2000a, b, c; Scholte and Vos, 2000). A trap crop has to stimulate the hatch of juvenile PCN from their cysts by its root diffusates, while preventing subsequent reproduction of the hatched PCN. The reproduction can be prevented by resistance of the trap crop or by destruction of the crop before adult female PCN have developed. Scholte (2000a) showed that potato itself can be used, but it is not ideal as a trap crop. Problems included the exact timing of crop destruction, the stimulation of other potato diseases and the tubers that, if not all removed completely, can become volunteers in the next year. If not controlled, volunteer potato plants are point sources of PCN multiplication. After extensive screening of non-tuber bearing *Solanaceae* for their potential as trap crops, *Solanum sisymbriifolium* (Lam.) was selected as a promising candidate (Scholte, 2000b). This species reached a level of hatch stimulation slightly less than that of potato cv. Bintje and, although its roots are invaded by PCN (Roberts and Stone, 1983), it is resistant to

PCN, meaning no progeny cysts were detected at all (Scholte, 2000b). Reduction of 50 to 80% of the population of PCN was observed (Scholte, 2000c; Scholte and Vos, 2000), and further research on the crop seemed relevant.

S. sisymbriifolium is an exotic plant species originating from Latin-America. In South Africa it is reported to be an invasive weed species (Byrne *et al.,* 2002; Hill and Hulley, 2000). Other research focussed on using its root extracts in pharmacology (Ibarrola *et al.,* 2000) and eggplant is grafted on it to promote resistance against *Verticillium dahliae* (Bletsos *et al.,* 2003). In The Netherlands, it was envisaged to produce the seed needed for commercial application abroad. Hitherto, the risk of establishment as a weed was considered not to exist under Dutch conditions. *S. sisymbriifolium* is not very sensitive to night frost. Inclusion of *S. sisymbriifolium* in cropping systems was not expected to present major problems (Scholte and Vos, 2000), because the species does not act as a host plant for important (polyfagous) pests and diseases.

Explorative research yielded merely qualitative, but promising data on the plants' performance in the field (Scholte and Vos, 2000). However, for the introduction of *S. sisymbriifolium* as a trap crop in The Netherlands, quantitative information on the ecology and agronomy of the plant was necessary, and crop management recommendations needed to be established. Quantitative information on the relation between emergence rate and temperature was needed. To explore the range of conditions under which effective growth of *S. sisymbriifolium* as a trap crop was to be expected, dry matter accumulation in relation to temperature and radiation needed to be quantified, and quantification of the relations between above-ground crop growth, root dynamics and hatching were necessary. Also, information on the tolerance of *S. sisymbriifolium* to PCN was necessary in order to assess possible PCN density related plant damage in infested fields. While cultivating *S. sisymbriifolium* in the field, an infection with *Phytophthora infestans* (Mont.) de Bary occurred in the end of the growing season in 2001. Although the crops were not severely damaged and recovered from initial infection, general concern arose, and data on the susceptibility of *S. sisymbriifolium* was needed.

The general objectives of the current research:

(i) Quantification of the emergence rate of the crop in relation to environmental factors, particularly temperature and soil moisture (Chapter 1).

(ii) Quantification of the growth of the plant in relation to environmental factors, particularly temperature and radiation (Chapter 2).

(iii) Quantification of root growth (density per soil layer, rooting depth) in relation to above-ground crop biomass (Chapter 3).

(iv) The analyses of hatching of larvae of cyst nematodes in relation to root growth dynamics (Chapter 4).

(v) Quantification of possible growth-reducing effects of PCN on *S. sisymbriifolium*, i.e., the tolerance of the plant to PCN (Chapter 5).

(vi) Investigation into the susceptibility of *S. sisymbriifolium* to potato late blight (*Phytophthora infestans*) (Chapter 6).

CHAPTER 2

Germination rates of *Solanum sisymbriifolium*: Comparison of four temperature response models, effects of temperature fluctuations and soil water potential

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Summary

Four models were compared describing the germination or emergence rate of *Solanum sisymbriifolium* (Lamarck) over a broad range of sub-optimal temperatures and at different soil water potentials.

In the laboratory, the effects on emergence rate of constant (9.1 – 21.8 °C) and diurnally fluctuating temperature and of soil water potential were tested. Linear, Q10, expolinear and quadratic models were fitted to the data on rate of emergence against temperature. For model validation, field emergence was monitored in 16 experiments conducted in 2001 – 2004.

Germination rate increased with temperature, and was not influenced by soil water potential in the range of –1.26 to –0.003 10^{-3} MPa or by diurnal temperature fluctuations. Goodness of fit was best for the quadratic and the expolinear models. These two models had root mean square errors for predicting field emergence rates (5.8 – 66.5 days) of 2.3 and 2.4 days, respectively.

The expolinear and the quadratic models adequately described the temperature effect on germination, especially for crop species germinating near their base temperature.

Key words: *Solanum sisymbriifolium,* germination, model, nonlinear, thermal-time, fluctuating temperatures, soil-water potential, Q10.

Introduction

Solanum sisymbriifolium (Lamarck) was introduced in The Netherlands as a trap crop for potato cyst nematodes, PCN, following research that identified the species as the most promising candidate among a diversity of potential trap crops tested (Scholte, 2000a,b,c; Scholte and Vos, 2000). The plant was found resistant to PCN (Scholte, 2000a), and showed levels of hatch stimulation between 50% and 80%, i.e. slightly lower than that of potato (Scholte, 2000c; Scholte and Vos, 2000). *S. sisymbriifolium* originates from South America. Performance of this plant species is being studied for West-European field conditions (Chapter 3). Germination and field emergence have been found to be critical processes as temperatures below 12 °C appear to result in slow and irregular emergence (Chapter 3). The quantification of the rate and final percentage of emergence is therefore a key element in predicting the potential of the crop in different cropping systems in temperate potato production areas. Early in the season soil temperatures can be expected to be below 10 °C. This is close to the base temperature often observed in crops from warmer ecosystems, which is e.g. 6.0 – 9.5 °C for rice (Sié *et al.*, 1998) and 10 °C for most maize cultivars (Ruiz *et al.*, 1998). Furthermore, rather high temperature fluctuations can be expected in the top few centimeters of the soil of freshly planted fields. Also soil moisture conditions close to the surface can be expected to range from near saturated to rather dry depending on rainfall, radiation and wind.

Several models have been suggested to predict rates of developmental processes such as emergence under field conditions: the linear thermal time model (e.g. Covell *et al.*, 1986; Trudgill *et al.*, 2000; Steinmaus *et al.*, 2000; Alvarado and Bradford, 2002; Jame and Cuttforth, 2004), the Q10 model (Watts, 1971; Johnson and Thornley, 1985) and the bell-shaped beta function (Yin *et al.,* 1995; Jame and Cuttforth, 2004). The latter can only be fitted when also measurements of emergence rates are available from the supra-optimal range of temperatures. This makes that model more difficult to validate under typical field conditions. Instead, two new models were tested that have a comparable non-linear behaviour over the lower range of temperatures, i.e. the log-linear or 'expolinear' model (Goudriaan and Monteith, 1990; Lee *et al.*, 2003) and a quadratic model.

The objectives of the current study are: (i) to assess effects of constant and diurnally fluctuating temperatures and several constant soil moisture levels on the germination and emergence rates and final percentage germination or emergence of *S. sisymbriifolium*; (ii) to select a temperature response model that predicts well the germination and emergence rates of *S. sisymbriifolium* seeds under controlled

conditions; (iii) to test the model's accuracy for predicting field emergence, measured in independent field experiments.

Materials and methods

Seeds of *Solanum sisymbriifolium* were harvested from September to November in 2000, 2001, 2002 and 2003. Red berries were handpicked, washed, crushed and rinsed. Seeds were collected on a sieve, and air-dried before storage at 12 °C and 50% relative humidity.

All data on emergence and germination are expressed as percentage of the number of sown seeds. When germinated on paper (Expt 2), a seed was considered to have germinated when the root tip was visible. Emergence (all other experiments) was defined as the first visible appearance of plant tissue at the soil surface.

Experiment 1. Effect of different constant temperatures and soil water potential on the rate of emergence

Seeds harvested in 2003 were sown in July 2004 in plastic containers (100 seeds per container) (Greiner Bio-one, Kremsmuenster, Australia) at 1 cm depth, in a mixture of sand (grain size 0.18 − 1.00 mm) and clay powder (Kaolin, WBB Devon Clays Ltd, Newton Abbot, Devon, UK). The proportion sand : clay was 6 : 1 on volume base, and the pF-curve of the sand-clay mixture was measured to convert volume percentages of water into water potentials. Treatments included five different soil water potentials ranging from ~ −10 to -1.8×10^{-3}MPa (the pF-curve was steep at very low volume percentages of water and the value of −10 MPa is an estimate) and five different (constant) temperatures, ranging from 9.1 to 21.8 °C. Five replicates of 100 seeds were monitored for each of the 25 temperature by soil water potential treatments. Emergence was scored twice a day at the beginning of the experiment, and thereafter the scoring frequency was adapted to the rate of seed emergence per treatment.

Experiment 2. Effect of fluctuating temperatures on germination

Seeds harvested in 2003 were placed in petri dishes (100 seeds per dish; dish diameter 9 cm) on two layers of germination paper (Filter paper circles, 4.1 g each, diameter 85 mm, product nr. 3621, Schleicher and Schuell, Dassel, D) moistened with 14 ml tap water. Petri-dishes were placed in an experimental facility consisting of 100 separate, thermally isolated cells (diameter 102 mm, height 33 mm) to each of which an independent temperature regime can be applied using a computerized control system. Twelve different daily temperature regimes (Fig. 1) were applied in five replicates each. Six treatments were defined with mean daily temperatures of 15 °C and six

treatments with mean daily temperature of 9 °C. Treatments within series differed in maximum amplitude of the daily sine wave, ranging from 0 °C (constant temperature) to 10 °C. However, in order to prevent chilling or freezing effects, the sine waves in the 9 °C series were truncated with minimum temperatures set at 4 °C (Fig. 1). Germination was scored twice a day at the beginning of the experiment; later the scoring frequency was adapted to the rate of seed germination per treatment.

Experiment 3. Field emergence of* S. sisymbriifolium *seeds

A total of 16 sowings of *S. sisymbriifolium* were carried out in the field in the years 2001 – 2004 on sandy soil near Wageningen (51° 58' N, 5° 40' E), using a Hege precision-sowing machine (Hege Maschinen, Eging am See, D). Sowing depth was 2 – 4 cm and row distance 12.5 cm. Non-limiting amounts of nutrients were applied.

Temperature was recorded (Datataker DT600, Datataker Data Loggers, Cambridgeshire, UK) during the emergence process at sowing depth under bare soil using thermocouples (type T, TempControl Industrial Electronic Products, Voorburg, NL).

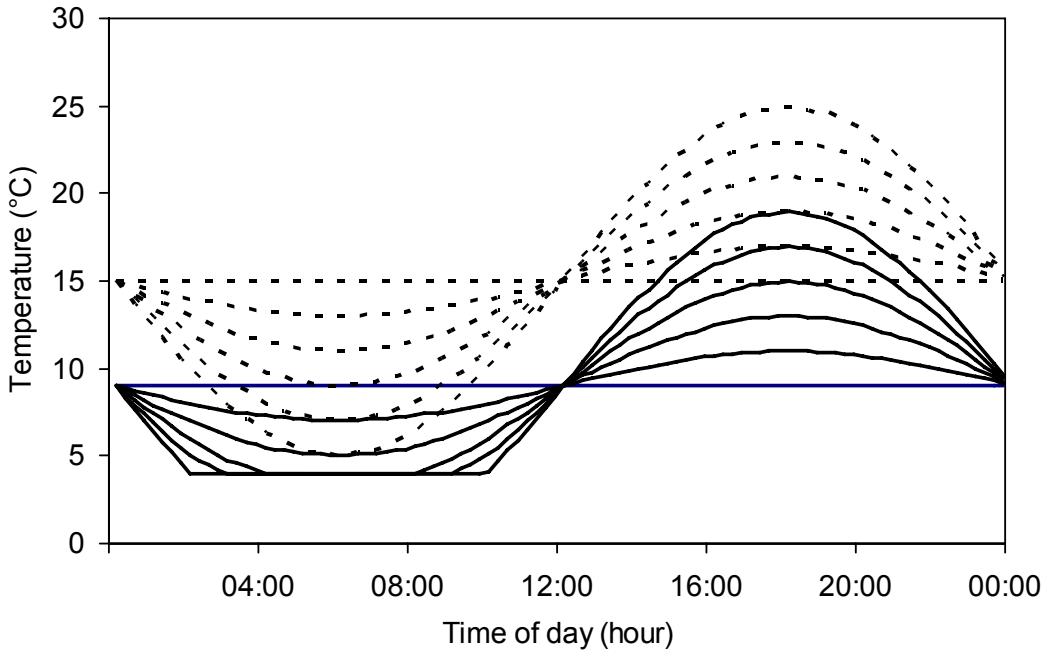


Figure 1. Diurnal temperature courses as applied in the twelve different temperature treatments in Experiment 2. Curves were sine functions (of one day wavelength) with daily means of 9 °C (solid lines) and 15 °C (dotted lines), respectively, with maximum amplitudes ranging from 0 to 10 °C. Functions were truncated at 4 °C, thus avoiding potential damage due to too low temperatures.

In 2001, four replicate fields were sown at a density of 400 seeds m^{-2} on seven different dates: first weeks of March, April, May, June, July, August and September. In each of the replicates, three row sections of 1 m (with 50 sown seeds each) were marked with plastic pegs, and within these row sections, emergence counts were made (frequency of scoring was adapted to the rate of seed emergence). In the years 2002 and 2003, a total of five sowings were repeated with 200 seeds m^{-2} (four replicates): the first week of May and the first week of June in 2002, and first week of May, third week of June and the first week of August in 2003. Emergence was monitored on row sections with 25 sown seeds each. In 2004, seeds were sown at a density of 200 seeds m^{-2} in six replicates on four dates: the fourth week of April, the fourth week of May, the last week of June and the first week of August. In each replicate, two areas of 0.5 × 0.5 m (with 50 sown seeds each) were marked and emergence counts were made in these areas.

Data processing and models

In all experiments, for each temperature treatment the progression of cumulative germination with time was fitted to the logistic model $y = a + b/(1 + e^{-c \times (x-d)})$; a, b, c and d being the parameters. With the fitted equation, the time to 50% emergence was calculated (unit: d). The rate of germination was then calculated as the reciprocal of the time to 50% germination (unit: d^{-1}), the same procedure was used to calculate the rate of emergence. Four different models were fitted to these rates of germination or emergence from different temperature treatments: linear, Q10, expolinear and quadratic models, using Slidewrite Plus for Windows version 5.01. Further statistics were performed using GenStat 7th edition version 7.1.0.205.

The widely used 'linear thermal time model' starts from fitting a simple linear regression of the rate of germination or emergence (y; unit: day^{-1}) on temperature (T; unit: °C) of the form (e.g. Kebreab and Murdoch, 1999a):

$$y = aT + b \qquad (1)$$

in which a (d^{-1} °C^{-1}) and b (d^{-1}) are parameters

The positive intercept with the T-axis is called the base temperature for germination, T_b, below which the rate of development is zero. T_b equals –b/a. Hence, Eqn. (1) can be rewritten to become:

$$y = a\,(T - T_b) \qquad (2)$$

which can be rewritten as:

$$1/a = \theta = 1/y\ (T - T_b) \quad (3)$$

where θ (unit: °C d) is the thermal time coefficient, the product of time (1/rate) and the temperature above the base temperature (T_b). θ has a constant value for the range of temperatures where the linear model holds.

In the Q10 model (Johnson and Thornly, 1985) the rate of germination was fitted to:

$$y = a^{(T-b)/10} \times c \quad (4)$$

in which a (-) , b (°C) and c (d^{-1}) are parameters.

The quadratic model is mathematically equivalent to the 'square root model' used to quantify the temperature dependency of biological processes such as mineralization of organic matter and bacterial growth at suboptimal temperatures (Ratkowsky *et al.*, 1982; Bernearts *et al.*, 2000). In this model, the rate to 50% germination was fitted to

$$y = aT^2 + bT + c \quad (5)$$

in which a (d^{-1} °C^{-2}) , b (d^{-1} °C^{-1}) and c (d^{-1}) are parameters.

The fourth model we used is the expolinear equation (Goudriaan and Monteith, 1990; Lee *et al.*, 2003):

$$y = a \ln(1 + \exp(b\,(T - c))) \quad (6)$$

in which a (d^{-1}), b (°C^{-1}) and c (°C) are parameters.

For all models (1, 4, 5 and 6) the fraction, f_t, of completion of the germination process to 50% germination is given by:

$$f_t = \int_0^t y \times \Delta t \quad (7)$$

where t is time and Δt the increment of time.

The relative performance of the four models was assessed with different criteria, including R^2 of fits and plots of residuals. An additional criterion is derived from rescaling the time axis. If a response model fits perfectly to the data, data points from treatments with constant or daily fluctuating temperatures will fall on one common curve when plotted against f_t. For the condition of perfect fit the point of 50% germination is reached for $f_t = 1$. The linear model is a special case. Here, the model also holds if the cumulative germination from different temperature treatments form

one single curve when plotted against thermal time, the latter being the product: time · (T – T_b). For treatments with fluctuating temperatures, daily increments of f_t were obtained from integrating Δf_t calculated for time steps of 15 minutes.

Simulations of emergence with the four models were compared with measurements obtained in the field. Simulated time from sowing to 50% emergence was calculated as follows: daily averages of the measured temperatures at sowing depth of each field sowing were calculated. From these average temperatures daily increments of f_t were calculated (Eqn 7). The time of 50% emergence is reached when the accumulated value of f_t equals 1. For each field sowing, the logistic model was fitted through the observations on cumulative emergence over time. All R^2 values of these fits were within 0.92 – 1.00. Using these fits, observed time from sowing to 50% emergence was calculated and plotted against simulated time from sowing to 50% emergence.

Two goodness of fit criteria were used to assess the agreement between model predictions of emergence and observed data, namely the root of the mean square error, RMSE (unit: d), and the squared bias, SB (unit: d^2). The first is defined by:

$$\mathrm{RMSE} = \sqrt{\frac{\sum_{i}^{n} (y_i - x_i)^2}{n}} \qquad (8)$$

in which x_i is the i^{th} value observed, y_i is the i^{th} simulated value and n is the number of data pairs. SB is defined as:

$$\mathrm{SB} = (y_{av} - x_{av})^2 \qquad (9)$$

in which x_{av} is the average of all observations and y_{av} is the average of all simulations.

Results

Emergence rates at constant temperatures (Expt 1)

The time to maximum percentage of germination (note: read ‘emergence’ when applicable but not explicitly mentioned) increased from 12 days at 21.8 °C to 24 days at 16.5 °C to 39 days at 13.0 °C and to more than 90 days at 9.1 °C. In all temperature treatments, more than 90% of all sown seeds emerged, with exception of the 9.1 °C treatment, in which only 68% of the sown seeds emerged (Fig. 2).

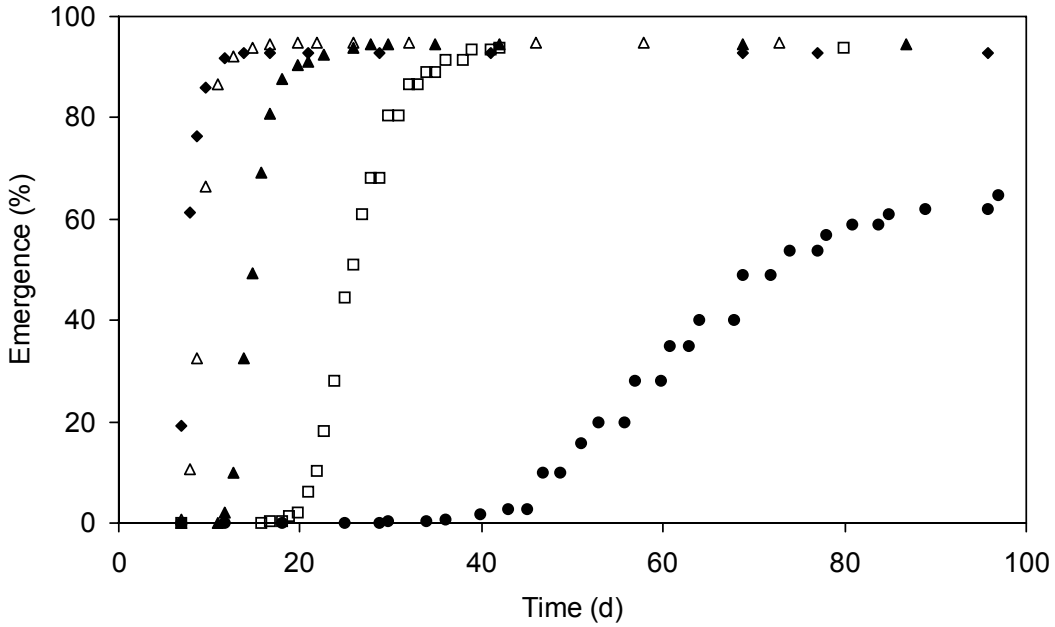


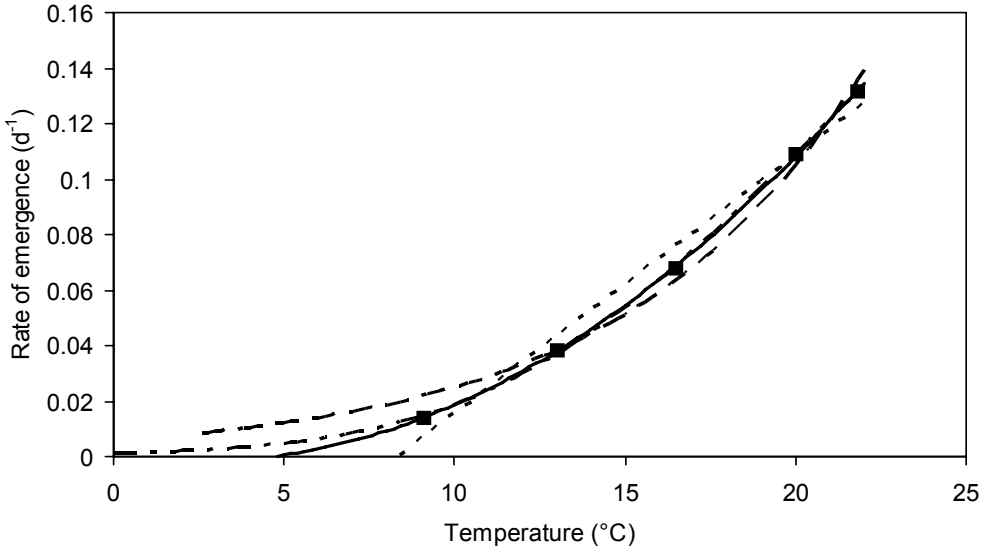
Figure 2. Time course of cumulative emergence (percentage of sown seeds) in Experiment 1, at water potential 0.016 MPa, for constant temperatures of 21.8 °C (♦), 20.0 °C (Δ), 16.5 °C (▲), 13.0 °C (□) and 9.1 °C (●).

The four temperature response models for germination fitted well to the experimental data of germination rates (Fig. 3a, Table 1). However, R^2 of the quadratic and the expolinear model were higher than those of the linear thermal time and the Q10 model. The residuals of the quadratic and the expolinear models showed no systematic trend with temperature (Fig. 3b) and were smaller than the residuals of the other models. The residuals of the linear thermal time model and the Q10 models showed parabolic, but opposite relations with treatment temperature (Fig. 3b), indicating inferior fit to the data over the entire range of temperature in comparison with quadratic and expolinear models.

Table 1. Overview of the parameter values of the four fitted temperature response models of germination with standard errors (SE).

Model	**a**	**SE a**	**b**	**SE b**	**c**	**SE c**	**R^2**
Linear (Eqn. 1)	0.0093	0.0007	–0.078	0.012	-	-	0.9814
Q10 (Eqn. 4)	4.180	$7.3 \cdot 10^{-9}$	13.1	$3.5 \cdot 10^{+11}$	0.039	$1.9 \cdot 10^{+9}$	0.9868
Quadratic (Eqn. 5)	0.00036	0.00002	–0.0018	0.0007	0.0004	0.005	0.9998
Expolinear (Eqn. 6)	0.044	0.009	0.3	0.04	12.11	0.86	0.9995

(a)



(b)

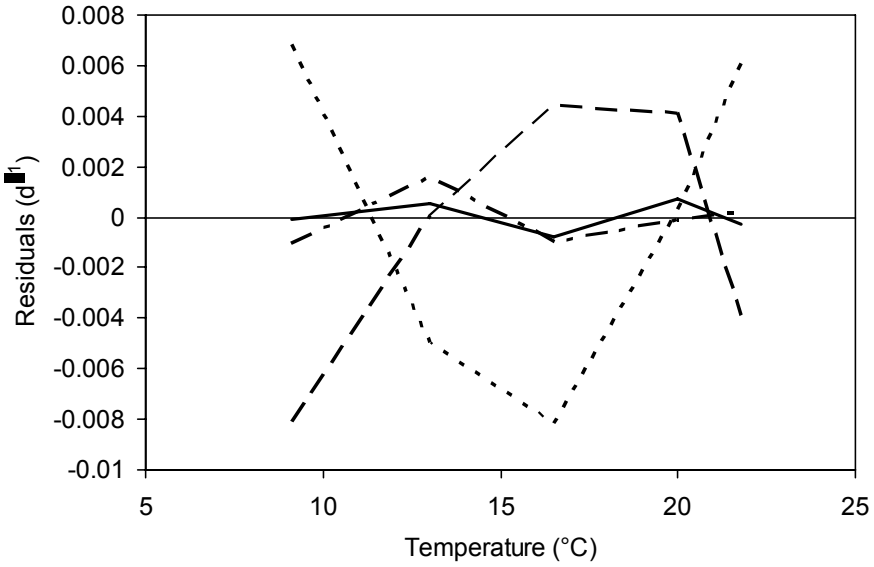


Figure 3. (a) Rate of emergence (t_{50}^{-1}) at water potential –0.016 MPa, as a function of treatment temperature in Experiment 1 (■). Standard errors were always smaller than 0.0015 d^{-1} and are left out. Linear (dotted line), Q10 (broken line), expolinear (dot-stripe-dot line) and the quadratic (solid line) models were fitted on rates to 50% emergence. (b) Residuals of the fits of the four models as a function of treatment temperature. The four models are indicated with the same line types as in (a).

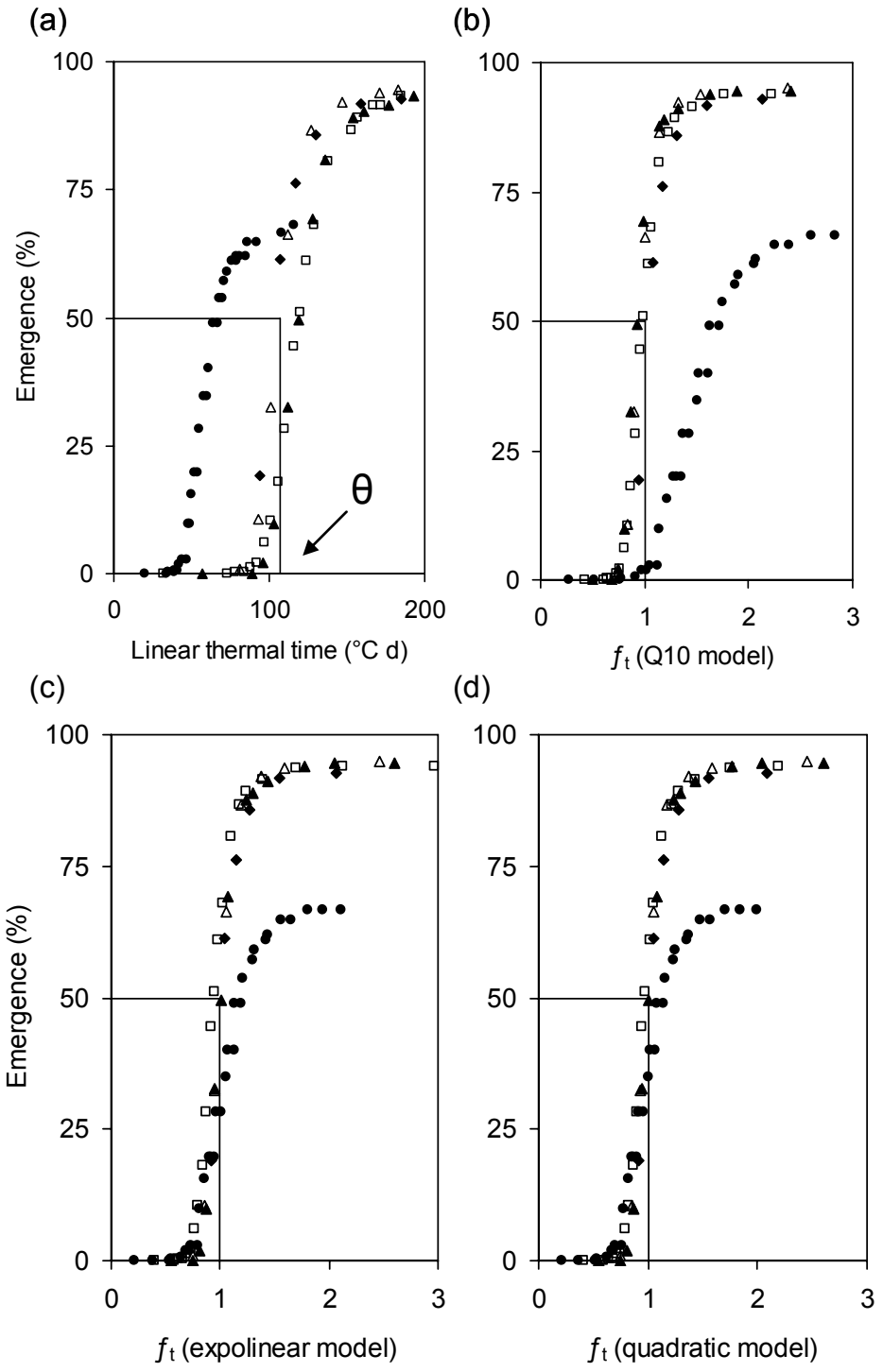


Figure 4. Cumulative emergence (percentage of sown seeds) for constant temperatures of 21.8 °C (♦), 20.0 °C (Δ), 16.5 °C (▲), 13.0 °C (□), and 9.1 °C (●) as function of the linear thermal time (a), and f_t calculated with the Q10 model (b), the expolinear model (c) and the quadratic model (d). Lines indicate the point on the x-axis where 50% germination should be reached if model fit was perfect. This is θ (107 °C days) for the linear model and a fraction $f_t = 1$ for the other three models. Data are from Experiment 1.

Plots of the observed cumulative emergence versus f (Fig. 4) showed that the seemingly small deviations from data of the linear thermal time and Q10 models in the low temperature range led to large errors in the accuracy of prediction of 50% germination. If a data series fits perfectly to the model the cumulative germination curve passes through the point (1, 50). For the 9.1 °C temperature treatment the linear thermal time model grossly underestimated and the Q10 model overestimated the progress in germination (for the linear model f accumulates faster than is justified in reality and the curve for 9.1 °C in Fig. 4 is shifted to the left of (1, 50); the opposite is true for the Q10 model).

Germination at fluctuating temperatures (Expt 2)

Germination rates for constant and fluctuating temperatures (Expt 2) showed a significant ($P < 0.001$, $R^2 = 0.99$) linear relation with daily accumulated f, calculated with the expolinear model (Fig. 5). Since f represents the fraction of the germination process that is completed in one day, there must be a linear relation between the rate of germination (= 1/time to 50% germination (day)) and f, provided f is calculated with a model that adequately describes the temperature response. Because data points from all treatments (whether fluctuating or constant temperature) fell on one common linear line (Fig. 5), it follows that differences in f calculated with the expolinear model for fluctuating temperature regimes could fully account for the variation in germination rates, and no additional effects of the fluctuations *per se* were observed. Also, the temperature fluctuations did not have an effect on final percentage of germination (data not shown).

Effects of soil water potential on emergence (Expt 1)

The rate of seed emergence (t_{50}^{-1}) was not strongly influenced by soil water potential between –0.003 MPa and –1.26 MPa (Fig. 6a). At water potentials as high as –0.0018 MPa and as low as –10 MPa almost no seedlings emerged and hence emergence rates were practically zero. Also, final emergence percentages in Expt 1 were not influenced by soil water potential for water potentials ranging between –0.003 MPa and –1.26 MPa (Fig. 6b).

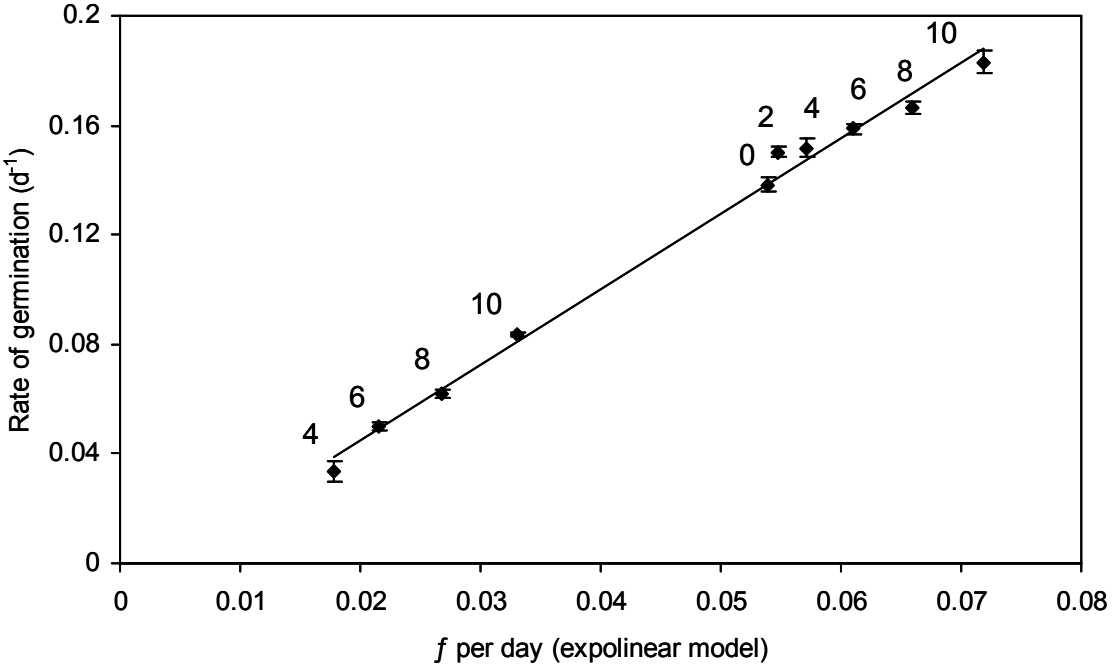
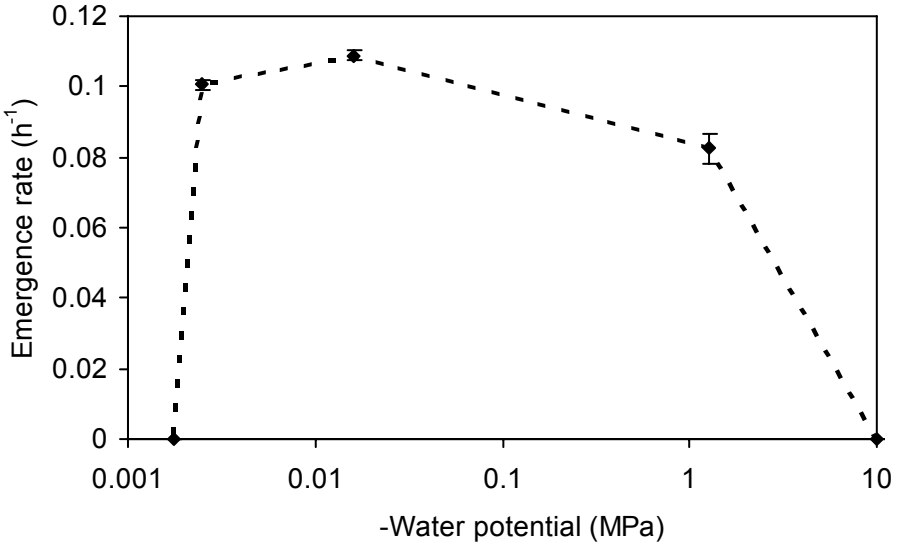


Figure 5. Rate of germination (t_{50}^{-1}) as a function of the *f* per day for each temperature treatment, calculated with the expolinear model, for data from Experiment 2. The equation of the regression line is 2.76 (standard error 0.09) ×*f* per day –0.010 (standard error 0.004)) (R^2 = 0.99, P < 0.001). Numbers indicate the amplitude (°C) of the associated sine function of each of the data points (disregarding truncation for the 9 °C series). For the 9 °C series, data from the treatments with constant temperature of 9 °C and with amplitude of 2 °C were left out as these ultimately reached only 21 and 35% germination, respectively. Error bars indicate standard error of the mean.

Comparison of model predictions and observations of field emergence (Expt 3)

Due to different timing of the 16 sowings and associated differences in weather, the time from planting to 50% emergence ranged from 5.9 to 66.5 days (Fig. 7). Time from planting to 50% emergence simulated with the expolinear model (Eqn 6, coefficients in Table 1) closely agreed with that measured in field plots. Both indicators for goodness of fit, RMSE and SB, showed little differences (less than 1 day) between the four models (Table 2). RMSE was highest (2.9 days) for the Q10 model, and lower (2.3 – 2.4 days) for all three other models. Squared bias (SB) was highest (2.8 days) for the linear model, and lower (1.9 – 2.0 days) for the other three models.

(a)



(b)

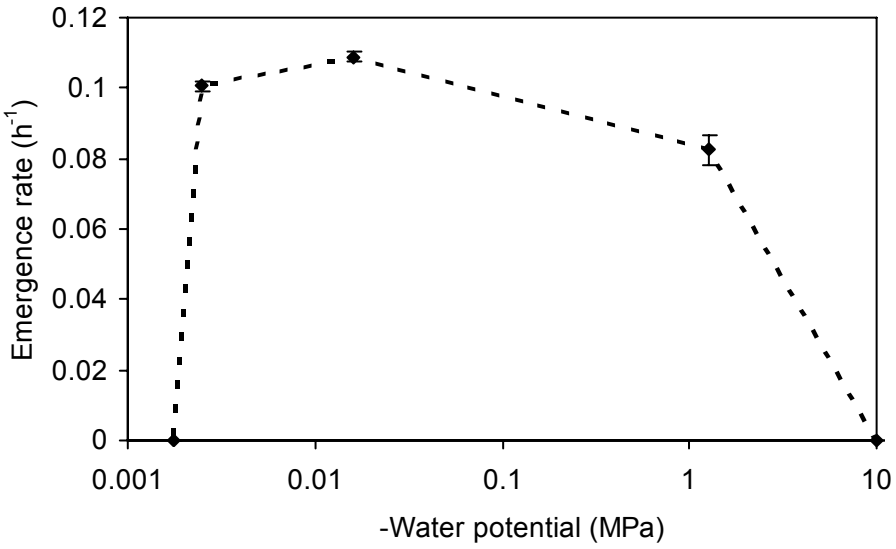


Figure 6. (a) Rate of emergence, at 20 °C, as a function of water potential (range: −10 to −1.8 × 10^{-3} MPa) in Experiment 1. (b) Final percentage emergence of sown seeds at 20.0 °C, as a function of water potential in Experiment 1. Error bars indicate standard error of the mean.

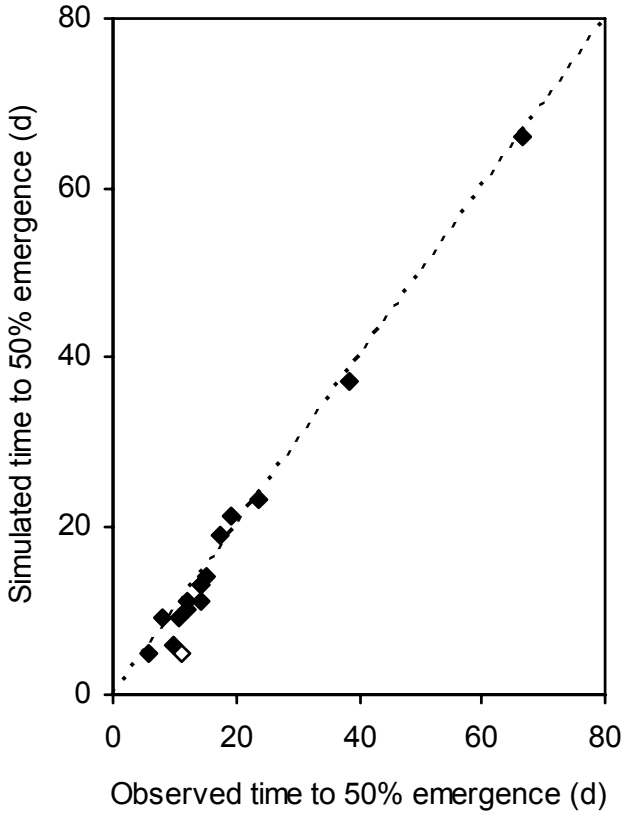


Figure 7. Simulated (using the expolinear model) and observed time from sowing to 50% of emergence of sown seeds in field sowings in 2001, 2002, 2003 and 2004 in Experiment 3. Dotted line indicates y = x. Open symbols indicate data points from a sowing under extreme hot and dry conditions. In few cases (1st, 6th and 7th sowing in 2001 and 1st sowing in 2003) field emergence did not reach 50% and time until 50% emergence of all emerged seeds was used.

Table 2. Root mean square error, RMSE (Eqn. 8) and squared bias, SB (Eqn. 9) calculated to compare goodness of fit between simulated and observed field emergence rates for the sowings in 2001, 2002, 2003 and 2004 (Expt 3).

Temperature response model	**RMSE ($^{-1}$)**	**SB (d^2)**
Linear (Eqn. 1)	2.4	2.8
Q10 (Eqn. 4)	2.9	2.0
Quadratic (Eqn. 5)	2.4	2.0
Expolinear (Eqn. 6)	2.3	1.9

Discussion

Especially for the early sowings of *S. sisymbriifolium* in temperate climates, it can be crucial to predict emergence rates at temperatures around ca 10 °C. Linear thermal time or hydrothermal time models are most commonly used to predict germination rates in several species (e.g. Grundy *et al.*, 2000; Alvarado and Bradford, 2002; Allen, 2003; Larsen *et al.*, 2004). The linear models can be applied only to the range of temperatures for which the data points of rate versus temperature follow a linear relation. This is a limitation of the linear thermal time model, since data points are bound to deviate from the linear relation near the base end of the response and near the optimum temperature at the high end of the response. For *S. sisymbriifolium*, emergence rates at the base end of its temperature response were not accurately predicted by the linear thermal time model and the quadratic and expolinear models clearly performed better near the 'base temperature'. Virtually all variation in emergence rates of *S. sisymbriifolium*, associated with temperature treatments in the range of 9.1 °C to 21.8 °C, could be explained using the quadratic and the expolinear models. Perhaps the expolinear model should be preferred over the quadratic model, as the former behaves linearly at higher temperatures and does not rise quadratically, which is bound to become unrealistic above a certain temperature range.

The concept of nonlinear temperature dependency of development rates in plant development is not new (Holshouser *et al.*, 1996; Yin *et al*., 1995). However, models used are often more intricate (beta functions or weibull functions) and include a decline in rate at temperatures above an optimum temperature. For *S. sisymbriifolium*, no such decline in germination rate was found for temperatures up to 33 °C (unpublished additional results) and the simpler, expolinear model sufficed in describing most of the variations in (field) emergence rates.

Fluctuating temperature regimes were not found to exert specific effects on rate (Fig. 5) and final percentage of germination. Current findings contrast with rate accelerating effects of fluctuating temperature reported for several other species (*Chenopodium album* and *Panicum maximum*, Murdoch *et al.*, 1989; *Orobanche* spp., Kebreab and Murdoch, 1999b); the stimulating effect of temperature fluctuations are regarded as an adaptation of seeds to germinate only when positioned close to the soil surface.

Strikingly, the soil water potential between −0.003 MPa and −1.26 MPa had no noticeable effect on the emergence rate or final percentage of emergence of *S. sisymbriifolium*. This means that in situations that are not extremely wet or dry (as in Expt 1 and most field sowings), temperature is the dominant factor influencing the rate of the germination and emergence of *S. sisymbriifolium* seeds, and no modification of

thermal time on the basis of soil water potential (hydrothermal time) is needed as proposed for other species, e.g. *Solanum tuberosum* (Alvarado and Bradford, 2002) and *Stellaria media* (Grundy *et al.*, 2000).

In the application of the four models to predict field emergence date, goodness of fit criteria for models differed only slightly (Table 2). This implies that the field experiments did not include prolonged periods with temperatures around 7 − 10 °C. However, such conditions may occur, especially early in the season, and then the predictions with the quadratic or expolinear models are expected to be superior to those obtained with the other models. For *S. sisymbriifolium* the current expolinear temperature response model of germination rate suffices to predict field emergence rates (under fluctuating field conditions) without further modifications (Fig. 7). Unexplained variation in rate of emergence in the field originated presumably from differences in sowing depth and soil compaction that inevitably occur during sowing.

It is concluded that the expolinear and the quadratic model are the preferred models to quantify germination and emergence, especially for crop species that are grown near the cold limit of their geographical distribution. These species include maize, soybean, sunflower and also *S. sysimbriifolium*.

CHAPTER 3

Field performance of *Solanum sisymbriifolium*, a trap crop for potato cyst nematodes. I. Dry matter accumulation in relation to sowing time, location, season and plant density

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Summary

Solanum sisymbriifolium (Lam.) is an interesting trap crop to control potato cyst nematodes. A series of field experiments were carried out in The Netherlands between 2001 and 2003 to test its performance under field conditions. Experimental factors included sowing time, sowing density and site. Rate of germination, plant establishment and change over time in light interception were monitored. Growth analysis was performed at 7 and 14 weeks after emergence and dry weight of component plant parts was determined. Time to 50% emergence was 36 – 38 days for planting early April and declined to minimum values of ca 8 – 11 days when planting took place in June, July or the first week of August. When planted later, time to 50% germination increased again. Time to 50% light interception showed a similar trend with sowing time; minimum time was 35 – 40 days for planting between June and half July. Planting before May did not advance crop growth. Crop performance was very variable across years and sites when planted later than the end of July – beginning of August. Therefore, the time window for planting in The Netherlands is between early May and end of July. Dry matter accumulation up to 400 g m^{-2} was found at 7 weeks after emergence and up to 1040 g m^{-2} after 14 weeks. At 7 weeks after emergence dry matter production increased with planting density (range 50 – 400 m^{-2}), but no statistically significant differences were found after 14 weeks. A seed rate of 100 m^{-2} seems generally sufficient. Radiation use efficiency was 1.69 (SE = 0.0208) g MJ^{-1} PAR. Dry matter accumulation (2002 – 2003) was somewhat higher in Wageningen (51° 58' N) on light sandy soil than in Flevoland (52° 31' N) on clay soil and in Drenthe (52° 51' N) on reclaimed peat soil.

Key words: Biological pest control, biomass, light interception, radiation use efficiency

Introduction

Since their introduction in Europe in the beginning of the 20th century (Wollenweber, 1923; Van Riel and Mulder, 1998) potato cyst nematodes (PCN) have caused substantial yield reductions in many potato production areas (e.g. Haydock and Evans, 1998). In a structured survey in England and Wales PCN were detected in 64% of the sampled fields (Minnis *et al*., 2002). In financial terms, current annual PCN damage to potato crops in the European Union is estimated at € 440 million (Ryan *et al*., 2000).

Whitehead and Turner (1998) mention the following control measures: (i) crop rotation; (ii) resistant and tolerant cultivars of potato; (iii) nematicides; (iv) soil solarization; (v) trap cropping; and (vi) integrated control. However, there are disadvantages and limitations to some of these: host resistances are broken, and resistant cultivars are not available for all market outlets. Control by means of nematicides (Oldekamp, 1991; Whitehead and Turner, 1998) is expensive and less desirable from an environmental point of view and therefore, lately restricted by legislation. For all these reasons, there is scope for additional control measures.

One of the options is the introduction of a new effective trap crop. PCN are plant parasitic nematodes that feed themselves in the roots of a potato plant, and then reproduce. The female nematode body, filled with embryonated eggs (juveniles), dies off at the end of the season and forms a survival structure (cyst). Each year, juveniles from part of these cysts hatch spontaneously (Been and Schomaker, 1998, 1999; Devine *et al*., 1999). Non-hatched juveniles can survive within their cysts in soil without a host potato crop for long periods, even up to 20 years (Jones *et al*., 1998). However, if stimulated by root exudates from a potato crop or from a related plant species, the major part of the PCN will hatch (Fenwick, 1952; Clarke and Shepherd, 1968; Jones *et al*., 1998; Devine *et al*., 1999). This principle is exploited by the use of a trap crop. *Solanum sisymbriifolium* was found to stimulate hatching of two years old *Globodera pallida* cysts until 77%. The corresponding percentage of hatch for one year-old cysts was 60%, while 52% was found in very severely infested soil (Scholte and Vos, 2000). Hatching levels in potato controls were 87%, 74% and 60%, respectively. *S. sisymbriifolium* proved resistant to a wide range of PCN pathotypes (Scholte, 2000b, c; Scholte and Vos, 2000). The latter is an essential requirement for a trap crop as no new cysts should be added to the population in a field.

S. sisymbriifolium is an annual plant species originating from South America. Common English names include sticky nightshade, dense thorned bitter apple and wild tomato. It is a highly branched shrubby plant of up to 1.5 m height. Its ovate leaves are deeply lobed. All parts of the plant except for the fruits are densely pubescent with stellate and glandular trichomes. Flower petals are blue or white and anthers bright

yellow. Fruits are tomato-like berries, 1.2 – 2.6 cm diameter and encased in large, spiny calyces (Hill and Hulley, 2000). Explorative work (Scholte 2000c; Scholte and Vos, 2000) showed that *S. sisymbriifolium* can be grown as a crop in climates such as in The Netherlands, and farmers have begun to use it as a trap crop. However, little quantitative information is available on the ecophysiology of the plant or on the crop management practices to optimize cyst hatching. The current study sets out to collect the ecophysiological information needed to make an assessment of the growth conditions for successful cultivation of *S. sisymbriifolium*, addressing seed germination, shoot growth, root growth and the relation between root growth and cyst hatching. The current chapter focuses on (i) field performance (dry matter accumulation) in relation to season, site, sowing date and plant density, (ii) dry matter distribution over component shoot organs, (iii) light interception and radiation use efficiency. The root growth will be treated in Chapter 4.

Materials and methods

Solanum sisymbriifolium seeds were multiplied in the field in the years 2000, 2001 and 2002 in Wageningen (51° 58' N). At the end of each growing season, red berries were harvested, crushed and rinsed. Seeds were collected on a sieve, and air-dried before storage at 12 °C and 50% relative humidity, for use in the next season's experiments.

Experimental series 1: Sowing times

Experiments were done in 2001, 2002 and 2003 to explore the effect of season and sowing date on emergence and growth of *S. sisymbriifolium*. In 2001, *S. sisymbriifolium* crops were sown on sandy soil in Wageningen on eight different dates: first weeks of March, April, May, June, July, August and September, and also the third week of July. The experiment was laid out as a randomized block design with four replicates. Plot sizes were 3 m by 6 m and sowing density was 400 seeds m^{-2} in rows with 12.5 cm distance, using a precision-sowing machine (Hege Maschinen, Eging am See, D). Fertilizer applications included 44 kg P ha^{-1} and 54 kg K ha^{-1} at sowing time, 100 kg N ha^{-1} (as calcium ammonium nitrate) three weeks after emergence and another 50 kg N ha^{-1} seven weeks after emergence. Plots were hand-weeded, but if weed pressure became too high while the *S. sisymbriifolium* plants were still small, the plots were sprayed with rimsulfuron (Titus, 250 g kg^{-1} rimsulfuron, Dupont Holland, Agricultural Products, spray rate active ingredient 10 g ha^{-1} and 0.3 liter ha^{-1} surfactant AgralLN).

Temperature was continuously measured at +150, −5, −10 and −20 cm from the

soil surface using thermocouples. In each plot, three transects of 1 m length were marked with plastic pegs and emergence of seeds was counted daily in the first period of emergence and less frequent when number of emerging seedlings per day declined. The time, at which half of the seeds that ultimately would emerge had done so, was called "T50" and was regarded as the date of emergence.

In each plot two sub-plots of 1 m^2 were marked using plastic pegs. Light interception measurements were done weekly on these sub-plots using a Sunscan light interception measurement system (Delta-T Devices, Cambridge, UK). The plots were harvested at 7 and 14 weeks after emergence. At each harvest, the crop was cut at ground level in one of the 1 m^2 sub-plots, plant number was counted and plant height and leaf area index (LAI) were measured. Harvested plant material was separated into stem, leaf, flower and berry, and was dried in a stove at 70 °C, to determine dry weights. The experimental period was limited to 14 weeks because (i) ideally it should not be needed to grow the trap crop throughout the whole growing season to obtain the maximum effects on hatching, thus leaving time for other economically more interesting crops, and (ii) the largest hatching effect occurs within that period of time (Scholte, 2000c).

In 2002 and 2003, the experiment was repeated with only three sowing times: the first week of May, the first (2002) or third (2003) week of June and the first week of August, on fields with a sandy soil near Wageningen (2002) and Achterberg (2003) (51° 57' N). Planting density was 200 seeds m^{-2}, and 6 or 7 (2002) and 4 (2003) successive samples from 1 m^2 were taken to perform growth analysis. Further experimental procedures were the same as described for 2001.

Experimental series 2: Sowing densities

Effects of sowing density on (early) growth were examined in a separate experiment in 2001. On the 17th of July 18 m^2 plots were sown at 50, 100, 200 and 400 seeds m^{-2} in a randomized block design with four replicates. Two successive harvests of each 1 m^2 were done, at 7 and 14 weeks after emergence. Experimental procedures were the same as described above for the experimental series 1.

Experimental series 3: Site differences

In 2002 and 2003, additional experiments were conducted in Flevoland (Lelystad) and in Drenthe (Valthermond). For soil types and greographic information see Table 1. On these locations, plants were sown on three (2002) and two (2003) dates on fields infested with PCN. Sowing density was 50 seeds m^{-2}, and row distances varied from 12.5 to 25 cm. Experimental designs were randomized block designs, with three (2002) or four (2003) replicates. Fertilizer N was applied at a rate of 80 kg N ha^{-1}. As

Table 1. Location and soil type of fields where *S. sisymbriifolium* was grown in 2002 and 2003.

Location name	Wageningen 2002	Flevoland (Lelystad) 2002	Drenthe (Valthermond) 2002 and 2003	Wageningen (Achterberg) 2003	Flevoland (Swifterband) 2003
Latitude (N)	51° 58’	52° 31’	52° 51’	51° 57’	52° 35’
Longitude (E)	5° 40’	5° 31’	6° 55’	5° 37’	5° 58’
Soil type	sand	marine-clay	reclaimed peat	sand	marine-clay

in Experimental series 1, rimsulfuron (Titus, identical application rates and dose) was sprayed when weeds threatened to outcompete the small crop plants. At all sites, above ground dry weight was measured once between September and November, except in some cases when plants were too small to harvest.

Calculation radiation use efficiencies and data analysis

Logistic curves were fitted to light interception data measured over time. These equations yielded fitted values for daily percentage light interception. Multiplication of these percentages with the daily records of the amount of photosynthetically active radiation (PAR, measured in the Wageningen weather station) yielded daily and cumulative estimates of intercepted PAR. Above ground dry weight of all harvests was plotted against cumulatively intercepted PAR, and radiation use efficiency (Monteith, 1977) was derived from linear regression (forced through the origin). Thermal time was based on temperature recordings at 150 m height, assuming a base temperature of 9 °C. All statistics were performed using GenStat 7th edition version 7.1.0.205.

Results

Experimental series 1: Sowing times

Dry matter accumulated exponentially with thermal time until ca 400 degree-days (Fig. 1); here after dry matter increased linearly with thermal time. The amount of dry matter in leaves increased during the exponential growth phase, but remained constant during the subsequent linear growth phase, indicating growth of new leaves balanced shedding of old leaves. The stem dry matter continued to increase throughout the experimental period until final harvest after 14 weeks.

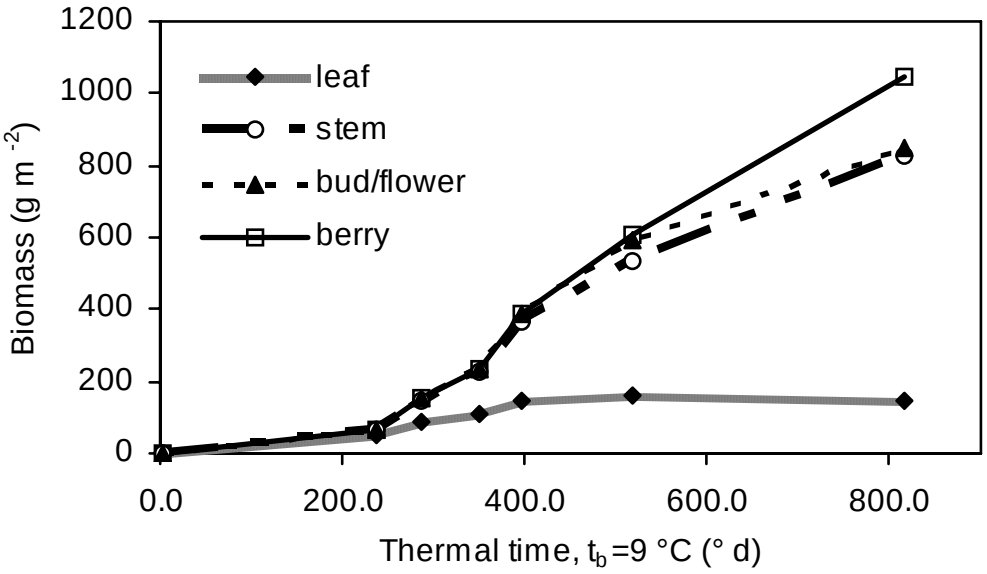

Figure 1. Representative example of dry matter accumulation in *S. sisymbriifolium*, plotted against thermal time (calculated with a t_{base} of 9 °C). Solid line: total amount of dry matter; lines for leaf, stem and bud/flower indicate the stacked contribution of the dry matter in the specific organ to the total. Data from 2002, crop sown on 10 May.

Data on dry matter production on periods 0 – 7 weeks from emergence and 0 – 14 weeks from emergence, obtained in 2001, 2002 and 2003, showed a linear association with cumulative light interception (Fig. 2). The slope, forced through the origin provides an estimate of the radiation use efficiency (RUE) which amounted to 1.69 (SE 0.0208) g MJ^{-1} of PAR, ($P<0.001$, $R^2=0.914$).

Seasonal variation in crop growth

The change over time of the percentage light interception reflects the growth performance of the crops sown on different dates in 2001 (Fig. 3). Seeds sown in March and April emerged at the end of May, simultaneously with the seeds sown in the first week of May and similar time courses of increase in percentage light interception were observed for the first three sowing dates. Clearly, sowing before May did not advance crop growth. Crops sown from July onward still emerged, but subsequent growth became slower the later the crop was sown. Whereas the mid-July sowing resulted in a reasonable crop that reached about 80% of light interception, the sowing in the first week of August 2001 resulted in a crop with only small plants (about 40% light interception reached, plant height ca 20 cm).

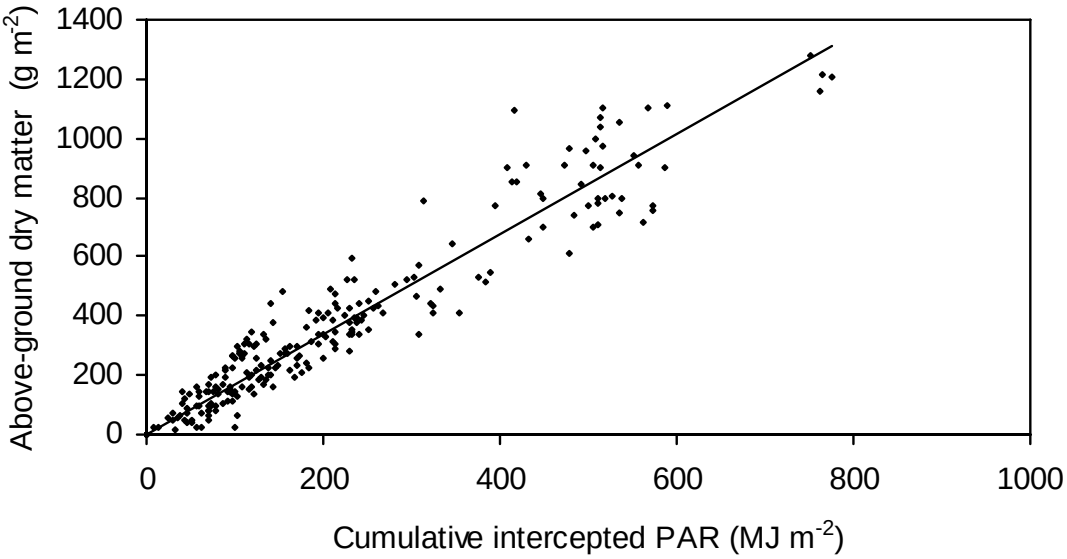

Figure 2. Above-ground dry matter and cumulative intercepted PAR for *S. sisymbriifolium* crops sown in densities of 50 – 400 seeds m^{-2} in 2001, 2002 and 2003 and sampled 7 and 14 weeks after emergence. Line: result of linear regression forced through the origin; the slope represents the radiation use efficiency and was 1.69 g MJ^{-1}, (R^2=0.914, standard error 0.0208 g MJ^{-1}). Data of lodged crops were left out as they did not have representative growth but lost a major part of their leaves.

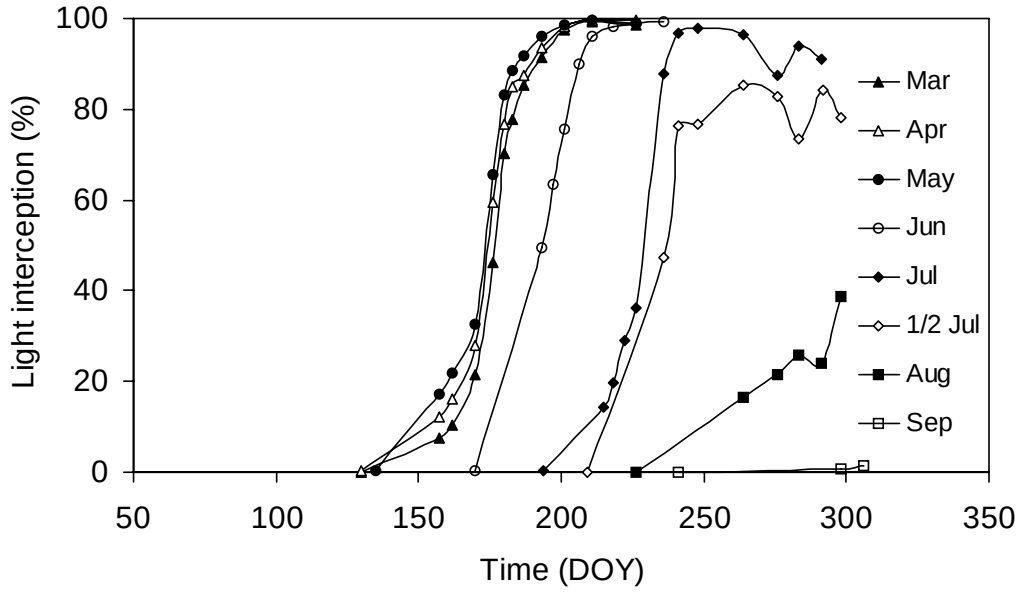

Figure 3. Change in percentage light interception over time, expressed as day number of year (DOY). Data from different sowing times in 2001. Lines represent linear interpolations between data points.

The plants sown in September 2001 still emerged, but there was almost no growth and the crop did not even reach 2% light interception.

The length of the period from sowing till T50 (Fig. 4) decreased from ca 65 days for sowing at day number of year (DOY) 64 to less than 10 days for crops sown between DOY 150 and 220. For the first part of the season, the period between sowing and 50% light interception also decreased from more than 110 days for sowings at DOY 65 to ca 38 days for sowings between DOY 150 to 200. When sown after DOY 200, the length of the period from sowing till 50% light interception increased again. This increase started at lower day numbers than the increase in time from sowing till T50. Furthermore, not all crops sown after DOY 200 reached 50% light interception.

Plotted as a function of sampling date, dry biomass measured at 5 – 7 weeks after emergence increased from 160 g m^{-2} on DOY 184 to maximally 410 g m^{-2} on DOY 220 and then decreased again to 30 g m^{-2} on DOY 293 (Fig. 5). Corresponding figures for dry biomass 14 weeks after emergence were 800 g m^{-2} measured on DOY 230, maximally 1043 g m^{-2} on DOY 241 and only 41 g m^{-2} on DOY 313.

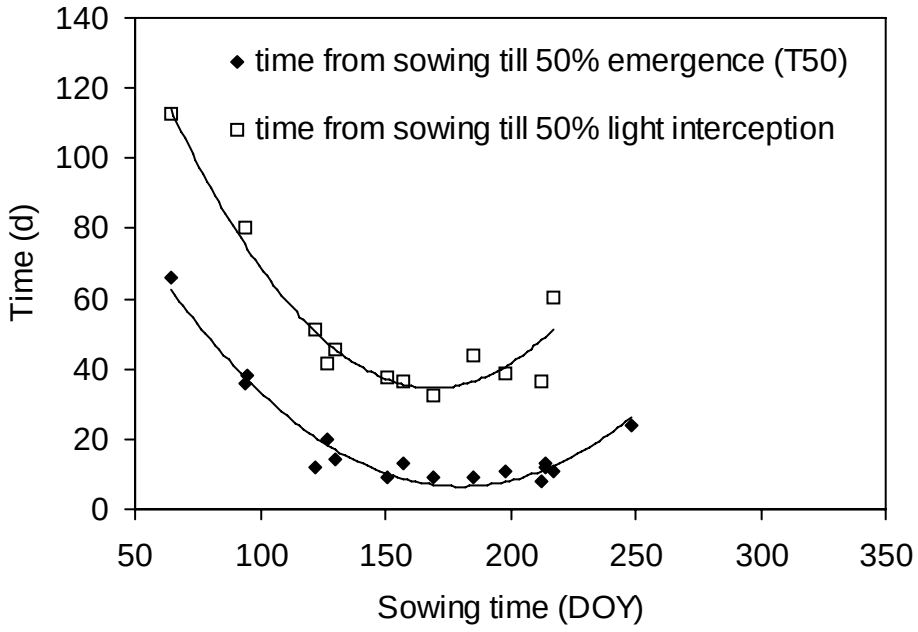

Figure 4. The time between sowing and 50% emergence of seeds (♦) and the time between sowing and 50% light interception (□) against day number of year (DOY) of sowing; data from Experimental series 1, 2001 – 2003. The trend lines are added to improve the visualization of the responses, but have no statistical meaning.

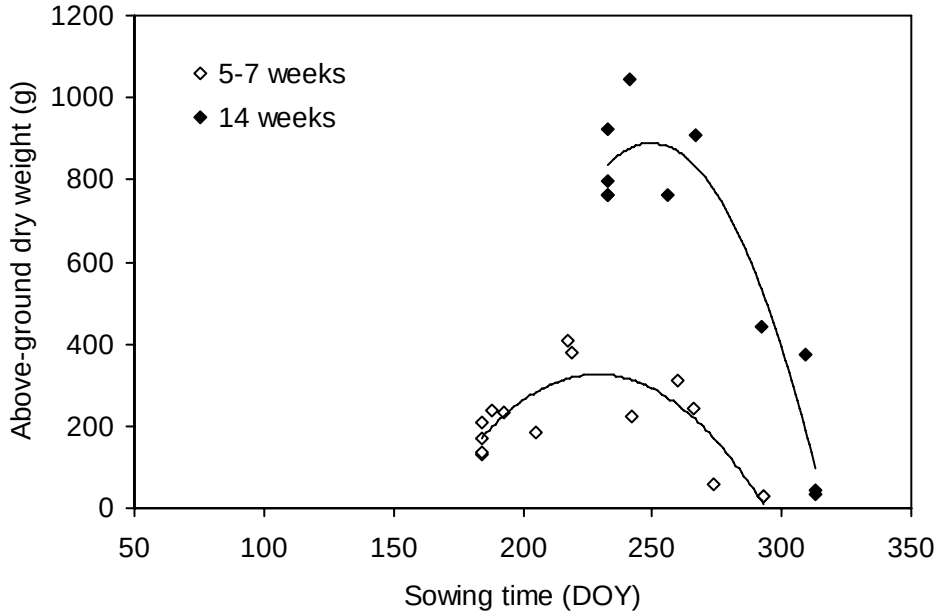

Figure 5. Dry weight plotted against sowing time in the sowing time experiments, after 5 – 7 (◊) and after 14 (♦) weeks of growth following emergence; data from 2001, 2002 and 2003. The trendlines are added to improve the visualization of the responses, but have no statistical meaning.

There was a large year to year variation in crop performance after planting in August (Fig. 6). In 2001, after emergence the plants did grow very slowly, and reached only 38% light interception and a plant height of ca 20 cm (measured after 72 days of growth). In 2002, light interception increased more rapidly, and reached an average maximum of 55% and a plant height of ca 50 cm. The high standard error (Fig. 6) in 2002 can be explained by a heavy shower that occurred one day after sowing. Large parts of the field were waterlogged for 1.5 weeks. This caused crop failure on part of the plots and, hence, high standard errors of the means of crop attributes over replicates. After this relatively wet period weather became warm and dry and seeds on the drier parts of the field germinated well and plants grew vigorously for some time. In 2003, the autumn was extraordinary warm and the August sowing near Wageningen resulted in a crop of ca 80 cm height that intercepted up to 96% of incoming light. Data presented indicate that at some stage after mid August growth conditions apparently rapidly switch from conditions allowing good crop performance to conditions resulting in a reasonable degree of germination but poor dry matter production.

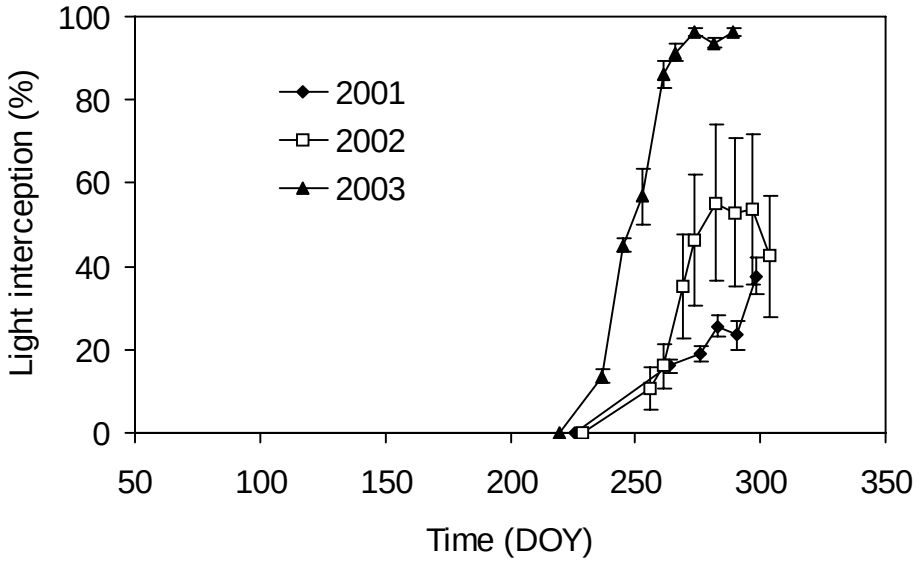

Figure 6. Light interception plotted against time (day number of the year) for the crops sown in the first week of August, in the years 2001 (♦), 2002 (□) and 2003 (▲). Error bars indicate standard error of the mean.

Experimental series 2: Plant densities

For all sowing densities ca 75% of the sown seeds emerged (Fig. 7). Over the first 7 weeks after emergence, seedling survival rates were near 100% at densities of 50 and 100 sown seeds m^{-2}. However, seedling survival decreased to 92% and 73% for, respectively, 200 and 400 sown seeds m^{-2}. The latter means only 57% of sown seeds survived as crop plants after 7 weeks at a sowing density of 400 m^{-2}. Survival over the second 7 weeks of growth was less density dependent, and ranged from 72% (at 100 and 200 seeds m^{-2} planted) to 76% at 400 seeds m^{-2} and up to 84% (at 50 seeds m^{-2}).

The initial rate of increase in percentage light interception with time after emergence was faster the higher the plant density (Fig. 8). However, after 3 months, the lowest sowing density (50 seeds m^{-2}) had 72% of light interception, which was close to the 78% recorded for the highest sowing density (400 seeds m^{-2}). The density effect on above-ground dry matter production (Fig. 9) showed the same pattern. After 7 weeks of growth, the total above-ground dry matter roughly doubled from 149 g m^{-2} for planting density 50 m^{-2} (41 or 82% plants m^{-2} established) to a maximum of 309 g m^{-2} for seed density 400 m^{-2} (228 or 57% plants m^{-2} established).

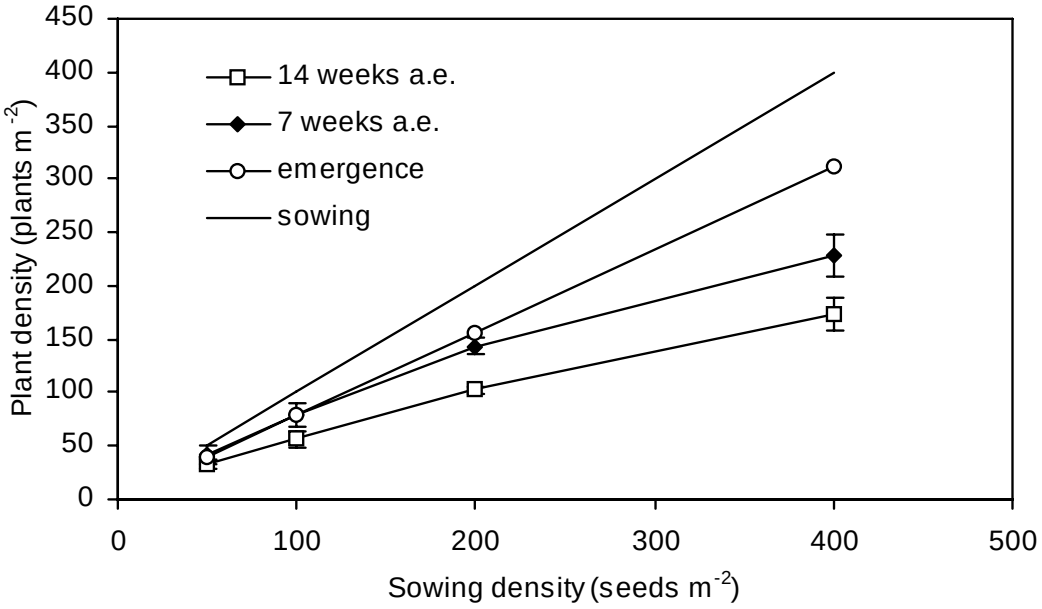

Figure 7. Plant density at sowing (-), emergence (○), 7 weeks after emergence (♦) and 14 weeks after emergence(□) plotted against the sowing density. Error bars indicate standard error of the mean. Data from Experimental series 2, 2001.

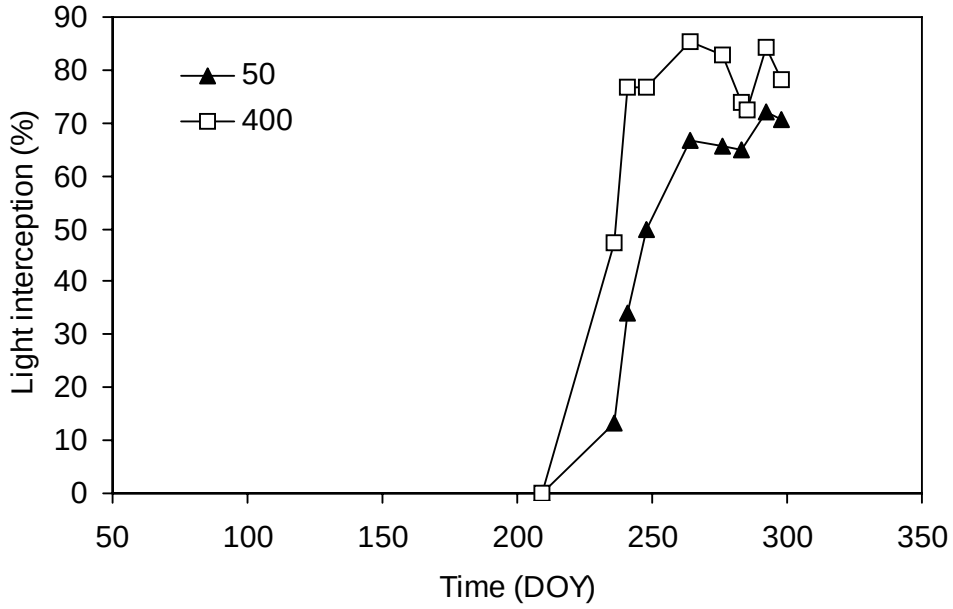

Figure 8. Light interception versus day number of year (DOY) for 50 seeds m^{-2} (▲) and 400 seeds m^{-2} (□). Intermediate sowing densities gave intermediate light interception curves over time. Data from Experimental series 2, 2001.

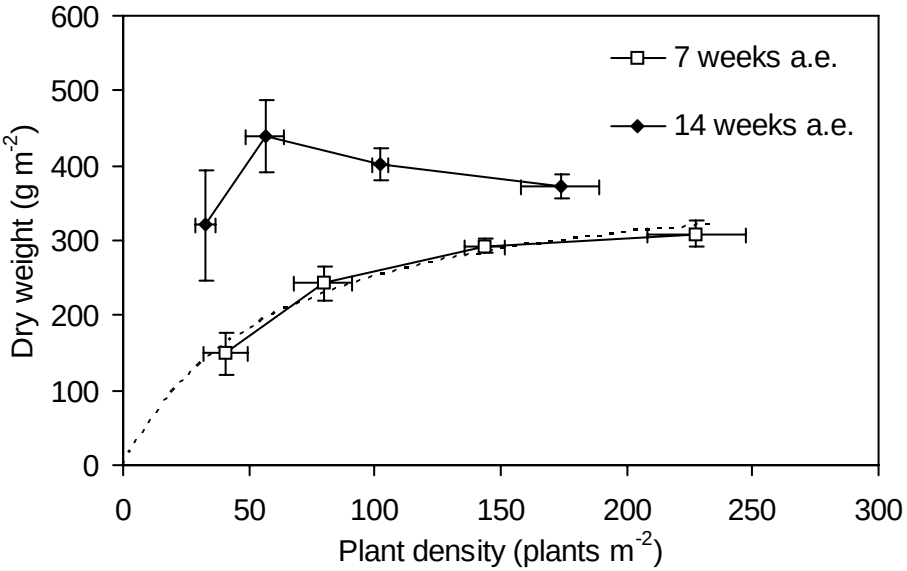

Figure 9. Above-ground dry weight (g m^{-2}) at 7 (□) and at 14 (♦) weeks after emergence, plotted against plant density at harvest. Dotted line indicates fitted model (De Wit, 1960) for density dependent yield per unit area ($Y = K \times \text{plant density} \times ((K - 1) \times \text{plant density} + 1)^{-1} \times M$, with K= 1.02 (standard error 0.0047) and M=6.56 (standard error 1.202), R^2=0.96). Error bars indicate standard error of the mean. Data from Experimental series 2, 2001.

The classical model of De Wit (1960) for density dependent crop yield per unit area was fitted (dotted line, r-square value: 0.96). There were no significant differences in dry matter between the density treatments when measured 14 weeks after emergence. This shows that, as for light interception, the initial rate of dry matter accumulation was faster at higher plant density, but this effect was only transient.

Experimental series 3: Site differences

Crops sown before DOY 155 produced higher above-ground dry weights in Wageningen (averages 1043 – 1434 g m^{-2}, Table 2a, with the highest measurement of 2034 g m^{-2} harvested in October 2003) than crops in Flevoland and Drenthe (averages 354 – 721 g m^{-2}, Table 2a). The same was observed for the crops sown after DOY 190 (Table 2b): in 2002, crops sown around DOY 200 accumulated 287 – 336 g m^{-2} in Flevoland and Drenthe, whereas crops sown on DOY 225 – 228 could not be harvested because plants were too small.

Table 2. Crop performance at different sites in 2002 and 2003 (experimental series 3), for crops sown before DOY 155 (a) and crops sown after DOY 190 (b). Several sowings in Flevoland (DOY 287) and Drenthe (DOY 228) in 2002 could not be harvested because plants were too small.

(a) Early season sowing (before DOY 155)

	Location and year						
	Wageningen 2002	Wageningen 2002	Flevoland 2002	Drenthe 2002	Wageningen 2003	Flevoland 2003	Drenthe 2003
Sowing date (DOY)	130	151	141	143	127	153	135
Time from sowing till harvest (days)	111	105	111	117	166	102	119
Above ground dry matter (g m^{-2})	1043 (60) [a]	761 (122)	721 (26)	699 (145)	1434 (482)	479 (67)	354 (30)

(b) Late season sowing (after DOY 190)

	Location and year							
	Wageningen 2002	Flevoland 2002	Flevoland 2002	Drenthe 2002	Drenthe 2002	Wageningen 2003	Flevoland 2003	Drenthe 2003
Sowing time (DOY)	217	197	225	196	228	212	196	195
Time from sowing till harvest (days)	87	92	- [b]	94	- [b]	85	93	94
Above-ground dry matter (g m^{-2})	145 (26)[a]	287 (27)	- [b]	336 (32)	- [b]	469 (128)	315 (54)	403 (17)

[a] in brackets: standard error of mean

[b] plants too small to harvest

A crop sown in Wageningen as late as DOY 217 (2002) still had 145 g m^{-2}. In the year 2003, crops sown in Flevoland and Drenthe on DOY 196 and 195, yielded only 315 and 403 g m^{-2} above-ground dry matter respectively, whereas a crop sown in Wageningen at DOY 225, so later, still resulted in 469 g m^{-2} above-ground dry matter.

Discussion

General characteristics of S. sisymbriifolium *grown as a field crop*

The current experiments confirm that *S. sisymbriifolium* can be grown successfully as a crop in temperate climates such as in The Netherlands. When sown from May until mid July the risk of crop failure is small. Above-ground dry matter accumulation is apparently directly associated with cumulative intercepted light (Fig. 2). *S. sisymbriifolium* is a plant with indeterminate growth such as tomato (*Lycopersicum esculentum* Mill., Katerji *et al*., 1998), the stems keep on growing larger (and heavier) until the end of the season. Consequently, if the crop is sown early with a long favourable growing season as in 2003, the crop grows very tall and shows average above ground dry matter yields of around 1400 g m^{-2}. The highest dry matter yield measured was even 2034 g m^{-2}. This is comparable to the dry matter yields of silage maize of up to ca 2000 g m^{-2} (Van Dijk and Brouwer, 1998).

The part of the season in which *S. sisymbriifolium* can be grown successfully is clearly restricted. If the crop is sown before May, emergence is slower than with later sowings (Fig. 4). This delay of seed emergence was seen up to DOY 120 (end of April) in the three experimental years. Such behaviour could be explained with a high base temperature (T_b) for germination (GarciaHuidobro *et al*., 1982; Chapter 2). A T_b high enough to cause the delay of emergence till DOY 120 would be around 9 °C. This would not be an exceptional value in the Solanaceae family. Values for T_b between 7.5 °C and 10 °C were found for *Solanum nigrum* (Del Monte and Tarquis, 1997) while ca 12.5 °C was observed for *Solanum physalifolium* (Del Monte and Tarquis, 1997). Indeed, in the field experiments, crops sown up to two months before DOY 120 did not emerge earlier then crops sown just after DOY 120, indicating there was apparently almost no accumulation of thermal time before DOY 120 (average measured daily temperatures were 5.8 °C and 8.3 °C for March and April, respectively).

The second restriction in the duration of the suitable season for *S. sisymbriifolium* appeared when sowing was later than DOY 210 (end of July). Although seedlings emerged in the field until September, subsequent growth end of August and September was very slow. Crops sown at the beginning of August accumulated very different amounts of biomass in the three experimental seasons. When sown in September,

crops accumulated very little biomass (1 – 2 g m^{-2} after 14 weeks). At present we have no satisfactory explanation for the sharp decline in crop performance in late summer. We hypothesize that leaf expansion and radiation use efficiency may be sensitive to temperature. After mid-September fast declining daily solar radiation becomes a strong co-limiting factor for growth.

Light interception and radiation use efficiency

The current data show that the relation between accumulated above-ground dry matter and cumulatively intercepted radiation could be adequately summarized by one single value of RUE, namely 1.69 g MJ^{-1} (PAR). This result does not exclude the possibility of systematic effects of environmental variables, such as the temperature regime, on RUE; it only indicates that such effects could not be discerned in the current data set, obtained in a variable field environment with rather long time intervals between samplings (7 weeks). The overall value of RUE of *S. sisymbriifolium* is low compared to RUEs for potato (1.93 – 2.79 g MJ^{-1} PAR depending on cultivar, nitrogen level and year) or wheat (2.11 – 2.77 g MJ^{-1} PAR) (Van Delden, 2001).

Once well established, the plant reached heights of 100 – 150 cm or more. This implies that established crops have a strong competitive ability against weeds. The duration of the period between planting and emergence, and particularly the duration of the period between emergence and the point of 50% light interception (Fig. 4) is an important determinant of the risk on establishment of weeds that can outcompete *S. sisymbriifolium*. From the viewpoint of weed prevention, these periods should be as short as possible, and in this respect planting in May – June should be preferred above earlier or later planting.

Plant density

Higher sowing densities resulted in proportionally higher plant densities at emergence, but a lower percentage of the seedlings survived the first 7 weeks. Higher loss rates at higher plant numbers can be interpreted as a manifestation of self thinning (Yoda *et al*., 1963). Initial growth, in terms of rate of increase in light interception (Fig. 8) was faster with more plants, and dry matter production at 7 weeks after emergence (Fig. 9) clearly responded in a classical fashion to plant density (De Wit, 1960). Statistically, there was no effect of plant density after 14 weeks of growth; but standard errors were high and there was a tendency for lower dry matter production at a seed rate of 50 m^{-2}. Hence, if the whole season is available for crop growth (no preceding crop or succeeding crop), there seems little need to plant at densities higher than 100 seeds m^{-2}, provided at least ca 70% of planted seeds results in established plants. In addition, less dense crops had fewer, but thicker stems that were stronger and had less

inclination to lodge, whereas lodging occurred often in the plots with high plant densities (i.e. > 150 plants m^{-2}). Higher seed rates can be considered when the weed pressure is high. It still needs to be established whether higher plant density results in more even distribution of roots and more even hatching of PCN in the top layers of the soil.

Site differences

The period with suitable conditions for growth of *S. sisymbriifolium* was apparently somewhat shorter in Flevoland and Drenthe than in Wageningen. Also during the optimal growing period the crop accumulated less biomass in Flevoland and Drenthe than in Wageningen. The sites differ in the first place in their soil types: sandy soil around Wageningen, with relatively light and dry conditions, heavier and wetter clay soil in Flevoland, and reclaimed peat in Drenthe, lighter than in Flevoland but possibly a soil that warms more slowly in spring than the soils in Wageningen. In the three years we have been growing the crop we observed that the plants grow better if conditions are initially not too moist. It is our impression that large amounts of rain, particularly when causing temporary ponding, are detrimental to the emergence and establishment of the crop.

Cropping options

Ideally, *S. sisymbriifolium* would be included as an extra crop into the rotation, without sacrificing the cultivation of a crop with commercial value. The current experiments indicate that the window of opportunity for successful planting and good crop performance in the tested agro-ecological zone is between early May and end of July. It remains to be established how long an early-planted *S. sisymbriifolium* crop needs to be on the field to achieve its maximum hatching effect. If the crop could be removed mid-July, there would be some opportunity to plant short season vegetables such as spinach after *S. sisymbriifolium*. If sufficient hatching would be achieved when planted early mid-July, farmers would have the option to use the land for a short season crop or early maturing biennial crop until that time. Whether such options exist in practice needs to be examined.

Conclusion

Based on the current study we expect that *Solanum sisymbriifolium* (Lam.) can be grown successfully in the field in The Netherlands, and probably so in Western Europe. The radiation use efficiency of the crop is low (1.69 g MJ^{-1} PAR). Yet, when sown early May, its indeterminate growth habit allows it to accumulate up to 14 ton

ha^{-1} dry matter. Planting before beginning of May did not advance crop development compared to planting early May; this points at a high minimum temperature for germination. Successful sowing was possible until the end of July, whereas performance of later plantings was very variable across years and sites. In other words the window for successful sowing in The Netherlands is limited from early May till end of July.

CHAPTER 4

Field performance of *Solanum sisymbriifolium*, a trap crop for potato cyst nematodes. II. Root characteristics

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Summary

Hatching of potato cyst nematodes is induced by root exudates of *Solanaceae*, such as *Solanum sisymbriifolium*, and is therefore related to root length distribution of this crop. A mathematical model was derived to relate the hatching potential to root length density. A series of field experiments was carried out to study actual root length distribution of *S. sisymbriifolium* in relation to shoot properties and to provide input into the model. Using a modified Poisson distribution formula for the three dimensional distribution of roots in a volume of soil, the relation between the zone of influence of hatching agents and the root length density could be derived. On this basis, the minimal root length density was estimated that is needed to expose 75, 90 or 95% of cysts to root exudates as a function of the length of the zone of influence of hatching agents on cysts. The logarithm of the total root length showed a linear relation with the logarithms of above-ground biomass and with leaf area index. Root diameter distribution was the same for all crops examined, and independent of soil depth. Fine roots (<0.4 mm diameter) constituted around 50% of total root length. Using a zone of influence of 1.00, 0.75 and 0.50 cm around the centre of each root, a minimal root length density for sufficient soil exploration (75%) was estimated. Depth to that minimal root length density was linearly related to total root length (km m^{-2}) and to above-ground crop biomass, enabling estimations of the potential hatching efficacy as related to measurable properties of *S. sisymbriifolium* crops. The proposed approach to derive potential hatching effects from crop properties needs further validation; particularly the distance of influence of root exudates is a critical factor.

Key words: *Solanum sisymbriifolium*, potato cyst nematodes, trap crop, hatching, root growth, minimal root length density.

Introduction

In recent years, *Solanum sisymbriifolium* (Lamarck) has been introduced in The Netherlands as a trap crop for potato cyst nematodes (PCN). In several pot and field experiments, the plant showed hatch stimulation slightly less than that of a susceptible potato crop, while it proved to be completely resistant, i.e. no progeny cysts were formed (Scholte, 2000b, c; Scholte and Vos, 2000).

As the crop has no economical value itself, the time it occupies the land should be reduced to a minimum and its effect on the nematode population should be high and reliable. The effect on the cyst population depends on the degree to which hatching agents penetrate the soil. The penetration into the soil depends on many factors, including the distribution of roots in the soil, the rate of exudate release from the root, the rate of diffusion and the rate of metabolization by soil organisms.

Particularly in young crops, growth of roots and shoot are correlated. For several crops linear relations have been described between the natural logarithms of the total root length beneath a unit of surface area and shoot dry weight or leaf area index, LAI (Greenwood *et al.*, 1982; Vos *et al*., 1998). The decline in root length density (RLD) with depth is described in literature with negative exponential decline functions (Greenwood *et al*., 1982; Vos *et al*., 1998). If such relations exist, root length and RLD can be estimated from shoot biomass or leaf area index (LAI), as obtained from measurements or from a dedicated crop model.

The question arising next is how much roots are required to stimulate a sufficient part of all nematodes to hatch. This question has analogy to questions on the relations between sorghum roots and germination of *Striga* seeds (Van Ast *et al*., 2000) and crops stimulating germination of microsclerotia of *Verticillium dahliae* (Mol, 1995). The answer to this question provides a method to derive potential efficacy in terms of hatching stimulation from root distribution data. The adjective ‘potential’ is used because the actual degree of hatching also depends on other factors including soil moisture content and the degree of hatchability of PCN (diapause). In literature, theories are present on minimal or optimal root densities required for the depletion of water and nutrients from the soil (Barley, 1970; De Willigen and Van Noordwijk, 1987; Dardanelli *et al*., 2004). Approaches to analyse soil exploration for water and nutrients in relation to root density may be applicable to the current problem even though water and nutrients move to the roots while hatching agents move away from the roots.

The first objective of this chapter is to examine whether the common quantitative relationships between shoot and root growth and of the root length densities over depth also apply to *S. sisymbriifolium*. The second objective of this chapter is to answer the

question: what root length density is theoretically minimally required to include a particular fraction P of the cysts in the zone of influence of the hatching agents of the nearest root? Since the radius of the hatching agent zone of influence around each root is not known, the question is answered for several assumed radius lengths. Finally, it is deduced to which soil depth 75% of all cysts reside in the zone of influence of the hatching agents, in relation to above-ground *S. sisymbriifolum* biomass or total root length.

Materials and methods

Experimental arrangements

In the 2001 experiment, three *S. sisymbriifolium* crops were sown: on May 2, June 6 and July 4, respectively, as part of a larger experiment including 8 sowing times. Plots were sown in a randomized block design with four replicates, on a field with a sandy soil in Wageningen. Plot sizes were 3 × 6 m and *S. sisymbriifolium* was sown at a density of 400 seeds m^{-2} in rows with 12.5 cm distance. Emergence of seeds was counted daily. The date, at which half of the seeds that ultimately would emerge had done so, was regarded as the date of emergence. Additional details on crop management are given in Chapter 3.

In 2003 *S. sisymbriifolium* crops were sown on May 7, June 18 and July 31 (Chapter 3). Sowing density was 200 seeds m^{-2} at a row distance of 12.5 cm. Plot size was 6 × 6 m. Experimental design, number of blocks, fertilizer application, weeding and spraying were done as in the 2001 experiment. In the summer in 2003 irrigation was applied on August 8 (42 mm), August 9 (22 mm) and August 15 (23 mm).

Root sampling

In 2001, roots were sampled in sowings 1 (S1), 2 (S2) and 3 (S3) on August 16, i.e. 94, 58 and 34 days after emergence in S1, S2 and S3, respectively. In S3, roots were sampled also on August 30, so at 48 days after emergence. All root sampling was done with an auger (diameter 7.5 cm, length 10 cm) to a maximum depth of 60 cm, taking a set of 6 samples per sampling point. At each sampling point paired augerings were made for two positions: one at the centre of a plant row and one in the middle between rows. In each of the four blocks, two replicates of such sampling sets were collected, leading to a total of eight individual samples per position per treatment and depth horizon. Since row spacing was narrow (12.5 cm) the within and between row samples had comparable root length densities and were averaged to calculate overall root length densities of the crops (cf. Bengough *et al.*, 2000).

In 2003, four successive root samplings were done in each of the three crops of *S. sisymbriifolium*. At each sampling date, a spot of 1 m^2 was randomly chosen in each plot. Then, 3 to 4 sampling sets were collected on randomly selected sampling points in this square metre area. At each point an augering was made on two sampling positions, one in the row and one between the rows. This procedure resulted in 12 – 16 individual samples per treatment and per position with respect to the planting row.

Roots were stored at −18 °C until processing. After thawing, roots were washed out of the soil by a hydropneumatic elutriation system, developed by Smucker *et al*. (1982). Primary sieve mesh was 0.4 mm. Next, the major part of organic debris was removed manually from the root samples, and the roots were painted with malachite green oxalate (Sigma-Aldrich Chemie GmbF, Schnelldorf, D). Finally, total root lengths and root lengths per diameter class were measured using the WinRhizo Pro root analysing system (Regent Instruments, Canada).

Sampling of above-ground plant material

In combination with all root samples, the above-ground crop was harvested. In 2001, an area of 25 × 25 cm was selected for all sampling sets, resulting in a total harvested area of 50 × 25 cm *S. sisymbriifolium* crop in each plot. In 2003, 1 m^2 of the crop was harvested in each plot before collecting the 3 – 4 sets of root samples in the same square metre. In both years, harvested above-ground biomass was separated into stem, leaf, bud, flower and berry, and dry weights were determined after drying to constant weight at 70 °C. In 2003 leaf area was measured on fresh samples using an electronic leaf area meter (Li-3100, Li-Cor, Lincoln, Nebraska).

Auxiliary growth chamber pot experiment

The root samples from the field experiments did not allow determination of the number of root tips per unit root length as roots were cut during sampling. Therefore, a pot experiment was conducted to estimate the number of root tips per unit root length. *S. sisymbriifolium* plants were grown in 5-litre pots in growth chambers at different temperatures (range: 13.3 – 22.3 °C). Whole root systems of 22 pots were recovered and analysed using Whinrhizo to calculate the root length, the number of root tips per unit length and root diameter distribution.

Theory

Hatching agents diffuse from the roots of a potato plant along their entire length (Rawsthorne and Brodie, 1986). We assume the same holds for *S. sisymbriifolium*. As the hatching agents are unstable when diffusing through the soil (Fenwick, 1956) the distance they reach from the roots (the zone of influence) will be limited. It is currently

assumed that the zone of influence of hatching agents around a root is in the order of 1.0 to 1.5 cm (Dr. T. Been, Plant Research International, The Netherlands, personal communication, 2004). For any given zone of influence (D) and root length density it is possible to derive the probability that cysts are positioned at distances smaller than D from the nearest root. We assume that cysts at distances < D will potentially hatch. The outcome of the analysis presented in section Results is the root length density minimally required to affect a given proportion of cysts as a function of the value of D.

Roots in a particular volume of soil can be characterized by an average root length density RLD, an average root diameter R_0 and the average number of root tips per unit of root length Λ. When the roots are dispersed randomly in this soil volume, the frequency distribution of three dimensional distances of randomly chosen points in the soil to the nearest root can be derived from a modified Poisson distribution (Barley, 1970; De Willigen and Van Noordwijk, 1987). The probability P ($0<P<1$) of the distance d (cm) to the nearest root being smaller than a given zone of influence of hatching agents D (cm) is given by:

$$P(d < D) = 1 - \pi \, RLD \, R_0^2 - \exp (- \pi \, RLD (D^2 + 4/3 \, \Lambda \, D^3)) \quad (1)$$

The second term in the right hand side of the equation is a correction for the volume occupied by the roots, which is normally negligible (De Willigen and Van Noordwijk, 1987) and is therefore not considered hereafter. It is assumed that cysts of potato cyst nematodes are randomly distributed through the upper part of the soil. A minimal fraction of cysts (P) is defined that has to be reached by the hatching agents for the trap crop to be effective. Given this value of P, the relationship between the zone of influence of the hatching agent, D, and minimal root length density, RLD_p, can now be derived:

$$P = 1 - \exp (- \pi \, RLD_p (D^2 + 4/3 \, \Lambda \, D^3)) \quad (1a)$$

This Equation can be rewritten as:

$$\exp (- \pi \, RLD_p (D^2 + 4/3 \, \Lambda \, D^3)) = 1 - P \quad (2)$$

and

$$- \pi \, RLD_p (D^2 + 4/3 \, \Lambda \, D^3) = \ln (1 - P) \quad (2a)$$

Finally the equation for the minimal root length density is:

$$RLD_p = \ln (1 - P) / (- \pi (D^2 + 4/3 \Lambda D^3)) \quad (3)$$

Models fitted to field data and statistics

Simple linear regression models were fitted to the natural logarithm of total root length beneath a unit land area (km m^{-2}) and the natural logarithm of either above-ground dry biomass (g m^{-2}) or leaf area index (m^2 m^{-2}) (Greenwood *et al.*, 1982). The decline in RLD with depth was fitted to the model (Greenwood *et al.*, 1982; Vos *et al.*, 1998):

$$\ln RLD = \ln RLD_0 - qX \quad (4)$$

where RLD_0 is the root length density at zero depth, q is the decline coefficient and X the depth. The midpoint of each soil layer was inserted for X.

Root diameters were grouped in classes of 0.08 mm; diameters > 1.52 mm were lumped into one class. Statistical analyses of results were done with GenStat (seventh edition, version 7.1.0.205, VSN International Ltd).

Results

Theoretical relations between root length density and potential hatching effect

In the auxiliary pot experiment a value of 1.23 tips cm^{-1} of root was found (Λ in Eqns 1 – 3). Next, Eqn 3 was solved for different values of P and D. Results were plotted with the minimally required RLD on the ordinate and the assumed values for D on the abscissa (Fig. 1). The different hyperbolic curves display solutions for different values of P (0.99, 0.95 and 0.75).

The (theoretical) meaning of the curves in Fig. 1 can be explained with the following examples. Under the assumption of D = 0.75 cm, i.e. the zone of influence of hatching agents extends to 0.75 cm from the root in all directions, the minimal RLD required to reach 75% of all cysts (P = 0.75), RLD_{75}, is 0.35 cm cm^{-3}. Likewise, for D = 1.00 cm, and D = 0.50 the values of RLD_{75} are 0.12 and 0.97 cm cm^{-3}, respectively.

Root characteristics measured in the field

In the first 50 – 60 days of crop growth, the total root length increased simultaneously with the above-ground dry matter (Fig. 2). After this period, the above-ground dry matter continued to increase, whereas the total root length did not increase in all crops. In the crops of the first and the third sowing the total root length even decreased between the last two harvests.

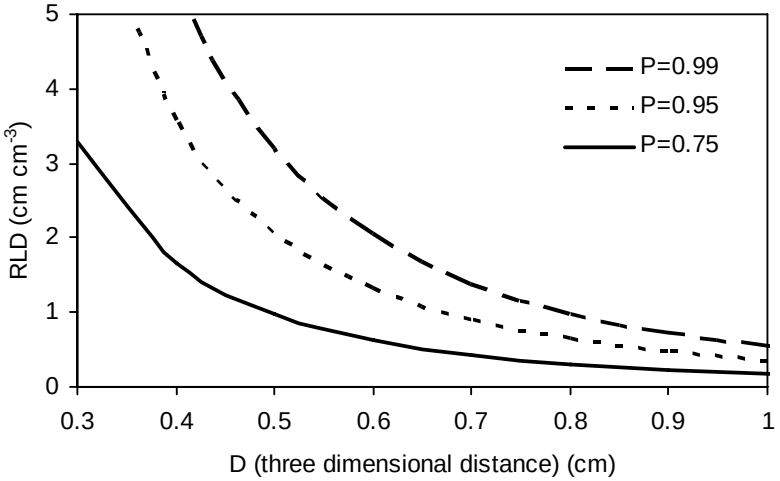


Figure 1. The relationship (Eqn 3) between the three dimensional zone of influence of root diffusate in soil (D) and root length density (RLD) for different fractions of cysts reached (P).

The natural logarithm of the total root length (km m^{-2}) (Y) had a significant linear relation with the natural logarithm of the above-ground dry matter (X) (Fig. 3), described by Y = –1.84 (SE 0.397) + 0.54 (SE 0.066) X, with P<0.001 and R^2=0.72. The natural logarithm of the total root length showed also a significant linear relation (P<0.001, R^2=0.80) with the natural logarithm of the leaf area index (Y), described by Y =0.32 (SE 0.181) + 0.96 (SE 0.158) X (Fig. 4; data from 2003).

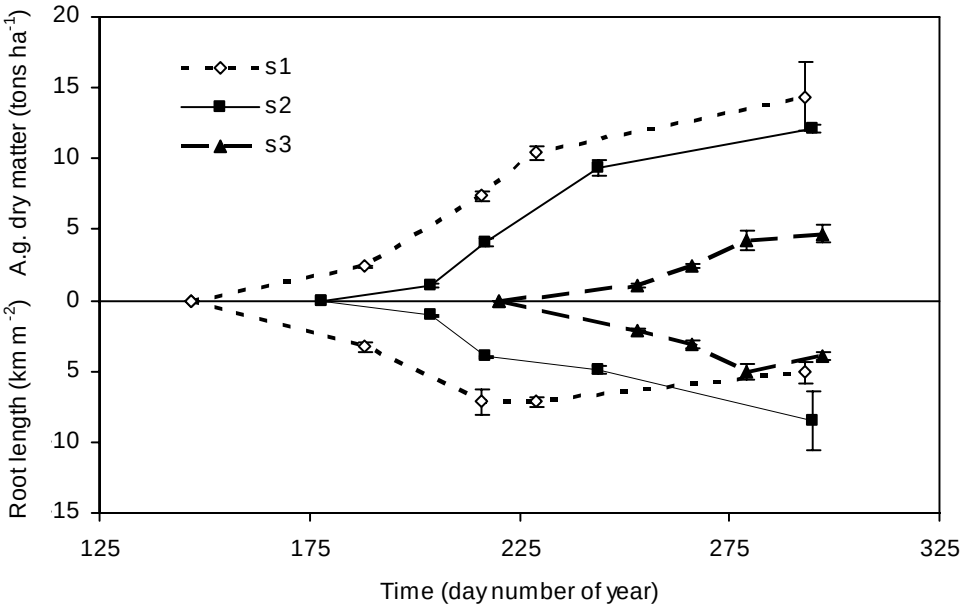


Figure 2. Total root length (km m^{-2}, negative part of Y-axis) and above-ground dry matter (tons ha^{-1}, positive part of Y-axis) plotted versus time, expressed as day number of year, for the *S. sisymbriifolium* crops in the year 2003 (S1 = sowing time 1, S2 = sowing time 2, S3 = sowing time 3). Vertical bars represent standard error of the mean.

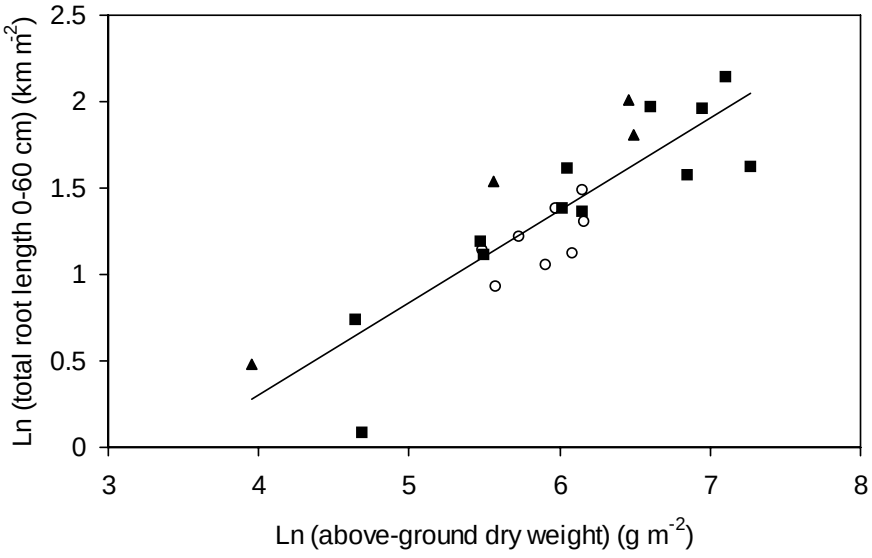


Figure 3. The natural logarithm of the total root length plotted against the natural logarithm of the above-ground dry matter for *S. sisymbriifolium* crops of 2001 (triangles sowing 1; open circles sowing 2) and 2003 (squares), (data from time series up to the point of maximum RLD in the top soil layer). The fitted line represents y = 0.54 (SE 0.066) x – 1.84 (SE 0.397); P<0.001), $R^2 = 0.74$).

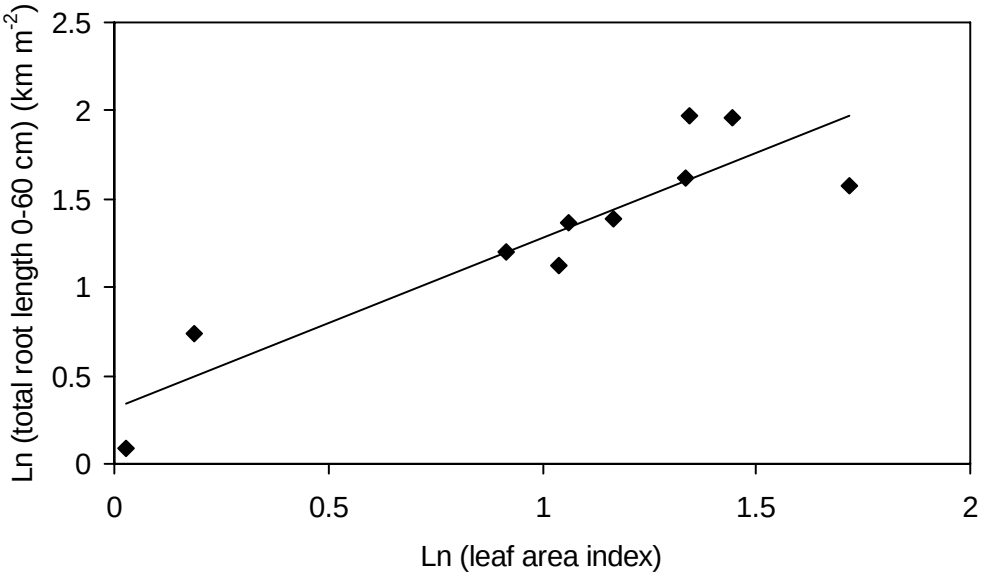


Figure 4. The natural logarithm of total root length plotted against the natural logarithm of the leaf area index, for *S. sisymbriifolium* crops in 2003. The fitted line represents y = 0.96 (SE 0.158) x + 0.32 (SE 0.181); P<0.001), adjusted $R^2 = 0.80$.

In young crops, the decrease in root length density with depth was exponential (Fig. 5, Figs 6a – c). Significant negative exponential relations were found for 10 of the 16 RLD profiles over depth, values of the exponential decline coefficient, q (Eqn 4; cm^{-1}) ranging between 0.03 and 0.12 (average 0.07). However, for crops of advanced age, exponential decline with depth was not always apparent. In the 2001 crop at 58 DAS (Fig. 5), there was no monotonous decline of RLD over depth and a peak in root length density was observed at 20 – 30 cm depth. Also in sowing 1 in 2003 on 69, 82 and 146 days after emergence (Fig. 6a), and sowing 2 in 2003 on 38 and 66 days after emergence (Fig. 6b), there was no continuous decline in RLD with depth, but peaks in root length density at 30 – 40 cm depth were observed.

The RLD_{75} for zones of influence, D, of 1.00, 0.75 and 0.50 cm were represented by vertical lines in the RLD profiles (Fig. 5, 6a – c). The depth at which the actual RLD was smaller than RLD_{75}, for a 1.00, 0.75 and 0.50 cm zone of influence of the hatching agents, was related to above-ground dry matter (Fig. 7a) and total root length (Fig. 7b).

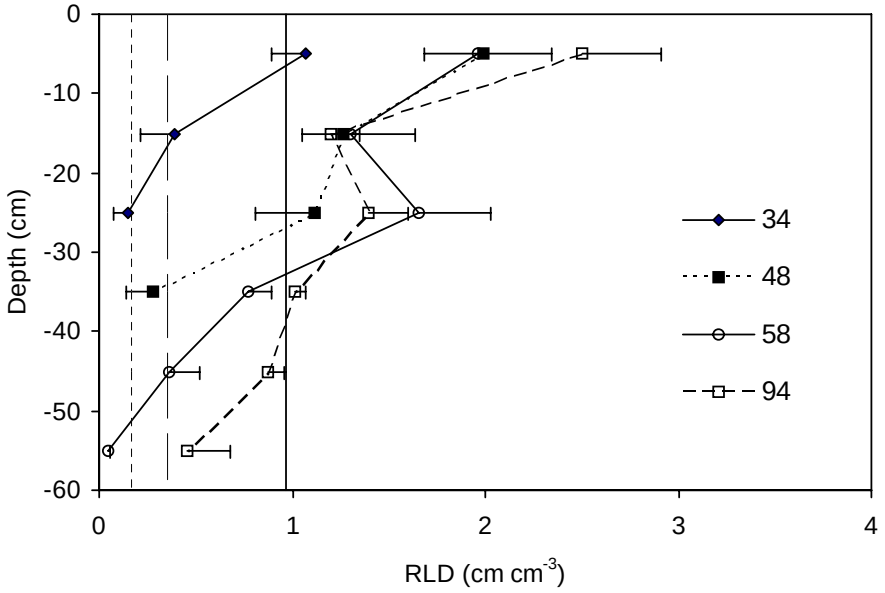
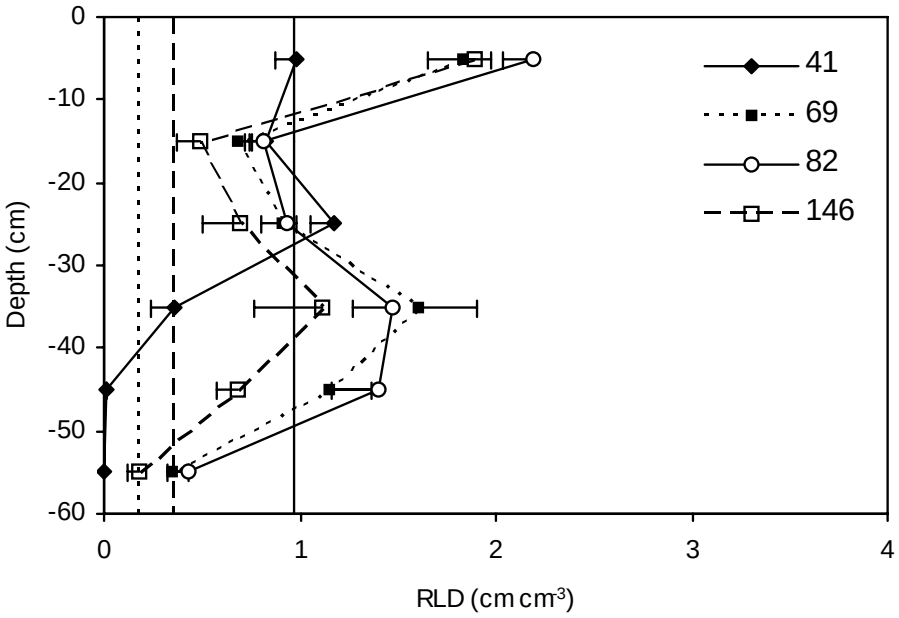
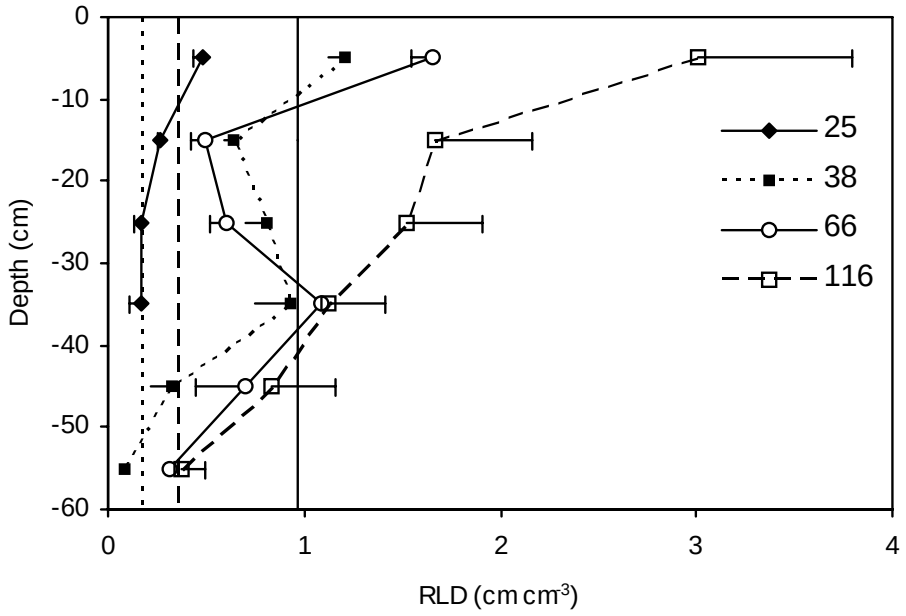


Figure 5 Root length densities as a function of depth, in *S. sisymbriifolium* crops of different ages (represented by the different series, age in days after emergence) in the year 2001 Vertical lines indicate minimal root length densities required for hatching agents to reach 75% of all cysts, with the assumption of a zone of influence of hatching agents of 1.00 cm (dotted line), 0.75 cm (broken line) and 0.50 cm (solid line).

(a)


0
-10
-20
-30
-40
-50
-60
Depth (cm)
0
1
2
3
4
RLD (cm cm-3)
41
69
82
146

(b)


0
-10
-20
-30
-40
-50
-60
Depth (cm)
0
1
2
3
4
RLD (cm cm-3)
25
38
66
116

(c)

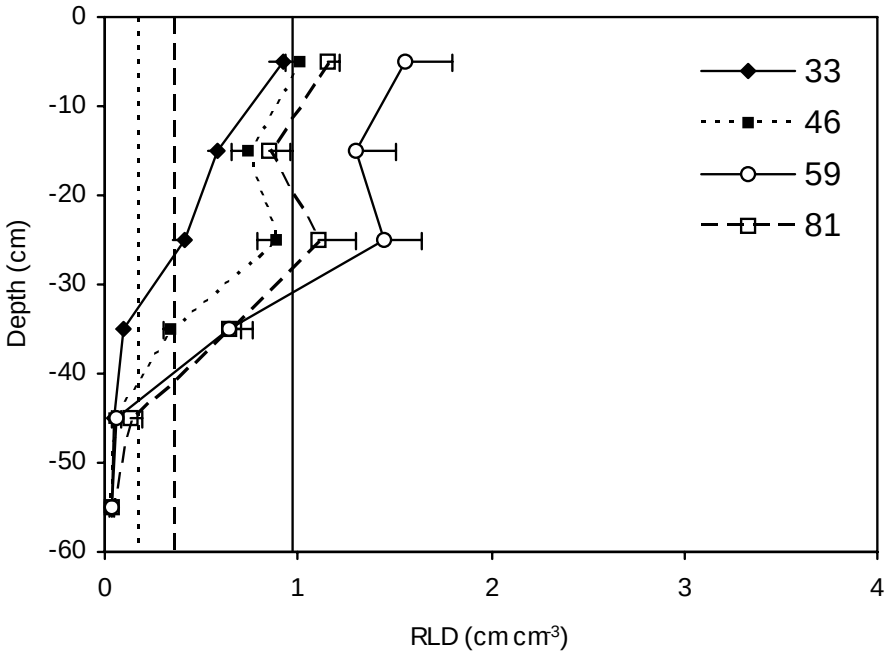
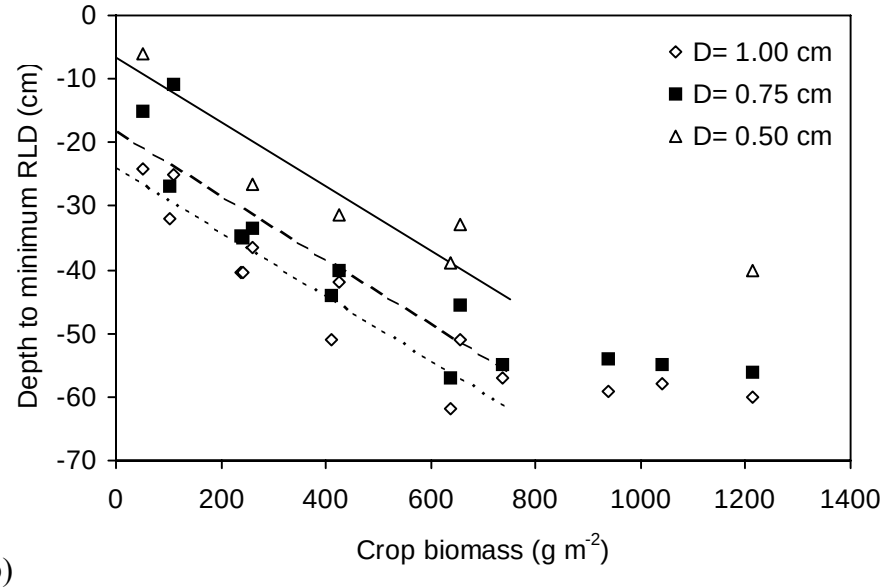


Figure 6. Root length densities as a function of depth under *S. sisymbriifolium* crops with different plant ages (33 – 81 days after emergence, represented by the different series in each panel). (a) Crop sown on May 7, 2003, (b) crop sown on the June 18, 2003 and (c) crop sown on the July 31, 2003. Each data point relates to a soil core of 10 cm depth and is entered in the midpoint of the depth interval it represents. Vertical lines indicate minimal root length densities required for hatching agents to reach 75% of all cysts, with the assumption of a zone of influence of hatching agents of 1.00 cm (dotted line), 0.75 cm (broken line) and 0.50 cm (solid line). Horizontal bars indicate standard error of the mean.

Linear regression of depth of RLD_{75} on above-ground crop biomass (excluding data points with crop biomass above 800 g m^{-2}) resulted in a model with three parallel lines that best fitted the data ($P<0.001$, $R^2 = 0.85$) with a slope of −0.051 (SE 0.005) cm (g $m^{-2})^{-1}$ and intercepts of −24.1 (SE 2.3), −18.4 (SE 2.3) and −6.6 (SE 3.1) cm for a zone of influence of the hatching agents of 1.00, 0.75 and 0.50 cm, respectively. Also, linear regression of depth of RLD_{75} on total root length was significant ($P<0.001$, $R^2 = 0.88$) with a slope of −5.8 (SE 0.82) cm (km $m^{-2})^{-1}$, and intercepts of −18.0 (SE 2.4), −12.2 (SE 2.4) and 1.5 (SE 3.1) cm for a zone of influence of the hatching agents of 1.00, 0.75 and 0.50 cm, respectively.

(a)



(b)

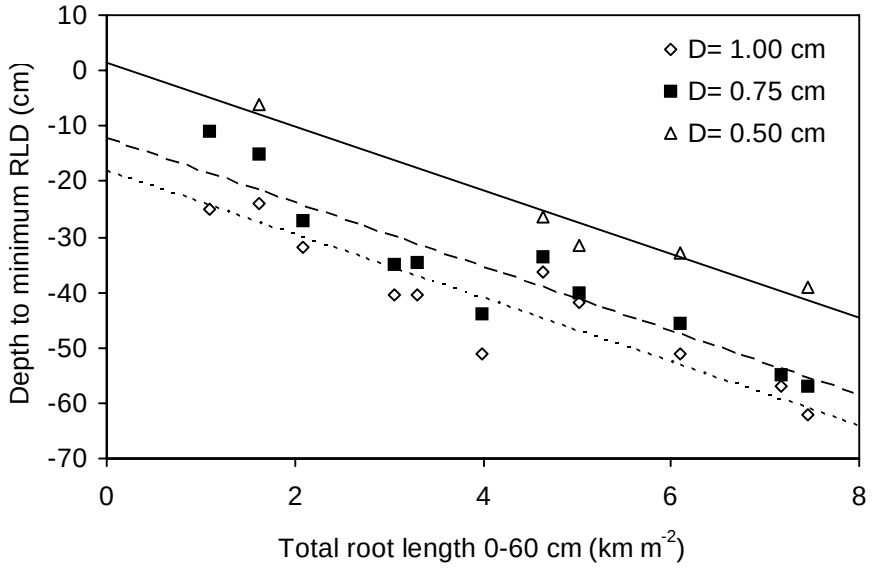


Figure 7a. Depth of the soil to RLD_{75}, plotted against the above-ground crop biomass (a) and against total root length (b) for a zone of influence of the hatching agents of 1.00, 0.75 and 0.50 cm as indicated in each panel. Linear regressions with three parallel lines best fitted the data, with R^2 values of 0.85 for crop biomass (excluding points above 800 g m^{-2}) and 0.88 for total root length. Fitted lines in Fig. (a) have a common slope of –0.051 (SE 0.005) cm (g m^{-2})$^{-1}$ and intercepts of –6.6 (SE 3.1), –18.4 (SE 2.3) and –24.1 (SE 2.3) cm for a zone of influence of the hatching agents of 0.50 (solid line), 0.75 (broken line) and 1.00 cm (dotted line), respectively. Fitted lines in Fig. (b) have a common slope of –5.8 (SE 0.5) cm (km m^{-2})$^{-1}$ and intercepts of 1.5 (SE 3.1), –12.2 (SE 2.4) and –18.0 (SE 2.4) cm for zones of influence of hatching agents of 0.50, 0.75 and 1.00 cm, respectively, shown by same line types as in (a).

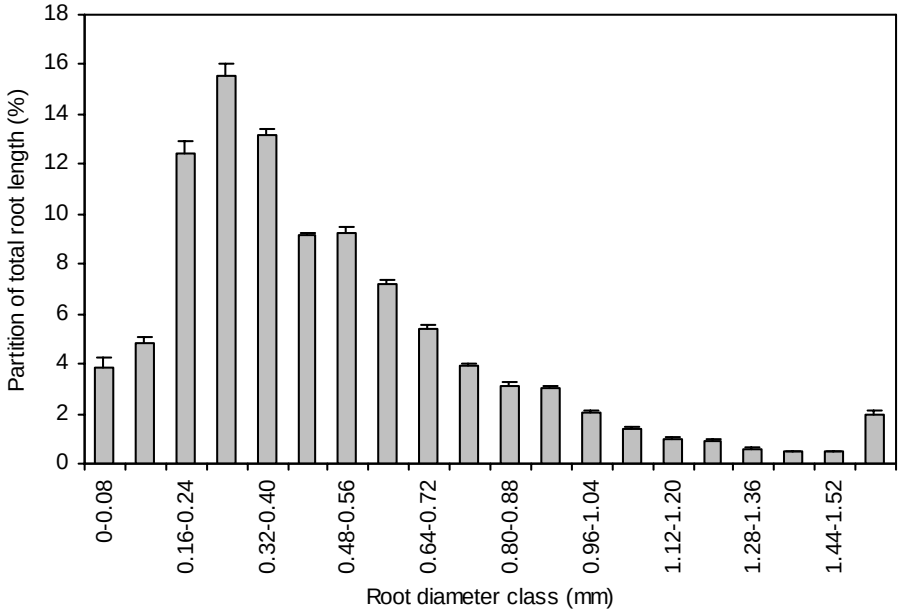


Figure 8. Partition of total root length (%) of root diameters in classes of 0.08 mm; data from all crops in 2003. Horizontal bars indicate standard error of the mean.

Important is to mention, that for calculations with length of the zone of influence of 0.5 cm, the soil depth at which the actual RLD was smaller than RLD_{75} could not be determined in profiles where RLD did not decline monotonously with depth.

The frequency distribution of root diameters (Fig. 8) did not show a normal distribution; the distribution was skewed to the larger diameter classes. The peak in frequencies (15.5%) was in the diameter class 0.24 – 0.32 mm. Fifty per cent of the roots had diameters less than 0.4 mm.

Discussion

The total root length under *S. sisymbriifolium* crops could be estimated using above-ground dry matter and LAI, as was suggested by several authors (Greenwood *et al*., 1982; Vos *et al*., 1998). Comparison of the current regression results to those of several nitrogen catch crops (winter rye, forage rape and oil radish, Vos *et al*., 1998) indicates that *S. sisymbriifolium* had much lower total root length for a given amount of above-ground biomass or LAI than these catch crops. For instance, the fitted value for total root length at 100 g m^{-2} biomass is 1.90 km m^{-2} for *S. sisymbriifolium*, whereas it is 9.27 km m^{-2} for the catch crops. For 200 g m^{-2} biomass these numbers

are 2.78 and 12.37 km m^{-2}, respectively. For LAI = 1, the fitted value for total root length is 1.38 km m^{-2} for *S. sisymbriifolium* and 5.93 km m^{-2} for the catch crops; corresponding numbers for LAI = 2 are 2.68 and 7.77 km m^{-2}. These differences between the species in relations between shoot attributes and total root length may be associated with the differences in dry matter distribution among shoot organs. The catch crops showed leaf weight ratios of 0.6 – 0.7 (Vos and van der Putten, 1997), whereas this ratio is decreasing from 0.8 to 0.2 during crop growth of *S. sisymbriifolium* (Chapter 3).

As was shown for other crops by Greenwood *et al*. (1982) and Vos *et al*. (1998), the decline of RLD over depth could be represented by a first order decay function, especially for young *S. sisymbriifolium* crops. The calculated exponential decline parameter q had values slightly higher than those found for nitrogen catch crops (0.03 – 0.07, average 0.05, Vos *et al*., 1998). However, in six of 16 RLD profile measurements, a pattern with a peak of high RLD at 25 – 40 cm depth was found. Such deviations might indicate non-uniformity in the soil profile of properties that affect root density (e.g. mechanical resistance, availability of water and nutrients, texture, pH). Visual observations revealed a transition in soil colour at the same depth, from dark brown (upper part) to light yellow (bottom part), that might be associated with soil qualities, that affect root density in those soil layers. However, at present we can not point at obvious factors and for the time being we suggest that in principle the root distribution under a *S. sisymbriifolium* crop is well described by a negative exponential model provided soil characteristics over the rooting depth are homogenous.

It is important to develop guidelines for agricultural practices on the use of *S. sisymbriifolium* as a trap crop, particularly on the duration of crop growth and the density of roots required to achieve a particular degree of hatching. To that end a theoretical approach was used to derive the relation between the zone of influence of the hatching agents in the soil and the associated required root length density to reach a major fraction (75%) of the cysts in the soil (Eqn 3; Fig. 1). With this relation, it is possible to calculate minimal root length densities for assumed zones of influence of the hatching agents around each root, and use these to calculate the potential effect of the roots on the cysts in a given volume of the soil.

This approach needs to be applied with caution. Clearly, without the presence of hatching agents, only few PCN juveniles will hatch spontaneously (Jones *et al*., 1998). The hatching of *G. pallida* juveniles is even more dependent on the presence of hatching agents than that of *G. rostochiensis* juveniles (Den Nijs and Lock, 1992). However, the penetration of hatching agents in the PCN cysts is only one of the requirements for hatching. The embryonated eggs within cysts may not always be

sensitive to the hatching agents due to diapause (Muhammad, 1994; Jones *et al.*, 1998). Scholte and Vos (2000), however, reported complete hatchability throughout the season that is suitable for cultivation of *S. sisymbriifolium*. Another point of caution is that potato cyst nematodes are known to affect root growth, root number and root morphology of infected potato plants (Van der Wal *et al*., 1997; De Ruijter and Haverkort, 1999). Scholte (2000c) has shown that total root weight of *S. sisymbriifolium* was not affected by increasing PCN infestation densities (0 to 56 juvenile PCN ml^{-1} soil), although the proportion of lateral roots was slightly increased in his experiments.

The depth in the soil, to which the actual root length density exceeded the calculated minimal root length densities, was correlated to total above-ground biomass and total root length. These associations can be used as a tool to link these crop traits, measured in the field or calculated by a dedicated crop growth model, to the potential PCN hatching effect of the trap crop. More than 90% of the potato cyst nematodes normally are located in the top 40 cm of the field soil (Been and Schomaker, 1998). Using our relations (Fig. 7a and b) it can be deduced that for 75% hatching a crop would be needed with 3.8, 4.8 or 7.2 km m^{-2} total root length when the zone of influence declines from 1.00 to 0.75 and 0.50 cm, respectively. Analogously, crops of 310, 430 and 660 g m^{-2} above-ground dry weight would be needed to expose 75% of the cysts to hatching agents for a zone of influence declining from 1.00 cm to 0.75 cm and 0.50 cm, respectively.

The interpretation of the required minimal root length density depends critically on the assumption regarding the zone of influence of hatching agents in the soil. Generally it is considered to be in the order of about 1 cm (Dr. T. Been, personal communication, 2004), although other unpublished results suggest it to be somewhat smaller. A small zone of influence (e.g. 0.5 cm) leads to a high minimal root length density required for 75% of the cysts to be located within this zone of influence. This could cause parts of the soil with lower root length densities to have insufficient hatching.

However, in literature horizontal zones of influence of up to 50 cm were mentioned for pot experiments (Rawsthorne and Brodie, 1987) and at least 10 – 20 cm for field conditions (Devine and Jones, 2003). Combining these much larger zones of influence with Eqn. 3 would imply that almost no roots are needed for the hatching agents to reach all cysts. This seems unrealistic as this would lead to the possibility of very high hatching under small or low density potato crops or trap crops. An explanation for part of this high variation in estimated size of zones of influence could be that methods to study root properties may have differed, perhaps resulting in underestimation of the presence and length of thin roots in the vicinity of the hatched cysts. We conclude that

when more critical information on the zone of influence of hatching distance will become available, the approach developed in this chapter can be applied with more certainty. The time required for the hatching agents to diffuse through the zone of influence and the time or concentration required for the bulk of the cysts to react to the hatching agents are not taken into consideration in the approach so far. Data on these aspects could further underpin calculations on effectiveness of cyst hatching for given crop characteristics.

CHAPTER 5

Growth duration and root length density of *Solanum sisymbriifolium* (Lam.) as determinants of hatching of *Globodera pallida* (Stone)

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Summary

Solanum sisymbriifolium is a trap crop for potato cyst nematodes (PCN). In this study, we quantified the effect of different periods of growth of *S. sisymbriifolium* and root length density on hatching of PCN, using potato and fallow treatments as references.

One and two-year-old *Globodera pallida* cysts were used in two years in greenhouse experiments carried out in containers. Two methods were used to study hatching. In the first method 7.5 cm diameter soil cores were removed and backfilled with infested soil. In the second method cysts were buried in nylon bags. The soil cores infested with cysts used in the first method had characteristics slightly different from those of the surrounding bulk soil and this textural difference was suspected to be the cause of very low root colonization of the cores as compared to bulk soil. Therefore, the effect of *S. sisymbriifolium* was strongly underestimated by the soil core method.

Hatching of PCN cysts, measured with the nylon bag method, increased with the duration of growth of *S. sisymbriifolium* from 47% after 6 weeks of crop growth up to 75% after 21 weeks of crop growth. Reductions per depth layer were also correlated to root length density and varied between 42.6% at 0.26 cm cm^{-3} and 85.3% at 5.8 cm cm^{-3}. Based on a single exponential decay function, a general method is presented to estimate for any PCN control measure the average reduction in the number of years needed to reach sanitation below a given PCN population density. Calculated reductions in duration of the sanitation period ranged from 2.3 years for 59% hatching (equivalent to 90 days of *S. sisymbriifolium*) to 4.4 years for 75% of hatching (equivalent to 150 days of *S. sisymbriifolium*). These reductions were independent of initial and final population density. In conclusion, these results corroborate the hatch inducing effect of *S. sisymbriifolium*, underline the importance of growth duration and root length density as determinants of the decimating effect on PCN, and draw attention to methodical pitfalls in the study of hatch stimulation.

Key words: Trap crop, nematode control, nylon bag, sampling method, *Solanum tuberosum*.

Introduction

In many potato growing areas, potato cyst nematodes (PCN) are a serious problem. For example a field study in England and Wales showed that PCN were present in 64% of all sampled sites (Minnis *et al.*, 2002). To control PCN, one of the available measures is a trap crop (Halford *et al.*, 1999; Scholte, 2000a, b, c). Such a trap crop has to stimulate the hatch of juvenile PCN from their cysts by chemicals exuded from its roots. Subsequently, reproduction of the hatched PCN has to be prevented, either by resistance or incompatibility of the used crop or by destruction of the crop before adult female PCN have developed. Scholte (2000a) has shown that potato itself can be used, but he argued potato is not an ideal trap crop. Problems include determination of the exact time when the potato crop must be killed, the stimulation of other potato diseases, and the tubers that, if not all removed completely, can become volunteers in the next year, which again form a potential point source for PCN reproduction. After extensive screening of non-tuber bearing *Solanaceae* for their potential as trap crops, *Solanum sisymbriifolium* (Lamarck) was selected as a promising candidate. This species reached a level of hatch stimulation slightly lower than that of potato cv. 'Bintje' and no progeny cysts were detected (Scholte, 2000b, c).
S. sisymbriifolium originates from South America. Studies on the performance of this plant species under West European field conditions are in progress (Chapter 3). In several pot and field trials, *S. sisymbriifolium* had reductions of 50 to 80% of the population of PCN juveniles (Scholte, 2000b; Scholte and Vos, 2000). However, the determinants of the degree of hatching need to be examined. A previous chapter explored theoretically the minimum root length density required to expose particular fractions of nematodes to exudates in relation to the distance of diffusion of exudates from the root surface (Chapter 4). The present work addresses the following issues: (i) quantification of the hatch stimulation, expressed as reduction in cyst content, in relation to growth period of the plant and (ii) investigation of the relation between root length density (RLD) and average cyst content reduction. Finally (iii), the reduction in duration of the sanitation period (hereafter denoted as 'time gain'), evolving from the reductions in the nematode population, is deduced from the current data.

Materials and methods

Nematodes

Cysts of *Globodera pallida* population E425 were bought from the Hildebrands Laboratory (Wijster, The Netherlands) in 2002, and multiplied in one litre pots in a sand mixture planted with the sensitive potato cv. Elkana in 2002. After multiplication,

pots were left to dry and nematodes were stored at 5 °C in the dried sand mixture until use, resulting in one-year-old cysts in the 2003 experimental season and two-year-old cysts in the 2004 experimental season. Additionally, part of the cysts were multiplied a second time in the same sand mixture with the sensitive potato cv. Bintje in 2003. After multiplication pots were left to dry and the nematodes were stored at 5 °C in the dried sand mixture, resulting in one-year-old cysts in 2004. All cysts were kept in the original sand mixture till needed in the hatching experiments in order to avoid artefacts in nematode behaviour.

To measure the effects of *S. sisymbriifolium* on hatching of cysts of PCN, two testing methods have been used in earlier studies: sampling of sand cores containing cysts, using an auger (Van der Wal *et al.*, 1997; Devine *et al.*, 1999; Halford *et al.*, 1999; Scholte, 2000c) and monitoring of cysts buried in nylon bags (Hominick *et al.*, 1985; Rawsthorne and Brodie, 1987; LaMondia *et al.*, 1987; Scholte, 2000c). Both methods are supposed to give reliable estimates of nematode cyst hatching. For the sand core method, the original soil in which nematodes were multiplied was diluted with soil of different texture in order to reduce the population density to 150 cysts per liter soil. For the nylon bag method, cysts were recovered from the original sand mixture by manual sieving, without using water or chemicals. Research with nylon bags containing differing amounts of sand and striga seeds showed density dependence of longevity of *Striga* seeds (Van Mourik *et al.*, 2005). Out of concern for comparable effects on PCN cysts, nylon bags were relatively large (4 – 5 cm long) and 50 cysts were spread through each bag as evenly as possible.

Experiment 1 (2003): The effect of S. sisymbriifolium *crops on one-year-old cysts of* G. pallida.

Expt 1 was conducted in a temperature controlled greenhouse (temperature 20 °C day, 15 °C night, natural daylength). Plastic containers (112 cm long, 72 cm wide and 65 cm high) were filled with the sandy soil that was also used to dilute the sand mixture as mentioned above and compacted per layer of 15 cm to prevent the soil from being too loose. On 25 – 27 June 2003, 60 cm deep holes (diameter 7.5 cm) were drilled with an auger and filled with the sandy soil mixture containing one-year-old *G. pallida* cysts. On 30 June 2003, first crops were planted in the containers and this date was considered as the start of the experiment (day 0).

Treatments (Table 1a) included a control without a crop (Fallow), susceptible potato cv. Bintje (Potato) and several planting times of *S. sisymbriifolium* (Ss P1, Ss P2 and Ss P3); *S. sisymbriifolium* plants were planted in five rows (12.5 cm row distance), with a plant density of 100 m^{-2} (8 cm planting distance in the row). Potato plants were planted in three rows (24 cm row distance) with a stem density of 26 m^{-2} (16 cm

planting distance in the row, potato eyes were cut out and planted in the containers to control final stem density of the potao crop). The first sowing of *S. sisymbriifolium* was done directly in the containers, but for practical reasons the second and third sowing were done in pots, and the seedlings were transplanted in the containers immediately after emergence. All crops were harvested 133 days after the start of the experiment, and above-ground dry weight was measured after drying at 105 °C to constant weight. Cores were sampled from the infected soil ('sand cores') until 60 cm depth, in four replicates per container using an auger of 30 cm length with a diameter of 5 cm, i.e. narrower than the backfilled soil core. To collect the cysts, each extracted core was divided into samples of 10 cm length and washed using a 'Kort funnel' (Kort, 1960) with an upward water flux of 5 liter minute^{-1}, over a 180 μm sieve. Debris was further cleaned using alcohol and isolated cysts were counted, crushed and finally the second stage juveniles (J2) as embrionated eggs or larvae were counted.

Roots were separated from infected cores on the top sieve of the 'Kort funnel' containing pores of 1.1 mm diameter. Most of the organic debris was removed manually from the root samples, and the roots were painted with malachite green oxalate (Sigma-Aldrich Chemie GmbF, Schnelldorf, D). Total root length and root length per diameter class were measured using the WinRhizo (version Pro 2001a) root analyzing system (Regent Instruments, Canada). Root density was measured in non-infested bulk soil in randomly taken soil samples. These samples were washed with a hydro pneumatic elutriation system and sieve mesh of 0.4 mm (Smucker *et al.*, 1982) to separate roots from soil. Organic debris removal, painting, and determination of total root lengths and root lengths per diameter class were done as for the roots from the infected cores.

***Experiment 2 (2004): The effects of* S. sisymbriifolium *crops on one- and two-year old-cysts of* G. pallida.**

The experiment was a repetition of Expt 1. The containers were filled and on 2 April 2004 holes (diameter 7.5 cm) were drilled and backfilled with soil cores infected with two-year-old cysts. Additionally, nylon bags containing one- or two-year-old cysts were buried in the containers at three depths (10, 25 en 40 cm) on 5 April 2004. This date was taken as the start of the experiment (day 0) as also the first crops were planted in the containers. Seeds were planted in pots and immediately after emergence seedlings were transplanted in two replicate containers. Planting density was 64 seedlings m^{-2} (12.5 cm between and within row distance).

Treatments (Table 1b) included a control without a crop (Fallow), susceptible potato cv. 'Bintje' (Potato) and several planting dates of *S. sisymbriifolium* (Ss P1, Ss P1 cut and Ss P2). In the treatment 'Ss P1 cut', the above-ground *S. sisymbriifolium*

Table 1. Details on treatments in Experiments 1 (a) and 2 (b); dates of transplanting, harvesting, sampling and resulting periods of treatment duration, in days after start of the experiment (day 0) on June 30, 2003 (Expt 1) and April 5, 2004 (Expt 2).

(a)

Treatment code	Transplanting	Removal of above-ground dry matter	Sand core sampling	Resulting period of treatment to sand core sampling (days)
Fallow	.	.	133	133
Potato	0	133	133	133[a]
Ss P1[b]	0	133	133	113[ad]
Ss P2	52	133	133	81[a]
Ss P3	81	133	133	52[a]

(b)

Treatment code	Trans-planting	Removal of above-ground dry matter	Removal nylon bag			Resulting period of treatment to nylon bag removal (days)			Sand core sampling
			Sampling 1	Sampling 2	Sampling 3	Sampling 1	Sampling 2	Sampling 3	
Fallow	.	.	44	95	153	44	95	153	178
Potato	0	150	44	95	153	44	95	150[a]	178
Ss P1[b]	0	150	44	95	153	44	95	150[a]	158
Ss P1 cut[c]	0	44	44	95	153	44	-	-	178
Ss P2	49	150	.	95	153	.	46	101[a]	158

[a] Numbers indicate the time of presence of *S. sisymbriifolium* or potato plants.

[b] Ss P1 – P3 represent first, second and third planting of *S. sisymbriifolium*, respectively.

[c] Ss P1 cut represents a treatment in which the above ground crop was harvested on day 44, whereas roots were not removed.

[d] On the first planting date *S. sisymbriifolium* was directly planted on the container, and plants emerged after 20 days. To make a fair comparison with the other plantings, only the period from emergence to crop removal is given in the Table.

crop was harvested on day 44, after which roots were not removed. Further experimental setup, number of replicates and experimental procedures were the same as in Expt 1.

Cysts in nylon bags were removed on three dates. Soil samples of infected soil (sand cores) were taken on two dates at the end of the experimental period (Table 1b). Around each nylon bag a soil sample (diameter 5 cm, 15 cm length) was taken with an auger, extending from 10 cm above the nylon bag to 5 cm below it (e.g. for the bags at 10 cm depth a sample was taken from 0 – 15 cm depth). Roots were recovered from the soil using a hydro pneumatic elutriation system with a sieve mesh of 0.4 mm (Smucker *et al*., 1982). Organic debris removal, painting, and measurements of total root length and root length per diameter class were done as in Expt 1.

Theory on nematode loss

The annual decrease in the number of nematodes present in an infected field is described by first order rate kinetics, similar to the exponential decay of the amounts or activities of radioactive elements (Harms and Jerome, 2004) or of decomposing soil organic matter (Römkens *et al*., 1999). The general equation for this process is:

$$A_t = A_0 \times 2^{\frac{-t}{T_{1/2}}} \quad (1)$$

in which A_t is the amount of radioactive substance or - in our case - the number of nematodes present at time t, A_0 the number of nematodes at the time the calculation is started, t the time (y) and $T_{1/2}$ the half life time (y) of the population of nematodes. This equation can be rewritten to:

$$A_t = A_0 \times e^{\frac{-t \times \ln(2)}{T_{1/2}}} \quad (2)$$

For known A_t and A_0, it is possible to calculate $T_{1/2}$ with:

$$T_{1/2} = (-t \times \ln(2)) / \ln(A_t / A_o) \quad (3)$$

Further the equation can be rewritten to:

$$t = \ln(A_1 / A_0) \times T_{1/2} \times (1 / - \ln(2)) \quad (4)$$

in which t is the time required to reach sanitation, meaning decline of the PCN population below a certain population density, A_1.

Calculation of time required to reach sanitation below given PCN population densities

The benefit of trap cropping with *S. sisymbriifolium* can be expressed as time gain to sanitation, i.e. the difference between a reference treatment and trap cropping in the number of years required before the PCN declined to a certain low threshold level. Three scenarios will be compared. The reference scenario is continuous fallow or cultivation of a non-host/non-trap crop, representing natural decay of the population. In two other scenarios *S. sisymbriifolium* is grown in the first year, followed by fallow or non-host/non-trap crops in subsequent years. The difference between these trap crop scenarios is growth duration of the trap crop, which is reflected in different percentages of PCN hatching

The initial PCN infestation density was set at 50 eggs g^{-1} dry soil, which is a realistic, moderately to severe infestation density (Schomaker and Been, 1999; Scholte and Vos, 2000). Experiment 2 yielded the fraction of nematodes that hatched in the first year under fallow and under *S. sisymbriifolium* crops of various growth durations. With these fractions and the initial population density of 50 eggs g^{-1} soil, population densities after the first year can be calculated for various treatments. In the second and subsequent years the annual hatch of nematodes was assumed to be a constant fraction typical for non-hosts or fallow, being equivalent to the spontaneous hatch of two-year-old cysts in Expt 2, i.e. 20.6%. With this fraction $T_{1/2}$ for continuous non-hosts or fallow can be calculated using Eqn (3). The time to sanitation after the first year, t, can now be culculated for several scenarios using Eqn (4) and the calculated value of $T_{1/2}$.

Time gain by S. sisymbriifolium cultivation over fallow or non-host cultivation

After the first year of different treatments, e.g. fallow, 90 and 150 days of trap cropping with *S. sisymbriifolium* (equivalent to 59% and 75% hatching, respectively) all infestations follow the same fractional decrease per year. This means that at the beginning of the second year onlyA_0 (Eqn 3) differs among treatments. If the fractional decrease for *S. sisymbriifolium* and fallow are defined as fd_s and fd_f, respectively, Eqn (4) for the fallow situation can be written as:

$$t_{fallow} = \ln(A_t / (A_0 \times fd_f)) \times T_{1/2} \times (1 / -\ln(2))$$

Which is equal to:

$$t_{fallow} = \ln(A_t / A_0) \times T_{1/2} \times (1 / -\ln(2)) - \ln(fd_f) \times T_{1/2} \times (1 / -\ln(2)) \qquad (5)$$

Equivalent to this, the equation for *S. sisymbriifolium* in the first year and fallow or non-hosts in subsequent years can be written as:

$$t_{S.\ sisymbriifolium} = \ln(A_t / A_0) \times T_{1/2} \times (1 / -\ln(2)) - \ln(fd_s) \times T_{1/2} \times (1 / -\ln(2)) \quad (6)$$

The time gain to sanitation, TG, to reduce the PCN below an assumed detection threshold (A_1) follows from equation (5) and (6), being:

$$TG = \ln(fd_f) \times T_{1/2} \times (1 / -\ln(2)) - \ln(fd_s) \times T_{1/2} \times (1 / -\ln(2))$$

which equals:

$$TG = \ln(fd_f / fd_s) \times T_{1/2} \times (1 / -\ln(2)) \quad (7)$$

It is import to note that this shows that the time gain by cropping *S. sisymbriifolium* is independent of A_0, which was the initial population density of the PCN infestation, and also independent of A_1, which is the final population density below which sanitation is regarded complete.

PCN detection threshold

In the statutory sampling method, specified in the regulations issued by the Dutch Plant Protection Service, 200 ml bulk soil per 0.33 ha is sampled (Been and Schomaker, 2000). The field is declared PCN infested if at least one cyst with living eggs is found in such a sample. Assuming that a full cyst contains an average of 260 eggs, and the soil bulk density is ~1.3 g ml^{-1}, this means that the detection threshold is ~1 egg g^{-1} soil. If final population size of a homogenously infested field would decrease below this threshold, it would theoretically not be detected by the sampling method. However, sampling in this statutory or other sampling methods, whether more intensive or not, is done in a grid of subsamples. Since most of the PCN infestations occur in a patch or focus in the field (Schomaker and Been, 1999), the number of subsamples taken in the infestation focus is a matter of chance. The chance to be detected depends on the size of the infestation focus, the number and grid of subsamples. Also, the number of cysts in the final subsample that is analyzed depends on chance, dependent on the volume of the each subsample and on the population density and spatial pattern of the PCN infestation. For this reason, a series of final population densities ranging from 1 egg g^{-1} soil (detection threshold, for homogenous infected field if the statutory sampling method is used) to 18 eggs g^{-1} soil was used in the calculations of sanitation time. The higher the final population density, the higher

the chance on detection of the infestation, but the exact chance depends on the methods used.

Results

The effect of S. sisymbriifolium *crops on cysts of* G. pallida *in sand cores (Expts 1 and 2)*

The one-year-old *G. pallida* cysts sampled in sand cores showed 22% spontaneous hatching at 133 days after the start of the experiment, whereas hatching increased to 42 – 43% after 52−113 days of growth of *S. sisymbriifolium* (Fig. 1).

The two-year-old *G. pallida* cysts showed 30% spontaneous hatching at 178 days after start of the experiment. Hatching under *S. sisymbriifolium* crops increased with growth duration and rose ultimately to 50% after 150 days (Fig. 1).

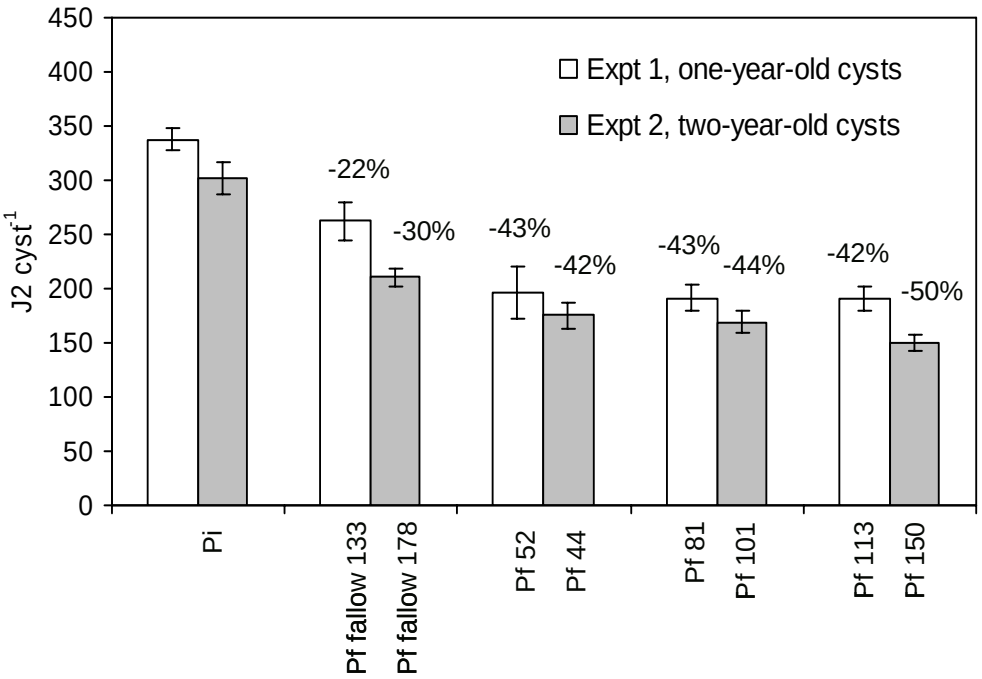

Figure 1. Cyst content (second stage juveniles: eggs and larvae) as obtained from sampling infected sandcores in Expt 1 (white bars) and Expt 2 (grey bars). Treatments included fallow, and growth durations of 44, 52, 81, 101, 113 and 150 days of *S. sisymbriifolium* plants on infected soil. Pi indicated the average cyst content, measured at the beginning of the experiment; Pf denotes the final average cyst content, measured 133 days after start of the experiment for all treatments for one-year-old cysts and 178 days after start of the experiment for two-year-old cysts. Numbers indicate hatching percentages; error bars indicate standard errors of the mean.

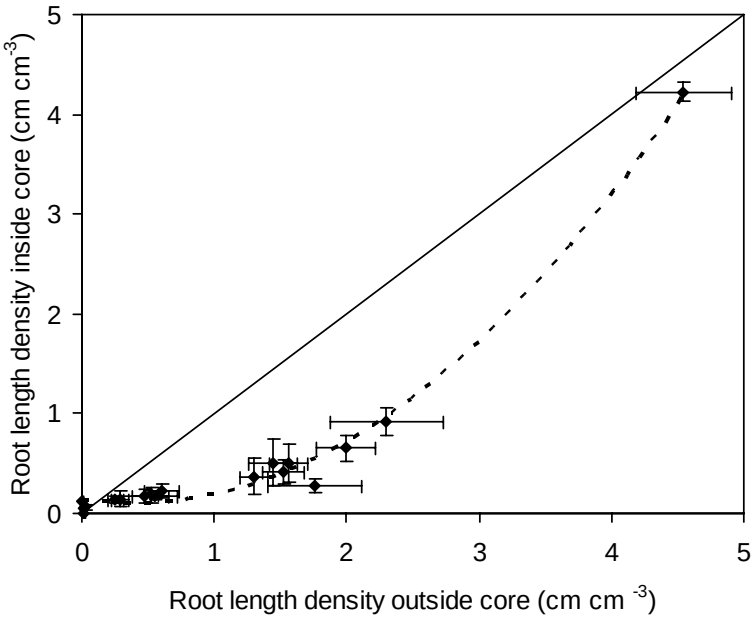

Figure 2. Root length densities (RLD) in corresponding samples taken in bulk soil outside sand cores and inside infected sand cores; data from Expt 1, 2003. The solid line indicates y = x and the dotted line represents a polynomial trend line.

RLD measured in infected soil cores was always lower than RLD measured in the same depth layer in uninfected bulk soil (Expt 1; Fig. 2). Only for the point of the highest RLD in bulk soil (4.5 cm cm^{-3} at 0 − 10 cm depth after 113 days of crop growth), the corresponding RLD inside the infected sand cores was high too (4.2 cm cm^{-3}). Therefore, the general message from Fig. 2 is that root proliferation was substantially lower in PCN infected cores than in bulk soil.

The effect of* S. sisymbriifolium *crops on cysts of* G. pallida *in nylon bags (Expt 2)

One-year-old *G. pallida* cysts buried in nylon bags showed 32% of spontaneous hatching after 153 days while 77% had hatched after 150 days of *S. sisymbriifolium* cultivation (Fig. 3). Two-year-old cysts buried in nylon bags showed 21% of spontaneous hatching after 153 days, while 62% had hatched after 101 days of *S. sisymbriifolium* crop growth and 69% had hatched after 150 days of *S. sisymbriifolium* crop growth. After 150 days under a susceptible potato crop, one and two-year-old cysts buried in nylon bags showed 95% and 90% hatching, respectively.

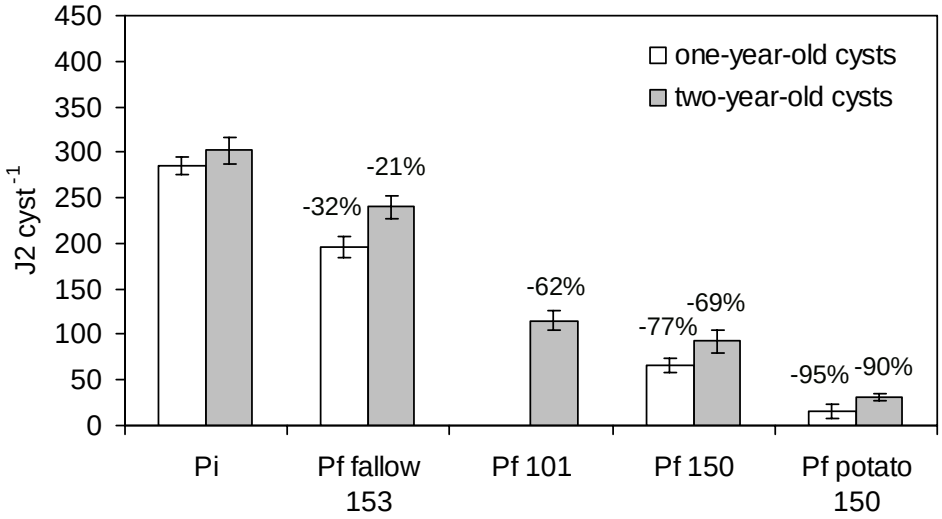

Figure 3. Cyst content (second stage juveniles: eggs and larvae) as obtained from cysts buried in nylon bags. Data from Expt 2, with one- and two-year-old cysts of *Globodera pallida*. Treatments included fallow and growth durations of 101 and 150 days for *S. sisymbriifolium*, and 150 days for potato (cv. 'Bintje'). Nylon bags were removed 153 days after start of the experiment in all treatments.

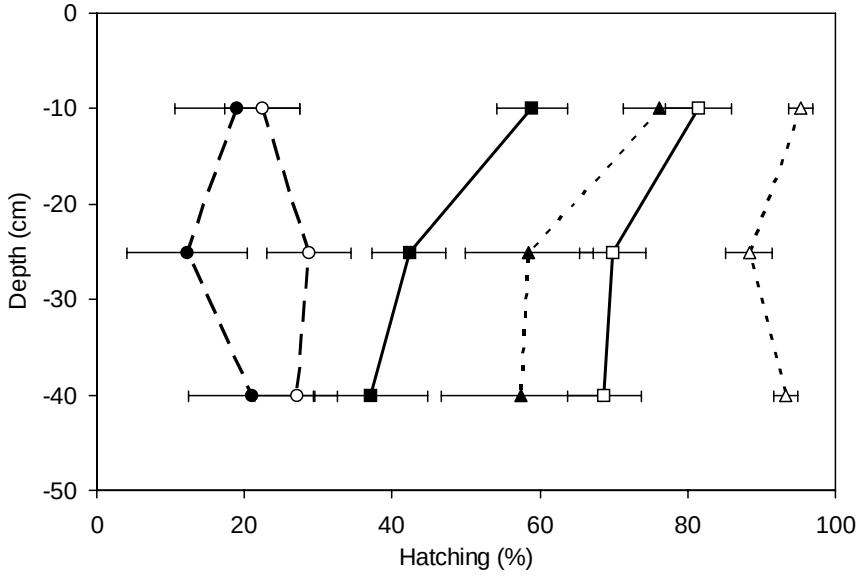

Figure 4. Hatching profiles over depth, measured in nylon bags in Expt 2. Data from day number day 44 (closed symbols) and day number 150 (open symbols) for fallow (broken lines, circles), *S. sisymbriifolium* (solid lines, squares) and potato (dotted lines, triangles). Error bars indicate standard error of the mean.

In the *S. sisymbriifolium* treatments hatching declined with depth on day numbers 44 and 150 (Fig. 4). Hatching ranged from 58% at –10 cm to 37% at –40 cm after 44 days, and from 81% at –10 cm to 69% at –40 cm after 150 days. Similarly, hatching for potato ranged from 76% at –10 cm to 58% at –40 cm after 44 days (Fig. 4). Hatching was as high as 88% to 95% after 150 days. Hatching of the fallow treatment did not decline with depth on both sampling dates. It ranged from 12% to 21% after 44 days and was slightly higher after 150 days, ranging from 22% to29 %.

Hatching as related to crop parameters (Expt 2)

There was a significant linear relation between the percentage of hatching of larvae and eggs from cysts buried in nylon bags and RLD (Fig. 5), with $P < 0.001$ for both potato and *S. sisymbriifolium*. Stepwise regression analysis showed that a model with parallel lines best described the data with $R^2 = 0.62$, a slope of 7.71% (SE 1.14%) hatching per unit of RLD and intercepts of 55.5% (SE 3.52%) hatching for potato and 40.6% (SE 3.6%) hatching for *S. sisymbriifolium*. The difference in intercepts indicates that for any RLD hatching is 14.9% lower for *S. sisymbriifolium* than for potato.

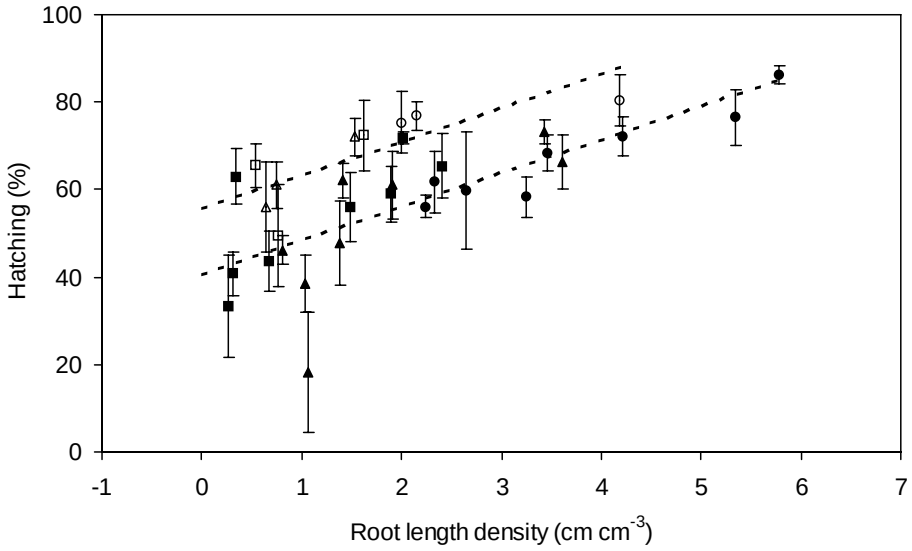

Figure 5. Hatching percentages for one- and two-year-old *Globodera pallida* cysts, buried in nylon bags in *S. sisymbriifolium* crops (closed symbols) and potato cv. Bintje (open symbols) crops as a function of RLD measured in a soil sample taken near each bag. Different symbols indicate measurement depths: circles –10 cm, triangles –25 cm and squares –40 cm. A linear model with parallel lines best fitted the data for *S. sisymbriifolium* and potato; regression equations being $y = 7.7$ (SE 1.14) $x + 40.6$ (SE 3.6) for *S. sisymbriifolium* and $y = 7.7$ (SE 1.14) $x + 55.5$ (SE 3.5), for potato ($P < 0.001$, $R^2 = 0.62$), dotted lines in the graph represent the fitted lines.

Percentage of hatching of larvae and eggs from cysts buried in nylon bags increased with growth duration of potato and *S. sisymbriifolium* crops (Fig. 6). Stepwise regression analysis showed that a model with parallel lines best fitted the data with $P < 0.001$, $R^2 = 0.71$, a slope of 0.26% (SE 0.03%) hatching per day and intercepts of 52.6% (SE 4.1%) hatching for potato cv. 'Bintje' and 36.0% (SE 3.2%) hatching for S. sisymbriifolium. The differences in intercept indicate ca 17% systematically lower hatching at any time during the experimental period for *S. sisymbriifolium* than for potato. Hatch under fallow did not significantly increase with time ($P = 0.35$).

Theoretical time to sanitation below a set final population density of PCN.

Time to sanitation below a final population density of PCN of 1 egg g^{-1} soil after a first year of fallow or non-host crop, or a year with 90 and 150 days of *S. sisymbriifolium* (and in all cases non-host in subsequent years) was 15.3, 13.0 and 10.9 years, respectively (Fig. 7). Logically, this sanitation time decreased with increasing final population density, e.g. to 6.1, 3.9 and 1.8 years at 5 eggs g^{-1} soil.

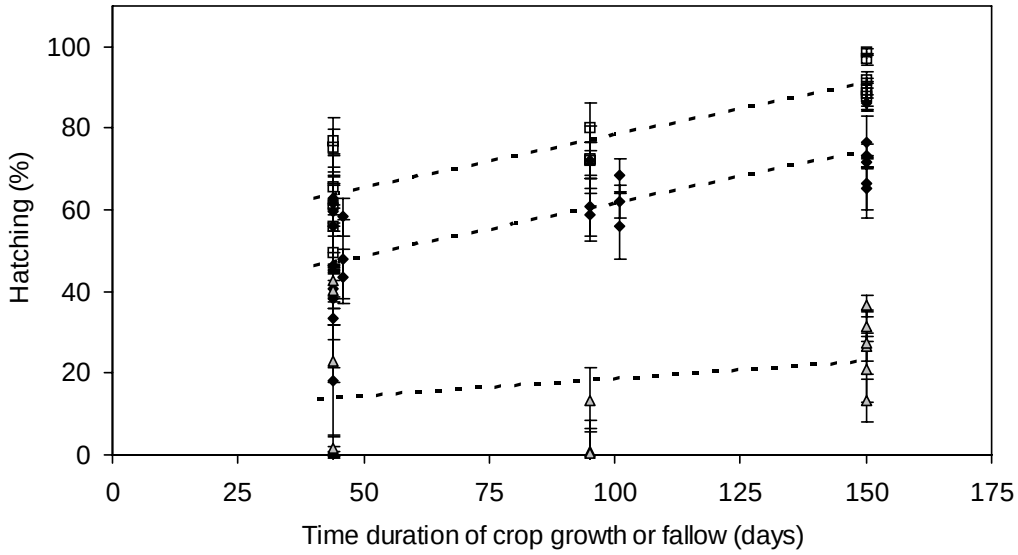

Figure 6. Hatching percentages for one- and two-year-old *Globodera pallida* cysts, buried in nylon bags under fallow (grey symbols), *S. sisymbriifolium* crops (closed symbols) and potato cv. 'Bintje' (open symbols) as a function of the duration of the treatment (growth duration or fallow). A linear model with parallel lines best fitted the data for *S. sisymbriifolium* and potato ($P < 0.001$, $R^2 = 0.72$, y= 0.26 (SE 0.03) x + 36.0 (SE 3.2) for *S. sisymbriifolium* and for potato y= 0.26 (SE 0.03) x + 52.6 (SE 4.1)), indicated as dotted lines in the graph. Linear regression on the control (fallow) treatment was not significant ($P = 0.35$).

The difference in time duration to sanitation between fallow and *S. sisymbriifolium* was independent of initial and final population density (Eqn 7), and ranged from 2.3 years for 59% hatching, equivalent to 90 days of growth of *S. sisymbriifolium* and 4.4 years for 75% hatching, equivalent to 150 days of growth of *S. sisymbriifolium*.

Discussion

The hatching of nematodes by *S. sisymbriifolium* was seriously underestimated with the sand core method, because the RLD in infected sand cores was systematically lower than the RLD in the same soil layer outside the infected sand cores (Fig. 2). Reduced root proliferation in sand cores was not caused by the presence of PCN, as in the vicinity of the nylon bags no reduction in root proliferation was observed. Furthermore, evidence is present that PCN cause increased root growth of host plants (De Ruijter and Haverkort, 1999).

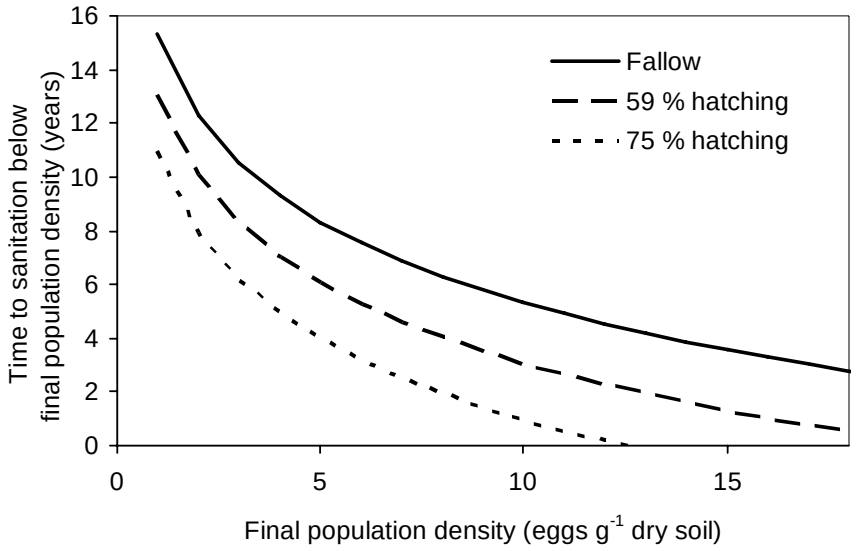

Figure 7. Time to sanitation below a set final PCN population density, plotted against this set final population density, for an initial PCN infestation density of 50 eggs g^{-1} dry soil. The solid line represents fallow or non-host crop in all years. The broken line represents the scenario with 90 days of growth of *S. sisymbriifolium* in the first year (equivalent to 59% hatching) and fallow or non-hosts in subsequent years; the dotted line represent the comparable treatment with a growth period of 150 days of *S. sisymbriifolium* (equivalent to 75% hatching) in the first year followed by non-host or fallow in subsequent years.

The reduced root proliferation in the sand cores, as compared to bulk soil, could be due to slight textural differences. We expected that these textural differences would be unimportant when planning the experiments. Apparently the transition of one soil type into another at the fringe of the infected sand cores presented an obstacle to root penetration. In this chapter the evidence was presented to demonstrate how small differences in experimental setup can have large unexpected effects. Had observations with another independent method not been available, this could easily have led to wrong conclusions on the hatch stimulation of *S. sisymbriifolium*. Questions can arise whether measuring hatching of cysts in nylon bags is a reliable method. Evidence collected on studies of weed seed longevity in nylon bags has shown that density dependent processes occurred (Van Mourik *et al.*, 2005). The responsible mechanism (fungal consumption of the seeds) is not expected to play a role in the current measurements of hatching. Nevertheless, to prevent other, possibly occurring density dependent processes, the size of the nylon bags used in the current study was relatively large (4 – 5 cm) allowing the 50 cysts inside each bag to be spread. In general, hatching studies should always be performed using proper blank controls and preferably using two different methods, e.g. cores and nylon bags. Since hatch stimulation of potato is invariably high, potato should be included in hatching studies to provide a check on viability and behaviour of cysts.

Preceding studies (Scholte 2000b, c; Scholte and Vos, 2000) collected a lot of evidence on the sanitating effect of *S. sisymbriifolium* on PCN infections. However, recently evidence found in another study (Hartsema *et al*., 2005) reported variable results with *S. sisymbriifolium* in practice; across experiments, average hatching percentages ranged from 0 to almost 90%. The study was carried out on farmers' fields, and for practical reasons it was not possible to include susceptible potato or fallow treatments in all experiments. Therefore, the interpretation of the variable results remains difficult. A newly designed field study with accurate experimental design, crop cultivation including fallow and susceptible potato as controls, and detailed measurements of root development and environmental conditions should be carried out. It could help to understand whether the variating results originated from differences in experimental designs applied (e.g. different soil types, nematode populations), from methodological pitfalls as described above or from differing environmental conditions (e.g. soil moisture contents or microorganisms present in soils).

The current results (Fig. 5) suggest almost complete sanitation if the duration of cultivation of *S. sisymbriifolium* plants was prolonged (by extrapolation 208 – 227 days would be needed to reach 90 – 95% hatching). However, in practice crop growth periods are limited (Chapter 3). Furthermore, the initial study in pots on hatch

stimulation of *S. sisymbriifolium* (Scholte, 2000c) showed strong levelling off of hatching stimulation in the period between 11 and 14 weeks after sowing, both for potato (achieving ca 90% reduction of PCN juveniles) and *S. sisymbriifolium* (achieving ca 80% reduction). These observations raised hope that a crop of *S. sisymbriifolium* would need to be present in the field only a limited period of time to achieve a high degree of sanitation. If only part of a growing season would need to be devoted to trap crop cultivation this would provide options for commercial crops being grown before or after *S. sisymbriifolium* in the same season. However, Scholte (2000c) made the observation in a greenhouse study with five litre pots, which became crowded with roots more rapidly, resulting in small distances between roots and cysts at an earlier stage, and probably also resulting in high concentrations of hatch stimulants. In the current study plants were grown in large containers, allowing a more natural development of root architecture than in pots. In field studies (Chapter 4) maximum RLD values of 2 – 3 cm cm^{-3} were observed in the top 10 cm soil layer. In the present study the range of RLD values (X-axis of Fig. 2) extended to higher values than is common under field conditions. Therefore, though more representative than pot experiments, caution is needed when extrapolating the current results to field conditions. Yet, an important inference is that, also in the field, the maximum sanitation effect of *S. sisymbrifolium* can be expected if growth duration is maximized, and not restricted to 14 weeks as thought initially. As noted by Scholte and Vos (2000) hatchability of cysts remained the same throughout the spring and summer season and seasonal change in hatchability is not a factor that needs be taken into account.

Reductions in cyst content were correlated with RLD in the soil around the cysts (Fig. 4). An explanation from this correlation could be that the higher the RLD in a particular volume of soil, the higher the amount or concentration of hatching factors and the higher the chance of triggering juveniles to hatch. However, the following remark needs to be made. Root sampling was made with 15 cm long cores. Given the vertical gradients in RLD in the field (Chapter 4), and the variation inherent to RLD, the association between RLD and hatching (Fig. 4) could be refined if many more small samples were taken near the nylon bags. Such very labour intensive, destructive sampling would require a much larger number of containers and nylon bags per container. In any particular volume of soil, RLD increases with time to some maximum density, followed by decline in RLD (Chapter 4). Hence, if a volume of soil A has a higher RLD than a volume B this most likely means that roots were present also for a longer duration in volume A. In other words, effects of growth duration and RLD are to some extent confounded. The present experimental setup did not allow disentangling these effects.

Unlike the differences in the temporal pattern in hatch stimulation (Fig. 5), the systematic difference in hatch stimulation for any given RLD between *S. sisymbriifolium* and potato (Fig. 4) seems to point at an inherent difference in hatch stimulation between the two plant species. Other studies showed evidence for the same phenomenon (Hartsema *et al*., 2005; Scholte, 2000c) Probably the analysis of differences in the flux of exudates and exudate composition in terms of hatch stimulating and hatch suppressing compounds could shed more light on differences between these plant species.

In the current study, *S. sisymbriifolium* was successful in reducing the population densities of *G. pallida* resident in nylon bags under the crop. Averaged over the soil profile, the hatching percentages ranged from 47% after 44 days of crop growth to 75% after 150 days of crop growth. Similar reductions of potato cyst nematodes by *S. sisymbriifolium* were reported by Scholte and Vos (2000) (52 – 77%) and on average by Hartsema *et al*. (2005) (52%). Such levels of reduction in *G. pallida* cyst content are comparable to or even exceed those of fumigants such as 1,3-dichloropropene, which ranged between 48% and 72% as reported by Been and Schomaker (1999). The advantages of *S. sisymbriifolium* over fumigants are that the ecological balance in the soil is not disturbed and its roots colonize the soil to a greater depth than fumigants penetrate. If a very intensive sampling method is used, the calculated time duration to sanitation is surprisingly high (ranging from 15 years for natural decay without control measures to still 11 years when one single control treatment is applied, resulting in 75% hatch stimulation, e.g. growing *S. sisymbriifolium* for 150 days). If effects of *S. sisymbriifolium* in the field are comparable to those in the current experiments, the difference in time to sanitation between fallow or non-host and *S. sisymbriifolium* ranges from 2.3 to 4.4 years for 90 – 150 days of trap crop cultivation (59 – 75% hatching, respectively), and is independent of initial or final population density. The implication of this independency is that the timing of growing the trap crop is unimportant for its effectiveness.

The method presented in this chapter to estimate the time gain to sanitation is a general one and can be used to compare effects of all kinds of control measures and cropping scenarios, provided valid estimates of decay coefficients are available.

CHAPTER 6

Effect of population density of *Globodera pallida* (Stone) on growth of *Solanum sisymbriifolium* (Lam.)

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Summary

Solanum sisymbriifolium (Lamarck) is a resistant trap crop for potato cyst nematodes (*Globodera* spp.). This chapter reports on a study in which the tolerance of the plant was evaluated for a range of inoculum densities (0 – 578 nematodes g^{-1} dry soil). Pre-soaked *S. sisymbriifolium* seeds were sown in 3 l pots, containing artificial soil. Plant height was monitored over time and plant dry weight was measured after a growth period of 103 days in the greenhouse. Two models were used to describe the relations of *S. sisymbriifolium* plant height or dry weight with nematode inoculation density, i.e. an exponential model and a hyperbolic model. At the two highest inoculation densities (288 and 578 nematodes g^{-1} soil) above-ground dry matter of *S. sisymbriifolium*, measured at day 103, was significantly lower than in the uninfected control. The exponential model revealed a tolerance limit, below which no reduction in performance of *S. sisymbriifolium* was present at all, of 24 and 36 nematodes g^{-1} dry soil for dry matter production and maximum plant height, respectively. Compared to potato varieties tested in previous experiments, this tolerance limit is extremely high. Also the hyperbolic model revealed very high tolerance of *S. sisymbriifolium* to potato cyst nematodes, showing that at 369 and 1120 nematodes g^{-1} dry soil above-ground dry weight and maximum plant height were halved, respectively. Both models adequately described the measured response. In principle both models can help to assess the risk of plant damage in field foci, but it is concluded that the tolerance of *S. sisymbriifolium* is so high that extensive plant damage is not likely to occur under current agricultural conditions.

Key words: Tolerance, trap crop, potato cyst nematodes, Model, exponential, hyperbolic, growth, plant height.

Introduction

Potato cyst nematodes and lately especially *Globodera pallida* (Stone) are a severe threat in many potato production areas (Ryan *et al.*, 2000; Minnis *et al.*, 2002). The nematodes inhabit the soil of an infected field, waiting until host plants, e.g. potato plants, are grown. Then, hatching agents present in root exudates of the potato plants trigger the nematodes to hatch from their eggs, after which the nematodes invade the roots of the potato plants (Fenwick, 1952; Clarke and Shepherd, 1968; Jones *et al.*, 1998; Devine *et al.*, 1999). Inside the roots, the nematodes induce the formation of a syncytium (Jones and Northcote, 1972), a giant transfer cell from which they extract their needs. Invasion and feeding can have strong influences on potato plants. De Ruijter and Haverkort (1999) and Van Oijen *et al.* (1995) report lower leaf nutrient concentrations in infected plants, accelerated leaf senescence, and slower root and overall growth. Mulder *et al.* (1997) found a correlation between infection density and decrease in potato growth and dry matter production. Seinhorst (1981, 1998) discriminated two mechanisms of growth reduction: the first mechanism operating at all nematode densities and a second mechanism of growth reduction additional to the first mechanism with a noticeable effect only at medium and high nematode densities. The first mechanism causes no visible symptoms except retardation of plant growth and occasionally increases of plant height. The second mechanism reduces water consumption per unit plant weight and the (active) uptake or excretion of K^+ and Na^+ whilst it increases the (passive) uptake of Ca^{2+} and the dry matter content of plants (Been and Schomaker, 1986).

In the late 1990s, *Solanum sisymbriifolium* (Lam.) was introduced in The Netherlands as a promising trap crop for potato cyst nematodes (Scholte, 2000b, c; Scholte and Vos, 2000). Although no progeny cysts of *G. pallida* or *G. rostochiensis* were found on *S. sisymbriifolium*, some effects were seen on the growth of the plants at high nematode densities (Scholte, 2000c). It was known that the roots of *S. sisymbriifolium* can be invaded by potato cyst nematodes and part of the developmental process of the nematodes can occur, which is presumably the cause of the growth reduction (Roberts and Stone, 1983).

A thorough study of plant tolerance and its dependence on nematode density is lacking. Therefore, the objective of this chapter was to quantify the growth reduction or tolerance in terms of dynamics of dry matter accumulation and plant height in relation to a range of *G. pallida* inoculation densities; indications for the manifestation of the two damage mechanisms were deduced from the dry matter concentration of plant material. Models of Seinhorst (1982b, 1998) and Elston *et al.* (1991) were fitted

to the data and could serve as a tool to assess possible growth reductions in actual field situations.

Material and methods

Cysts of *Globodera pallida* population E425 were bought at the Hildebrands Laboratory (Wijster, The Netherlands) in 2002, and multiplied in 1 litre pots in a sand mixture planted with the sensitive potato cv. 'Elkana' in 2002. After multiplication, nematodes were left in the dried sand mixture and stored at 5 °C. The cysts were multiplied a second time in the experimental setup with the sensitive potato cv. 'Bintje' in 2003. After multiplication the nematodes were left in the dried sand mixture and stored at 5 °C, resulting in 1 year old cysts in 2004. In that year, cysts were crushed and rinsed with tap water to prepare a series of inoculum densities (Pi), containing eggs and larvae. The Pi is expressed in nematodes (nem.) g^{-1} dry soil, which includes both the eggs and larvae (although not mentioned explicitly).

Artificial soil was made by mixing humus poor 'silver' sand (BouwCenter 't Kachelhuus Gijs Verschuur b.v., Ede, The Netherlands) with crushed hydro pellets (diameter < 4 mm) and clay powder (Kaolin, WBB Devon Clays Ltd, Newton Abbot, Devon, UK) as 6:1.5:1 on a dry mass basis. Through this artificial soil, 2 g l^{-1} controlled release fertilizer (Scotts Osmocote©, containing 13% N, 5.7% P, 10.8% K, 1.2% Mg and 1% Fe) was mixed. Additionally, during the rest of the experiment once every two weeks 100 ml trace element solution (containing 30 g H_3BO_3, 85 g $MnSO_4 \cdot 4H_2O$, 4.6 g $ZnSO_4 \cdot 4H_2O$, 0.8 g $CuSO_4 \cdot 5H_2O$ and 1.3 g $Na_2MoO_4 \cdot 2H_2O$ per litre) was added per pot. Water was added to the artificial soil resulting in a soil moisture content of 15% of the total volume. Three litre pots (top diameter 19 cm) were filled with soil. Inoculation was done on day 0 of the experiment using ten 5 ml syringes with needles (25 cm length, 2 mm diameter) in each pot: first the needles were inserted in a circle of nine (3 cm distances from pot rim) and one in the middle. Then the 5 ml inoculum was injected, simultaneously pulling the needle slowly upwards, to disperse the inoculum evenly over all depths. After inoculation, exact Pi was measured and amounted to 0, 1.1, 2.2, 4.3, 8.2, 18.5, 36.4, 65.2, 151, 288 and 578 nem. g^{-1} of dry soil. All Pi were applied in 6 replicate pots. Then, on top of each pot a porous plastic foil was placed, reducing water loss by evaporation. Pots were placed in a temperature controlled greenhouse (temperature 20 °C day, 15 °C night, natural day length) and were kept at constant soil moisture content.

On day 0 of the experiment after PCN inoculation, seeds of *S. sisymbriifolium* were placed on moist filter paper at 20 °C, and pre-soaked. On day 4 of the experiment one pre-soaked seed of *S. sisymbriifolium* was sown in each pot. All 66 pots were

randomized and moved around in three groups once a week to prevent systematic effects of different positions in the greenhouse on plant performance.

Measurements of plant height were done on day 24, 33, 41, 47, 54, 63, 75 and 103 of the experiment to follow the time dynamics of plant growth. After at least 14 weeks (which is the minimum time duration used for potato resistance tests), on day 103 all plants were cut at soil surface and above-ground plant biomass was dried at 70 °C to constant weight, to measure total above-ground dry matter.

Data analysis

Dry weight of plants

Two models were used to describe the relation between above-ground dry weight of *S. sisymbriifolium* plants and the inoculation density of *G. pallida*, enabling comparison of tolerance properties of *S. sisymbriifolium* with literature data. The first model is an exponential equation developed by Seinhorst (1982b; 1998):

$$\begin{aligned} Y &= Y_{max} && \text{for } Pi \le T \\ Y &= Y_{max} \times \{(m + (1-m) \times 0.95^{(Pi/T)-1}\} && \text{for } Pi > T \end{aligned} \qquad (1)$$

in which Y is the yield or above-ground dry weight (g plant^{-1}) and Pi is the inoculation density with *G. pallida* (nem. g^{-1} soil). The tolerance limit T (nem. g^{-1} soil) is the density of nematodes below which no effects on the dry weight are noticeable, Y_{max} (g plant^{-1}) is the average dry weight at nematode densities below T and m (g) is the ratio between minimum (Pi=∞) and maximum (Pi=0) dry weight. Secondly, the hyperbolic model of Elston *et al.* (1991) was fitted to the data:

$$Y = Y_{max} \times \{(1 - (1-m) \times Pi/(c + Pi))\} \qquad (2)$$

in which Y is the yield or above-ground dry weight (g plant^{-1}), Pi is the inoculation density of *G. pallida* (nem. g^{-1} soil), Y_{max} is the expected dry weight (g plant^{-1}) at Pi=0, m is the same parameter as in Eqn (1) and parameter c (nem. g^{-1} soil) represents the nematode density at which plant dry weight is half the expected dry weight Y_{max} at Pi=0.

To investigate if besides the first mechanism of growth reduction also the second mechanism, causing mechanical damage, was active, percentage dry matter was calculated from the above-ground fresh and dry plant weights. Significant differences in the percentage of dry matter were tested on a 5% level, and Eqn (1) was fitted to the

data to find the nematode density above which the second mechanism of growth reduction was active.

Plant height

The increase in haulm length or plant height (h) for each nematode density during the growing period was modelled with a logistic model:

$$h = \frac{C}{1 + \exp(-b \times (t - a))} \tag{3}$$

Where t is the time (d), C (cm) is maximum plant height which is reached at large values of t, and b (d^{-1}) is the maximum relative growth rate. The parameter a represents the time (d) when plants reach half their maximum height C.

Next, maximum plant height (C) was related to nematode density Pi with an exponential equation, similar to Eqn (1):

$$\begin{aligned} C &= C_m && \text{for } Pi \le T_c \\ C &= C_m \cdot \{(m_c + (1 - m_c) \cdot 0.95^{(Pi/T_c)-1})\} && \text{for } Pi > T_c \end{aligned} \tag{4}$$

where C_m (cm) is the maximum plant height at Pi= 0; T_c (nem. g^{-1} soil) is the critical nematode density below which plant height did not decrease and m_c (cm) is the minimum plant height at Pi $\rightarrow\infty$.

The maximum plant height (C) was also related to nematode density Pi using an equation similar to Eqn (2):

$$C = C_{max} \cdot \{(1 - (1 - m_c) \cdot Pi/(c_c + Pi))\} \tag{5}$$

in which C_{max} is the expected maximum plant height (cm) at Pi=0, m_c is the same parameter as in Eqn (4) and parameter c_c (nem. g^{-1} soil) represents the nematode density at which maximum plant height is half the expected maximum plant height C_{max} at Pi=0.

The relation between nematode inoculation density (Pi) and the parameter a was described by an exponential function

$$\begin{aligned} a &= a_{min} && \text{for } P \le T_a \\ a &= a_{min} + (1 - z^{(Pi/T_a)-1}) && \text{for } P > T_a \end{aligned} \tag{6}$$

where a_{min} is the minimal value for a, calculated as the average of all values of a for nematode densities smaller than the tolerance limit T_a and *z* is a parameter determining increase of a with nematode density Pi at densities larger than the tolerance limit T_a.

The relation between nematode inoculation density (Pi) and the parameter b was not described by regression. Instead, analysis of variance was performed to investigate if the differences between PCN density treatments were statistically significant.

All statistics (including non-linear regressions) were performed using least squares (in GenStat 8th edition version 8.1.0.155 in and a program written in R2.1.1). Since Eqns (1) and (4) are discontinuous, fitting was done in the following sequence: A first fit was made with the second part of the equations to estimate the starting values of the parameters. Thereafter, Y_{max} and C_{max} (for Eqn (1) and (4), respectively) were calculated as the average of measurements for Pi $\leq T_{estimated}$. Next, a second fit was made using parameter values of Y_{max} and C_{max}, yielding a value for T. If this value deviated from the $T_{estimated}$, a new fit was made with adapted values of Y_{max} and C_{max}. During the iterative regression process this procedure was repeated for adjusted estimates of the parameters.

Results

Dry weight of plants

At Pi=288 and Pi=578 g^{-1} dry soil one of the six plants died in the second and the fourth week after planting, respectively. These plants were excluded from further analysis of the dry weights and plant heights. Dry weight per plant, measured after 103 days, differed significantly from the uninfected controls at the two highest nematode densities (288 and 578 nem. g^{-1} soil, Table 1). The goodness of fit of Eqn (1) and Eqn (2) was almost the same (R^2 was 0.74 and 0.76, respectively) and there was little difference in the values found for Y_{max} (Fig. 1, Table 2). Fitting Eqns (1) and (2) to the data resulted in negative values for m, which is unrealistic and both were adjusted to m=0. For Eqn (1) a tolerance limit T of 24 (SE 4.9) nem. g^{-1} dry soil was found, below which no effect of the nematodes on dry weight was observed. For Eqn (2) a value for c of 369 (SE 118) nem. g^{-1} dry soil was found, representing the nematode density at which the above-ground dry weight was half that of plants at Pi=0.

Percentage dry matter of the plants varied from 25.5 (standard error, SE 0.5) at Pi=1.1 nem. g^{-1} dry soil) to 21.6 (SE 0.7) at Pi=578 nem. g^{-1} dry soil (Fig. 2). Analysis of variance (data not shown) revealed that differences were on the border of significance (P=0.05). Regression analysis was done with Eqn (1) and a tolerance limit T of 20 (SE 16) nem. g^{-1} dry soil was found, below which no differences in dry matter percentage were observed.

Table 1. Average above-ground dry weight for all eleven inoculation densities measured on day 103 of the experiment. Significant differences were tested on a 5% level.

Inoculation density (nem. g^{-1} soil)	0	1.1	2.2	4.3	8.2	18.5	36.4	65.2	151	288	578
Above-ground dry weight (g)	19.8 (cde)	23.6 (de)	21.4 (de)	22.5 (de)	25.4 (e)	20.8 (de)	19.6 (cde)	14.2 (bc)	19.0 (cd)	11.8 (b)	5.5 (a)

Table 2. The results of the non-linear regression analysis using Eqns (1) and (2) to describe above-ground dry matter data measured 103 days after start of the experiment in relation to Pi. Standard error is indicated in brackets.

Model	Parameters				
	Y_{max} (g)	T (nem. g^{-1} soil)	m (g)	c (nem. g^{-1} soil)	R^2
Eqn (1)	22.26 (0.83)	24 (4.9)	0	-	0.74
Eqn (2)	22.23 (1.1)	-	0	369 (118)	0.76

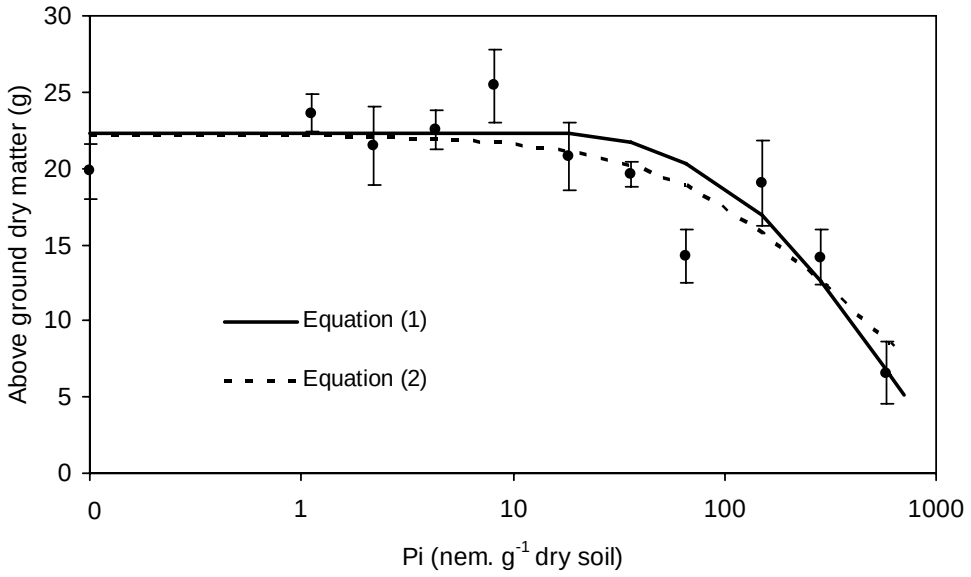

Figure 1. Above-ground dry weight of *S. sisymbriifolium* harvested 103 days after start of the experiment as a function of inoculation density of *G. pallida*. Solid and dotted lines represent non-linear models, i.e. Eqn (1) (Seinhorst, 1982b; 1998) and Eqn (2) (Elston *et al.*, 1991). Vertical bars represent standard error of each data point.

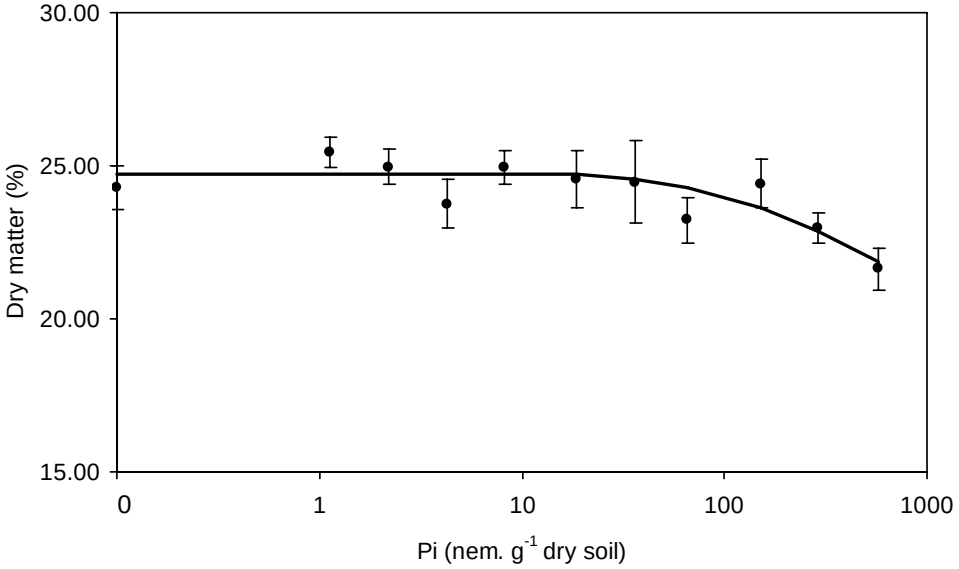

Figure 2. Dry matter percentage as a function of nematode inoculation density (Pi). Vertical bars represent standard error. Analysis of variance revealed that differences were on the border of significance (P= 0.05). The solid line is the result of nonlinear regression with Eqn (1), which yielded a value for T of 20 (SE 16) nem. g^{-1} dry soil, above which the second mechanism of damage was active.

Plant height

The logistic model (Eqn 3) fitted well to the data (Fig. 3): for all nematode densities the R^2 values were higher than 0.98. Parameter C in the logistic model, being the estimated maximum plant height at $t \rightarrow \infty$, had a significant relation with nematode density (Fig. 4). Both Eqn (4) and (5) described this relation well (both had R^2 values of 0.84, Table 3). Eqn (4) revealed a tolerance limit T_c of 36 nem. g^{-1} dry soil, below which no effects on maximum plant height was observed. Fitting Eqn (5) yielded a value of 1120 nem. g^{-1} dry soil for parameter c, representing the nematode density at which maximum plant height was decreased by a factor two. The minimum plant height m_c at $Pi \rightarrow \infty$ was estimated to be zero. Parameter a in the logistic model (Eqn 3), representing the time that plant height was half the maximum plant height, significantly differed for different Pi. Eqn (6) described the relation well (R^2 value of 0.88) (Fig. 5, Table 4) and yielded a tolerance limit of 29 nem. g^{-1} dry soil, below which no increase in parameter a was observed. Parameter b in the logistic model (Eqn 3), being the maximum relative growth rate, ranged between 0.071 and 0.092 d^{-1} for the different Pi, and differences were not significant (Table 4).

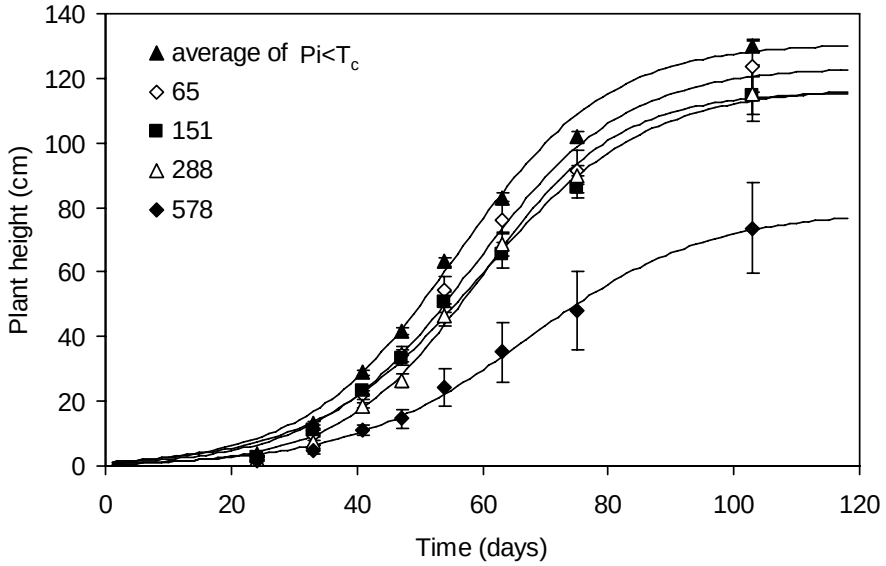

Figure 3. Plant height of *S. sisymbriifolium* plants measured over time since day 0 of the experiment for the different nematode inoculation densities. Vertical bars represent standard error of each data point. Lines indicate fits of Equation (3): for Pi < 36 nem. g^{-1} dry soil (=T_c) and for Pi= 65, 151, 288 and 578 nem. g^{-1} dry soil.

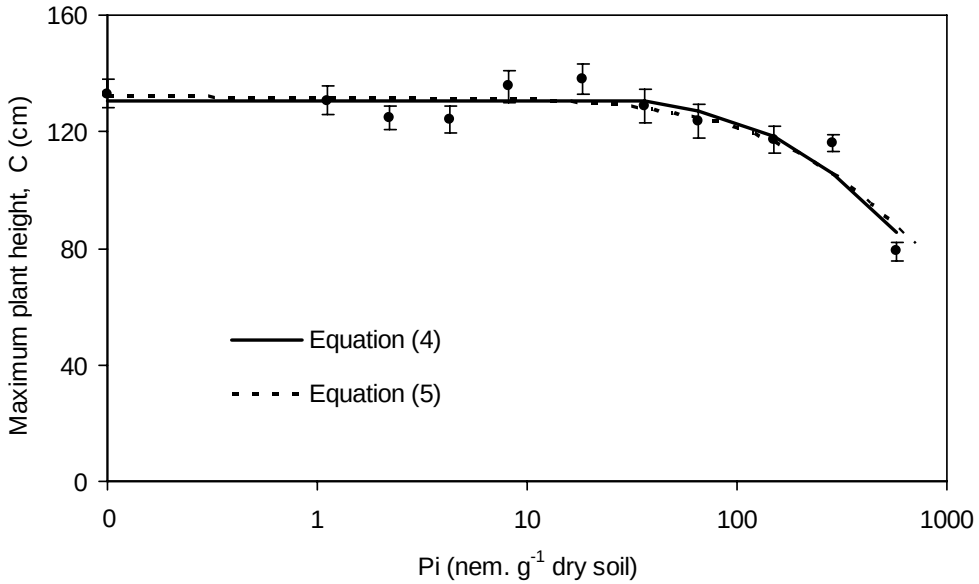

Figure 4. Maximum plant height (parameter C, Eqn 3) in relation to nematode inoculation density (Pi). The solid line and the dotted lines represent Eqn (4) (Seinhorst, 1982b; 1998) and Eqn (5) (Elston *et al.*, 1991), respectively. Vertical bars represent standard error of each data point.

Table 3. The results of the non-linear regression analysis using Eqns (4) and (5) to describe maximum plant height C (Eqn 3) in relation to Pi. Standard error is indicated in brackets.

	Parameters				
Model	C_{max} (cm)	T_c (nem. g^{-1} soil)	m_c (g)	c_c (nem. g^{-1} soil)	R^2
Eqn (4)	130.8 (2.0)	36	0.36	-	0.84
Eqn (5)	132.2 (2.5)	-	0	1120 (212)	0.84

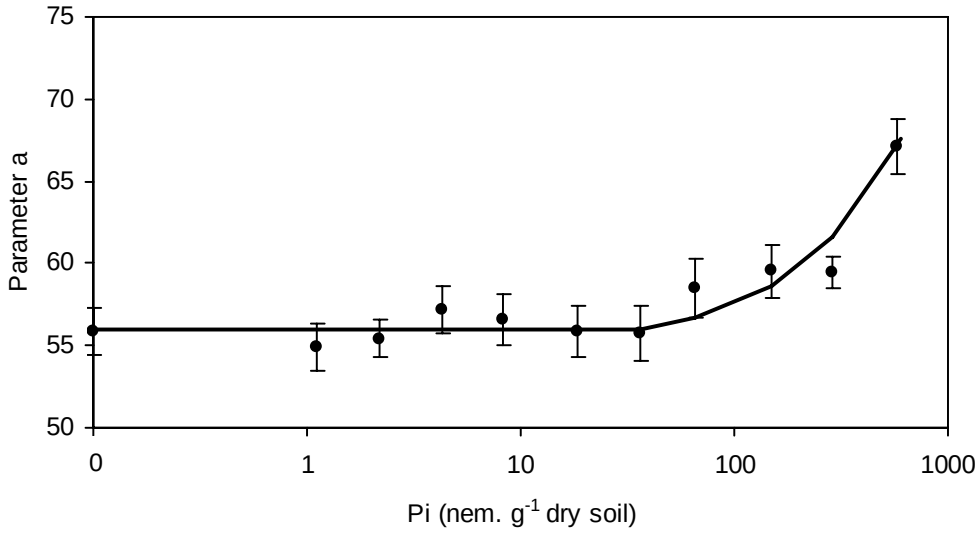

Figure 5. Parameter a (Eqn 3) in relation to nematode inoculation density (Pi). Solid line results from non-linear regression using Eqn (6). Vertical bars represent standard error of each data point.

Table 4. The results of the non-linear regression analysis using Eqn (6) to describe parameter a (Eqn 3), representing the time at which half the maximum plant height is reached, in relation to Pi. Standard error is indicated in brackets.

	Parameters			
Model	a_{min} (d)	T_a (nem. g^{-1} soil)	z (-)	R^2
Eqn (6)	55.92 (0.28)	29	0.99	0.88

Discussion

Eqns (1) and (4) revealed tolerance limits T of 24 nem. g^{-1} dry soil for dry matter accumulation and of 36 nem. g^{-1} dry soil for estimated maximum plant height. These equations were fitted on 35 other nematode – plant combinations (Seinhorst and Van den Ouden, 1971; Seinhorst, 1998) including potato cyst nematodes and potato cultivars, but the values found for T in the literature varied between 0.05 and 6 nem. g^{-1} soil (the highest estimate was found for plant weight of potato cv. Multa 12 weeks after planting). Also, the estimate of parameter c of the model of Elston *et al.* (1991) (Eqns 2 and 5), which was 369 (SE 118) nem. g^{-1} dry soil for dry matter accumulation and 1120 (SE 212) nem. g^{-1} dry soil for estimated maximum plant height, indicates high tolerance compared to that of most potato cultivars. In comparison, the most tolerant potato genotype (genotype '12243') in the experiments of Elston *et al.* (1991) had an estimate of 398 (SE 88) for c. Evidence from former experiments (Schomaker *et al.*, 1995) indicated that the tolerance limit T varies little between experiments, whether they are done in the greenhouse or in experimental fields and it can be concluded that *S. sisymbriifolium* is very tolerant for potato cyst nematodes. The minimum yield m, on the other hand, is sensitive to external conditions and varies strongly between experiments. The small values for m found in the current greenhouse experiment do not necessarily mean that in other experiments, especially field experiments, minimum yields may not be higher. To estimate the minimum yield and its variance, field experiments are needed at a sufficient range of nematode densities.

The tolerance limit of 29 nem. g^{-1} of soil for the time a when plants reach half their maximum height supports the suggestion of high tolerance of *S. sisymbriifolium* for potato cyst nematodes. The fact that dry matter content does not increase but rather decreases means that the second mechanism of growth reduction is not manifest and that plants are not mechanically damaged. Dry matter content at the highest densities is probably/perhaps decreased because plants are delayed in development. Therefore, we can conclude that *S. sisymbriifolium* is very tolerant to the second mechanism of growth reduction and therefore to mechanical damage caused by potato cyst nematodes.

The importance of the current findings for practice become clearer when our inoculum densities are compared with nematode densities found in actual field infestations. Schomaker and Been (1999) report average maximum population densities of *G. rostochiensis* and *G. pallida* on susceptible potato cultivars of 150 and 300 nem. g^{-1} of soil, respectively, which may be smaller in potato production areas on heavy clay soils (personal communication, ir. L.P.G. Molendijk, PPO-AGV). In the starch potato area, where mostly resistant cultivars are grown, these densities are never

reached. In areas where seed and ware potatoes are grown, infestations with potato cyst nematodes often occurs in foci with high densities only in the centre of the focus. Percentage growth reduction is negligible in these infestations. For scenarios with extremely high infestation densities of potato cyst nematodes, the currently deduced relations could be used to predict the size of effects on the growth of *S. sisymbriifolium*, but more information is needed about parameter values - also on population dynamics - under field conditions. However, for estimates of damage in practice, precise information on the local population density is needed, which requires extensive sampling or modelling of the infestation foci as was done by Schomaker and Been (1999).

CHAPTER 7

How important is *Solanum sisymbriifolium* as a host for *Phytophthora infestans*?

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Summary

Two experiments were carried out to study susceptibility of *Solanum sisymbriifolium* (Lam.) to potato late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary. In a laboratory experiment, detached leaves of *S. sisymbriifolium* and of *Solanum tuberosum* (L.) cv. Bintje were inoculated with two isolates (90128, IPO428-2), to compare infection efficiency and increase in lesion radius. In a field experiment plants of *S. sisymbriifolium* and of *S. tuberosum* cv. Bintje and cv. Désirée were inoculated with isolate WAU01001 originally collected on a *S. sisymbriifolium* field crop, to compare leaf area loss. Results showed that *S. sisymbriifolium* is fairly resistant to the *P. infestans* isolates used. Infection efficiencies ranged from 20 – 36%, compared with 100% for potato cv. Bintje. If infection occurred, the lesions hardly increased in radius. In the field, potato plants had lost 100% of their leaf area 28 – 42 (cv. Bintje) and 56 (cv. Désirée) days after inoculation, respectively. *S. sisymbriifolium* plants had lost around 9 – 13% of their leaf area after 42 days. Relative area under the disease progress curve was 0.80 for potato cv. Bintje, 0.71 for potato cv. Désirée, and ranged from 0.08 to 0.11 for the examined *S. sisymbriifolium* cultivars.

In conclusion, the susceptibility of *S. sisymbriifolium* to *P. infestans* is very small, and the risk that *S. sisymbriifolium* could act as a source of *P. infestans* for potato crops or the risk of the development of virulent isolates can be managed through a correct timing of crop destruction.

Key words: Potato late blight, *Solanum sisymbriifolium*, host, trap crop, infection efficiency, area under the disease progress curve, resistance.

Introduction

Phytophthora infestans (Mont.) de Bary, causing potato late blight, is considered to be the most important disease of the potato crop (Drenth *et al.*, 1993; Hijmans *et al.*, 2000; Flier *et al.*, 2003b). Already present in Europe shortly after introduction of potato itself (Andrivon, 1996), it constantly adapts to new resistant potato varieties and to new fungicides (Fry and Smart, 1999; Grünwald *et al.*, 2002). Lately, mating type A2, that was restricted to Mexico until the 1980s (Spielman *et al.*, 1991), appeared in Western Europe and sexual reproduction of *P. infestans* mating types A1 and A2 is possible in West European fields (Drenth *et al.*, 1993). The resulting oospore formation is presumably leading to more rapid adaptation of the oomycete to new resistant potato varieties.

In the late 1990s, *Solanum sisymbriifolium* (Lam.) was selected after screening of some hundred non-tuber bearing Solanaceae species as the most promising candidate trap crop for potato cyst nematodes (Scholte 2000b, c; Scholte and Vos 2000). Breeding companies became interested in the crop and in the years 2003 – 2005 a few hundreds of hectares of *S. sisymbriifolium* were grown on farms in The Netherlands.

Flier e*t al.* (2003b) showed that *S. sisymbriifolium* can be infected with *P. infestans*, and this raised serious concern and led to the current study. In these experiments we wanted to extend investigations on the degree of susceptibility of *S. sisymbriifolium* to *P. infestans.* The first question concerns the chance on infection of *S. sisymbriifolium*. Therefore, the infection efficiency of *P. infestans* on *S. sisymbriifolium* was compared with that on *Solanum tuberosum* (L.) cv. Bintje. The second question concerned the lesion growth rate on *S. sisymbriifolium* compared to the rate on potato cv. Bintje. Thirdly, the progress of the disease in field stands of *S. sisymbriifolium* was compared with that of potato cv. Bintje and cv. Désirée.

Materials and methods

Experiment 1: Laboratory test

Two *P. infestans* isolates (90128 and IPO428-2) were used (Table 1), of which the second was known to be virulent to *S. sisymbriifolium* (Flier *et al.*, 2003b). Isolates were stored in liquid nitrogen storage facilities until a week before inoculation. Isolate 90128, kindly supplied by V.G.A.A. Vleeshouwers (Plant Research International, Wageningen UR, Wageningen) was plated on rye agar medium supplemented with 20 g l^{-1} sucrose and incubated at 18 °C in the dark. After one week the plates were covered with sporulating mycelium. Cold water (4 °C) was added and the resulting sporangial suspension was pipetted into a test tube. Isolate IPO428-2, kindly supplied

by G.B.M. Van den Bosch (Plant Research International, Wageningen UR, Wageningen) was sub-cultured on tuber slices of potato cv. Bintje until abundant sporulation occurred. Cold water (4 °C) was added and resulting sporangial suspension was pipetted into a test tube. For both isolates, sporangia were counted and used to estimate and adjust resulting zoospore concentration to 5×10^4 zoospores ml^{-1}. Sporangial suspensions were then incubated at 4 °C for 3 hours to allow zoospore formation, after which remaining sporangia were not removed.

Leaves of *S. sisymbriifolium* and susceptible potato cv. Bintje were collected from plants grown in a greenhouse in August and September 2001, at a temperature regime of 15 °C at night and 25 °C during the day and 12 hour day length. Full-grown leaves were detached on the day of inoculation and placed on moist filter paper in plastic trays, with their abaxial side upwards. Leaves were inoculated placing four drops of 10 µl inoculum (containing 5×10^4 zoospores ml^{-1}) on the abaxial side of each leaf. Each drop was placed on a different leaflet of the leaves, to have sufficient uninfected space around each droplet to allow monitoring of lesion growth. The terminal leaflet was never used.

Ten trays containing each two leaves of *S. sisymbriifolium* and 5 trays containing each two leaves of potato cv. Bintje were inoculated per isolate. Additionally, one tray containing two leaves of potato cv. Bintje were inoculated with four 10 µl drops of tap water and served as a control. Each plastic tray was placed inside a closed transparent plastic bag to prevent leaves from drying. Trays were placed on randomised positions in a growth chamber at a constant temperature of 20 °C and 12 hours day length using artificial lights. To prevent too high light intensities, trays were covered with a shade cloth.

On the 3rd, 4th, 5th, 6th, 7th and 10th days after inoculation the following measurements were made: firstly, infection efficiency was measured counting the number of drops resulting in an infection. Every visible change of colour at the location of a drop of inoculum was counted as an infection. Secondly, the maximal diameter of all lesions was measured and considered as length. Perpendicular to this length the width was measured and the ellipse area ($A = ¼ \times \pi \times$ length $\times$ width) was calculated. This area was square root transformed to obtain the lesion radius (Vleeshouwers *et al.*, 1999).

Experiment 2: Field inoculation

Preliminary information from Experiment 1 suggested the isolates were not very virulent on *S. sisymbriifolium*. Therefore the *P. infestans* isolate WAU01001 (Table 1), collected on a *S. sisymbriifolium* crop in Wageningen in the autumn 2001, was used for this second experiment. The isolate was stored in liquid nitrogen storage facilities

until a week before inoculation. Prior to the experiment, the isolate was used to infect leaves of several potato varieties in the lab and appeared very virulent (W.G. Flier, personal communication, 2001). One week before inoculation, isolate WAU01001 was sub-cultured on detached leaves of potato cv. Bintje until abundant sporulation occurred. Further preparation of the inoculum was done as in Experiment 1. Estimated zoospore concentration was (1.7×10^4 zoospores ml^{-1}).

Four accessions of *S. sisymbriifolium* were used: the original plant material used by Scholte (2000b, c) and Scholte and Vos (2000) multiplied by harvesting seeds in 2001, and cultivars Sharp, Domino and Pion provided by VanDijke Semo BV, Scheemda, Groningen, The Netherlands. Controls consisted of the highly susceptible potato cv. Bintje and the less susceptible potato cv. Désirée. The experimental setup was a randomised complete block design with four blocks each containing two plants of all plant species and cultivars. *S. sisymbriifolium* plants were grown in 50 cc pots for around 4 weeks and then transplanted into a field near Wageningen, The Netherlands. After transplanting the plants were grown for another 4 weeks before potato plants were placed in the field. Potato plants were grown in 5 litre pots for two months in a greenhouse. Potato plants were not grown in the field directly to avoid untimely and uncontrolled infection. Pots were placed in plots in between the *S. sisymbriifolium* plants one week before inoculation. Interplant distance was 50 cm after the potato plants were placed in the field.

Plots were sprayed with 7 ml m^{-2} of the inoculum on 23 July 2002, at 20:30 p.m. Weather conditions during the inoculation were cloudy, moist (light drizzle), and temperatures that day had been between 15.0 °C and 20.3 °C. Directly after inoculation, the plots were covered with a single sheet of transparent plastic foil for one night. During the rest of the experimental period, conditions favouring the spread of the disease were created by sprinkler irrigating the plots, twice a day, at 9:00 a.m. and at 20:30 p.m.

Table 1. Description of the *Phytophthora infestans* isolates used in this study.

Isolate	Race	Host	Year of collection	Origin	Mating type
IPO428-2	1.2.3.4.5.6.7.8.9.10.11	*S. tuberosum*	1992	Ede, The Netherlands	A2
90128	1.3.4.6.7.8.10.11	*S. tuberosum*	1990	Geldrop, The Netherlands	A2
WAU01001	1.2.3.7.8.11	*S. sisymbriifolium*	2001	Wageningen, The Netherlands	A1

The percentage of the total leaf area of the plants that was infected or lost was estimated 14, 21, 28, 42 and 56 days after inoculation. Resistance of the plants was also characterised by calculating the relative area under the disease progress curves (AUDPC) according to Shaner and Finney (1977) and Fry (1978).

GenStat software (eighth edition, version 8.1.0.155, VSN International Ltd) was used for all statistics.

Results

Experiment 1: Infection efficiency

All inoculum droplets placed on potato cv. Bintje of both isolates (90128 and IPO 428-2) led to a successful infection with *P. infestans* (Figs 1a, b), i.e. an infection efficiency of 100%. The isolates showed lower infection efficiency on *S. sisymbriifolium*: inoculation with 90128 resulted in 36.3% of all cases in an infection and inoculation with IPO428-2 in 20%. Additional visual observations were that infection on *S. sisymbriifolium* leaves took the form of a number of very small dark spots (< 1 mm) close to each other, on the place of inoculation which resembles a hypersensitivity reaction. Such a reaction was always counted as an infection. Droplets placed on leaves of *S. sisymbriifolium* dispersed over the leaf surface after a few (15-30) seconds, which did not happen on leaves of potato.

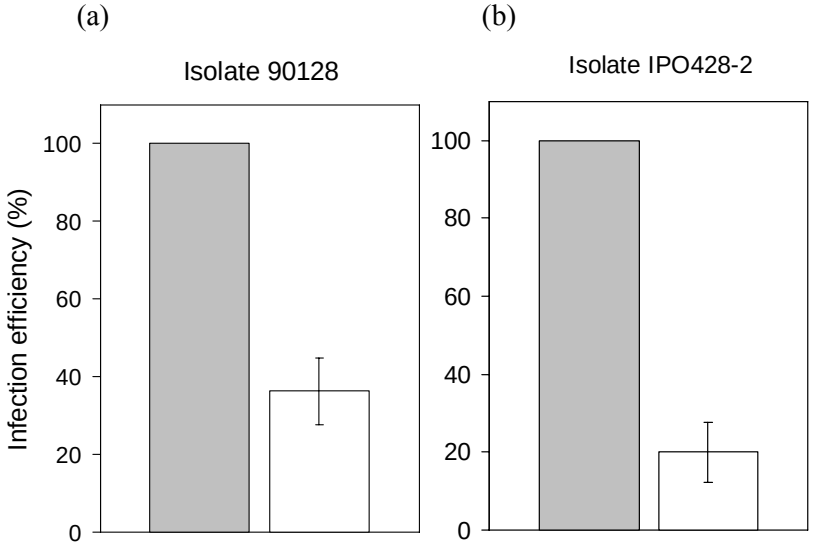


Figure 1. The infection efficiency of *Phytophthora infestans* (the percentage of drops placed on the abaxial side showing symptoms of infection) on leaves of potato cv. Bintje (grey) and *S. sisymbriifolium* (white), for isolate 90128 (a) and for isolate IPO428-2 (b). Data from Expt 1; Bars indicate standard errors of the mean.

Lesion radius and growth

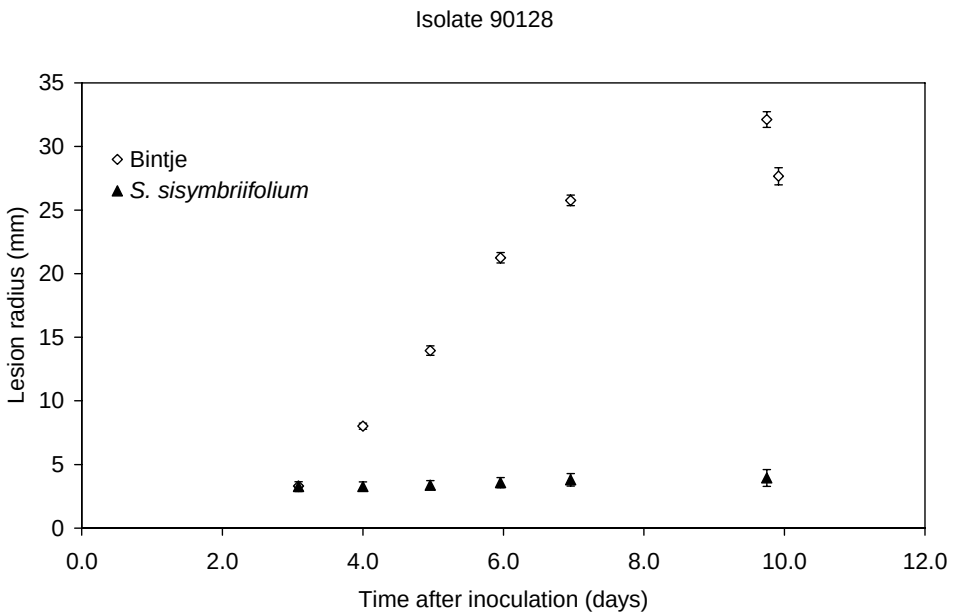
For both isolates (90128 and IPO428-2), lesion radii on leaves of potato cv. Bintje were larger and faster growing than those on *S. sisymbriifolium* leaves (Figs 2a, b). On potato leaves radii ranged from 3.3 mm 3.1 days after inoculation to 32.1 mm 9.8 days after inoculation for isolate 90128, and from 2.7 mm 3.1 days after inoculation to 28.8 mm 9.8 days after inoculation for isolate IPO428-2. For both isolates, lesion radii increased significantly ($P<0.001$) in time. Lesion growth rates were 6.0 (standard error, SE, 0.2) mm d^{-1} for isolate 90128 and 4.8 (SE 0.3) mm d^{-1} for isolate IPO428-2 between days 3 – 7 after inoculation. Measurements of lesion radii on day 10 after inoculation were excluded from the regression analysis because at that time lesion growth was limited by the size of the leaflets.

On *S. sisymbriifolium* leaves lesion radii for isolate 90128 ranged from 3.3 mm 3.1 days after inoculation to 3.9 mm 9.8 days after inoculation, showing a small but significant increase in lesion radius with time ($P = 0.002$). Regression resulted in a lesion growth rate of 0.12 (SE 0.02) mm d^{-1}. For isolate IPO428-2, the lesions on leaves of *S. sisymbriifolium* were larger, ranging from 4.6 mm 4 days after inoculation to 6.4 mm 9.8 days after inoculation. However, the increase in lesion radius (and thus lesion growth rate) between 4 and 9.8 days after inoculation was not significant.

Experiment 2: Field inoculation

At 14 days after inoculation, plants of potato cv. Bintje had already lost 65% of their leaf area, and another 14 days later this was 99% (Fig. 3). Potato cv. Désirée had lost around 50% of its leaf area 14 days after inoculation, this increased to 85% another 14 days later and finally reached 100% after 56 days. For *S. sisymbriifolium*, 5 to 6% of the leaf area was lost 14 days after inoculation, thereafter the area slowly increased, to between 9 and 13% after 42 days (average for the four genotypes, which did not differ in response). In the last 14 days of the experiment, there was a slight rise in leaf area loss in all of the *S. sisymbriifolium* accessions, ultimately reaching values of 17.1 – 27.7%. The relative area under the disease progress curves (Table 2) was the highest for potato cv. Bintje (0.80), followed by that of potato cv. Désirée (0.71). Relative AUDPC of the four cultivars of *S. sisymbriifolium* was much lower than that of the two potato cultivars (0.08 – 0.11), and did not significantly differ among cultivars.

(a)



(b)

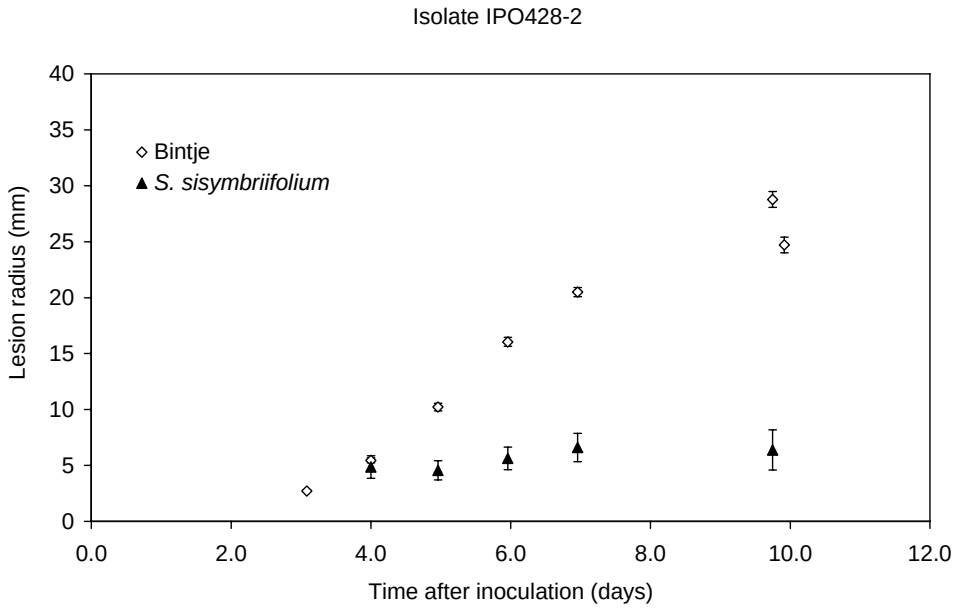


Figure 2. Lesion radius of *Phytophthora infestans* on leaves of potato cv. Bintje and *S. sisymbriifolium* plotted against time after inoculation, for isolate 90128 (A) and for isolate IPO428-2 (B). Error bars indicate standard error of the mean.

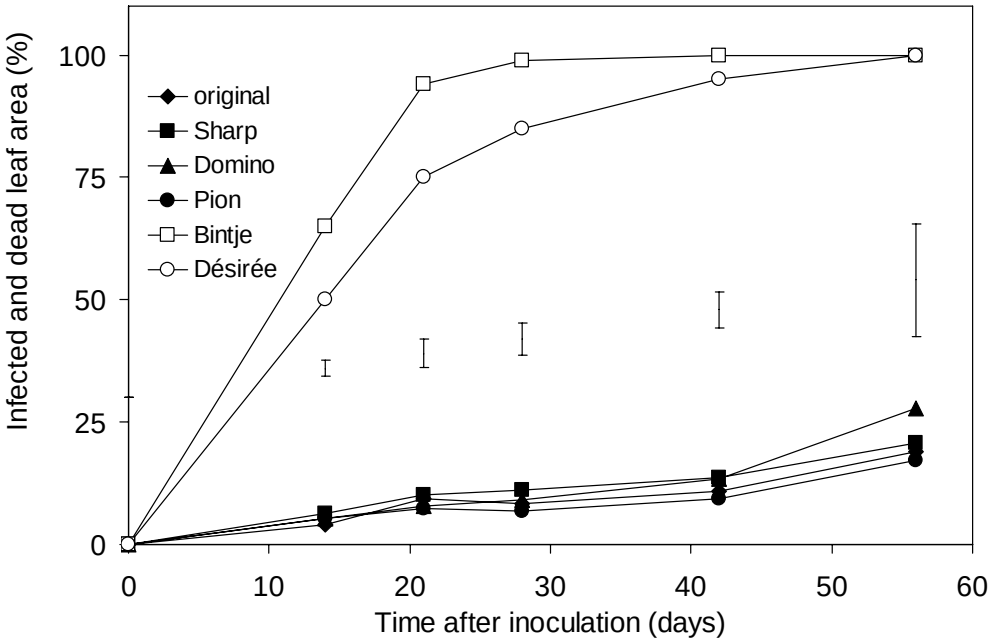


Figure 3. Percentages of infected or dead leaf area plotted against time after inoculation with *Phytophthora infestans*, isolate WAU01001 for *S. sisymbriifolium* (the original seed material, and three cultivars Sharp, Domino and Pion), and potato (cvs. Bintje and Désirée) (Expt 2). Bars indicate least significant difference ($P<0.05$) between potato and *S. sisymbriifolium* treatments, resulting from analysis of variance (data not shown).

Discussion

The results indicate a much lower susceptibility of *S. sisymbriifolium* to *P. infestans* than potato cultivars. Firstly, the infection efficiency of *P. infestans* on *S. sisymbriifolium* was very low. In comparison, lowest measured infection efficiencies for isolate CO-42 on potato cultivars (including cv. Malinché and cv. Tollocan, which are Mexican cultivars containing major R genes) were 60% (Rubio-Covarrubias *et al.*, 2005). Secondly, on *S. sisymbriifolium* lesion growth was very slow in the laboratory experiment. For isolates 90128 and IPO428-2 on potato cv. Bintje, comparable lesion growth rates (of around 6 and 5 mm d^{-1}, respectively) were found in other laboratory studies (Flier *et al.*, 2003a; Vleeshouwers *et al.*, 1999). In these studies, lesion growth rates reported on all used potato cultivars and on genotypes of several other *Solanum* species (with the exception of three *Solanum* genotypes that were know to be non-host or completely resistant) were higher (0.3 – 6.4 mm d^{-1}) than those measured on *S.*

sisymbriifolium. Thirdly, in the field experiment, the increase in infected leaf area of *S. sisymbriifolium* with time was also very slow, resulting in a very low relative AUDPC, in contrast with that of the two potato cultivars. Bisognin *et al.* (2002) reported higher relative AUDPC than currently observed for *S. sisymbriifolium* in greenhouse and field studies for several resistant potato cultivars (except for potato cv. Tollocan in some plots).

In the current experiment, between 42 and 56 days after inoculation in the field, the percentage of infected or dead leaf area of *S. sisymbriifolium* increased to around 20 – 25%. It is questionable whether this late increase was the result of *P. infestans*. During this period the soil around the *S. sisymbriifolium* was waterlogged as a result of the two irrigations a day, thus growth conditions had become suboptimal and therefore the loss of leaves during this period may have been caused by the growing conditions also, rather than by *P. infestans* only. As the potato plants were already 100% dead long before that time, the waterlogging only affected *S. sisymbriifolium.* Therefore, it is possible that the relative AUDPC for *S. sisymbriifolium* were overestimated, and in fact were lower than 0.08 – 0.11.

Damage of *P. infestans* to *S. sisymbriifolium* is limited. Also in earlier field studies the crop appeared to recover from incidences of infection. However, it should be realized that differences in resistance between nonhost, highly resistant and less resistant *Solanum* species seems of quantitative (rather than qualitative) nature (Vleeshouwers *et al.*, 2000), and furthermore, that the expression of resistance is influenced by environmental conditions (Rubio-Covarrubias *et al.*, 2005). This means that in other environmental conditions (especially at other temperatures) the resistance of *S. sisymbriifolium* to *P. infestans* could differ.

Table 2. Relative area under the disease progress curve (AUDPC), calculated for potato cv. Bintje, potato cv. Désirée, the original seed material of *S. sisymbriifolium* (Scholte, 2000b) and *S. sisymbriifolium* cv. Sharp, cv. Domino and cv. Pion (Expt 2). Means followed by a different letter are significantly (P>0.05) different.

Plant	Relative AUDPC
Potato cv. Bintje	0.80 (c)
Potato cv. Désirée	0.71 (b)
S. sisymbriifolium original seed material	0.09 (a)
S. sisymbriifolium cv. Sharp	0.11 (a)
S. sisymbriifolium cv. Domino	0.11 (a)
S. sisymbriifolium cv. Pion	0.08 (a)

Additionally, it cannot be denied that sporulation was observed in some cases, especially in September (results not shown). Sporulation of *S. sisymbriifolium* crops relatively late in the growing season probably does not represent a substantial increase in *P. infestans* pressure on potato fields. Flier *et al.* (2003b), though, reported oospore formation on *S. sisymbriifolium*. Oospore formation is the result of interactions between the hyphea of opposite mating types of *P. infestans*, enabling sexual reproduction. It occurs if a leaf is infected with both mating types A1 and A2 (Drenth *et al.*, 1993; Strömberg *et al.*, 2001). Oospores can survive in the soil until the next year(s) and can possibly cause new infections (Andersson *et al.*, 1998; Flier *et al.*, 2003b) and, therefore, *S. sisymbriifolium* can potentially add to infection pressure on potato crops grown the following year.

Towards the end of the season when *P. infestans* infection pressure is high also *S. sisymbriifolium* may become infected. The possible oospore formation may lead to the development of new more virulent isolates, including isolates to which *S. sisymbriifolium* itself is susceptible. Though hatching of nematodes increases with growth duration of *S. sisymbriifolium* (Capter 5), farmers should therefore be advised to kill the crop when sporulation of *P. infestans* is observed in September. Based on the presented information it can be expected that development of new isolates can be avoided in this way.

CHAPTER 8

General discussion

During four years of working on the development of *S. sisymbriifolium* as a trap crop for potato cyst nematodes (PCN), data on various aspects of the crop were collected. Also, several aspects of trap crop management and efficiency were studied at PPO-AGV, Lelystad, NL (Hartsema *et al.*, 2005). In this chapter, several findings are combined to discuss the growth, effects on PCN, crop management and possible risks of the use of *S. sisymbriifolium*. Finally, the possibilities for growth of *S. sisymbriifolium* in other climatic regions, the possibilities for crop improvement and practical implications are addressed.

Optimum growing season of *S. sisymbriifolium*

One of the aims of the current project was to find the adequate growing season for *S. sisymbriifolium* in The Netherlands and Western Europe. Planting of *S. sisymbriifolium* was successful in The Netherlands from early May till the end of July. If sown earlier in the season, e.g. in March or April, germination and subsequent emergence were quite slow, and there was hardly any germination below ca 8 °C (Chapter 1). The minimal temperature for germination is comparable to that of crops evolved in warmer climates e.g. maize (Ruiz *et al.* 1998) and rice (Sié *et al.* 1998). Later in the season (late July, beginning of August or September) germination and emergence were successful (Chapter 2). However, growth conditions apparently became unfavourable during initial plant growth, resulting in a drastic inhibition of canopy expansion. This resulted in crops that intercepted less than 50% of light at the time of cessation of growth. These observations are supported by findings of Hartsema *et al.* (2005), who reported a large chance for insufficient above-ground crop growth if planting is later than the second week of July. The increased probability of *Phytophtora infestans* infection in September (Chapter 6) is an additional reason to avoid late sowings.

It was suggested that *S. sisymbriifolium* could be grown as trap crop for PCN either before or after a main season crop (Scholte and Vos, 2000). However, the genotype used in this study needs the full main season to grow. The best farming practice would be to incorporate the crop into the soil in September or October, after its growth has ceased, but before berries are ripe. Crop destruction in September is probably a good practice if the crop is at risk to be infested by *Phytophthora infestans* (Chapter 6).

Incorporation of the biomass represents a large input of organic matter into the system and probably yields indirect advantages associated with maintaining soil organic matter (Thomsen and Christensen, 2004).

Recommendations for an optimal cropping season of *S. sisymbriifolium* in other Western European countries have as yet to be established (see page 103, Possibilities for cultivation of *S. sisymbriifolium* in other climatic regions). On the basis of the here reported studies we initiated the development of a simple crop growth model in order to provide the tool for extension of the current data to a wider region.

Effects on potato cyst nematodes

General effects

In the greenhouse study (Chapter 4), hatching under *S. sisymbriifolium*, and therefore reduction in numbers of PCN, was related to crop cultivation time. Hatching increased from 45% after 35 days to 75% reduction after 150 days. Also, hatching was related to root length density, and increased from 43% hatching at 0.3 cm cm^{-3} to 85% hatching at 5.8 cm cm^{-3}. In theory (Chapter 3) a certain minimal root length density of *S. sisymbriifolium* roots could be calculated, that suffices for hatching agents to reach the majority of the cysts in the soil. The depth in the soil, above which the actual root length density exceeded this minimal root length density, was linearly correlated with the above-ground biomass and with total root length. This calculation critically depended on the size of the diffusion distance of hatching agents in the soil. Our results (Chapter 4) lead to the suggestion that this diffusion distance was relatively small. Estimations can be made by inverting the reasoning: if it is assumed that all cysts reached by the hatching agents will indeed hatch and that hatching in a soil volume will be 85% at root length density 5.8 cm cm^{-3} as was observed (Chapter 4), a hatching agent diffusion distance of around 0.3 cm can be deduced from Fig. 1 in Chapter 3. Although, the hatching percentage was probably still increasing when measurements of the root length density were made, and therefore the diffusion distance in reality must have been larger than 0.3 cm, diffusion distances in the order of 1 cm seem unrealistically high.

A diffusion distance of hatching agents smaller than 1 cm may not be unrealistic, although Rawsthorne and Brodie (1987) deduced a much higher hatching agent diffusion distance (50 cm!) from pot experiments. In other plant root pathogen systems with chemical triggering, comparable small distances were reported, e.g. germination of microsclerotia of *Verticillium dahliae* was found to decline significantly within 0.1 cm distance from the nearest potato root (Mol and Van Riessen, 1995). An explanation for such a small hatching agent diffusion distance can be that the concentration of

hatch stimulants declined with distance from the root surface, and might have dropped below the adequate concentration at a much shorter distance than 1 cm from the roots. For *Globodera rostochiensis*, it was shown that hatching agents are active at very low concentrations ($7 \times 10^{-7} - 7 \times 10^{-9}$ mg litre^{-1}) but hatching clearly reached an optimum at $0.7 - 7 \times 10^{-4}$ mg litre^{-1} (Devine *et al.*, 1996), while the response decreased again at higher concentration (Devine *et al.*, 1996, Devine and Jones, 2000a).

For *Striga asiatica* the situation is known to be more intricate: the activity of the germination stimulating chemical, xenognosin, in the root exudate of the host critically depends on the presence of other chemicals in the root exudate (Fate and Lynn, 1996). These co-factors increase the activity and lifetime of xenognosin in the root exudate. Therefore, the distance from the host root at which *Striga* seeds germinate is variable: if the co-factor is not formed, the xenognosin is not active for long. Such a more intricate situation could also be applicable for the hatching of PCN: it is already known that hatching agents actually are a small group of chemical compounds (Devine and Jones, 2000b) and also hatching inhibitors exist in potato root exudate (Byrne *et al.*, 1998).

Consequences of a small hatching agent diffusion distance in the soil are that irregularities in the root length density profiles under *S. sisymbriifolium* crops may become important in terms of effects on hatching, and more fundamental research is needed to elucidate quantitative consequences in practise.

Extrapolating hatching information to field situations

There was a relation between hatching and the duration of the growth period of *S. sisymbriifolium* crops in the two greenhouse experiments conducted in large containers (Chapter 4). Such a relation was also found in a pot experiment with five litre pots (Scholte, 2000c). Final percentages of hatching were comparable in Scholte's pot experiment and in the current study on potato and *S. sisymbriifolium*. In both studies also hatching in non-host or fallow treatments varied over the same range. However, the rise of the cumulative hatching curves in the smaller pots (Scholte, 2000c) occurred sooner after sowing than in the current greenhouse study with large containers. In the pot experiment hatching did not increase beyond 75 days after sowing irrespective of the crop type. This levelling-off did not occur in the current experiments with the large containers. The difference in dynamics over time between the two experiments can possibly be explained by the fact that root length density in the pots must have increased faster, due to space limitation. It can be assumed that the root colonisation in the large containers resembled a field situation more closely than in pots; however, root length densities at a specific depth under a *S. sisymbriifolium*

crop in the field were lower (Chapter 3) than those under a crop of the same age in the containers (Chapter 4).

Hatching of PCN was measured using the nylon bag method. The sand core data were less representative (Chapter 4) because there was insufficient root colonization in the infected sand cores. Additionally, the nylon bag method offers the advantage of measurement of hatching with a susceptible potato, as cysts buried in bags at the start of the experiment remain separated from newly formed cysts in the roots. However, in the nylon bags, the cysts are not embedded in the soil as in natural situations potentially leading to interactions between cysts. Development of protocols on good experimental practice in hatching studies is highly desirable.

We used one *G. pallida* population in the current study. This raises questions on the applicability of the current data for other PCN populations. Scholte (2000b) showed *S. sisymbriifolium* stimulated hatching of a broad range of *G. rostochiensis* and *G. pallida* populations. This is reassuring, but does not rule out some variation in hatching between PCN populations when exposed to *S. sisymbriifolium*.

Scholte's results (Scholte, 2000b) inspired us to the following remark: hatching on potato and *S. sisymbriifolium* was found to vary among the PCN populations, with low hatching in some cases. However, this variation in hatching always showed the same pattern for susceptible potato and *S. sisymbriifolium*. Such coupled responses could reflect diapause (Muhammad, 1994), or reduced viability of the cysts of some PCN populations used. Since healthy PCN cysts invariably show almost complete hatching when exposed to potato hatching agents, it is recommended to include potato as a control treatment in studies examining hatch stimulation of agents other than potato root exudates.

It can be concluded that the current study corroborated the potential effect of a *S. sisymbriifolium* trap crop on PCN. However, the limitations of the current methodology show the need for additional field experimentation, with different soil types and *Globodera* sp. populations. In order to minimize spatial variation in nematode density to the lowest possible level, infestation should start from artificial inoculation, followed by multiplication on divergent hosts (as in Scholte and Vos, 2000). Treatments should include crops of *S. sisymbriifolium* and of susceptible potato. Root length density in cores and around nylon bags would need to be measured over time. Hatching should be measured using nylon bags and core samplings. Problems with core samplings are not expected to occur, since cysts are present in a population in the field soil. Such experiments are time and money consuming, but data would validate whether the observed effects of a *S. sisymbriifolium* trap crops occur in practice.

Crop management

Seeds of *S. sisymbriifolium* are relatively small and require application of adequate seed bed preparation and sowing practices. In the current study a precision sowing machine was used, but several sowing machines common in Dutch agriculture are adequate (Hartsema *et al.*, 2005). Seeds need to be delivered shallow (1 – 2 cm depth), pressed into a compacted tilled layer (to ensure water transport to the seed), and covered by looser soil. Row distance of 12.5 cm is advisable, since larger row distances could cause parts of the soil to have lower root colonisation and nematode reduction. Seed rates of 200 – 400 seeds m^{-2} (= 6.2 – 12.4 kg ha^{-1}) were used in the current study, but 50 – 100 seeds m^{-2} (~ 1.5 – 3 kg ha^{-1}) were recommended as sufficient by Hartsema *et al.* (2005). Optimization of sowing density as proposed by Hartsema *et al*, though is based on total above-ground biomass at the end of a growing season. If for most effective hatching a quick early growth and thus long period with a high root length density is needed, as indicated by the results in Chapter 4, a higher seed density may be more effective.

Sowing of *S. sisymbriifolium* should be done from May onwards, because temperatures in the Dutch field conditions then become high enough for seed germination and emergence (Chapters 1 and 2). This relatively late sowing enables farmers to perform weed control by making a false seedbed: soil tillage to trigger weed seed emergence a few weeks before sowing. Some days before sowing the emerged weeds need to be killed, either mechanically of chemically, reducing the weed seed bank and weed emergence shortly after sowing of the crop. Such a practice is advisable, since initial growth of *S. sisymbriifolium* is slow. Therefore, *S. sisymbriifolium* will not easily overgrow early established, densely populated patches of weeds. Especially at low sowing densities this risk is present (Hartsema *et al.*, 2005).

The crop does not require high nitrogen rates: in the current study nitrogen was applied at a rate of 150 kg N ha^{-1} to ensure unlimited growth. However, in unpublished experiments rates of 80, 40 and 0 kg N ha^{-1} were applied and no differences in crop performance were seen. Also, Hartsema *et al.* (2005) recommended lower N application rates, of 40 – 80 kg N ha^{-1}, with the higher rate to be applied on crops sown early in the season.

A new weed?

S. sisymbriifolium is an invasive weed species in South Africa (Hill and Hulley, 2000; Byrne *et al.* 2002). However, the risk of *S. sisymbriifolium* becoming a weed in The

Netherlands is small. In practice it is advisable to incorporate the crop in the soil in September (see section Cropping season) to reduce risks of infection by *P. infestans*. Such a practice will also prevent the development of any mature berries. If for some reason crops are not incorporated in the soil, plants will not survive the Dutch winter (unpublished observations). Mature berries will be produced, but emergence and initial growth of plants in spring is very slow, resulting in poor competitive strength of any *S. sisymbriifolium* seedlings that would emerge as weed.

Host plant behaviour

S. sisymbriifolium has a high tolerance for PCN (Chapter 5). It can be grown successfully on infestations that cause economical damage in potato crops. If PCN infestation densities are extremely high (200 eggs g^{-1} soil and more), growth of *S. sisymbriifolium* can be somewhat reduced. However, such situations do not normally occur.

Sensitivity and host plant behaviour of *S. sisymbriifolium* towards other nematode species has been investigated too. Scholte and Vos (2000) reported that *S. sisymbriifolium* is resistant against *Meloidogyne hapla*. Hartsema *et al.* (2005) concluded from work by Visser and Korthals (2002) and Van Beers *et al.* (2001) that *S. sisymbriifolium* is a moderate host for *M. chitwoodi*, while it is a moderate or even bad host for *Pratylenchus penetrans*. Furthermore it is a moderate to bad host for *Trichodorus primitivus*, *T. similis* and *Paratrichodorus teres*. *S. sisymbriifolium* was mentioned as a good host to *P. pachydermis.*

In the field experiments of the current study it was clearly noticed that *S. sisymbriifolium* is a host plant for the Colorado beetle (*Leptinotarsa decemlineata*) (Chapters 2 and 3), as was already observed by Scholte (personal communication). The beetles attacked the plants already in very early stages, and caused some damage if present in sufficient numbers.

S. sisymbriifolium showed low sensitivity to *Phytophthora infestans* isolates used in the current study (Chapter 6). There is no risk of the crop being killed by *P. infestans*. Oospore formation on its leaves may occur and in this way cultivation of *S. sisymbriifolium* can contribute to the formation of new, potentially more virulent isolates. Furthermore, if oospores would be formed, these can survive the winter in the soil, and potentially infest potato crops in the years after *S. sisymbriifolium*. However, if cultivation of *S. sisymbriifolium* is interrupted in September, and the crop incorporated in the soil, there is acceptably low risk of aggravating *P. infestans* problems of the potato industry.

Brown rot, also known as bacterial wilt, is a serious potato disease, caused by the soilborne bacterium *Ralstonia solanacearum*. Brown rot has occurred first in The Netherlands in 1995 and efforts are made to eradicate the disease. There are reports in the literature showing that some genotypes of *S. sisymbriifolium* are sensitive to strains of *R. solanacearum* (e.g. Ali *et al.,* 1990). The pathogenic variety of *R. solanacearum* occurring in Europe is race 3, biovar 2. When *S. sisymbriifolium* plants (same genotype as in the current study) were inoculated with that strain (stem injection or irrigation of potted plants with bacterial suspension) no wilting symptoms were observed, but bacteria could be re-isolated from plant material. This led to the conclusion that *S. sisymbriifolium* is a potential latent host for *R. solanacearum* race 3, biovar 2 under European climatic conditions (Tjou-Tam-Sin and Janse, 2001). Being a quarantine disease, detection of brown rot in a potato field leads to prohibition of potato cultivation for a long time. Hence, there are limited practical consequences of *S. sisymbriifolium* being a potential latent host of *R. solanacearum.*

Possibilities for cultivation of *S. sisymbriifolium* in other climatic regions

With the results of the current study in mind, preliminary predictions can be made on the season adequate for cultivation of *S. sisymbriifolium* in different regions in Europe. In more northern regions, the suitable growing season for *S. sisymbriifolium* is expected to be shorter than in The Netherlands: the range of months between which successful planting can be done will probably begin later than May and end earlier than the end of July. As a consequence the crop always needs the main season for its growth. In more southern countries of Europe, probably possibilities exist to grow the crop before or after another main crop. Here, conditions seem more favourable to include *S. sisymbriifolium* in an existing rotation. On the other hand, these warmer areas may have limited options for a second crop due to the limited rainfall during the warmer part of the summer season. Furthermore, also the risk of *S. sisymbriifolium* becoming a weed is present in warmer regions. Currently, the plant seems to occur as a weed already in Sardinia (Brunu, 2004). In view of creating weed problems the plant should be used with care in warmer regions and before red berries are formed cultivation should be interrupted and plant material could be incorporated in the soil. More research on the performance of *S. sisymbriifolium* in the different areas with PCN problems is recommended. Some work is in progress elsewhere in Europe. Modelling would be an efficient way to explore cropping options and hatch stimulation in different climates.

Crop improvement

The genotypes currently used in The Netherlands can be improved in many ways. First of all, seed priming (e.g. with nitrate, unpublished results) might result in accelerated and more uniform emergence. This would increase the chances of the crop to be grown successfully before a main crop. Secondly, screening *S. sisymbriifolium* genotypes for cold tolerance, with special focus on emergence and initial growth, would help to reach fast crop closure early in the season, and improve the crop's competitive strength against weeds. Also, it could enlarge the chances of using *S. sisymbriifolium* after a main crop in autumn. Thirdly, genotypes of *S. sisymbriifolium* could be screened for production of a higher root length density per unit above-ground biomass, and production of hatching agents (amount and composition), which could increase the effects of the crop on PCN.

Practical implications

In the current study, the effects of *S. sisymbriifolium* were comparable to those of fumigants such as 1,3-dichloropropene, which ranged between 48 and 72% as reported by Been and Schomaker (1999). The advantages of *S. sisymbriifolium* over fumigants are that the ecological balance in the soil is not disturbed and that its roots colonize the soil to a greater depth than fumigants penetrate. In the results reported by Hartsema *et al*. (2005) effectiveness of *S. sisymbriifolium* in reducing PCN were more variable, and the causes of this variation are not clear. Additionally, the current genotypes of *S. sisymbriifolium* need a full growing season to be effective. Although this seems unfavourable, during the last years Vandijke Semo BV (Scheemda, NL) has set up a seed production and supply chain. Three varieties were submitted for registration and plant breeding rights were granted in 2002. These varieties also need a full growing season to be effective, but are used in practice in combination with a fallow-premium (a few hundreds of hectares annually since 2002). Additionally, the Plant Protection Service has included *S. sisymbriifolium* in the list of PCN control measures, and grants farmers the rights on resampling after growing the crop. Chances of detecting an infection a second time after resampling are limited, and probably this contributes to the current success of the crop. However, on the long term, crop improvement seems necessary. A breeding programme was already started, and new varieties are expected to be released in the short run that exhibit a faster germination, quicker crop establishment and exudation of more effective hatching agents. These steps in the breeding program were partly triggered by the preliminary results of this study. For now it seems a valid conclusion that a trap crop of *S. sisymbriifolium* can be successful if a crop with a high amount of above- and below-ground dry matter is realized.

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Summary

Potato cyst nematodes (PCN) are a major pest in many potato growing regions. On a world scale they were estimated to account for 12% loss of annual potato production. Also, in Europe the problem is present. Here, two species of PCN were identified: *Globodera pallida* (Stone) and *G. rostochiensis* (Woll.), and a recent structured survey in England and Whales showed that 64% of all sampled fields were infested with at least one of them.

PCN are named after their soilborne survival structure, the cyst, which consists of the toughened integument of the dead female body, filled with embryonated eggs. Cysts remain in the soil after the host crop is harvested. Every year part of the cysts will hatch spontaneously, but the remaining part can survive in the soil for many years. If a potato crop is grown on infested soil, the nematodes close to a potato root are triggered to hatch from their cyst in great numbers by chemicals, so called hatching factors, exuded from the roots of the host crop. Juvenile nematodes invade the roots of the host crop, where they feed and reproduce themselves. The invasion by PCN has a strong effect on the host crop, e.g. reducing its overall growth.

Several control options for PCN are available, including crop rotation, use of resistant cultivars and nematicides. When relying on crop rotation only, potato can be grown on a field every six to seven years. However, such a long rotation is often undesirable from an economic point of view. Breeding for resistance has been successful, but did not bring a permanent solution. Resistances were broken, and especially in ware potatoes it has been difficult to combine PCN resistance with other desired potato traits. In The Netherlands, in the seventies and eighties of the last century nematicides (e.g. 1,3-dichloropropene) were promoted as a suitable solution to the problem. However, in rotations with a potato crop on the same field every second or third year, the ensuing reduction of PCN cysts was often not high enough to prevent the PCN populations from increasing.

Therefore there is scope for additional control measures, e.g. a trap crop. Such a trap crop has to produce root exudates that stimulate the hatch of PCN juveniles from their cysts. Subsequent reproduction has to be prevented, either by resistance of the crop, or by destruction of the crop before adult female development is completed. After extensive screening of non-tuber bearing *Solanaceae*, *Solanum sisymbriifolium* (Lam.) was selected as the most promising trap crop. In a preceding field experiment, *S. sisymbriifolium* was found to result in 50 – 80% reduction in soil infestation of PCN, i.e. slightly less than was observed for potato cv. Bintje, while the plant was found completely resistant against PCN. *S. sisymbriifolium* is an exotic plant species, originating from South America. Explorative research yielded merely qualitative

results, and little quantitative information was available on the ecology of the plant and appropriate crop management.

The general objectives of the current study included investigation of the rate of emergence in relation to temperature and soil water potential, and quantification of biomass production and root length density over depth. The next step was relating root growth and duration of crop growth to hatch stimulation. Finally, it was aimed to quantify the tolerance of plant growth to infestation density of PCN in the soil, and to investigate the susceptibility of *S. sisymbriifolium* to *Phytophthora infestans* (Mont.) de Bary.

The rates of seed germination and emergence were studied in a series of laboratory experiments (Chapter 2). Treatments consisted of constant (9.1 – 21.8 °C) and diurnally fluctuating temperatures and different soil water potentials. Linear, Q10, expolinear and quadratic models were fitted to data on the temperature response of emergence and germination. For model validation, field emergence was monitored in 16 sowings made over the years 2001 – 2004. Germination (and thus emergence) rate increased with temperature, and was not influenced by soil water potential in the range of –1.26 to –0.003 10^{-3} MPa. The relation between germination rate and temperature was not influenced by diurnal temperature fluctuations. Goodness of fit was best for the quadratic and the expolinear model. For these two models the root mean square error for predicting the length of the period between sowing and field emergence was 2.3 and 2.4 days, respectively for observations ranging from 5.8 to 66.5 days. The expolinear and the quadratic models adequately describe the temperature dependency of field emergence, especially for crops species germination near their base temperature.

To study performance of *S. sisymbriifolium* in The Netherlands, a series of field experiments were carried out between 2001 and 2003 (Chapter 3). Experimental factors included sowing time, sowing density and site. Rate of emergence, crop growth and change over time in light interception were monitored. Growth analysis was performed at 7 and 14 weeks after emergence and dry weights of component plant parts were determined. Time to 50% emergence was 36 – 38 days for planting early April and declined to minimum values of ca 8 – 11 days when planting took place in June, July or the first week of August. When planted later, time to 50% emergence increased again. Time to 50% light interception showed a similar trend with sowing time; minimum time was 35 – 40 days for planting between June and mid July. There was no advantage in planting before May in accordance with above mentioned relation of emergence with (soil) temperature. Crop performance was very variable across years and sites when planted later than the end of July – beginning of August. Therefore, the time window for planting in The Netherlands is between early May and

end of July. Above-ground dry matter accumulation up to 400 g m^{-2} was found at 7 weeks after emergence and up to 1040 g m^{-2} at 14 weeks. At 7 weeks after emergence dry matter production increased with plant density (range 50 – 400 seeds m^{-2}), but no statistically significant differences were found after 14 weeks. Radiation-use efficiency was 1.69 (SE =0.0208) g MJ^{-1} of intercepted Photosynthetically Active Radiation. Dry matter accumulation (2002 – 2003) was somewhat higher in Wageningen (51° 58' N) on light sandy soil than in Flevoland (52° 31' N) on clay soil and in Drenthe (52° 51' N) on reclaimed peat soil.

Hatching of potato cyst nematodes is induced by root exudates of *Solanaceae*, such as *S. sisymbriifolium*, and is therefore related to root length distribution of the crop. A mathematical model was derived to calculate the minimally required root length density to include a particular fraction P of the cysts in the zone of influence of the hatching agents of the nearest root (Chapter 4). Since the radius of the hatching agent zone of influence around each root is not known, the calculation was done for several assumed radius lengths. A series of field experiments was carried out to study actual root length distribution of *S. sisymbriifolium* in relation to shoot properties. The logarithm of the total root length showed a linear relation to the logarithms of above-ground biomass and leaf area index. Root diameter distribution was the same for all crops, and independent of soil depth. Fine roots (<0.4 mm diameter) constituted around 50% of total root length. The mathematical model, which consists of a modified Poisson distribution formula for the three dimensional distribution of roots in a volume of soil, was used to deduce to which soil depth 75% of all cysts reside in the zone of influence of the hatching agents, in relation to above-ground *S. sisymbriifolum* biomass or total root length. This was done for 0.50 cm, 0.75 cm and 1.00 cm assumed radius lengths of the zone of influence of the hatching agents around each root. The proposed approach to derive potential hatching effects from crop properties needs further validation; particularly the assumed diffusion distance of hatching agents is a critical factor.

Experiments were conducted to quantify the effect of different periods of growth of *S. sisymbriifolium* and root length density on hatching of PCN, using potato and fallow treatments as references (Chapter 5). One and two year old *Globodera pallida* cysts were used in two years in greenhouse experiments carried out in containers with non-infested soil. Two methods were used to study hatching. In the first method 7.5 cm diameter soil cores were removed and backfilled with infested soil. These infested cores were sampled over time to monitor hatching. In the second method cysts were buried in nylon bags, which were removed over time to monitor hatching. The soil cores infested with cysts used in the first method had characteristics slightly different from those of the surrounding bulk soil and these differences were suspected to be the

cause of very low root colonization of the cores as compared to bulk soil. Therefore, in our experiments the soil core method apparently grossly underestimated hatching. Hatching of PCN cysts (nylon bag method) increased with the duration of growth of *S. sisymbriifolium*, and ranged from 47% after 44 days of crop growth up to 74% after 150 days of crop growth (averaged across all depths of insertion of bags). Reductions per depth layer were also correlated to root length density and varied between 42.6% at 0.26 cm cm^{-3} and 85.3% at 5.8 cm cm^{-3}. Based on a single exponential decay function, a general method is presented to estimate for any PCN control measure the average reduction in the number of years needed to reach sanitation below a given PCN population density. For *S. sisymbriifolium* trap cropping, calculated reductions in duration of the sanitation period ranged from 2.3 years for 60% hatching (equivalent to 90 days of growth of *S. sisymbriifolium*) to 4.4 years for 75% of hatching (equivalent to 150 days of growth of *S. sisymbriifolium*). These reductions were independent of initial and final PCN population density. The current results corroborate the hatch inducing effect of *S. sisymbriifolium*, underline the importance of growth duration and root length density as determinants of the decimating effect on PCN, and draw attention to methodical pitfalls in the study of hatch stimulation.

A greenhouse experiment was conducted to test the tolerance of the plant for a range of PCN infestation densities (0 – 578 nematodes g^{-1} dry soil) (Chapter 6). Pre-soaked *S. sisymbriifolium* seeds were sown in 3 l pots, containing artificial soil. Plant height was monitored over time and plant dry weight was measured after a growth period of 103 days in the greenhouse. Two models were used to describe the found relations of *S. sisymbriifolium* height and dry weight with nematode inoculation density: an exponential model and a hyperbolic model. At the two highest inoculation densities (288 and 578 nematodes g^{-1} soil) above-ground dry matter of *S. sisymbriifolium*, measured at day 103, was significantly lower than in the uninfected control. The exponential model revealed a tolerance limit, below which no growth reduction of *S. sisymbriifolium* was present at all, of 24 and 36 nematodes g^{-1} dry soil for dry matter production and maximum plant height, respectively. Compared to potato varieties tested in previous experiments, this tolerance limit is extremely high. Also the hyperbolic model revealed very high tolerance of *S. sisymbriifolium* to potato cyst nematodes, showing that at 369 and 1120 nematodes g^{-1} dry soil above-ground dry weight and maximum plant height were halved, respectively. Both models adequately described the measured response. In principle both models can help to assess the risk of plant damage in field foci, but it is concluded that the tolerance of *S. sisymbriifolium* is so high that extensive plant damage is not likely to occur under current agricultural conditions.

Finally, two experiments were carried out to study susceptibility of *S. sisymbriifolium* to potato late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary (Chapter 7). In a laboratory experiment, detached leaves of *S. sisymbriifolium* and of *Solanum tuberosum* (L.) cv. Bintje were inoculated with two isolates (90128, IPO428-2); infection efficiencies and increase in lesion radii were compared. First results indicated low virulence of the used isolates on *S. sisymbriifolium* and therefore the ensuing study was performed with isolate WAU01001, which was originally collected on a *S. sisymbriifolium* field crop and seemed the most virulent isolate for *S. sisymbriifolium* obtainable. In a field experiment plants of *S. sisymbriifolium* and of *S. tuberosum* cv. Bintje and cv. Désirée were inoculated with isolate WAU01001, to compare leaf area loss. Results of the laboratory experiment showed that *S. sisymbriifolium* is fairly resistant to the *P. infestans* isolates used. Infection efficiencies ranged from 20 – 36%, compared with 100% for potato cv. Bintje. If infection of *S. sisymbriifolium* occurred, the lesions hardly increased in radius. In the field, potato plants had lost 100% of their leaf area 28 – 42 (cv. Bintje) and 56 (cv. Désirée) days after inoculation, respectively. *S. sisymbriifolium* plants had lost around 9 – 13% of their leaf area after 42 days. Relative area under the disease progress curve was 0.80 for potato cv. Bintje, 0.71 for potato cv. Désirée, and ranged from 0.08 to 0.11 for *S. sisymbriifolium*. In conclusion, the susceptibility of *S. sisymbriifolium* to *P. infestans* is very low, and the risk that *S. sisymbriifolium* could act as a source of *P. infestans* for potato crops or the risk of the development of virulent isolates can be managed through a correct timing of crop destruction. In September, the crop cultivation should be ended to decrease the risk on *P. infestans* infection and avoid oospore formation. *S. sisymbriifolium* can be incorporated in the soil as a green manure. Observations from several other sources indicate that the cultivation of *S. sisymbriifolium* does not aggravate other soilborne pests and diseases that are a threat to potato production.

In the ideal situation, *S. sisymbriifolium* would be sown before or after an economically attractive main crop. However, current results showed that in The Netherlands, planting was successful between May and the end of July. This limits possibilities to cultivate *S. sisymbriifolium* in the same season as another main crop. Further it raises the expectation that in more Northern, colder regions, the crop will always need the main season to grow, whereas in more southern, warmer regions there may be more possibilities to grow *S. sisymbriifolium* as a trap crop before or after a main season crop with relatively short crop cycle (e.g. some vegetables). Efficient trap crops against PCN offer many advantages compared to other control measures such as nematicides and breeding for resistance in potato. Therefore, *S. sisymbriifolium* clearly deserves further development as a trap crop for potato cyst nematodes.

Samenvatting

Aardappelcysteaaltjes (ACA), de veroorzakers van aardappelmoeheid, zijn een belangrijke ziekte in veel gebieden waar aardappels worden geteeld. Op wereldschaal wordt geschat dat ze een verlies veroorzaken van 12% van de jaarlijkse aardappelproductie. Ook in Europa is het probleem aanwezig. Hier zijn twee soorten aaltjes geïdentificeerd: *Globodera pallida* (Stone) en *G. rostochiensis* (Woll.). Een recent uitgevoerd gestructureerd onderzoek in Engeland en Wales liet zien dat daar 64% van alle velden geïnfecteerd waren met tenminste één van de twee soorten.

ACA zijn genoemd naar hun overlevingsstructuur in de bodem, de cyste, die bestaat uit het verharde restant van het dode vrouwelijke aaltje, gevuld met bevruchte eitjes. De cyste blijft in de bodem achter nadat een aardappelgewas is geoogst. Ieder jaar zal een deel van de cysten, dat in de bodem achterblijft, spontaan uitkomen. Maar het gedeelte van de cysten dat niet uitkomt, kan vele jaren in de bodem overleven. Als een aardappelgewas verbouwd wordt op besmette grond, dan worden de ACA dicht bij de wortels van het gewas door chemische stoffen die uit de wortels van het gewas lekken (zogenaamde hatching factoren) gestimuleerd uit hun cyste te komen. De juveniele ACA dringen dan binnen in de wortels van het gewas, waar ze zich voeden en voortplanten. Een dergelijke invasie door ACA heeft sterke effecten op een gastheergewas, onder andere een reductie van de groei.

Verschillende beheersingsmaatregelen voor ACA zijn beschikbaar, onder andere gewasrotaties, het gebruik van resistente aardappelcultivars en nematiciden. Met alleen gewasrotatie als beheersmaatregel kunnen aardappels slechts eens in de zes of zeven jaar op een perceel geteeld worden. Een dergelijke rotatie is vaak niet gewenst vanuit een economisch oogpunt. Het veredelen op resistentie tegen ACA is succesvol geweest, maar heeft ook geen permanente oplossing opgeleverd. Resistenties werden doorbroken, en vooral in consumptieaardappels is het moeilijk gebleken resistentie tegen ACA te combineren met andere, gewenste eigenschappen van aardappels. In de jaren zeventig en tachtig van de afgelopen eeuw zijn in Nederland nematiciden aanbevolen als de oplossing voor het probleem. Het is echter gebleken dat in rotaties met eens in de twee of drie jaar aardappels de reductie van de ACA vaak niet groot genoeg was om te voorkomen dat de ACA populaties toenamen.

Om deze redenen is er ruimte voor andere beheersmethoden, zoals een vanggewas. Een dergelijk vanggewas moet wortelexudaten produceren die de ACA stimuleren uit hun cyste te komen. Vervolgens moet de voortplanting van de ACA voorkomen worden, bv. doordat het gebruikte gewas resistent is, of door het gewas te vernietigen voordat de ontwikkeling van volwassen vrouwtjes is voltooid. Na een uitgebreid onderzoek van niet-knoldragende *Solanaceae* is *Solanum sisymbriifolium* (Lam.)

uitgekozen als het meest veelbelovende vanggewas. In een veldproef bleek *S. sisymbriifolium* een reductie van 50 – 80% van de ACA in de bodem te bewerkstelligen. Deze reductie was slechts een beetje lager dan het percentage dat de aardappelcultivar Bintje stimuleert om uit de cyste te komen. *S. sisymbriifolium* bleek echter volledig resistent tegen ACA. *S. sisymbriifolium* is een exotische plantensoort, die van oorsprong afkomstig is uit Zuid Amerika. Het eerste onderzoek leverde vooral kwalitatieve resultaten op en weinig kwantitatieve gegevens waren beschikbaar over de ecologie en de teeltaspecten van de plant.

De algemene doelstellingen van de huidige studie waren om de opkomstsnelheid in relatie tot de temperatuur en bodemwaterpotentiaal te onderzoeken en om de drogestofproductie en wortellengtedichtheid over diepte te kwantificeren. Vervolgens werden wortelgroei en duur van de aanwezigheid van het gewas gerelateerd aan de stimulatie van de ACA om uit hun cyste te komen. Tenslotte werd de tolerantie van het gewas zelf voor ACA gekwantificeerd, en werd de gevoeligheid van het gewas voor *Phytophthora infestans* (Mont.) de Bary onderzocht.

Snelheid van kieming en opkomst zijn onderzocht in een reeks van laboratoriumproeven (Hoofdstuk 2). Behandelingen bestonden uit constante temperaturen (9.1 – 21.8 °C), dagelijks fluctuerende temperaturen en bodemwaterpotentialen. Een lineair, een Q10, een expo-lineair en een kwadratisch model is vereffend op de gegevens van de opkomstsnelheid als functie van de temperatuur. Om de modellen te valideren is de veldopkomst gemeten in 16 proeven in het veld, gedaan in de jaren 2001 – 2004. Kiemsnelheden en dus ook opkomstsnelheden namen toe met toenemende temperatuur, maar werden niet beïnvloed door bodemwaterpotentialen tussen –1.26 en –0.003 10^{-3} MPa. Verder werd de relatie tussen kiemsnelheid en temperatuur niet beïnvloed door dagelijkse temperatuurfluctuaties. De kwadratische en expo-lineaire modellen waren het meest nauwkeurig in het beschrijven van de data. Voor deze twee modellen was de ‘root mean square error’ tussen zaai en opkomst in het veld 2.3 en 2.4 dagen, voor observaties van perioden tussen zaai en veldopkomst die varieerden van 5.8 tot 66.5 dagen. De expo-lineaire en kwadratische modellen beschrijven de relatie tussen veldopkomst en temperatuur nauwkeurig, vooral voor gewassen die verbouwd worden onder omstandigheden dicht bij hun basistemperatuur voor kieming.

Een reeks van veldproeven is uitgevoerd in de jaren 2001 tot 2003 (Hoofdstuk 3) om het functioneren van *S. sisymbriifolium* in Nederland te bestuderen. Behandelingen waren zaaitijd, zaaidichtheid en locatie. Opkomstsnelheid, plantengroei en verandering van de lichtonderschepping in de tijd werden gemeten. Groeianalyse is uitgevoerd op 7 en 14 weken na opkomst. Drooggewichten van de verschillende plantorganen werden hierbij gemeten. Bij zaai in de eerste week van april was de tijd tot 50% opkomst 36 –

38 dagen. Deze tijdsduur nam af tot ongeveer 8 – 11 dagen bij zaai in juni, juli of de eerste week van augustus. Bij zaaitijden later in het jaar nam de tijd tot 50% opkomst weer toe. De tijd van zaai tot 50% lichtonderschepping liet een zelfde verloop met zaaitijd zien: de minimum tijd was 35 – 40 dagen voor zaaitijden van begin juni tot half juli. Er is geen voordeel in zaai eerder dan mei, in overeenstemming met de bovengenoemde relatie tussen opkomst en (bodem)temperatuur. De groei van gewassen die na het einde van juli gezaaid werden varieerde behoorlijk tussen de verschillende jaren en locaties. Daarom is het seizoen dat geschikt is voor het zaaien van *S. sisymbriifolium* in Nederland de periode tussen mei en juli. Gemeten bovengrondse drogestofhoeveelheden waren tot 400 g m^{-2} 7 weken na opkomst en tot 1040 g m^{-2} gemeten 14 weken na opkomst. De bovengrondse drogestof nam toe met plantdichtheid (variërend van 40 tot 230 planten m^{-2}) 7 weken na opkomst, maar 14 weken na opkomst waren geen significante verschillen in bovengrondse drogestof meer aanwezig. Stralingsgebruikefficiëntie was 1.69 (SE =0.0208) g MJ^{-1} voor onderschepte fotosynthetisch actieve straling. Hoeveelheden bovengrondse drogestof waren wat hoger in Wageningen (51° 58’ N) op een lichte zandgrond dan in Flevoland (52° 31’ N) op kleigrond en in Drenthe (52° 51’ N) op dalgrond.

Wortelexudaten van *Solanaceae*, zoals *S. sisymbriifolium*, stimuleren ACA om uit hun cyste te komen. Deze stimulering is gerelateerd aan de wortellengteverdeling onder een gewas. Er is een mathematisch model gemaakt om de minimum wortellengtedichtheid te berekenen, die nodig is om een bepaalde fractie P van alle cysten binnen de zone van invloed van wortelexudaten van de dichtstbijzijnde wortel te brengen (Hoofdstuk 4). Omdat de grootte van de straal van de zone van invloed van wortelexudaten rondom een wortel niet bekend is, zijn berekeningen gemaakt voor een aantal aannames van straalgrootte. Een serie veldproeven is uitgevoerd om de werkelijke wortellengteverdeling onder een *S. sisymbriifolium* gewas te bestuderen en te relateren aan bovengrondse gewaseigenschappen, om input te leveren voor het model. De natuurlijke logaritme van de totale wortellengte was lineair gerelateerd aan de natuurlijke logaritme van de bovengrondse biomassa en aan de natuurlijke logaritme van de bladoppervlakte per eenheid van grondoppervlakte (‘leaf area index’). Worteldiameter verdeling was hetzelfde voor alle gewassen, en onafhankelijk van diepte. Ongeveer 50% van de totale wortellengte bestond uit dunne wortels (<0.4 mm diameter). Het mathematische model, bestaande uit een aangepaste Poisson-verdelingsformule voor de driedimensionale verdeling van wortels in een bodemvolume, is gebruikt om af te leiden tot op welke diepte 75% van alle cysten zich binnen de zone van invloed van wortelexudaten van een wortel bevindenden. Deze diepte is vervolgens gerelateerd aan bovengrondse drogestof en totale wortellengte. Dit is gedaan voor zones van invloed van wortelexudaten van 0.50 cm, 0.75 cm en

1.00 cm rondom elke wortel. De hier gepresenteerde methode om lokking van ACA uit hun cyste te relateren aan gewaseigenschappen heeft verdere validatie nodig; vooral de aannames van de diffusieafstand van wortelexudaten is een kritiek punt.

Er zijn proeven uitgevoerd om verschillende perioden van gewasgroei van *S. sisymbriifolium* en wortellengtedichtheid te koppelen aan de stimulering van ACA om uit hun cyste te komen (Hoofdstuk 5). Hierbij zijn metingen onder braak en vatbare aardappel als referenties gebruikt. Eén en twee jaar oude cysten van *G. pallida* zijn hierbij gebruikt in twee jaren in kasexperimenten met bakken gevuld met onbesmette grond. Twee methoden zijn gebruikt om lokking van ACA te meten. In de eerste methode zijn ronde gaten van 7.5 cm diameter geboord in de grond en weer opgevuld met door ACA besmette grond. Deze besmette grond werd bemonsterd in de tijd om de lokking van ACA uit hun cysten te volgen. In de tweede methode werden cysten in nylon zakjes begraven, en weer opgegraven op verschillende tijdstippen om de lokking in de tijd te volgen. De besmette grond in de eerste methode had eigenschappen die een beetje verschilden van die van de omringende grond en deze verschillen waren waarschijnlijk de oorzaak van de veel lagere wortellengtedichtheden in de besmette grond dan in de omringende grond. Daarom werd de lokking van *S. sisymbriifolium* zwaar onderschat met de eerste methode. Lokking van ACA uit hun cysten (gemeten in de nylon zakjes) nam toe naarmate *S. sisymbriifolium* langer op de besmette grond groeide en varieerde van 47% na 44 dagen gewasgroei tot 75% na 150 dagen gewasgroei (gemiddeld over alle dieptes waarop zakjes begraven waren). Lokking per dieptelaag was ook gerelateerd aan wortellengtedichtheid en varieerde van 43% bij 0.26 cm cm^{-3} wortel tot 85% bij 5.8 cm cm^{-3} wortel. Een algemene methode is gepresenteerd: hiermee kan, op basis van een negatief exponentiele functie, voor elke beheersmaatregel van ACA de winst in tijd (jaren) worden berekend, tot populatieafname beneden een bepaalde gekozen minimum populatiedichtheid ten opzichte van een situatie zonder beheersmaatregelen. Voor *S. sisymbriifolium* varieerde de berekende tijdwinst ten opzichte van braak tussen de 2.3 jaar voor 60% lokking (wat gemeten werd na 90 dagen gewasgroei) en 4.4 jaar voor 75% lokking (gemeten na 150 dagen gewasgroei). Deze tijdwinst was onafhankelijk van de initiële en uiteindelijke populatiedichtheden van ACA. De gevonden resultaten bevestigen de effecten van *S. sisymbriifolium* om ACA uit hun cyste te lokken, onderstrepen het belang van duur van de gewasgroei en wortellengtedichtheid als bepalende factoren voor dit lokkende effect en vestigen de aandacht op mogelijke methodologische problemen bij het bestuderen van lokking van ACA.

Een kasproef is uitgevoerd om de tolerantie van *S. sisymbriifolium* voor een reeks van besmettingsniveaus met ACA (0 – 578 nematoden g^{-1} droge grond) te testen (Hoofdstuk 6). Voorgeweekte *S. sisymbriifolium* zaden werden in drie liter grote

potten met kunstgrond (mengsel van zilverzand, gemalen hydrokorrels en kleipoeder) gezaaid. Planthoogte werd op verschillende tijdstippen gemeten. Het bovengrondse drooggewicht van de planten werd na 103 dagen groei gemeten. Twee modellen werden gebruikt om de relatie tussen de bovengrondse drogestof en het besmettingsniveau met ACA te beschrijven: een exponentieel model en een hyperbolisch model. Bij de twee hoogste besmettingsniveaus (288 en 578 nematoden g^{-1} droge grond) was de bovengrondse drogestof lager dan de onbesmette controle. Het exponentiële model liet schadedrempels zien voor bovengrondse drogestof en planthoogte van respectievelijk 24 en 36 nematoden g^{-1} droge grond waar beneden geen reductie in groei werd waargenomen. In vergelijking met literatuurgegevens van aardappelcultivars is deze schadedrempel erg hoog. Ook het hyperbolische model liet erg hoge tolerantie van *S. sisymbriifolium* voor ACA zien: de bovengrondse drogestof en de planthoogte waren gehalveerd bij respectievelijk 369 en 1120 nematoden g^{-1} droge grond. Beide modellen beschrijven de gemeten relatie nauwkeurig genoeg. In principe kunnen beide modellen gebruikt worden om risico's op groeischade van *S. sisymbriifolium* in veldbesmettingshaarden te berekenen. Er wordt echter geconcludeerd dat de tolerantie van *S. sisymbriifolium* voor ACA zo hoog is, dat omvangrijke gewasschade, veroorzaakt door ACA, niet verwacht wordt op te treden.

Tenslotte zijn twee experimenten uitgevoerd om de gevoeligheid van *S. sisymbriifolium* voor de aardappelziekte, veroorzaakt door de oomyceet *Phytophthora infestans* (Mont.) de Bary, te bestuderen (Hoofdstuk 7). In een laboratoriumexperiment zijn afgeknipte bladeren van *S. sisymbriifolium* en *S. tuberosum* (L.) cv. Bintje geïnoculeerd met twee isolaten (90128, IPO428-2); infectie-efficiënties en toename in lesiestraal zijn vergeleken. Omdat uit de eerste resultaten bleek dat de virulentie van de gebruikte isolaten voor *S. sisymbriifolium* niet erg groot was, is besloten het vervolg van het onderzoek uit te voeren met isolaat WAU01001, dat oorspronkelijk was geïsoleerd uit een geïnfecteerd *S. sisymbriifolium* veldgewas en het meest virulente isolaat voor *S. sisymbriifolium* leek dat verkrijgbaar was. In een veldproef zijn hiermee planten van *S. sisymbriifolium* en van *S. tuberosum* cv. Bintje en cv. Désirée geïnoculeerd om het bladoppervlakteverlies te vergelijken. De resultaten van de laboratoriumproef lieten zien dat *S. sisymbriifolium* behoorlijk resistent was tegen de gebruikte isolaten. Infectie-efficiënties varieerden van 20 – 36%, terwijl die 100% waren voor de aardappelcultivar Bintje. Als wel infectie van *S. sisymbriifolium* optrad, groeiden de lesies niet of heel langzaam in de tijd. In de veldproef hadden de aardappelplanten 100% van hun bladoppervlak verloren na 28 – 42 dagen (cv. Bintje) en na 56 dagen (cv. Désirée). *S. sisymbriifolium* planten daarentegen hadden na 42 dagen slechts 9 – 13% van hun bladoppervlak verloren. De relative oppervlakte onder de ziektevoortgangscurve was 0.80 voor aardappelcultivar Bintje, 0.71 voor

aardappelcultivar Désirée en varieerde tussen de 0.08 en 0.11 voor *S. sisymbriifolium*. Het kan geconcludeerd worden dat de gevoeligheid van *S. sisymbriifolium* voor *P. infestans* erg klein is. Het risico dat *S. sisymbriifolium* een bron van *P. infestans* voor aardappelgewassen gaat vormen en het risico op de ontwikkeling van nieuwe virulente isolaten kan beperkt worden door een gewas tijdig te vernietigen. In september moet de groei van het gewas worden beëindigd om het risico op *P. infestans* infectie te verkleinen en oosporenvorming te voorkomen, bijvoorbeeld door *S. sisymbriifolium* onder te ploegen. Verschillende bronnen vermelden dat het verbouwen van *S. sisymbriifolium* verschillende andere bodemgebonden plagen en ziekten van aardappelgewassen niet verergert.

In de ideale situatie zou *S. sisymbriifolium* voor of na een ander, economisch interessant gewas verbouwd moeten kunnen worden. De huidige studie laat echter zien dat zaaitijden succesvol waren tussen mei en het einde van juli. Dit beperkt de mogelijkheden om *S. sisymbriifolium* in het zelfde seizoen als een ander, hoofdgewas te verbouwen. Verder roept het de verwachting op dat in noordelijker, koudere gebieden het gewas altijd het hoofdseizoen voor groei nodig zal hebben, terwijl in zuidelijker, warmere gebieden er meer mogelijkheden zullen zijn om *S. sisymbriifolium* te telen als een vanggewas voor of na een hoofdgewas met een relatief korte groei periode (bv. sommige groentes). Efficiënte vanggewassen tegen ACA hebben veel voordelen ten opzichte van andere beheersmaatregels zoals nematicides en het veredelen op resistentie van aardappels. Daarom verdient *S. sisymbriifolium* verdere ontwikkeling als vanggewas voor ACA.

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PE&RC PhD Education Statement Form

With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 22 credits (= 32 ECTS = 22 weeks of activities)

Review of Literature (4 credits)

- *Solanum sisymbriifolium*, a trap crop for potato cyst nematodes: general introduction (2005)

Writing of Project Proposal (1 credit)

- Functional-structural modelling of a Barley-*Capsella* system (2001)

Post-Graduate Courses (3 credits)

- Scientific writing (2001)
- Modelling and techniques in nematology (2005)

Deficiency, Refresh, Brush-up and General Courses (7 credits)

- Inleiding in nematologie (2002)
- Modelling potential crop growth (2002)
- Project and time management (2002)

PhD Discussion Groups (5 credits)

- Plant and crop ecology (2001-2005)

PE&RC Annual Meetings, Seminars and Introduction Days (1 credit)

- PE&RC day: “Food Insecurity” (2001)
- PE&RC day: “Ethics in Science” (2002)
- Risks of *P. infestance* to *Solanum sisymbriifolium* (2002)
- PE&RC day: “Global Climate Change & Biodiversity (2003)

International Symposia, Workshops and Conferences (5 credits)

- 15th Triennial conference of EAPR (2002)
- 55th International crop protection symposium, Ghent University (2003)
- 8th ESA conference (2004)
- 16th triennial conference of EAPR (2005)
- Nederlandse nematologen bijeenkomst (2005)

Laboratory Training and Working Visits (2 credits)

- NIOO Zeeland, Whinrhizo training (2001)
- PRI Nematology, Training potato cyst nematodes laboratory techniques (2002)

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Curriculum vitae

Bart Gerardus Hubertus Timmermans was born on the 12th of February 1978 in Sittard. The secondary school he attended was the “Bisschoppelijk College Schöndeln” in Roermond, and he graduated from the Gymnasium in 1996. Next, he started studying Biology at Utrecht University and finished his Propedeuse cum laude. In his further studies, he specialized in plant ecology. His first MSc thesis was conducted at the Plant Ecology group, under supervision of Dr. M. Soons and Dr. G.W. Heil. This thesis concerned the isolation of patches of blue grassland, that get rare in The Netherlands. He constructed a mathematical model to calculate the seed dispersal distance by wind of typical blue grassland species. Measurements of seed characteristics, wind velocity profiles and actual dispersal distances were performed to parameterize and validate the model calculations. He performed his second MSc thesis at the Landscape Ecology group, under supervision of Dr. ir. M. Hefting and Prof. dr. J. Verhoeven. It concerned the denitrifying functioning of wetlands at the border of fields grown with maize crops year after year. An existing model for C, N and P dynamics was adapted to two such wetlands. Measurements (including tree height and diameter, and soil water levels in time) were done to collect necessary parameter values. He finished his studies in 2001 and in that year he started a PhD-project at the Crop and Weed Ecology Group of Wageningen University, under supervision of Dr. ir. J. Vos, Dr. ir. T.J. Stomph and Prof. dr. M.J. Kropff. The project concerned the development of *S. sisymbriifolium* as a trap crop for potato cyst nematodes, which is described in this thesis.