

**Biotic and abiotic soil factors in the succession
of sea buckthorn, *Hippophaë rhamnoides* L.
in coastal sand dunes**

Ontvangen
12 MEI 1995
UB-CARDEX

F.C. Zoon



CENTRALE LANDBOUWCATALOGUS

0000 0577 1460

409332

40951

Promotoren: dr. L. Brussaard
 hoogleraar in de bodembiologie

 dr. ir. J.W. Woldendorp
 hoogleraar in de biologie van de rhizosfeer

Co-promotor: dr. A.D.L. Akkermans
 universitair hoofddocent

NN08201, 1981

Frans Zoon

**Biotic and abiotic soil factors in the succession
of sea buckthorn, *Hippophaë rhamnoides* L.
in coastal sand dunes**

Proefschrift

ter verkrijging van de graad van doctor
in de landbouw- en milieuwetenschappen
op gezag van de rector magnificus
dr. C.M. Karssen
in het openbaar te verdedigen
op maandag 22 mei 1995
des namiddags te vier uur in de Aula
van de Landbouwuniversiteit te Wageningen

ISBN: 907882

CIP-DATA KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Zoon, F.C.

Biotic and abiotic soil factors in the succession of sea buckthorn, *Hippophaë rhamnoides* L. in coastal sand dunes. /

F.C. Zoon, - [S.l. : s.n.]

Thesis Wageningen. - With ref. - With summary in Dutch.

ISBN

Subject leadings: soil biology / vegetation succession / *Hippophaë rhamnoides*

BIBLIOTHEEK
LANDBOUWUNIVERSITEIT
WAGENINGEN

This thesis contains results of a research project of the Netherlands Institute of Ecology, Centre of Terrestrial Ecology, PO Box 40, 6666 ZG Heteren, The Netherlands. The study was supported by the Netherlands Integrated Soil Research Programme.

The authors present address is:

DLO - Research Institute for Plant Protection (IPO-DLO)

Postbox 9060, 6700 GW Wageningen, The Netherlands.

STELLINGEN

1. Degeneratie van *Hippophaë rhamnoides* is in het algemeen te wijten aan fosfaatgebrek en niet aan stikstofgebrek.

Dit proefschrift.

2. *Tylenchorhynchus microphasmis* versnelt de degeneratie en successie van *H. rhamnoides*.

Dit proefschrift.

3. In de periode kort na de vestiging van de duindoorn in helmduinen wordt de populatieontwikkeling van *Tylenchorhynchus microphasmis* beperkt door de lage stikstofbeschikbaarheid.

Dit proefschrift.

4. Schade aan duindoornwortels ten gevolge van nematoden in vroege stadia van duindoornvegetatie vertraagt de stikstofaccumulatie, voorkomt daarmee verbetering van de voedselkwaliteit voor deze nematoden en stelt successie door andere plantesoorten uit. Dit is de oorzaak van de lange duur van het duindoornstadium in de successie van duinvegetatie.

Doing H., 1988. Landschapsoecologie van de Nederlandse Kust. Stichting Duinbehoud. Leiden, 228 pp.; Dit proefschrift.

5. Verstuiving leidt tot 'terug naar af' voor duindoornvegetatie.

Dit proefschrift.

6. De gedurende de successie toenemende bezetting van duindoorn met VA-mycorrhiza en de ogenschijnlijk lage effectiviteit van deze symbiose rechtvaardigen nader onderzoek naar de rol van fungivore bodemfauna in het degeneratieproces.

Dit proefschrift.

7. Het zoeken naar *Rhizobium*-stammen die in staat zijn tot nodulatie in aanwezigheid van sedentaire endoparasitaire wortelnematoden heeft weinig zin.

Huang J.S., 1987. In: Vistas on Nematology. J.A. Veech & D.W. Dickson (eds.), Hyattsville, Society of Nematologists, pp. 301-306.

8. Indien het vermelden van volledige namen van tijdschriften in de literatuurlijst bij publicaties ter verheldering moet dienen, moet worden voorkomen dat de argeloze lezer door verzinsels als 'Revue Nématologique' of 'Animals of applied biology' op een dwaalspoor wordt gebracht.

Fasan T. & Haverkort A.J., 1991. Neth. J. Pl. Path. 97: 151-161.

9. Gelukkig voor schaatsliefhebbers en slootleven heeft ijs een veel groter warmtegeleidingsvermogen dan water.

Barkman J.J. & Stoutjesdijk P., 1987. Microklimaat, Vegetatie en Fauna. Wageningen, Pudoc, 223 pp.

10. Maatregelen die verdroging tegengaan, kunnen bijdragen aan het voorkomen van overstromingen.

11. Genetische modificatie van planten wordt al millennia toegepast door bepaalde bladluizen en nematoden die door het inbrengen van virussen in de plant de voedingswaarde voor zichzelf verhogen.

Hijner J.A. & Martinez Cordon F., 1953. Med. Inst. Rationele Suikerproductie 23: 251-270.; Ayala A. et al., 1970. J. Agric. Univ. Puerto Rico 54: 341-369.

12. Vrijlevende wortelaaltjes bestaan niet.

Maas P.W.Th., 1976. Gewasbescherming 7: 141-144.

Stellingen behorende bij het proefschrift van Frans Zoon: 'Biotic and abiotic soil factors in the succession of sea buckthorn, *Hippophaë rhamnoides* L., in coastal sand dunes.' Wageningen, .. mei 1995.

1998
XZ 02/12-80

CONTENTS

| | |
|---|-----|
| ABSTRACT | 7 |
| VOORWOORD | 9 |
| CHAPTER 1. General introduction | 11 |
| CHAPTER 2. Ecology of the plant feeding nematode fauna associated with sea buckthorn (<i>Hippophaë rhamnoides</i> L. ssp. <i>rhamnoides</i>) in different stages of dune succession. | 23 |
| CHAPTER 3. Interactive effects of rhizosphere fungi and nematodes on the actinorhiza and growth of <i>Hippophaë rhamnoides</i> . | 49 |
| CHAPTER 4. A device for automatic soil moisture control and water use registration in pot experiments. | 65 |
| CHAPTER 5. A rapid measurement of root length and -branching by microcomputer image analysis. | 73 |
| CHAPTER 6. The effect of soil moisture on the relationship between the nematode <i>Tylenchorhynchus microphasmis</i> Loof, and sea buckthorn (<i>Hippophaë rhamnoides</i> L. ssp. <i>rhamnoides</i>). | 85 |
| CHAPTER 7. Belowground herbivory on actinorhizal sand dune plants: Effects of <i>Tylenchorhynchus microphasmis</i> Loof on growth and nodulation of <i>Hippophaë rhamnoides</i> L. ssp. <i>rhamnoides</i> . | 93 |
| CHAPTER 8. The influence of the nematode <i>Tylenchorhynchus microphasmis</i> Loof and the root fungus <i>Cylindrocarpon destructans</i> Zinns. Scholten on growth and nodulation of sea buckthorn (<i>Hippophaë rhamnoides</i> L. ssp. <i>rhamnoides</i>). | 111 |
| CHAPTER 9. General discussion | 127 |
| SUMMARY | 137 |
| SAMENVATTING | 139 |
| CURRICULUM VITAE | 143 |

ABSTRACT

Zoon, F.C. 1995. Biotic and abiotic soil factors in the succession of sea buckthorn, *Hippophaë rhamnoides* L. in coastal sand dunes. Ph.D. Thesis, Wageningen Agricultural University, Wageningen, The Netherlands. 143 pp.

Decline and succession in dune scrub vegetation of sea buckthorn, *Hippophaë rhamnoides* L. in the Netherlands was previously found to be stimulated by biotic soil factors. In the present study, the role of plant-parasitic nematodes and root fungi was investigated. A field survey showed that during the ageing of *H. rhamnoides* vegetation there was a change in the plant-feeding nematode fauna. Certain nematode species, such as *Tylenchorhynchus microphasmis* Loof, were present in different stages and their population increased from stage to stage when expressed per unit of root length. The fraction of root length infected by VA-mycorrhizal fungi increased, as did soil concentrations of nitrogen and organic matter. Pot experiments with selective inhibition of groups of soil organisms indicated that soil fungi could be harmful to *H. rhamnoides* in the presence of nematodes, although fungi were not harmful on their own. The impact of various densities of *T. microphasmis*, either alone or in combination with the nitrogen-fixing actinorhizal symbiont *Frankia* or the fungus *Cylindrocarpon destructans* Zinnschm. Scholten, was assessed in pot experiments with controlled soil moisture conditions. Results showed inhibitory effects of the nematode on growth and on the acquisition of certain nutrients (especially phosphate). Although root nodulation and nitrogen fixation by *Frankia* were reduced, this did not appear to be the primary cause of growth reduction. No synergic interaction was found between *T. microphasmis* and *C. destructans* in their effect on growth of *H. rhamnoides*. The results of this study support the hypothesis that the increase in the concentration of soil nitrogen in the field stimulates nematode activity which in its turn impedes the acquisition of phosphate by *H. rhamnoides*. This reduces the competitive ability of this shrub and gives way to other plant species in the vegetation which are less sensitive to root damage.

Key words: *Cylindrocarpon*, *Frankia*, *Hippophaë rhamnoides* (Eleagnaceae), plant-parasitic nematodes, nitrogen fixation, nutrient acquisition, sand dune vegetation, soil-borne pathogens, succession, *Tylenchorhynchus microphasmis*.

VOORWOORD

Bij het gereedkomen van dit proefschrift wil ik graag iedereen bedanken die in verschillende fasen en op verschillende plaatsen aan het totstandkomen ervan hebben bijgedragen. De gemoedelijke en zeer stimulerende sfeer in die fantastische jaren op Weevers' Duin en in Heteren hebben veel betekend voor mijn wetenschappelijke vorming. Het is onmogelijk iedereen te noemen van wie ik zeker weet dat ze in die sfeer en vorming de hand hebben gehad.

In het bijzonder dank ik mijn promotoren en begeleiders Jan Woldendorp, Lijbert Brussaard, Antoon Akkermans, Sep Troelstra, Cees van Dijk, Paul Maas, Riks Laanbroek en Ton van der Wal voor hun inzet, commentaren, en het eindeloze geduld bij de afronding van de publicaties en het proefschrift.

Jan, Lijbert en Antoon, jullie bereidheid om de klus in de laatste fase te begeleiden heb ik als een enorme rijkdom ervaren.

Dirk Koudijs, Arie de Zwart, Willy Keultjes en Ab Wijlhuizen: bedankt voor jullie inzet bij het maken van verscheidene technische hoogstandjes; Fred Hage en Peter van Tienderen voor hard- en software en theoretische onderbouwing voor artikelen; Ron de Goede voor introductie in CANOCO; Tom Bongers en Henk Doing voor hun interesse en discussies over de ecologie en successie van respectievelijk aaltjes en duindoorn; Gerrit Bollen voor de mycologische en bodemecologische basis; Henk Brinkman en Pieter Loof voor hulp bij nematoden-determinaties; Wilma Akkermans voor het verstrekken van *Frankia*-materiaal en een gaschromatograaf en Wim Verholt voor fotografische assistentie.

Het verzetten van bergen werk was mede mogelijk door Lindian Heindijk, Leonie van de Voort, Anja van Herp en Inez Scheurleer, die in het kader van een stage of afstudeeronderwerp aan het onderzoek meewerkten.

Dit promotieonderzoek heeft lang op haar afronding moeten wachten. Dit bracht een extra belasting voor mijn naaste omgeving en mijn begeleiders met zich mee. Ik ben erg dankbaar voor al die mensen die met geduld en vertrouwen het verloop hebben gevolgd en me steeds weer stimuleerden. Liesbeth, Thijs, Pepijn en Jet, familie en vrienden, ik was er te weinig voor jullie. Fijn dat we er zijn.

Frans

CHAPTER I.

General introduction

Succession in the vegetation of coastal sand dunes

Vegetation succession has been in people's awareness as long as mankind inhabits its dynamic environment. Major development of the theory of succession as a universal and ongoing process in vegetation was gained by the beginning of this century (Cowles, 1911; Clements, 1916). Clements (1904: cited in Miles, 1987) distinguished between *primary succession* on newly exposed surfaces which had never borne vegetation before, and *secondary succession* after disturbance of the vegetation on developed soils. The initial attempts to devise an elaborate universal concept of succession were bound to fail, because of the wide range of processes regulating plant growth and dispersal in the vegetation. The mechanisms involved in succession may be quite diverse, even within a relatively uncomplicated coastal dune ecosystem. Recent studies and theoretical textbooks are generally devoted to the understanding of these component processes and mechanisms, such as competition, dispersal and nutrient cycling (Gray *et al.*, 1987; Grace & Tilman, 1989; Olff, 1992).

The stability and formation of coastal dune soils is greatly dependent on the presence of vegetation. Higher plants reduce wind speed, catch drifting sand, improve the cohesion of soil aggregates by their roots, mycorrhiza and rhizosphere bacteria (Koske & Polson, 1984; Forster & Nicolson, 1981; Forster, 1990) and stimulate weathering of soil minerals. Plant species differ in their ability to establish and grow in the various zones of the very dynamic dune landscape, due to differences in dispersal, morphology, growth characteristics, ecophysiology and biotic interactions. Thus, a sequence of vegetation types can be found on transects from sea to inland dunes often concurring with the increasing age of the soil. It is often assumed that the geographic transect from the sea towards inland dunes (*toposequence*) reflects the course of primary vegetation succession (*chronosequence*) (Olsen, 1958; Crawley, 1993) and in some cases such pathways have indeed been elucidated by vegetation monitoring (e.g. Londo, 1974; Van Dorp *et al.*, 1985; Olff, 1992). Proportionality of time and space on such a transect

(correlation between chronosequence and toposequence) is best in situations with a steadily advancing coastline and absence of severe disturbance. However, these ideal situations are rare and thus long-term monitoring of vegetation and soil factors is often indispensable for an accurate description of processes and sequences of succession.

Various models have been postulated according to which succession can proceed (see Miles, 1987). Connell & Slatyer (1977) emphasized the effects and interactions of plants in succession and distinguished models of facilitation, tolerance and inhibition. *Facilitation* is the modification of a site by biotic factors, which make it suitable for succeeding species. This mechanism certainly plays a role in dune vegetation, because without the effects of the early sand-binding vegetation no dunes would exist for plants to inhabit (Crawley, 1993). The accumulation of nitrogen from N_2 -fixing root symbionts of plants may also facilitate the growth of succeeding species. *H. rhamnoides* is the first and most dominant plant species with symbiotic N_2 -fixation in dune succession in the Netherlands and many other places in Western Europe (Stewart & Pearson, 1967; Akkermans, 1971). In dune areas in other parts of the world other leguminous or non-leguminous plant species may occupy a similar niche (Koske & Halvorson, 1981), but sometimes these plants occur only scarcely or rather late in the succession (Olson, 1958). Plant pathogens may retard facilitation, and in some cases cause a cyclic succession of two interacting plant species, such as clover and grass in a mixture (Ennik *et al.* 1965; Korteweg & s'Jacob, 1980).

The *tolerance* model includes succession due to introduction of plant species without strong competition with the present vegetation. This may occur during early primary succession and after disturbances which remove a large part of the vegetation and create vacant niches. Such disturbances are common in secondary succession, but remaining plant propagules may strongly influence the initial development there.

Inhibition is generally related to competition between plant species. A gradual change in resources (e.g. light, nutrients) will lead to a shift in the relative abundance of plant species with different competitive ability for these resources (Tilman, 1985; Olff, 1992). However, soil biota may affect resource uptake and utilization and thus competitive ability is not an intrinsic plant characteristic. Beneficial soil organisms, such as mycorrhizal fungi, may determine the balance between competing plant species in a mixture (Grime *et al.*, 1987; Newman, 1991). Soil-borne

pathogens may reduce the competitive ability of a plant species in the vegetation (Clements, 1928; Crawley, 1993) and thereby affect the rate and direction of succession (Van der Putten *et al.*, 1993). Although soil-borne diseases and pests are a major topic in agriculture, relatively few examples are available of their role in mixed vegetation and natural ecosystems (Ennik *et al.*, 1965; Oremus, 1982; Weste, 1986; De Nooij *et al.*, 1986; Van der Putten *et al.*, 1988; Brown & Gange, 1991; Seliskar & Huettel, 1993). The mechanisms by which harmful soil organisms affect the growth of plant species within a mixed vegetation are often unknown, especially when also beneficial soil organisms play a role, as in the case of the non-leguminous nitrogen-fixing and VA-mycorrhizal dune shrub *Hippophaë rhamnoides*. Pathogens and pests are suggested to be more important in a vegetation with a few dominant species, rather than in species-rich seral stages (Crawley, 1993), but this may depend on the specificity of their effects on different plant species.

Abiotic and biotic development of dune soils

Succession in dune vegetation coincides with changes in abiotic and biotic characteristics of the soil. Development of abiotic soil factors is only in part an autonomous chemico-physical process. Effects of the vegetation on soil development are often obvious, but rather poorly quantified. The main abiotic developments in coastal sand dune systems are 1. reduction of wind speed and subsequent smaller particle size of sand deposits inland, 2. reduction of salt spray, 3. accumulation of organic matter and nitrogen in the top soil (Olson, 1958; Willis *et al.*, 1959; Vitousek & Walker, 1987; Wallén, 1980; Olf, 1992), 4. weathering of calcium carbonate and eventual soil acidification (Salisbury, 1925; Olson, 1958; Stuyfzand, 1984) and 5. reallocation and leaching of other minerals (Syers & Walker, 1969a, b; Walker *et al.*, 1981). All of these abiotic soil processes may, directly or indirectly, affect plant growth and composition of the vegetation.

Relatively little is known about the development of the wide variety of biotic components of coastal sand dune soils and even less is known about the causes and effects of the development of soil biota. Yet, there are a few descriptive studies on fungal flora, mites, collembola and nematodes and on the role of mycorrhizal fungi and N₂-fixing organisms in dune soils. Differences in fungal flora of bulk soil and rhizosphere have been documented for different successional stages (Webley

et al., 1952; Brown, 1958; Pugh, 1970). Soil-borne fungi were suggested to play a role in the decline of marram grass, *Ammophila arenaria* (Van der Putten *et al.*, 1990).

Soil mesofauna is present in dune soil even before plants have become established. Various successional stages of lime-poor sand dunes on the North Sea and Baltic coast have characteristic species of Gamasina (Acari) and Collembola (Koehler & Weidemann, 1993). Nematodes are amongst the very early pioneers in coastal dune soils and the species-rich nematode fauna develops with dune age (Yeates, 1968; Bussau, 1990a and 1990b; Kisiel, 1970). Similarly, a clear development of the total nematode community was found in successional transects on inland drift sands (De Goede *et al.*, 1993) and inland sand dunes (Wasilewska, 1970 and 1971). The typical nematode fauna of successional stages seems to be determined by characteristics of vegetation and soil and by the rate of dispersal of the various nematode species. Plant-feeding nematodes may be involved in the decline of the dune pioneers *Ammophila arenaria* (Van der Putten & Troelstra, 1990), *Ammophila breviligulata* (Seliskar & Huettel, 1993) and *H. rhamnoides* (Oremus, 1982; Maas *et al.*, 1983).

Studies on the role of VA-mycorrhiza in dune soils indicate effects of extramatrical fungal hyphae on soil stability due to increased cohesion of sand grains (Koske & Polson, 1984). In Addition, VA-mycorrhiza are very important for plant growth in the nutrient-poor dune soils (Nicolson & Johnston, 1979; Ernst *et al.*, 1984; Gardner & Clelland, 1984). A gradual shift of VAM-fungal species was observed during succession (Rose, 1988).

Also, nitrogen-fixing organisms may be crucial for early colonizing plant species in coastal sand dunes, especially behind the seaward dune ridges where the input of organic nitrogen from the sea has diminished (Fay & Jeffrey, 1992). Free-living N₂-fixing bacteria (*Azotobacter*, *Bacillus*) were found in the rhizosphere and on leaves of *Ammophila arenaria* and other pioneer dune plants (Hassouna & Wareing, 1964; Akkermans, 1971; Abdel Wahab & Wareing, 1980), but their contribution to the nitrogen supply is rather small (Akkermans, 1971). Symbiotic N₂ fixation by the actinomycete *Frankia* occurs in *H. rhamnoides* root nodules and may account for a yearly input between 2 and 58 kg N per ha (Akkermans, 1971; Stewart & Pearson, 1967; Oremus, 1982). N₂ fixation in *H. rhamnoides* apparently covers only a small part of the nitrogen budget and is less efficient than uptake of mineral N (Troelstra, *et al.*, 1987). Deposition of atmospheric nitrogen in coastal dune areas in the Netherlands is estimated at 20 kg.ha⁻¹.year⁻¹ (Stuyfzand, 1984).

Sea Buckthorn in coastal dune vegetation in the Netherlands

The Sea Buckthorn, *Hippophaë rhamnoides* L. is a dioecious perennial shrub belonging to the family Elaeagnaceae. The species has its distribution from Central China all along Southern Eurasian mountainous areas on to the coastal areas in Western Europe, including the British isles (Pearson & Rogers, 1962). In *H. rhamnoides* nine subspecies are distinguished (Rousi, 1971), two of which are native to Western Europe. The subspecies *fluviatilis* occurs inland, especially in mountainous areas and on river banks. The subspecies *rhamnoides* is found along the coasts of North-Western Europe. The present distribution of *H. rhamnoides* in Western Europe is considered to be a relict of its common presence in late glacial times (Hafsten, 1987; Bohnke *et al.*, 1988). This thesis deals with *H. rhamnoides* L. ssp. *rhamnoides* in the Netherlands. In the course of this thesis, the genus name *Hippophaë* and the species name *H. rhamnoides* are used to indicate *H. rhamnoides* L. ssp. *rhamnoides*, unless stated otherwise.

H. rhamnoides is the dominant plant species in the pioneer scrub vegetation on calcareous coastal sand dunes. Due to N-fixation by the symbiotic actinomycete *Frankia* in its root nodules and the subsequent accumulation of nitrogen *Hippophaë* is likely an important facilitating factor in the succession in dune vegetation. The phytosociological position of *H. rhamnoides* (ssp. *rhamnoides*) has been discussed by a number of authors (Boerboom, 1960; Rogers, 1961; Beeftink, 1965; Skogen, 1972; Sloet van Oldruitenborgh, 1976; Van Dorp *et al.*, 1985; Van der Maarel *et al.*, 1985). Westhoff & Den Held (1969) recognized 5 associations with *H. rhamnoides* in the Netherlands. *H. rhamnoides* invades the *Elymus-Ammophila* dunes and may form dense mono-species thickets for a decade or more. On the calcareous soils of the *Dune District* these scrubs gradually degenerate to a poor grass vegetation or become dominated by other shrub species, such as *Ligustrum vulgare* L., *Sambucus nigra* L., *Rosa rubiginosa* L. and *Crataegus monogyna* L. (Sloet van Oldruitenborgh, 1976; Van Dorp *et al.*, 1985; Van der Maarel *et al.*, 1985). Continued mixed-shrub development seems to occur mainly at undisturbed sites where water availability and accumulation of organic matter is high (Van der Meulen & Wanders, 1985). In the *Wadden District*, where the initial lime content is lower, *H. rhamnoides* is succeeded by *Empetrum nigrum* L. and *Polypodium vulgare* L. vegetation (Westhoff & Van Oosten, 1991) or *Rosa pimpinellifolia* L. on the southern part of the isle of Texel (F. Zoon, pers.obs.).

H. rhamnoides is a clonal species which proliferates by means of rhizomes. Individual shoots (ramets) rarely become older than 25 years, although in Scandinavia a few 80 year old ramets have been reported (Skogen, 1972). Effective emergence from bird-dispersed seeds occurs in moist and open habitats (F.C. Zoon, personal observation). The capacity to grow in nutrient-poor dune soils is in part merited by root symbioses with nitrogen-fixing *Frankia* actinomycetes (Akkermans, 1971; Stewart & Pearson, 1967; Oremus, 1982; Troelstra *et al.*, 1987) and vesicular-arbuscular mycorrhizal (VAM) fungi (Gardner *et al.*, 1984). Actinorhizal and mycorrhizal symbioses of *H. rhamnoides* are mutually supportive under nutrient-poor conditions in sand culture (Gardner *et al.*, 1984). Various quantitative aspects of the actinorhiza of *H. rhamnoides* in dune soil were studied by Oremus (1982). It was observed that the age of the root nodules rarely exceeds 3 to 4 years. This means that nodulation must take place continuously, in order to maintain the nitrogen-fixing capacity. The amount of infective propagules may be a limiting factor to nodulation in colonization stages (Oremus, 1980). In later stages, growth and nodulation decrease, although sufficient *Frankia* propagules are present. Scrubs of *H. rhamnoides* tend to lose vigour some decades after colonization. The rate of this degeneration varies with the location. Succession of degenerated *H. rhamnoides* vegetation is generally slower on dry calcareous dunes than on calcium-poor dunes and dune slacks. On the former, less vigorous *H. rhamnoides* may remain present for a long period (Van Dorp *et al.*, 1985; Doing, 1988).

Outline of the thesis

Degeneration of *Hippophaë* scrub on the former isle of Vorne, the Netherlands, was found to be associated with the presence of plant-feeding nematodes of a species later described as *Longidorus dunensis* Brinkman, Barbez & Loof (Oremus & Otten, 1981). Inoculation of this species together with *Tylenchorhynchus microphasmis* Loof in a pot experiment caused growth reduction (Maas *et al.*, 1983). There appeared to be an interaction with other soil biota, because in non-sterilized soil the harmful effect per inoculated individual nematode was much stronger than in sterilized soil.

The following hypotheses formed the basis of the present study:

1. Plant-feeding nematodes play a role in the succession of *H. rhamnoides* in Dutch coastal dune areas in general. More than one nematode species may be at stake (Oremus, 1982; Maas *et al.*, 1983).
2. Other soil biota (root pathogens) and abiotic factors (moisture stress, nutrient limitation) aggravate nematode damage (Maas *et al.*, 1983).
3. The mechanism of growth reduction by nematodes is reduced uptake of phosphate and, consequently, reduced N-fixation (Oremus, 1982).

The research was started with a survey on the occurrence of species of plant-feeding nematodes, VA-mycorrhiza and *Frankia* nodulation on roots of *H. rhamnoides* in relation to shrub vigour and soil chemical and physical factors (chapter 2). The data of this survey were analyzed using ordination and regression techniques. Pot experiments were conducted in the greenhouse to determine the potential role of groups of fungi and nematodes in the disease complex by using selective inhibitors (chapter 3). Moreover, the pathogenicity of several fungus isolates from *H. rhamnoides* rhizosphere and roots was tested by inoculating them in a pot experiment (chapter 3).

In order to be able to mimic the rather dry natural soil moisture regimes in pot experiments with dune sand, an apparatus was developed for automatic watering and registration of water use (chapter 4). Also, a method was developed to estimate root length and branching using image analysis (chapter 5).

Both methods were used in greenhouse studies on the effects of the nematode *Tylenchorhynchus microphasmis* on growth of *H. rhamnoides*. This plant-parasitic nematode species was the most frequently and numerously occurring one in the field survey. The relationship between *H. rhamnoides* and *T. microphasmis* was studied under different moisture regimes (chapter 6) and further pot experiments were conducted to clarify mechanisms behind the effects of *T. microphasmis*, in the presence or absence of the nitrogen-fixing root symbiont *Frankia* (chapters 7 and 8). Possible interactions between *T. microphasmis* and the dominant root fungus *Cylindrocarpon destructans* in causing damage to *H. rhamnoides* were studied as well (chapter 8).

References:

- Abdel Wahab A.M. & Wareing P.F. 1980 Nitrogenase activity associated with the rhizosphere of *Ammophila arenaria* (L.) and effect of inoculation of seedlings with azotobacter. *New Phytol.* 84: 711-721.
- Akkermans A.D.L. 1971 Nitrogen fixation and nodulation of *Alnus* and *Hippophaë* under natural conditions. PhD. Thesis Univ. of Leiden, The Netherlands, 85 pp.
- Beeftink W.G. 1965. De zoutvegetatie van ZW-Nederland beschouwd in Europees verband. *Meded. Landb.hogesch.*, Wageningen 65(1): 1-167.
- Boerboom J.H.A. 1960. De plantengemeenschappen van de Wassenaarse duinen. *Meded. Landb.hogesch.*, Wageningen 60(10): 1-135.
- Bohnke S., Wijmstra J., Van der Woude J. and Sohl H. 1988 The late glacial infill of three lake successions in the Netherlands: regional vegetation history in relation to NW European vegetation developments. *Boreas* 17: 385-402.
- Brown J.C. 1958. Soil fungi of some British sand dunes in relation to soil type and succession. *J. Ecol.* 46: 641-664.
- Brown V.K. & Gange A.C. 1991. Effects of root herbivory on vegetation dynamics. In: Atkinson D. (ed.) *Plant Root Growth: An Ecological Perspective*. Blackwell, London: 453-470.
- Bussau C. 1990a. Freilebende Nematoden aus Küstendünen und angrenzenden Biotopen der deutschen und dänischen Küsten. I. Gesamtüberblick und Chromadorida (Nematoda). *Zool. Anz.* 225: 161-188.
- Bussau C. 1990b. Freilebende Nematoden aus Küstendünen und angrenzenden Biotopen der deutschen und dänischen Küsten. II. Monhysterida, Enoplida und Trefusiida (Nematoda). *Zool. Anz.* 225: 198-209.
- Clements F.E. 1916. *Plant succession: an analysis of the development of vegetation*. Carneghie Inst. of Washington Publication, 242.
- Clements F.E. 1928. *Plant Succession and Indicators*. Wilson, New York, 234 pp.
- Connell J.H. and Slatyer R.O. 1977. Mechanisms of succession in natural communities and their role in community stability and organisation. *Am. Nat.* 111: 1119-1144
- Cowles H.C. 1911. The causes of vegetative cycles. *Botanical Gazette* 51:161-183
- Crawley M.J. 1993. Succeeding in the sand dunes. *Nature* 362: 17-18.
- De Goede R.G.M., Georgieva S.S., Verschoor B.C. & Kamerman J.W. 1993 Changes in nematode community structure in a primary succession of blown-out areas in a drift sand landscape. *Fundam. appl. Nematol.* 16: 501-513.
- De Nooij M.P., Troelstra S.R. & Wagenaar R. 1986 Growth reduction in *Plantago lanceolata* in relation to biotic factors in the soil environment. *Oecologia (Berlin)* 70: 266-272.
- Doing H. 1988. *Landschapsoecologie van de Nederlandse Kust*. Stichting Duinbehoud, Leiden, 228 pp (with maps).

- Ennik G.C., Kort J. and Van der Bund C.F. 1965. The clover cyst nematode (*Heterodera trifolii* Goffart) as the probable cause of death of white clover in a sward. *J. Brit. Grassland Soc.* 20, 258-262
- Ernst W.H.O., Van Duin W.E. & Oolbekking G.T. 1984. Vesicular-Arbuscular Mycorrhiza in dune vegetation. *Acta Bot. Neerl.* 33: 151-160.
- Fay P.J. & Jeffrey D.W. 1992. The foreshore as a nitrogen source for marram grass. In: Carter R.W.G., Curtis T.G.F & Sheehy-Skeffington M.J. (eds.) *Coastal Dunes*. Balkema, Rotterdam: 177-188.
- Forster S.M. & Nicolson T.H. 1981 Microbial aggregation of sand in a maritime dune succession. *Soil. Biol. Biochem.* 13: 205-208.
- Forster S.M. 1990 The role of microorganisms in aggregate formation and soil stabilization.: Types of aggregation. *Arid Soil Res. & Rehab.* 4: 85-98.
- Gardner I.C., Clelland D.M. & Scott A. 1984. Mycorrhizal improvement in non-leguminous nitrogen-fixing associations with particular reference to *Hippophaë rhamnoides* L. *Plant and Soil* 78: 189-201.
- Grace J.B. & Tilman D. (eds.) 1989 *Perspectives on Plant Competition*. Academic Press, New York.
- Gray A.J., Crawley M.J. & Edwards P.J. (eds.) 1987. *Colonization, Succession and Stability*. Blackwell, Oxford.
- Grime J.P., Mackey J.M.L., Hillier S.H. & Read D.J. 1987. Floristic diversity in a model system using experimental microcosms. *Nature* 328: 420-422.
- Hafsten U. 1987. Vegetatsjon, klima og landskaps-utvikling i Trondelag etter siste istid. *Norsk Geografisk Tidsskrift* 41: 101-120.
- Kisiel M. 1970. Studies on the ecology of nematodes inhabiting *Ammophila arenaria* plant community on beaches and dunes on Baltic sea shores. *Akademia Rolnicza* 34: 111-150.
- Koehler H. and Weidemann G. 1993 Biogenic dune sand stabilization. European Union Coastal Conservation Congress, Jastrzebia Gora, Poland, september 1993.
- Korteweg C.R. and s'Jacob J.J. 1980. Influence of *Heterodera trifolii* and nitrogen on white clover in a clover-grass mixture. *Meded. Fac. Landbouww. Rijksuniv. Gent* 45(3), 761-767.
- Koske R.E and Halvorson W.L. 1981. Ecological studies of vesicular-arbuscular mycorrhizae in a barrier sand dune. *Can. J. Bot.* 59: 1413-1422.
- Koske R.E and Polson W.R. 1984. Are VA mycorrhizae required for sand dune stabilization ? *Bioscience* 34:420-424.
- Londo G. 1974. Successive mapping of dune slack vegetation. *Vegetatio* 29: 51-61
- Maas P.W.Th., Oremus P.A.I. & Otten H. 1983. Nematodes (*Longidorus* n.sp. and *Tylenchorhynchus microphasmis* Loof) in growth and nodulation of sea buckthorn (*Hippophaë rhamnoides* L.). *Plant and Soil* 73: 141-147.
- Miles J. 1987. Vegetation succession: past and present perceptions. In: A.J. Gray et al. (eds.), *Colonization, Succession and Stability*, Blackwell Sci. Publ., Oxford, 482 pp

- Newman E.I. 1991. The role of root-inhabiting fungi in mixed species plant communities. In: Atkinson D. (ed.) Plant Root Growth: An Ecological Perspective. Blackwell, London: 437-451.
- Nicolson T.H. and Johnston C. 1979. Mycorrhiza in the Gramineae. III. *Glomus fasciculatus* as the endophyte of pioneer grasses in a maritime sand dune. Trans Br. mycol. Soc. 72: 261-268.
- Olf H. 1992. On the Mechanisms of Vegetation Succession. PhD. Thesis, Rijksuniversiteit Groningen, The Netherlands. 211 pp.
- Olson J.S. 1958. Rates of succession and soil changes on southern Lake Michigan sand dunes. Bot. Gaz. 119: 125-170.
- Oremus P.A.I. 1980. Occurrence and infective potential of the endophyte of *H. rhamnoides* ssp. *rhamnoides*. Plant & Soil 56: 123-139.
- Oremus P.A.I. 1982. Growth and Nodulation of *Hippophaë rhamnoides* L. in the coastal Sanddunes of The Netherlands. Ph.D. Thesis, Univ. of Utrecht NL, 118 p.
- Oremus P.A.I. and Otten H. 1981. Factors affecting growth and nodulation of *Hippophaë rhamnoides* L. ssp. *rhamnoides* in soils from two successional stages of dune formation. Plant & Soil 63: 316-331.
- Pearson M.C. and Rogers J.A. 1962. *Hippophaë rhamnoides* L. J.Ecol. 50:501-513.
- Pugh G.J.F. 1970. A study of fungi in the rhizosphere and on root surfaces of plants growing in primitive soils. In: J. Phillipson (ed) Methods of Study in Soil Ecology. Unesco, Geneva.
- Rogers J.A. 1961. The autecology of *Hippophaë rhamnoides* L. Ph.D. Thesis, Univ. of Nottingham.
- Rose S.L. 1988. Above and belowground community development in a marine sand dune ecosystem. Plant & Soil 109: 215-226.
- Rousi A. 1971. The genus *Hippophaë* L.: A taxonomic study. Ann. Bot. Fenn. 8: 177-227.
- Salisbury E.J. 1925. Note on the edaphic succession in some dune soils with special reference to the lime factor. J. Ecol. 13: 322-328.
- Seliskar D.M. and Huettel R.N. 1993. Nematode involvement in die-out of *Ammophila breviligulata* (Poaceae) on the mid-atlantic coastal dunes of the United States. J. Coastal Res. 9: 97-103.
- Skogen A. 1972. The *Hippophaë rhamnoides* alluvial forest at Leinöra, central Norway. A phytosociological and ecological study. K. Nor. Vidensk. Selsk. 4: 1-115.
- Sloet van Oldruitenborgh C.J.M. 1976. Duinstruwelen in het Deltagebied. PhD Thesis, Landb.hogeschool, Wageningen, 111 pp.
- Stewart W.D.P. and Pearson M.C. 1967. Nodulation and nitrogen fixation by *Hippophaë rhamnoides* L. in the field. Plant and Soil 26: 348-360.
- Stuyfzand P.J. 1984. Effecten van vegetatie en luchtverontreiniging op de grondwaterkwaliteit in kalkrijke duinen bij Castricum. H₂O 17: 152-159.

- Syers J.K. and Walker T.W. 1969a. Phosphorus transformations in a chronosequence of soils developed on wind-blown sand in New Zealand I. total and organic phosphorus. *J. Soil Sci.* 20: 57-64.
- Syers J.K. and Walker T.W. 1969b. Phosphorus transformations in a chronosequence of soils developed on wind-blown sand in New Zealand. II. inorganic phosphorus. *J. Soil Sci.* 20: 318-324.
- Syers J.K. *et al.* 1970. Accumulation of organic matter in a chronosequence of soils developed on wind-blown sand in New Zealand. *J. Soil Sci.* 21: 146-153.
- Tilman D. 1985. The resource-ratio hypothesis of plant succession. *Am Nat.* 125: 827-852.
- Troelstra S.R., Blacquièrre T., Wagenaar R. and Van Dijk C. 1987. Ionic balance, proton efflux, nitrate reductase activity and growth of *Hippophaë rhamnoides* L. ssp. *rhamnoides* as influenced by combined-N nutrition or N₂-fixation. *Plant and Soil* 103: 169-183.
- Van der Maarel E., Van Dorp D. and Rijntjes J. 1985. Vegetation succession on the dunes near Oostvoorne, The Netherlands; A comparison of the vegetation in 1959 and 1980. *Vegetatio* 58: 137-187.
- Van der Meulen F. and Wanders E.A.J. 1985. Dynamics and management of some coastal dune woodlands near The Hague, The Netherlands. *Vegetatio* 62: 457-465.
- Van der Putten W.H., Van Dijk C. and Troelstra S.R. 1988. Biotic soil factors affecting the growth and development of *Ammophila arenaria*. *Oecologia* 76: 313-320.
- Van der Putten W.H. and Troelstra S.R. 1990. Harmful soil organisms in coastal foredunes involved in degeneration of *Ammophila arenaria* and *Calammophila baltica*. *Can. J. Bot.* 68: 1560-1568.
- Van der Putten W.H., Maas P.W.Th., Van Gulik W.J.M. and Brinkman H. 1990. Characterization of soil organisms involved in the degeneration of *Ammophila arenaria*. *Soil. Biol. Biochem.* 22: 845-852.
- Van der Putten W.H., Van Dijk C. and Peters B.A.M. 1993. Plant-specific soil-borne diseases contribute to succession in foredune vegetation. *Nature* 362: 53-55.
- Van Dorp D., Boot R.G.A. and Van der Maarel E. 1985. Vegetation succession on the dunes near Oostvoorne, The Netherlands, since 1934, interpreted from air photographs and vegetation maps. *Vegetatio* 58: 123-136.
- Vitousek P.M. and Walker L.R. 1987. Colonization, succession and resource availability: ecosystem-level interactions. In: Gray A.J., Crawley M.J. & Edwards P.J. (eds.) 1987. *Colonization, Succession and Stability*. Oxford, Blackwell Sci. Publ.: 207-223.
- Wallén B. 1980. Changes in structure and function of *Ammophila* during a primary succession. *Oikos* 34: 227-238.

- Walker J., Thompson C.H., Fergus I.F. and Tunstall B.R. 1981. Plant succession and soil development in coastal dunes of subtropical Eastern Australia. In: D.C. West, H.H. Shugart & D.B. Botkin (eds.) *Forest Succession, Concepts and Application*. New York, Springer Verlag, pp 107-131.
- Walker T.W. and Syers J.K. 1976. The fate of phosphorus during pedogenesis. *Geoderma* 15: 1-19.
- Wasilewska L. 1970. Nematodes of the sand dunes in the Kampinos forest. I. Species structure. *Ekol. Polska*, 18: 429-443.
- Wasilewska L. 1970. Nematodes of the dunes in the Kampinos forest. II. Community structure based on numbers of individuals, state of biomass and respiratory metabolism. *Ekol. Polska*, 19: 651-688.
- Webley D.M., Eastwood D.J. and Gimingham C.H. 1952. Development of a soil microflora in relation to plant succession on sand-dunes, including a 'rhizosphere' flora associated with colonizing species. *J. Ecol.* 40: 168-178.
- Weste G. 1986. Vegetation changes associated with invasion by *Phytophthora cinnamomi* of defined plots in the Brisbane Ranges, Victoria. *Austr. J. Bot.* 34: 633-648.
- Westhoff V. and Van Oosten M.F. 1991. *De Plantengroei van de Waddeneilanden*. Stichting Uitgeverij Kon. Ned. Nat.hist. Vereniging, Utrecht, 420 pp.
- Westhoff V. and Den Held A.J. 1969. *Plantengemeenschappen in Nederland*. Thieme, Zutphen NL, 324 pp.
- Willis A.J., Folkes B.F., Hope-Simpson J.F and Yemm E.W. 1959. Braunton Burrows: The dune system and its vegetation I. *J. Ecol.* 47: 1-24.

CHAPTER 2.

Ecology of the plant-feeding nematode fauna associated with sea buckthorn (*Hippophaë rhamnoides* L. ssp. *rhamnoides*) in different stages of dune succession.

F.C. Zoon, S.R. Troeistra & P.W.Th. Maas
Fundam. appl. Nematol. 16: 247-258 (1993).

SUMMARY

A field survey was carried out to investigate the development of the plant parasitic nematode fauna under *Hippophaë rhamnoides* ssp. *rhamnoides* from the colonization to the degeneration stage. Samples from different sand dune locations along the Dutch coast were analysed and the occurrence and densities of different nematode taxa were studied in relation to soil parameters and plant productivity, using ordination and regression techniques. There appeared to be a succession of nematode taxa along with the development of soil and vegetation. The maturity index of the obligate plant-feeding nematode fauna and the mean number of species increased significantly. The characteristics of sites, with respect to soil parameters and nematode fauna, diverged as it reached the degeneration stage. *Tylenchorhynchus microphasmis* Loof and *Mesocriconema xenoplax* (Raski) were common in all stages. The abundance of plant feeding nematodes in the rhizosphere of *Hippophaë* increased towards later stages in a succession gradient, whereas population densities in the bulk soil remained practically constant. The status of *H. rhamnoides* as a host of several free living plant-feeding nematodes is discussed.

INTRODUCTION

Hippophaë rhamnoides L. ssp. *rhamnoides* is a pioneer shrub species on calcareous coastal sand dunes in north-western Europe (Pearson & Rogers, 1967). The dunes under *H. rhamnoides* vary in age from a decade to several centuries. Changes in the vegetation occur, although *Hippophaë* may remain the dominant

species over a long period (van der Maarel *et al.*, 1985). Different aspects of chemical and physical development of calcareous dune soils have been known for a long time (Salisbury, 1922; Olson 1958; Willis *et al.*, 1959) and were suggested to play a major role in the succession of vegetation. The main processes of development in calcareous dune soils are: *i*) accumulation of organic nitrogen, especially from symbiotic N₂-fixation by the actinomycete *Frankia* sp. in *Hippophaë* root nodules (Akkermans, 1971; Stewart & Pearson, 1967) and atmospheric deposition; *ii*) leaching of calcium and other cations (Salisbury, 1922; Stuyfzand, 1984; Rozema *et al.*, 1985); and *iii*) decreasing wind impact on locations behind a progressing coast line, leading to decreasing salt spray and a smaller particle size median of the sand.

The development of the nematofauna of dune soils has received little attention (Kisiel, 1970; Wasilewska, 1970, 1971). Nematofaunal changes in the calcareous sand dunes of Western Europe have hardly ever been studied. The colonization of young dunes by nematodes may depend on the rate of dispersal and reproduction, and on the availability of suitable niches. Presumably, some nematode species have a high dispersal rate or a broad ecological amplitude, whereas others are only slowly dispersed or have a relatively high degree of niche specialization. The stage of edaphic and vegetational development may determine habitat suitability for different nematode taxa.

Plant-feeding nematodes were suggested as a cause of the decline of *H. rhamnoides* (Oremus, 1982; Maas *et al.*, 1983). Especially, the occurrence of *Longidorus* n.sp. (*L. dunensis* Brinkman *et al.*, 1987) in the late successional stage was considered to be important. Inoculation of a mixture of *L. dunensis* and *Tylenchorhynchus microphasmis* Loof in a pot experiment caused severe growth reduction of *H. rhamnoides* (Maas *et al.*, 1983). It was not clear, however, if these nematode species and their effects on plant vigour are of more than local importance, in other regions other species may be involved. The results of a field survey on the occurrence of plant-feeding nematode taxa in different stages of *Hippophaë* succession are presented in this paper. An attempt was made to relate the presence and the density of nematode taxa to soil parameters and the productivity of the vegetation. Soil samples from vegetations preceding and succeeding those with *Hippophaë* were analysed in a study of a transect from young to old dunes. Furthermore, the nematode fauna of *Hippophaë* rhizosphere samples from different successional stages was analysed and compared to samples of the bulk soil to evaluate the importance of *Hippophaë* roots in the distribution of nematode taxa.

Table 1. Topography and some environmental parameters of sampling locations in the field survey.

| Site nr. | Region | Coordinates NW / EL | Stage 1) | pH | N 2) | OM/N 3) | NOD 4) | VI 5) |
|----------|-------------|------------------------|-------------|-----|---------|------------|-----------|----------|
| 1 | Texel I. | 53°08'/4°47' | D | 8.3 | 20.2 | 17.8 | 5 | 2 |
| 2 | id. | 53°08'/4°47' | So | 8.2 | 23.4 | 18.8 | 1 | 4.5 |
| 3 | Texel II. | 53°00'/4°45' | P | 7.9 | 10.8 | 19.4 | 9 | 5 |
| 4 | id. | 53°01'/4°44' | D | 5.8 | 21.0 | 19.0 | 2 | 1.5 |
| 5 | id. | 53°01'/4°44' | Sb | 7.6 | 17.0 | 19.4 | 0 | 5 |
| 6 | id. | 53°01'/4°44' | D | 6.9 | 22.2 | 22.1 | 8 | 1 |
| 7 | Castricum | 52°33'/4°38' | D | 8.2 | 35.1 | 18.8 | 40 | 1.5 |
| 8 | id. | 52°33'/4°38' | Ss | 8.0 | 44.8 | 22.1 | 11 | 4.5 |
| 9 | id. | 52°30'/4°36' | So | 8.2 | 39.3 | 21.6 | 4 | 5 |
| 10 | id. | 52°32'/4°36' | D | 8.3 | 23.1 | 22.9 | 14 | 2 |
| 11 | Oostvoorne | 51°55'/4°03' | P | 8.6 | 13.0 | 13.1 | 3 | 5 |
| 12 | id. | 51°54'/4°04' | D | 8.4 | 28.5 | 14.4 | 0 | 2 |
| 13 | id. | 51°54'/4°04' | D | 8.5 | 22.8 | 16.7 | 1 | 2 |
| 14 | id. | 51°54'/4°03' | D | 8.5 | 23.6 | 18.6 | 7 | 1.5 |
| 15 | id. | 51°55'/4°04' | P | 8.7 | 16.3 | 16.6 | 3 | 4.5 |
| 16 | Schouwen I | 51°44'/3°43' | D | 5.6 | 25.8 | 22.1 | 0 | 1 |
| 17 | id. | 51°44'/3°43' | Sd | 8.2 | 18.6 | 20.4 | 0 | 4 |
| 18 | id. | 51°44'/3°43' | Sd | 8.5 | 14.4 | 20.8 | 1 | 2 |
| 19 | id. | 51°42'/3°41' | P | 8.4 | 17.8 | 23.6 | 2 | 2 |
| 20 | id. | 51°42'/3°41' | P | 8.6 | 12.2 | 23.0 | 3 | 3.5 |
| 21 | Schouwen II | 51°42'/3°44' | D | 5.2 | 32.0 | 24.4 | 3 | 2 |
| 22 | id. | 51°42'/3°43' | So | 5.7 | 14.9 | 22.8 | 3 | 4 |
| 23 | id. | 51°42'/3°43' | S | 5.5 | 16.0 | 13.8 | 3 | 1 |

1) P: Early primary succession, <20 years of *H. rhamnoides*.

S: Early secondary succession after disturbance (o= overblown, b= blowout, d= digging activities, s= sludge deposit)

D: Degeneration on stable sites, >>20 years *H. rhamnoides*.

2) total N mg/100 g.

3) org. matter/N (w/w).

4) *Frankia*-nodules per seedling in pot bioassay.

5) visual *Hippophaë* vigour: 0=dead, 5=vigorous.

METHODS

Field survey

Sampling locations. 23 scrubs were selected in six different dune areas (Table 1) which were at the most 160 kilometer apart. Within each area both vigorous and degenerating *Hippophaë* vegetations were sampled in mid September 1985. The oldest shoots (ramets) in the selected scrubs were between 15 and 20 years old. The sites were classified into three successional stages on the basis of studies on the geomorphological and vegetation history of the area and its present vegetation: 1. sites of early primary succession with a first vegetation of *H. rhamnoides*; 2. sites of early secondary succession, which had a regenerated (but not always vigorous) *Hippophaë* vegetation after soil disturbance either by wind or by digging; These sites were surrounded by non-disturbed vegetation which had developed much further (e.g. with *Rosa pimpinellifolia* L. or deciduous forest). 3. late successional stages with degenerating scrubs on soil which had remained *in situ* for at least a few decades after primary or (more often) secondary colonization. These sites had mixed scrubs or a sparse *Hippophaë* vegetation with an undergrowth of grass or moss. Twenty soil cores (diam.: 2.5 cm) were taken from each site (about 20 m²) and divided into the 0-10 and 10-70 cm layers. The cores of each layer were carefully mixed together to form one sample per layer per site.

Plant parameters. The vigour of *Hippophaë* was estimated with an index for the visual shrub vigour (VI) (0=dead, 5=vigorous), which is significantly correlated with the mean biomass of current twigs and the xylem increment of one-year-old branches (Zoon, unpubl.). The percentage of fine root length of *Hippophaë* from the 10-70 cm soil samples infected with vesicular-arbuscular mycorrhiza (VAM) was assessed using the line-intersection method after staining with Chlorazole Black E (Brundrett *et al.*, 1984). The Frankia-nodulation potential (NOD) was estimated by counting the number of nodules formed after 10 weeks on *Hippophaë* seedlings planted in 500 ml of the 10-70 cm soil sample from each site.

Soil chemical and textural analysis. Soil samples from each of the two layers were analysed to determine soil chemical and textural parameters. After drying (35-°C) and sieving (2 mm), bulk soil samples were mechanically subdivided and part of each sample was ground in a mortar mill. The pH was measured potentiometrically

in 1:2.5 (W/V) suspensions in H₂O. Total P and total N were measured colorimetrically in single soil digests (Novozamsky *et al.*, 1984). The percentage organic matter (OM) was estimated from the weight loss on ignition at 430°C for 24 h. Percentage carbonates (mainly CaCO₃; LIME) was determined gasvolumetrically by treating samples with 4 M HCl. Exchangeable cations (Mg, K and Na) were determined by atomic absorption spectrophotometry after shaking soils with neutral ammonium acetate. Electrical conductivity (EC) was measured in 1:5 (w/v) suspensions in H₂O. The soil texture was analysed by dry sieving (fractions > 53 μ), and a pipette method (fractions < 53 μ). The particle size median (PSM) and the percentage clay (< 2 μ) were taken as characteristic parameters. Ratios P/N and OM/N were calculated. The latter was assumed to reflect the C/N ratio, which itself was not determined.

Nematode fauna. Analysis of the nematode fauna was done only for the 10-70 cm layer, because roots of *H. rhamnoides* are present mainly in this layer (Oremus 1982). Nematodes were extracted from a 500 ml subsample of the 20 mixed samples of a 20 m² plot by using the Oostenbrink elutriation method (Oostenbrink, 1960). The top sieve on the funnel was 2.5 mm mesh to improve the recovery of large nematodes. The funnel was emptied over sieves of 180 μm (2x) and 45 μm (3x) mesh, respectively. The fraction larger than 180 μm was extracted overnight through a 120 μm mesh gauze, the smaller sized fraction through a double cotton-wool filter. Plant-feeding nematodes were counted at genus or species level. A few specimen from each taxonomic group in a sample were fixed and mounted for species identification. For Tylenchida the classification by Siddiqi (1986) was followed, except for the genera *Macroposthonia* and *Dolichorhynchus*, which have been synonymized with *Mesocriconema* (Loof & De Grisse, 1989) and *Tylenchorhynchus* (Fortuner & Luc, 1987), respectively. The successional position of each taxon was characterised by its optimum on the imaginary succession gradient (stage 1 to 3). These successional optima (SOPs) take into account both the frequency of occurrence and the population density and were calculated as follows:

$$SOP = 0.5 \times \frac{\sum_{i=1}^3 (F_i \times i)}{\sum (F_i)} + 0.5 \times \frac{\sum_{i=1}^3 (N_i \times i)}{\sum (N_i)}$$

Where F_i is the frequency of occurrence (positive/total samples) in stage i, and N_i,

the mean number per 500 ml soil calculated for the samples in stage *i* where the taxon occurred. In addition, the 'maturity index' of the plant-feeding nematofauna was calculated for each sample following Bongers (1990) as the weighted mean of the 'maturity values' (1-5) of each individual present.

Statistical analysis. Simple correlations within and between environmental and nematode parameters were calculated. Canonical Correspondence Analysis (CCA) (Ter Braak, 1987a, 1987b) was used for analysis of the (unimodal) relations between nematode fauna and environmental parameters. The nematode data used were percentages of total plant-feeding nematodes. To obtain a small set of principal factors, the number of environmental parameters in the ordination was reduced by stepwise elimination of parameters which were correlated to others, or which were not correlated to the first four ordination axes. Significance of the eigenvalues of the first canonical axis and of the sum of the eigenvalues of all axes (trace) was tested by a Monte Carlo test with 99 random permutations of environment and nematofauna. For comparison, the data were also analysed by Redundance Analysis (RA), the linear analogue of CCA, and the use in CCA of nematode numbers instead of percentages was evaluated.

Transect sampling

Another series of samples was taken in September 1987 on a transect from beach to inner dunes on the former isle of Schouwen. A description of the sites, which represent successive vegetation types, is given in Table 2. Methods were the same as in the field survey, but now samples were collected from the 0 to 50 cm layer.

Table 2. Description of the succession transect at Schouwen (coordinates as sites 16-18; Table 1.)

| Site | Distance to sea (m) | Dune type | Dominant vegetation |
|------|---------------------|-----------------|---|
| A. | 200 | embryo dune | <i>Elymus arenarius</i> |
| B. | 500 | low foredune | <i>Ammophila arenaria</i> |
| C. | 530 | id. | <i>H. rhamnoides</i> invading |
| D. | 1160 | flat inner dune | <i>H. rhamnoides</i> degenerating in meadow |
| E. | 1220 | id. | <i>Ligustrum vulgare</i> |

Rhizosphere sampling

In order to find out to what extent nematodes concentrate in the rhizosphere of *H. rhamnoides* four-month-old seedlings were planted in May 1987 near sites 11 and 12 of the field survey. In September the plants were dug up carefully and -with the adhering soil- taken into the laboratory. Samples of bulk soil from the 0 to 25 cm layer (approximately seedling rhizosphere depth) were collected at the same time, at a few dm from the seedling roots. The amount of 'rhizosphere' soil attached to the roots was determined by subtracting the fresh weight of the roots from the total weight of roots plus soil. Nematodes were extracted and counted as described above.

RESULTS

Field survey

Plant-feeding nematode fauna. *Mesocriconema xenoplax* (Raski, 1952), *Tylenchorynchus microphasmis* Loof, 1960 and Tylenchidae were the most common taxa under *Hippophaë*. Less common or less numerous were *Rotylenchus goodeyi* Loof & Oostenbrink, 1958, *Paratylenchus* spp., *Meloidogyne* spp. (mainly *M. hapla* Chitwood, 1949 and *M. maritima* Jepson 1987), *Helicotylenchus pseudorobustus* (Steiner, 1914) and *Pratylenchus* spp. (mainly *P. crenatus* Loof, 1960 and *P. penetrans* (Cobb, 1917)). Some other taxa seemed to be restricted to certain regions. This was most clearly the case for *Longidorus dunensis* Brinkman *et al.*, 1987 and *Geocenamus arcticus* (Mulvey, 1969) (first record in The Netherlands), which were not present in samples from the isle of Texel and the area Schouwen II. *Paratrichodorus* spp. (*P. pachydermus* (Seinhorst, 1954), *P. nanus* (Allen, 1957) and *P. teres* (Hooper, 1962), in descending order of importance) and *Bitylenchus dubius* (Bütschli, 1873) were absent from the Texel samples. *Rotylenchus robustus* (De Man, 1876) was found at only one site, possibly introduced by nearby agricultural activities. Other 'minor' taxa in this survey were Heteroderinae (J2), *Telotylenchus ventralis* Loof, 1963, *Criconema annuliferum* (De Man, 1921) and *C. princeps* (Andrássy, 1962), *Criconemoides informis* (Micoletzky, 1922) and *C. amorphus* De Grisse, 1967, and *Hemicycliophora conida* Thorne, 1955.

Table 3. Frequency of occurrence and mean densities of plant feeding nematodes as related to the stage of succession of *H. rhannoides* vegetations. F= number of sites where taxon is present, N= mean number per 500 ml soil (mean of positive sites), SE= standard error of the mean, SOP= weighted optimum on succession scale 1-3. Different letters within the same line in the lower part of the table indicate significant differences ($p < 0.05$).

| STAGE OF SUCCESSION NEMATODE TAXON \ | 1. EARLY PRIMARY (n=5) | | | 2. EARLY SECONDARY (n=8) | | | 3. DEGENERATION (n=10) | | | SOP |
|---|------------------------|-----|------|--------------------------|-----|------|------------------------|-----|------|-----|
| | (F) | N | SE | (F) | N | SE | (F) | N | SE | |
| <i>Rotylenchus goodeyi</i> | (3) | 118 | 34 | (4) | 28 | 12 | (4) | 11 | 7 | 1.6 |
| <i>Paratylenchus</i> spp. | (5) | 9 | 4 | (4) | 5 | 2 | (7) | 6 | 2 | 1.9 |
| <i>Mesocriconema xenoplax</i> | (5) | 86 | 50 | (8) | 57 | 29 | (10) | 41 | 12 | 1.9 |
| <i>Tylenchorhynchus microphasmsis</i> | (4) | 174 | 66 | (7) | 115 | 27 | (10) | 105 | 16 | 2.0 |
| <i>Rotylenchus robustus</i> | (0) | | | (1) | 20 | 20 | (0) | | | 2.0 |
| Heteroderinae J2 | (2) | 1 | 0 | (3) | 5 | 4 | (3) | 2 | 0 | 2.0 |
| Meloidogyne spp. | (3) | 2 | 1 | (2) | 17 | 11 | (7) | 5 | 2 | 2.1 |
| Tylenchidae | (5) | 42 | 13 | (8) | 41 | 15 | (10) | 72 | 12 | 2.1 |
| <i>Telotylenchus ventralis</i> | (1) | 2 | 2 | (1) | 1 | 1 | (2) | 4 | 2 | 2.1 |
| <i>Pratylenchus</i> spp. | (3) | 7 | 3 | (5) | 38 | 15 | (8) | 25 | 11 | 2.2 |
| <i>Bitylenchus dubius</i> | (1) | 1 | 1 | (1) | 5 | 5 | (3) | 16 | 12 | 2.4 |
| <i>Criconema</i> spp. | (0) | | | (1) | 5 | 5 | (1) | 5 | 5 | 2.5 |
| <i>Paratrichodorus</i> spp. | (1) | 4 | 4 | (4) | 8 | 2 | (6) | 38 | 30 | 2.5 |
| <i>Helicotylenchus pseudorobustus</i> | (1) | 1 | 1 | (3) | 10 | 7 | (7) | 79 | 47 | 2.6 |
| <i>Criconemoides</i> spp. | (0) | | | (1) | 5 | 5 | (4) | 8 | 3 | 2.7 |
| <i>Longidorus dunensis</i> | (0) | | | (2) | 5 | 1 | (5) | 32 | 13 | 2.8 |
| <i>Geocanamus arcticus</i> | (0) | | | (0) | | | (5) | 30 | 17 | 3.0 |
| <i>Hemicyclophora conida</i> | (0) | | | (0) | | | (4) | 26 | 14 | 3.0 |
| TOTAL PLANT FEEDERS | 357 | a | 75 | 267 | a | 42 | 388 | a | 56 | |
| MEAN NUMBER OF TAXA | 6.60 | a | 0.67 | 6.63 | ab | 1.02 | 9.20 | b | 0.74 | |
| MEAN MATURITY INDEX | 2.87 | a | 0.03 | 2.88 | a | 0.03 | 2.88 | a | 0.02 | |
| IDEM EXCL. TYLENCHIDAE | 2.97 | a | 0.02 | 3.02 | ab | 0.01 | 3.12 | b | 0.04 | |

Table 4. Coefficients of correlation (R) of significant simple linear correlations between soil and plant parameters. See methods for explanation of parameter codes 1-19. N=23; if R>.41 then p<0.05, if R>.52 then p<0.01, if R>.67 then p<0.001. Non significant coefficients are not shown.

| Parameter | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | parameter sets used in CCA | |
|---------------------|------|------|------|------|------|------|-----|-----|------|------|-----|-----|------|------|----|----|----|----|----|------|----|----------------------------|-------------|
| 10-70 cm soil layer | | | | | | | | | | | | | | | | | | | | | | | |
| 1 PH | 1 | | | | | | | | | | | | | | | | | | | | | A B C D E F | |
| 2 P | .63 | 1 | | | | | | | | | | | | | | | | | | | | A B | |
| 3 N | | | 1 | | | | | | | | | | | | | | | | | | | A B C D E F | |
| 4 OM | | | | .95 | 1 | | | | | | | | | | | | | | | | | | |
| 5 LIME | .68 | .87 | | | 1 | | | | | | | | | | | | | | | | | | |
| 6 MG | .78 | .73 | | | .90 | 1 | | | | | | | | | | | | | | | | | |
| 7 K | | | | | .52 | .50 | 1 | | | | | | | | | | | | | | | | |
| 8 NA | .50 | .55 | .58 | .59 | .55 | .44 | .42 | 1 | | | | | | | | | | | | | | A C E | |
| 9 EC | .92 | .78 | | | .80 | .86 | .68 | | 1 | | | | | | | | | | | | | A | |
| 10 P/N | .57 | .53 | .56 | .59 | .52 | .50 | .46 | | | 1 | | | | | | | | | | | | A B | |
| 11 OM/N | | | | .51 | | | | | | | 1 | | | | | | | | | | | A B C D E F | |
| 12 PSM | -.49 | -.55 | | | -.65 | -.76 | | | | | .61 | 1 | | | | | | | | | | A C | |
| 13 CLAY | .52 | .79 | | | .74 | .70 | | | | | .70 | .75 | 1 | | | | | | | | | | |
| top soil | | | | | | | | | | | | | | | | | | | | | | | |
| 14 N | | | | .68 | .76 | | | | | | | | | | | | | | | | | | |
| 15 OM | -.42 | .60 | .70 | | | | | | | | | | | | | | | | | | | A B | |
| 16 EC | .46 | .65 | .42 | .43 | .70 | .67 | .44 | .66 | .71 | | | .48 | .45 | 1 | | | | | | | | A C | |
| 17 P/N | .47 | .49 | -.42 | -.49 | .43 | | | | .81 | | | | -.63 | -.59 | 1 | | | | | | | C D | |
| 18 OM/N | -.60 | -.44 | | | -.43 | -.47 | | | -.49 | -.43 | | | .70 | .78 | | | | | | | | | |
| plant | | | | | | | | | | | | | | | | | | | | | | | |
| 19 VI | | | | | | | | | | | | | | | | | | | | 1 | | | A B C D E F |
| 20 VAM | | | | | | | | | | | | | | | | | | | | -.44 | 1 | | A B C D |
| 21 NOD | | | | | | | | | | | | | | | | | | | | | | 1 | A B C D E F |

The successional optimum points (SOPs) of *R. goodeyi*, *Paratylenchus*, *M. xenoplax* and *T. microphasmis* were between stage 1 and 2 (Table 3), whereas those of other taxa were between stage 2 and 3. *G. arcticus* and *H. conida* were found only in the degeneration stage (Table 3). For taxa that were present in different stages of succession the coefficients of variation of the population density (as can be calculated from Table 3) generally increased in the later stages (e.g. *R. goodeyi* from 0.50 to 1.27). Only *T. microphasmis* and *M. xenoplax* showed decreased coefficients of variance in later stages (from 0.76 to 0.48 and from 1.30 to 0.92, respectively). The number of taxa per sample ranged from 4 (sites 18 and 23) to 14 (site 14). The mean was higher in the late *Hippophaë* stage than in the early primary stage (Table 3). The maturity index for the total plant-feeding nematode fauna ranged from 2.57 at site 4 to 3.19 at site 21 (both late *Hippophaë* stage). Means for each successional stage (Table 3) were not different. However, when calculated only for obligate plant feeders (by excluding Tylenchidae), the maturity index increased significantly towards the later stages.

Nematode - environment ordination. Parameter sets for Canonical Correspondence Analysis (CCA) indicated by the letters A, C and E (Table 4) exclude factors that were correlated by $R > 0.67$ ($p < 0.001$), $R > 0.52$ ($p < 0.01$) and $R > 0.41$ ($p < 0.05$), respectively. Substitution of factors by their excluded correlatives hardly affected the results of the analysis. Sets B, D and F were derived from the sets A, C and E by eliminating parameters which were not significantly ($p < 0.05$) correlated with one of the first four environmental axes of the CCA. This selection of parameters led to improved significance of the canonical axes, and a higher trace per parameter (Table 5). Thus, parameters which were eliminated appeared to be less important. Redundance analysis (RA) resulted in non-significant ordinations, which implies that most taxa had a unimodal rather than a linear response to the environmental gradients. The results of CCA using nematode numbers were inferior to those of percentages.

Passive ordination of excluded environmental parameters (Fig. 1A) shows the imaginary position of these parameters in the simplified force field. Nearly identical or opposite direction of vectors does not always imply correlation between two factors. It may also reflect a similar or inverse relation, respectively, with the nematofauna. The ordination of sites (Fig. 1B) shows that sites of the early primary succession type are all situated in the lower left quadrant. Sites with early secondary succession are below the first axis, except for site 8, which developed after

deposition of sludge from a drinking-water purification plant. The sites below the first axis are predominantly mono-vegetations of *Hippophaë*. Old stable sites with degenerating scrubs are above the first axis, except for site 6, which has developed from sand with a low lime content (< 2% CaCO₃). Above the axis only mixed vegetations with *Hippophaë* are found; in the top right quadrant are two damp slacks and a typical dune meadow site, whereas in the top left quadrant the mixed scrubs and sites with a poor vegetation are located. The environmental optima of the nematode taxa (Fig. 1C) are arranged roughly along the same lines as the sites.

Table 5. Quality of ordination by Canonical Correspondence Analysis (CCA) or Redundance Analysis (RA) using percentages or numbers of plant feeding nematode taxa. Parameter sets A to F include environmental parameters as indicated in Table 4. The quality is expressed as the level of the eigen value of the first canonical axis and of the sum of eigen values (trace) of all axes and the probability of achieving these levels in a Monte Carlo Random Permutation test *= $p \leq 0.05$ **= $p \leq 0.01$.

| Ordination Method | CCA | CCA | CCA | CCA | CCA | CCA | RA | RA | CCA | CCA |
|-------------------------------|------------|-----------|------------|------------|-----------|-----------|------------|------------|------------|------------|
| % / nrs. | % | % | % | % | % | % | % | % | nrs. | nrs. |
| parameter set | A | B | C | D | E | F | B | D | D | F |
| nr. of parameters | 13 | 9 | 8 | 7 | 6 | 5 | 9 | 7 | 7 | 5 |
| Eigen value of the first axis | 0.50 * | 0.50 * | 0.50 * | 0.49 ** | 0.40 * | 0.38 * | 0.19 ns | 0.17 ns | 0.48 ** | 0.36 * |
| Trace | 2.08 ns | 1.70 * | 1.70 ns | 1.36 * | 1.00 * | 0.94 * | 0.69 ns | 0.44 ns | 1.30 ns | 0.92 ns |

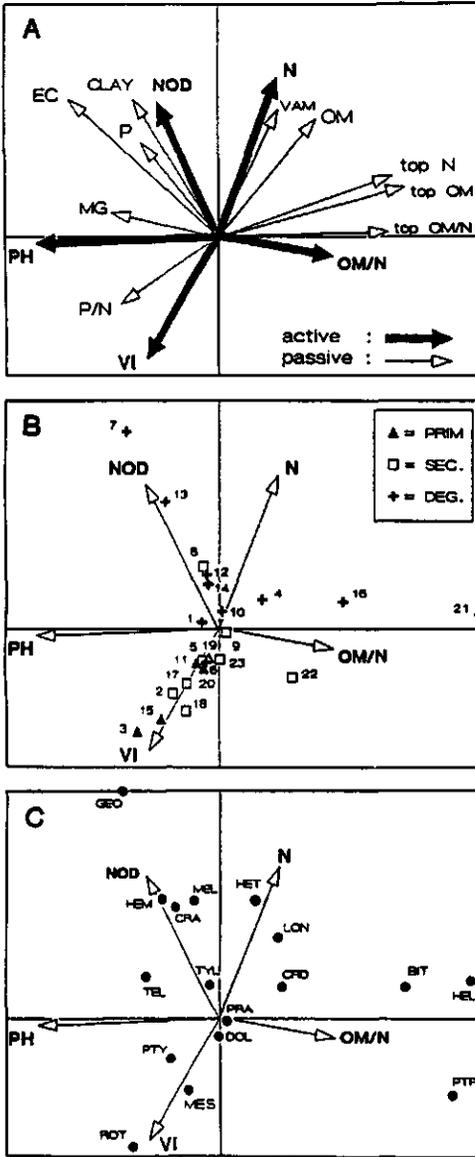


Fig. 1. Results of Canonical Correspondence Analysis of nematofauna and five selected environmental factors. Ordination of environmental parameters, sites and nematode optima on the first two canonical axes. **A.** Environmental parameters; for parameter codes see methods. **B.** Sampling sites; Symbols indicate successional stages: PRIM= early primary, SEC= early secondary, and DEG= degeneration stage; Site numbers refer to those in Table 1. **C.** Canonical optima of nematode taxa; Codes are the first three letters of the genus name, except for: Cra= *Criconeema*, Crd= *Criconemoides*, Pty= *Paratylenchus*, Ptr= *Paratrichodorus*, and Trh= *Tylenchorhynchus*.

Linear nematode - environment correlations. *R. goodeyi* was found in higher numbers under vigorous *Hippophaë* scrubs (Table 6), whereas numbers of *L. dunensis* tended to be higher under degenerating scrubs ($p=0.10$). *Meloidogyne* spp. and *L. dunensis* were correlated positively with total P and lime. Higher numbers of these two taxa and of *H. pseudorobustus* and *Pratylenchus* were found when total N or organic matter was higher. Most of the correlations which were found using nematode numbers (Table 6) were also found when relative abundances of taxa (percentage of total plant-feeding nematodes) were used. This indicates that effects on the total number of plant feeders did hardly affect the proportion of the major taxa.

Nematode - nematode correlations. Significant ($p<0.05$) negative correlations were observed between abundances of *R. goodeyi* and *T. microphasmis* and between *M. xenoplax* and *Pratylenchus* spp.. Positive correlations were found between *H. pseudorobustus* and *Pratylenchus* spp. ($p<0.05$), between *Meloidogyne* spp. and *Pratylenchus* spp. ($p<0.05$), and between *L. dunensis* and *Pratylenchus* spp. ($p<0.01$). *H. pseudorobustus*, *Paratrichodorus* spp. and total plant feeders were correlated due to their high numbers in an old slack (site 21).

Table 6. Nematode numbers - environmental factors correlations. Only coefficients of significant correlations are given; $n=23$, if $R>.41$ then $p<0.05$, if $R>.52$ then $p<0.01$. Nematode data were either original or $\log(x+1)$ transformed (largest R is shown). Taxa and factors that are not shown had no significant correlations.

| Taxon | pH | P | N | OM | LIME | K | Na | P/N | VI |
|----------------------------------|-------------------|-----|-----|-----|------|------------------|-----|------|-----|
| <i>Rotylenchus goodeyi</i> | . | . | . | . | . | .49 ¹ | . | . | .48 |
| <i>Mesocriconea xenoplax</i> | . | . | . | . | . | .48 ¹ | . | . | . |
| <i>Meloidogyne</i> spp. | .42 | .55 | .64 | .54 | .52 | . | .46 | . | . |
| <i>Pratylenchus</i> spp. | . | . | .51 | .45 | . | . | . | . | . |
| <i>Paratrichodorus</i> spp. | -.49 ¹ | . | . | . | . | . | . | . | . |
| <i>Helicotyl. pseudorobustus</i> | -.56 | . | .48 | .53 | . | . | . | -.48 | . |
| <i>Longidorus dunensis</i> | . | .48 | .43 | . | .43 | . | . | . | . |
| Total plant feeders | . | . | .42 | . | . | . | . | . | . |

¹) Correlation due to one deviating sample.

Table 7. Nematode densities (per 100 g soil) on a succession transect at Schouwen. Sites (see also Table 2.): A. *Elymus arenarius*; B. *Ammophila arenaria*; C. *H. rhamnoides* (invading); D. *H. rhamnoides* (degenerating); E. *Ligustrum vulgare*.

| NEMATODE TAXON \ SITE | A | B | C | D | E |
|---------------------------------------|------|------|------|------|------|
| <i>Rotylenchus goodeyi</i> | 0 | 1 | 0 | 5 | 0 |
| <i>Paratylenchus</i> spp. | 1 | 1 | 0 | 0 | + |
| <i>Mesocriconema xenoplax</i> | 0 | 3 | 10 | 3 | 6 |
| <i>Tylenchorhynchus microphasmis</i> | 0 | + | 2 | 29 | 1 |
| Heteroderinae (juv. 2) | 5 | 0 | 0 | 3 | + |
| Tylenchidae | 8 | 13 | 82 | 4 | 9 |
| <i>Telotylenchus ventralis</i> | 2 | + | 0 | 1 | 0 |
| <i>Pratylenchus</i> spp. | 1 | 1 | 3 | 4 | 9 |
| <i>Bitylenchus dubius</i> | 0 | 0 | 0 | 8 | 0 |
| <i>Paratrichodorus</i> spp. | 0 | 0 | 0 | 1 | 32 |
| <i>Helicotylenchus pseudorobustus</i> | 0 | 1 | 0 | 238 | 17 |
| <i>Longidorus dunensis</i> | 0 | 0 | 0 | 21 | 3 |
| <i>Hemicycliophora conida</i> | + | 0 | + | 0 | 0 |
| TOTAL PLANT FEEDERS | 15 | 19 | 97 | 317 | 78 |
| MATURITY INDEX | 2.48 | 2.28 | 2.15 | 3.12 | 3.37 |
| IDEM EXCL. TYLENCHIDAE | 2.94 | 2.86 | 3.00 | 3.14 | 3.54 |

(+ = present, but less than 1 per 100 g soil)

Transect sampling

Highest numbers of most taxa were found under the degenerating *Hippophaë* scrub at a meadow border (Table 7). *M. xenoplax* had a maximum in an earlier stage and *Paratrichodorus* spp. and *Pratylenchus* spp. in a later stage. *L. dunensis*, *Paratrichodorus* spp. and *B. dubius* were not found on the pre-*Hippophaë* sites.

Rhizosphere sampling

T. microphasmis was the dominant species in the area, which is in agreement with the field survey results. Densities of *R. goodeyi* and *M. xenoplax* in the bulk soil on the seaward dune ridge were lower than at comparable sites in the field survey. For most taxa, the density in the rhizosphere was much higher than in the bulk soil (Table 8). Generally, the ratio rhizosphere/bulk soil was higher in the inner dune sites compared to the sites on the seaward ridge. In a few cases (e.g. for *H. conida*) a lower density was found in the rhizosphere than in the bulk soil.

Densities in rhizosphere soil were higher at the inner dune sites, especially in the case of *T. microphasmis*, Tylenchidae and *Pratylenchus* spp. Population densities in bulk soil were not much different between sites, except for the higher level of *T. microphasmis* in the vigorous *Hippophaë* scrub and the absence of certain taxa at some locations. *Pratylenchus* spp. were very much concentrated in the rhizosphere, and thus their numbers may have been largely underestimated in the field survey and transect samplings.

Table 8. Plant feeding nematodes in *Hippophaë* seedling rhizosphere (R) and bulk (B) soil. Numbers per 100 g. soil. Locations: 1. seaward ridge, between degenerating *Ammophila*; 2. id. between vigorous *Hippophaë*; 3. inner dunes, between degenerating *Hippophaë*; 4. id., grasses post-*Hippophaë* stage.

| Taxon \ Location | 1 | 2 | 3 | 4 |
|--------------------------------------|--------|--------|--------|---------|
| | R / B | R / B | R / B | R / B |
| <i>Rotylenchus goodeyi</i> | 4/ 1 | 6/ 2 | 0/ 1 | 17/ 1 |
| <i>Mesocriconea xenoplax</i> | 0/ 1 | 3/ 4 | 0/ 2 | 17/ 2 |
| <i>Tylenchorhynchus microphasmis</i> | 196/37 | 245/72 | 498/28 | 503/34 |
| Tylenchidae | 5/ 1 | 44/ 3 | 36/ 1 | 1418/27 |
| <i>Pratylenchus</i> spp. | 22/ 1 | 15/ 1 | 102/ 1 | 0/ 0 |
| <i>Longidorus dunensis</i> | 0/ 0 | 0/ 0 | 32/17 | 4/ 4 |
| <i>Paratrichodorus</i> spp. | 3/ 0 | 0/ 1 | 12/ 2 | 0/ 2 |
| <i>Hemicyclophora conida</i> | 0/11 | 0/ 0 | 0/ 0 | 0/ 0 |
| Ratio rhizosphere/bulk soil | 4.4 | 3.8 | 13.1 | 28.0 |
| Final shoot biomass (g) | 4.7 | 4.0 ns | 3.9 *) | 3.5 ** |

1) significantly different from location 1.: * = $p < 0.05$; ** = $p < 0.01$

DISCUSSION

Environmental development. Development of environmental characteristics in *Hippophaë* vegetation proceeds along the vigour- and nitrogen vectors, rather than exactly along one of the nematode-environment axes (Fig. 1). From the primary succession stage with low soil-N concentrations and high *Hippophaë* vigour, locations develop (directly, or after disturbance and secondary succession) towards a stable state with higher concentrations of total N. There is a divergence of sites in the degeneration stage, either towards a lower pH and an increased ratio organic matter/N (dune slacks and meadows), or towards an increased *Frankia*-

nodulation potential (NOD) (mixed scrubs on calcareous sites). The ecological meaning of NOD is not clear, but it probably reflects the duration and intensity of the *Frankia* - *Hippophaë* symbiotic history of a certain site (Oremus, 1979).

Of the five most important environmental factors in the ordination, pH and total N are correlated with a number of other factors (Table 4), and thus positions of sites or nematode taxa might (albeit less strongly) also be attributed to those other factors.

Nematofauna development. Dune ageing and vegetational development are accompanied by development of the plant-feeding nematode fauna. The number of taxa and the maturity index of this trophic group increase (Table 3), because species with slower dispersal, longer generation period, and larger body size (e.g. *L. dunensis*) or less resistance to environmental stress (e.g. *Paratrichodorus*) become more abundant (Bongers, 1990). This may proceed partly at the expense of species with opposite characteristics (e.g. *Paratylenchus* spp., *R. goodeyi* and *M. xenoplax*). The significant difference between maturity indices of obligate plant-feeding nematofaunas of different *Hippophaë* stages shows the sensitivity of the maturity index as a measure of ecosystem stability and long-term development.

Paths of nematofaunal succession can be derived from taxon optima in the ordination (Fig. 1C). The 'average' development proceeds from *R. goodeyi* in the young primary stage soils, through successive optima of *M. xenoplax*, *Paratylenchus* spp., *T. microphasmis* and *Pratylenchus* spp. to *L. dunensis* and others under degenerating scrubs on old stable dunes. As in the edaphic development, there is also some divergence in the nematode fauna. This is mainly due to a few sites which deviate both in environment and in nematofauna. The ordination of *Paratrichodorus* spp. was largely affected by the high abundance of *P. pachydermus* in an old dune slack (site 21). *B. dubius* was found mainly at a decalcified dune meadow site (16). Conversely, *G. arcticus* was found almost exclusively under degenerating *Hippophaë* vegetations on dry calcareous soil (sites 7 and 13).

Ordination of environment, sites and nematofauna by canonical correspondence analysis (CCA) appeared a useful way to visualize successional changes and ecological associations. The better results of CCA compared to redundancy analysis indicate that nematodes have unimodal rather than linear responses to the selected environmental factors. Relative abundances of taxa (percentages) were more informative in CCA than numbers (Table 5), probably because the number of plant-feeding nematodes had more residual variation than the species composition

of the nematofauna. Comparison of numbers and percentages in linear nematode - environment correlation supports the conclusion from CCA that variation in the total number of plant-feeding nematodes hardly affects the species composition.

Nematode dispersal and niche differentiation. For plant-feeding nematodes, the presence and productivity of their host plants are the chief determinants of population densities (Yeates, 1987). However, in the relatively young dune soil the presence of nematode species for which *Hippophaë* is a good host seems largely determined by accidental arrival and subsequent colonization. Growth of *Hippophaë* is optimal in the early stages, and thus high densities of *Hippophaë*-feeding nematode species would be expected if they were present all over the site and if environmental constraints were not stronger than in later stages. In the present survey some taxa were not found in the early primary stage (Table 3). One could conclude that *R. goodeyi*, *M. xenoplax* and *T. microphasmis* are much better dispersed than *L. dunensis*. One of the reasons for this may be that *L. dunensis*, contrary to e.g. *T. microphasmis*, is almost absent from the top 10 cm of soil (F.C. Zoon, unpubl.). Taxa which are present in the top soil and which are more resistant to dehydration are more easily dispersed by wind (Gaur, 1988) or biotic vectors. Active dispersal through the soil generally takes place at a rate of less than a meter per annum. Dispersal by man, the major means of nematode colonization in new polders (Kuiper, 1977), may be important for nematode species present in the rhizosphere of Marram grass (*Ammophila arenaria* (L.) Link), which is used as planting material in coastal defence practice. The ability of species to colonize preceding vegetations may also contribute to their presence in early *Hippophaë* stages. Populations of *R. goodeyi*, *Pratylenchus* spp., *Paratrichodorus nanus* and Tylenchorhynchinae (*Telotylenchus ventralis* and *T. microphasmis*) were found on outer dune locations with a vegetation of *Ammophila arenaria* (van der Putten, 1989). These taxa, as well as *M. xenoplax*, *Helicotylenchus pseudorobustus* and *Hemicycliophora conida* were also found at some pre-*Hippophaë* sites in the present study. Species found at these sites presumably feed on *Ammophila*. *Pratylenchus* sp. was able to multiply on *Ammophila* in a pot experiment (van der Putten, 1989).

Coefficients of variation of the mean population density of taxa generally increased during succession, only in the case of *M. xenoplax* and *T. microphasmis* it decreased. The rise in variation may be due to an increasing difference between environmental constraints of sites, or to an increasing clustering of nematode

sub-populations within sites together with the development of mixed vegetations. The latter may result in site compartments with near maximal densities of preferentially *Hippophaë*-feeding nematodes, alternating with compartments having much lower densities. The decrease in variation observed for *M. xenoplax* and *T. microphasmis* may be attributed to incomplete colonization of many early stage sites, and little response to changing soil and vegetation characteristics in later stages. Both of these species remain present in fairly high densities in later *Hippophaë* stages. The possible role of changing environmental constraints can only partly be explained by the results of the present study. Plant-feeding nematode taxa exhibit a certain optimum during the course of succession as is illustrated by the SOP in Table 3 and in the results of CCA. The optimum for a taxon as found by CCA (Fig. 1C) corresponds to environmental factors, which are not necessarily functionally related to the presence or abundance of a taxon. The correlation of *Meloidogyne* and *H. pseudorobustus* with total N and organic matter (Table 6) may be due to the fact that better host plants (grasses) are present in the late *Hippophaë* stages. The development of the herbaceous undergrowth can determine the nematode fauna to a great extent as was found in tree nurseries (Niblack & Bernard, 1985). However, the positive correlation of different taxa and of the total number of plant feeders with total N (Table 6) may also have a functional background in the food quality (Yeates, 1987). The association between *Longidorus dunensis* and nitrogen may be related to its slow dispersal and late arrival under *Hippophaë*. On the isle of Texel, *L. dunensis* was not found, in spite of high ultimate levels of total N. It seems that the edaphic factors studied do not directly explain the presence or absence of the major taxa. Nevertheless, they do correspond to the relative abundance of taxa within the plant-feeding nematode community and they may be key factors in the regulation of population densities. Especially, the nitrogen availability, which in the early stages limits plant growth (Willis & Yemm, 1961; Vitousek & Walker, 1987) will be even more limiting to nematodes, as these have a lower C/N ratio than plants (White, 1984; Yeates, 1987). This may also explain the lower density of *T. microphasmis* in the rhizosphere of seedlings planted at the foredune sites (Table 8). The content of available nitrogen in soil at these sites was about 10 meq/kg, compared to 20 meq/kg at the inner-dune sites (Zoon unpubl.).

The negative correlations *R. goodeyi* - *T. microphasmis*, and *M. xenoplax* - *Pratylenchus* spp. in the survey may indicate interspecific competition or differential host preference. From what is known about the feeding habits of these or congen-

ric taxa, and their feeding sites on the root (Klinkenberg, 1963; Wyss, 1981) competition seems possible. For reasons discussed above, the statement that plant-feeding nematodes are usually not resource limited, and therefore seldom compete for common resources (Norton, 1989) appears to face exceptions in sand dune ecosystems.

Hippophaë rhamnoides as a host plant. Many of the plant-feeding nematode species found under *H. rhamnoides* are able to multiply on this shrub species. Populations of *T. microphasmis* increased by a factor 5 within four weeks after inoculation in sterilized sand in greenhouse pot experiments with *Hippophaë* seedlings (Maas *et al.*, 1983). For *L. dunensis* the same authors found multiplication by a factor 3.5 in four weeks, but a factor 9 was found if lower densities were inoculated in large soil columns (Zoon unpubl. res.). *M. xenoplax* is a parasite of various woody and herbaceous plants especially in sandy soils (Knobloch & Bird, 1978; Nyczepir & Lewis, 1984; Zehr *et al.*, 1986; Güntzel *et al.*, 1987). Pot experiments with different nematode species (Zoon, unpubl. res.) show that *M. xenoplax* is also able to multiply on *Hippophaë* (from 20 to 27000 per dm³ soil in 6 months). A population of *P. pachydermus* increased from 20 to 6000 within 6 months, but *H. pseudorobustus* failed to multiply substantially (from 20 to 70 in 6 months). In the present study, no root knots or cysts were observed on *Hippophaë* roots isolated from the field, indicating that the Heteroderidae species present do not feed on *H. rhamnoides*.

The ratio rhizosphere/bulk soil (Table 8) can also give an indication of the host preference of a nematode species. In addition to the host relations mentioned above, *Pratylenchus* spp. appear to prefer *Hippophaë*, whereas *H. conida* seems to avoid this plant. The latter confirms observations by Kuiper (1977) that woody plants, such as apple and pear trees are non-hosts for *H. conida*. Population densities of nematodes on a soil volume basis (Tables 3 and 7) were low in these dune soils when compared to agricultural soils. This is probably due to the very diffuse rooting in the poor dune sands and the low level of primary production. However, nematodes may be considered harmful on a root length basis. The sampling of bulk soil greatly underestimated the number of *Pratylenchus* spp. in the rhizosphere. Densities of *T. microphasmis* and *M. xenoplax* in bulk soil were relatively high on a site closely surrounded by vigorous *H. rhamnoides* (site 2 in Table 8), probably because there were many *Hippophaë* roots present. No other plant species were present at this site. Growth reduction of the *Hippophaë* seed-

lings (Table 8) seems to be related to the population density of *T. microphasmis* in the rhizosphere ($p=.14$). The pathogenicity of this nematode towards *H. rhamnoides* is evident from pot experiments (Zoon, in prep.). As discussed above, higher availability of soil N may be favourable for nematode populations, but in addition, root damage by nematodes and the subsequently reduced uptake of P by the host plant (Zoon, in prep.) might also increase the N concentrations in the root tissues, thus offering a better food quality for nematodes. It seems possible that nematodes and other root herbivores do not only increase the food quality for foliar feeding herbivores (Gange & Brown, 1989), but also for themselves. Nonetheless, the root damage may lead to degeneration or loss of competitive ability. The ordination (Fig. 1) shows that low vigour of *Hippophaë* is associated with high N, OM and VAM and with low P:N ratio. The increased mycorrhiza (VAM) is an indication for P-limitation (Menge *et al.*, 1978). Therefore, we suggest that the change in the balance of nutrient resources (N and P) may be the ultimate factor in this succession (Tilman, 1982), whereas plant-feeding nematodes may be the proximate factor by interfering with the plants nutrient uptake capacity.

Nematode - host relationships with other plant species of late- and post-*Hippophaë* stages were not studied extensively. It appears that *H. pseudorobustus* is associated with gramineous hosts which often form the undergrowth of degenerating *Hippophaë* scrubs. Multiplication on *Hippophaë* in pots was negligible. Numbers of *L. dunensis* were lower at sites of the post-*Hippophaë* stage than in the preceding degeneration stage (Tables 7, 8), but it is not clear whether this is due to the appearance of inferior hosts.

In conclusion, this study has demonstrated that during natural development of soil and vegetation there can be a considerable development of the plant-feeding nematode fauna, even if the dominant plant species remains the same. This process may be of importance for the decline of *H. rhamnoides* and for the rate and direction of vegetational succession in general.

REFERENCES

- Akkermans A.D.L. (1971). Nitrogen Fixation and Nodulation of *Alnus* and *Hippophaë* under Natural Conditions. Ph.D. Thesis, Univ. of Leiden, The Netherlands, 85 p.
- Bongers A.M.T. (1990). The maturity index: an ecological measure of environmental disturbance based on nematode species composition. *Oecologia* 83: 14-19.
- Brinkman H., Loof P.A.A. & Barbez D. (1987). *Longidorus dunensis* n. sp. and *L. kuiperi* n. sp. from the sand dune coastal region of the Netherlands (Nematoda: Longidoridae). *Revue Nématol.* 10: 299-308.
- Brundrett M.C., Piché Y. & Peterson R.L. (1984). A new method for observing the morphology of vesicular-arbuscular mycorrhizae. *Can. J. Bot.* 62: 2128-2134.
- Fortuner R. & Luc M. (1987). A reappraisal of Tylenchida (Nemata). 6. The family Belonolaimidae Whitehead, 1960. *Revue Nématol.* 10: 183-202.
- Gange A.C. & Brown V.K. (1989) Effects of root herbivory by an insect on foliar-feeding species, mediated through changes in the host plant. *Oecologia* 81: 38-42.
- Gaur H.S. (1988). Dissemination and mode of survival of nematodes in dust storms. *Indian J. Nematol.* 18: 94-98.
- Güntzel O., Klingler J. & Delucchi V. (1987). Tylenchids (Nematoda) extracted from soil of Swiss vineyards north of the Alps. *Revue Nématol.* 10: 361-368.
- Kisiel M. (1970). Studies on the ecology of the nematodes inhabiting *Ammophila arenaria* plant communities on beaches and dunes of Baltic sea shores. *Akad. Rolnicza* 34: 111-150.
- Klinkenberg C.H. (1963). Observations on the feeding habits of *Rotylenchus uniformis*, *Pratylenchus crenatus*, *P. penetrans*, *Tylenchorhynchus dubius* and *Hemicycliophora similis*. *Nematologica* 9: 502.
- Knobloch N. & Bird G.W. (1978). Criconematinae habitats and *Lobocriconema thornei* n.sp. (Criconematidae: Nematoda). *J. Nematol.* 10: 61-70.
- Kuiper K. (1977). Introductie en vestiging van planteparasitaire aaltjes in nieuwe polders in het bijzonder van *Trichodorus teres*. Meded. Landbouwhogeschool Wageningen 77 (4): 140 p.
- Loof P.A.A. & De Grisse A. (1989). Taxonomic and nomenclatorial observations on the genus *Criconemella* De Grisse & Loof, 1965 sensu Luc & Raski, 1981 (Criconematidae). *Meded. Fac. Landbouww. Rijksuniv. Gent* 54: 53-74.
- Maas P.W.Th., Oremus P.A.I. & Otten H. (1983). Nematodes (*Longidorus* sp. and *Tylenchorhynchus microphasmis* Loof) in growth and nodulation of Sea Buckthorn (*Hippophaë rhamnoides* L.). *Plant and Soil* 73: 141-147.
- Menge J.A., Steyrlé D., Bagyaraj D.J., Johnson E.L.V. & Leonard R.T. (1978). Phosphorus concentrations in plants responsible for inhibition of mycorrhizal infection. *New Phytol.* 80: 575-578.

- Niblack T.L. & Bernard E.C. (1985). Plant parasitic nematode communities in Dogwood, Maple and Peach nurseries in Tennessee. *J. Nematol.* 17: 132-139.
- Norton D.C. (1989). Abiotic soil factors and plant-parasitic nematode communities. *J. Nematol.* 21: 299-307.
- Novozamsky I., Houba V.J.G., Temminghoff E. & Van der Lee J.J. (1984). Determination of 'total' N and 'total' P in a single soil digest. *Neth. J. agric. Sci.* 32: 322-324.
- Nyczepir A.P. & Lewis S.A. (1984). Incidence of *Fusarium* and *Pythium* spp. in peach feeder roots as related to Dibromo-chloropropane application for control of *Criconebella xenoplax*. *Pl. Dis.* 68: 487-499.
- Olson J.S. (1958). Rates of succession and soil changes of southern Lake Michigan sand dunes. *Bot. Gaz.* 119: 125-170.
- Oostenbrink M. (1960). Estimating nematode populations by some selected methods. *In: Sasser J.N. & Jenkins W.R. (Eds), Nematology.* Chapel Hill, North Carolina Univ.Press: 85-102.
- Oremus P.A.I. (1979). A quantitative study of nodulation in *Hippophaë rhamnoides* ssp. *rhamnoides* in a coastal dune area. *Plant and Soil* 52: 59-68.
- Oremus P.A.I. (1982). Growth and nodulation of *Hippophaë rhamnoides* L. in the coastal sand dunes of the Netherlands. Ph.D. thesis, Univ. of Utrecht, The Netherlands, 116 p.
- Oremus P.A.I. & Otten H. (1981). Factors affecting growth and nodulation of *Hippophaë rhamnoides* ssp. *rhamnoides* in soils from two successional stages of dune formation. *Plant and Soil* 63: 317-331.
- Pearson M.C. & Rogers J.A. (1967). Flora of the British isles. *Hippophaë rhamnoides* L. *J. Ecol.* 50: 501-513.
- Rozema J., Laan P., Broekman R., Ernst W.H.O. & Appelo C.A.J. (1985). On the lime transition and decalcification in the coastal dunes of the province of North Holland and the island Schiermonnikoog. *Acta Bot. Neerl.* 34: 393-411.
- Salisbury E.J. (1922). The soils of Blakeney Point: a study of soil reaction and succession in relation to plant covering. *Ann. Bot.* 36: 391-431.
- Siddiqi M.R. (1986). Tylenchida, parasites of plants and insects. Commonwealth Inst. Parasitol. Farnham Royal, Slough, U.K., 645 p.
- Stewart W.D.P. & Pearson M.C. (1967). Nodulation and nitrogen fixation by *Hippophaë rhamnoides* in the field. *Plant and Soil* 26: 348-360.
- Stuyfzand P.J. (1984). Effecten van vegetatie en luchtverontreiniging op de grondwaterkwaliteit in kalkrijke duinen bij Castricum: lysimeterwaarnemingen. *H₂O* 17: 152-159.
- Ter Braak C.J.F. (1987a). Ordination. *In: Jongman R.H.G et al. (Eds), Data analysis in community and landscape ecology.* Wageningen, Pudoc: 91-173.
- Ter Braak C.J.F. (1987b). CANOCO - a FORTRAN program for canonical community ordination by [partial] [detrended] [canonical] correspondence analysis, principal components analysis and redundancy analysis (version 2.1). Agriculture Mathematics Group, Wageningen.

- Van der Maarel E., Boot R., Van Dorp D. & Rijntjes J. (1985). Vegetation succession on the dunes near Oostvoorne, The Netherlands; A comparison of the vegetation in 1959 and 1980. *Vegetatio* 58: 137-187.
- Van der Putten W.H. (1989). Establishment, growth and degeneration of *Ammophila arenaria* in coastal sand dunes. Ph.D. Thesis, Wageningen Agricultural University, The Netherlands, 152 p.
- Vitousek P.M. & Walker L.R. (1987). Colonization, succession and resource availability: ecosystem-level interactions. *In*: Gray A.J. *et al.* (Eds), *Colonization, Succession and Stability*. Oxford, Blackwell Sci. Publ.: 207-223.
- Wasilewska L. (1970). Nematodes of the sand dunes in the Kampinos forest. I. Species structure. *Ekol. Pol.* 18: 429-443.
- Wasilewska L. (1971). Nematodes of the sand dunes in the Kampinos forest. II. Community structure based on numbers of individuals, state of biomass and respiratory metabolism. *Ekol. Pol.* 19: 651-688.
- White T.C.R. (1984). The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia* 63: 90-105.
- Willis A.J., Folkes B.F., Hope-Simpson J.F. & Yemm E.W. (1959). Braunton Burrows: The dune system and its vegetation. Part I. *J. Ecol.* 47: 1-24.
- Willis A.J. & Yemm E.W. (1961). Braunton Burrows: mineral nutrient status of the dune soils. *J. Ecol.* 49: 377-390.
- Wyss U. (1981). Ectoparasitic root nematodes: feeding behaviour and plant cell responses. *In*: Zuckerman B.M. & Rohde (Eds), *Plant Parasitic Nematodes*, Vol. III. New York, Academic Press : 325-351.
- Yeates G. (1987). How plants affect nematodes. *Adv. Ecol. Res.* 17: 61-113.
- Zehr E.I., Lewis S.A. & Bonner, H.J. (1986). Some herbaceous hosts of the ring nematode (*Criconebella xenoplax*). *Pl. Dis.* 70: 1066-1069.

APPENDIX OF CHAPTER 2

Table 9. Chemical soil properties as related to the stage of succession of *Hippophaë rhamnoides*. Values of soil parameters are per 100 gram of dry soil from the 10-70 cm layer. Different letters per row indicate significant differences between stages ($p < 0.05$).

| stage or type of succession | Early H.r. Primary n=5 | | Early H.r. Secondary n=8 | | Late H.r. Fixed Dunes n=10 | |
|-----------------------------------|------------------------------|------|--------------------------------|------|----------------------------------|------|
| | Mean | SE | Mean | SE | Mean | SE |
| pH(H ₂ O) | 8.44 a | 0.14 | 7.49 a | 0.42 | 7.36 a | 0.43 |
| total P mg/100 g | 10.2 a | 1.15 | 10.0 a | 1.20 | 9.9 a | 1.35 |
| total N mg/100 g | 14.0 a | 1.30 | 23.5 ab | 4.20 | 25.4 b | 1.56 |
| Organic Matter % | 0.27 a | 0.04 | 0.48 ab | 0.10 | 0.50 b | 0.04 |
| Carbonate % | 2.74 a | 0.92 | 1.59 a | 0.61 | 2.06 a | 0.70 |
| Mg me/100 g | 0.24 a | 0.04 | 0.16 a | 0.03 | 0.19 a | 0.03 |
| K x100 me/100 g | 3.20 a | 0.73 | 2.25 a | 0.16 | 2.52 a | 0.22 |
| Na x100 me/100 g | 8.00 a | 0.63 | 7.63 a | 2.06 | 6.90 a | 1.28 |
| Cl me/100 g | 0.93 a | 0.07 | 0.78 a | 0.11 | 0.81 a | 0.07 |
| EC μ S | 72.6 a | 6.8 | 60.5 a | 8.7 | 59.1 a | 8.1 |
| Particle size median μ | 190 a | 7.5 | 197 a | 4.4 | 184 a | 6.1 |
| Clay % | 0.63 a | 0.10 | 0.65 a | 0.06 | 0.74 a | 0.09 |
| P/N ratio (PNR) | 0.74 a | 0.08 | 0.47 b | 0.07 | 0.39 b | 0.05 |
| Org.M/N (OMNR) | 19.2 a | 1.98 | 20.0 a | 1.02 | 19.7 a | 0.98 |

APPENDIX OF CHAPTER 2

Table 10. Plant parameters as related to the stage of succession of *Hippophaë rhamnoides*. Different letters per row indicate significant differences between stages ($p < 0.05$). For methods see chapter 2.

| stage or type of succession | Early H.r. Primary n=5 | | Early H.r. Secondary n=8 | | Late H.r. Fixed Dunes n=10 | |
|-----------------------------------|------------------------------|------|--------------------------------|------|----------------------------------|------|
| | Mean | SE | Mean | SE | Mean | SE |
| Vigour Index (VI) | 4.00 a | 0.57 | 3.75 a | 0.52 | 1.65 b | 0.13 |
| Frankia Potential (NOD) | 4.00 a | 1.26 | 2.88 a | 1.27 | 8.00 a | 3.81 |
| VAM Infection % | 35.6 a | 11.1 | 40.8 a | 7.3 | 61.8 b | 5.13 |

Table 11. Concentrations of nitrogen and phosphorus (mg/kg) at two field sites of different successional stage. Site numbers correspond to those in Table 8 of Chapter 2. Nutrient concentrations are means of 6 layers from depth 0-60 cm, each in four replicates.

| Successional stage site nr. | Hr optimal | Hr decline |
|--------------------------------|--------------|--------------|
| | 2 | 3 |
| total N | 252.0 | 350.0 |
| NH ₄ ⁺ N | 6.4 | 5.6 |
| NO ₃ ⁻ N | 10.5 | 19.3 |
| total P | 117.1 | 128.6 |
| Olsen P | 9.7 | 4.0 |

CHAPTER 3.

Interactive effects of rhizosphere fungi and nematodes on growth and nodulation of sea buckthorn, *Hippophaë rhamnoides*

F.C. Zoon

submitted to Soil Biol. Biochem.

SUMMARY

The natural decline and succession of *Hippophaë rhamnoides* (HR), an actinorhizal pioneer shrub species in coastal sand dunes, was previously suggested to be caused by biotic soil factors. The aim of this study was to assess effects of groups of soil biota and their possible interactions on nodulation and growth of HR. Effects of fungi and nematodes on growth and nodulation were studied by treating soils of three successional stages of HR vegetation, and a *Frankia*-amended sterilized soil, with the fungicides benomyl or propamocarb, the nematostaticum oxamyl, or combinations. Furthermore, the pathogenicity of some fungus isolates was tested in sterilized soil. Nodulation by *Frankia* was greatly enhanced by benomyl in the natural HR soils, but not in sterilized soil. Shoot growth was also increased when benomyl was applied, except in soil of an early successional stage. The dominant fungi isolated from roots and rhizoplane of HR in the natural soils were *Cylindrocarpon destructans* and *Fusarium oxysporum*. Their infection percentages were greatly reduced by benomyl. Benomyl also reduced populations of certain plant-feeding nematodes. Oxamyl increased fungal colonization of roots and rhizoplane, but had no or a stimulating effect on nodulation and shoot growth, probably due to the simultaneous decrease of plant parasitic nematodes. The abovementioned effects suggest that plant parasitic nematodes, apart from being harmful by themselves, predispose the plants to damage by fungi. Inoculation of fungi in sterilized sand with HR showed that only one *Cylindrocarpon* isolate was slightly pathogenic, when present alone. The results indicate that plant parasitic nematodes alone, or in combination with soil fungi may decrease the vigour of HR in the field, particularly when the availability of soil nitrogen and the inoculum potential of *Frankia* are both low.

INTRODUCTION

Sea buckthorn (*Hippophaë rhamnoides* L. ssp. *rhamnoides*) is a nitrogen-fixing pioneer shrub species in the calcareous coastal sand dunes of Western Europe (Pearson & Rogers, 1967). The decline of *H. rhamnoides* and its replacement in the succession sequence is thought to be stimulated by harmful soil organisms, because growth suppression was largely eliminated by gamma-irradiation of the soil (Oremus, 1982). So far, only a few examples of succession in natural vegetation have been shown to be associated with effects of soil-borne pathogens including nematodes (e.g. Weste, 1981; Van der Putten *et al.*, 1993).

The mycoflora of dune soils has been found to change during early primary succession of the vegetation (Webley *et al.*, 1952; Brown, 1958). However, Oremus (1982) found no significant differences in the mycoflora of *Hippophaë* roots between soils from two successional stages. The plant-feeding nematofauna associated with *H. rhamnoides* in the field appears to change gradually from the colonization stage towards the decline stage, although some species remain dominant (Zoon *et al.*, 1993). This might affect the decline of *Hippophaë*.

Certain plant parasitic nematodes have been shown to be harmful to *H. rhamnoides* and seem to interact synergically with unknown biotic factors in causing damage; in non-sterilized soil fewer nematodes were necessary to invoke a certain level of growth reduction than in sterilized soil (Maas *et al.* 1983). The present study explores the effects of, and possible interactions between nematodes and fungi in natural soil, making use of selective inhibition by pesticides. Moreover the pathogenicity of some isolates of dominant root fungi is tested in an inoculation experiment.

MATERIALS AND METHODS

Selective inhibition experiment

Sand dune soils (5-50 cm depth) were taken from three *Hippophaë* locations of different age. The *Hippophaë* vegetation was about 15, 60-80, and >100 years old on sites A, B, and C, respectively (site numbers 15, 12 and 16 in Zoon *et al.*, 1993). The vegetation of *H. rhamnoides* can be classified as optimal (A), or degenerating (B and C). These soils, and a 2.5 Mrad gamma-irradiated sand dune soil from an ante-*H. rhamnoides* stage were treated as follows:

1. CO: control, with addition of water instead of pesticide solution.
2. BE: benomyl (Benlate 50WP; against hyphomycetes) 15 mg a.i./kg, at t=0 and 45 days.
3. PR: propamocarb (Previcur N; against phycornycetes) 15 mg a.i./kg, at t=0 and 45 days.
4. OX: oxamyl (Vydate 10G/FL; against mesofauna), 20 mg a.i./kg Vydate 10G (at t=0) and Vydate FL (at t=20, 45 and 60 days)
5. OXBE: combination of oxamyl and benomyl treatment.
6. OXPR: combination of oxamyl and propamocarb treatment.

At t=0 the chemicals were mixed through the soil in concentrated form, all later treatments were injected in 60 ml water per pot. In the irradiated soil, the actinorhizal endophyte *Frankia* was mixed through the soil as a suspension of homogenized surface-sterilized nodules from a site with colonizing *H. rhamnoides* at Voorne, the Netherlands (0.7 g fresh nodule weight per kg soil).

Hydroponic *Hippophaë* seedlings (6-8 leaves) (Oremus, 1980) were weighed and planted in pots with 500 g of the four soils (5 replicates per treatment in a randomized block design) and kept in a greenhouse (September-November) which was set at 23 °C day and 15 °C night temperature. A daylength of 16 h was maintained with additional illumination ($150 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) in the morning and in the evening. The soil water potential was maintained between -3 and -10 Kpa (10 ± 3 % of dry weight) by replenishing with 1/2 strength Hoagland solution in which NO_3^- was replaced by an equivalent amount of Cl^- . The plants were harvested 12 weeks after planting. At harvest, plant biomass was weighed and root nodules larger than 2 mm were counted. Suspensions of fungal propagules from the rhizoplane of roots were obtained by shaking 40 white root pieces of one cm for 5 minutes in 10 ml sterile water with glass beads (diameter 1 mm). Dilution series of these suspensions were plated out on 1:20 potato dextrose agar/water agar with 150 ppm streptomycin for plate counts. In addition, 25 one-cm root segments of each treatment x soil origin combination were surface-sterilized (30 s in 1 % NaOCl), rinsed in sterile water and incubated on the same agar medium to determine the level of internal root infection. The number of infected pieces was assessed after incubation during 2 and 4 days at 20 °C. Differences in the percentage fungal infection were analyzed using a binomial test. Fungal isolates were classified according to colony form and radial growth rate and representatives were used for identification (Domsch *et al.*, 1980), in order to get an indication of possible changes in species composition due to the treatments.

Plant and nematode parameters were analyzed by ANOVA, with oxamyl and fungicides as separate treatments. Direct effects (e.g. phytotoxicity) of the chemical treatments on growth and nodulation were accounted for by adding the chemicals to plants in sterilized *Frankia*-amended soil. The results were used to calculate corrected effects of the treatments in the natural soils. Means were standardized to the untreated control in the gamma-irradiated soil. It was assumed that soil biota did not influence the direct effects of the chemicals on the plant and thus, these direct effects are assumed to be proportionally the same in all soils.

Fungus inoculation experiment

Seven fungal isolates from roots of the inhibition experiment were inoculated in gamma-sterilized dune sand as a homogenized sand-oat culture. The control soil received an equivalent amount of non-inoculated sand-oat medium. This resulted in a slight difference in available nitrogen (extracted in 2 M KCl); about 4.1 vs. 3.3 ppm NO₃ and 7.1 vs. 14.6 ppm NH₄ in the control and the fungus treatments, respectively, at the start of the experiment. The initial density of colony forming units per gram of soil (Table 4) was estimated by counting colonies in series of soil dilution plates (1:20 PDA:water agar). *Hippophaë* seedlings were planted as in the previous experiment in 5 replicates per isolate in a randomized block design (classes of initial seedling weight as blocks), cultured as described above and harvested after 45 days. The water lost by evapo-transpiration was recorded for each pot three times a week and replenished. To calculate cumulative water uptake, the intercept of the regression of the total water use on the biomass formed ($R^2=0.92$; $p<0.001$) was taken as cumulative evaporation and subtracted. Fungi were recovered from roots and rhizoplane in two replicates with the same methods as described for the previous experiment.

RESULTS

Selective inhibition experiment

Shoot growth. In sterilized soil, oxamyl appeared to be phytotoxic (Fig. 1a). This phytotoxic effect was also found in natural soil A (Fig. 1b). If phytotoxicity of oxamyl in gamma-sterilized soil is taken into account, a positive effect of oxamyl on growth is found in soil from site B (Fig. 1g). Benomyl had a significant positive effect on shoot growth in the non-sterilized soils B and C, also after correction (Fig. 1fg). The combination of oxamyl and benomyl stimulated growth in soil A, but did the opposite in soil B when compared with oxamyl alone. Propamocarb (PR) did not affect growth, neither added to the effect of oxamyl.

Nodulation. Nodulation in sterilized (*Frankia*-amended) soil was strongly inhibited by all treatments, but in all three natural soils there was a significant positive effect of benomyl, and in soil from site A also of propamocarb (Fig. 2b). If direct treatment effects in sterile soil are taken into account, positive effects of all treatments on nodulation are found in soils A and B, and only of benomyl in soil C (Fig. 2fgh).

Root infection. There was a significant reduction of the internal root infection when benomyl was applied to soils from sites A and B (Fig. 3). This effect was not significant in soil C. The treatment with oxamyl appeared to stimulate root infection in soils A and C, but not in B. This stimulating effect was also present when oxamyl was added in combination with propamocarb in soil C, but not in soils A and B. Propamocarb alone had no effect, except for the first observation of roots from soil B. A closer look at the fungal taxa isolated from surface-sterilized roots (Table 1), shows some differences between sites, which may partly explain the differences in the response to the treatments. In soils A and B *Cylindrocarpon destructans* (Zinns.) Scholten and *Fusarium oxysporum* Schlecht, emend. Sny. & Hans. were dominant, whereas soil C was dominated by *Pythium irregulare* Buisman. Among the other species isolated from different soils were a *Verticillium* sp., *Trichoderma* cf. *pseudokoningii* Rifai, *Gliocladium roseum* Bain., and *Penicillium* spp..

In general, *C. destructans*, *F. oxysporum* and *Verticillium* sp. were strongly inhibited by benomyl. They seem to be replaced by *P. irregulare* and *Penicillium* spp. *P. irregulare* was only partly inhibited by propamocarb in soil from site C, and this reduced occurrence was completely offset by the development of other fungi. The low number of root pieces used did not allow further statistical analysis.

SHOOT BIOMASS (GRAM DRY WEIGHT)

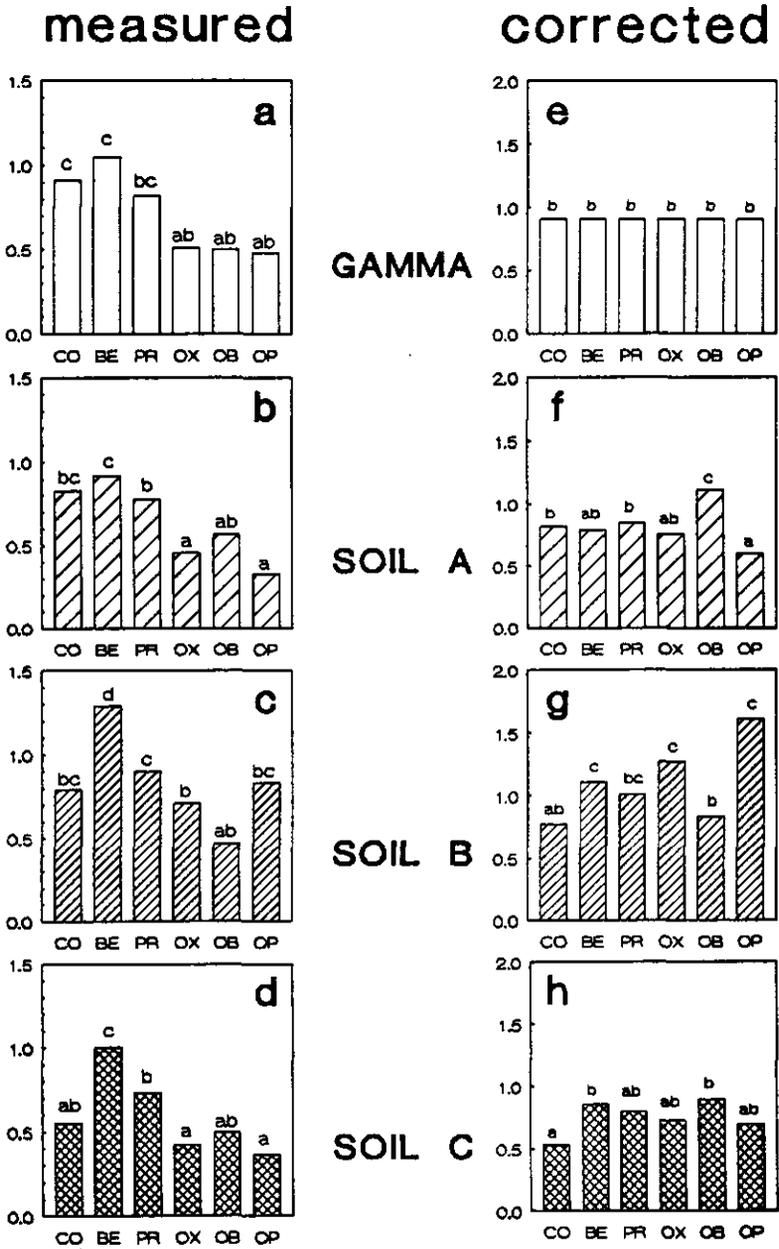


Fig. 1. The effect of treatments and soil origins on shoot biomass (1a-d), and the same after correction for treatment effects in sterilized soil (1e-h). GAMMA = irradiated and *Frankia*-inoculated reference soil. Sites A: about 15 years *Hippophaë* (vigorous), B: 60-80 years (degenerating) and C: >100 years (degenerating). Treatment codes CO = control, BE = benomyl, PR = propamocarb, OX = oxamyl, and combinations. Different letters within the data on the left or on the right indicate significant differences ($p < 0.05$) according to Tukey's test.

PERCENTAGE INFECTED ROOT PIECES

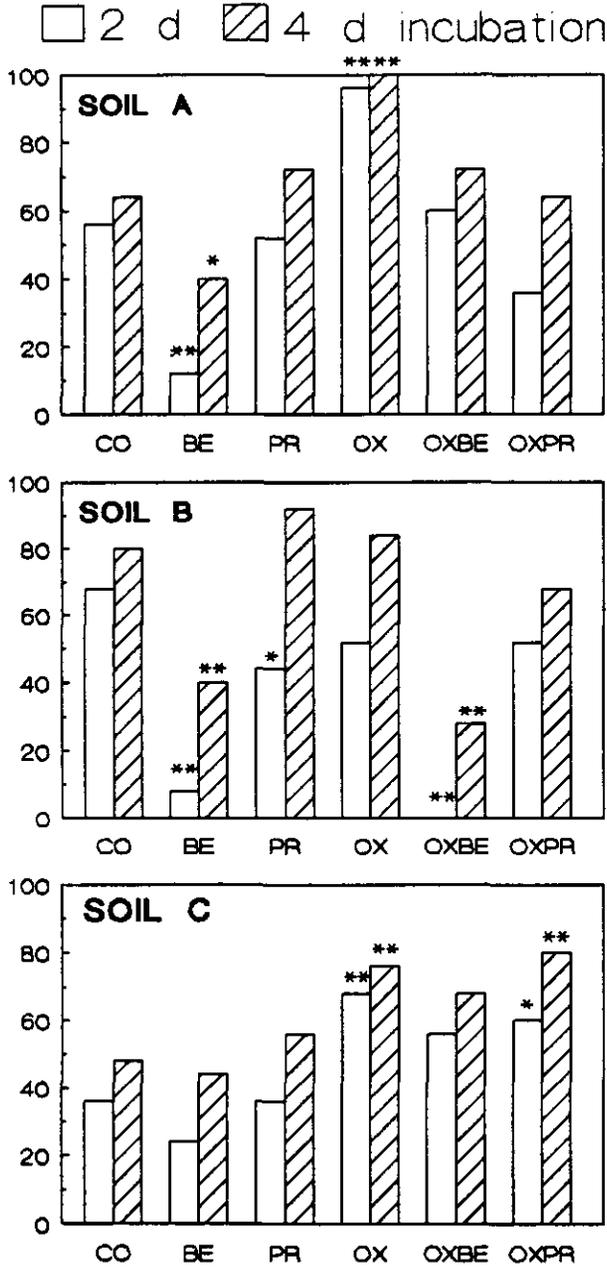


Fig. 3. The percentage of root pieces with internal fungal infection, counted after incubation during 2 and 4 days. Soils and treatments as in Fig. 1. Asterisks indicate significant differences from the control with $p < 0.05$ (*) and $p < 0.01$ (**).

Table 1. Fungal taxa isolated from roots of *H. rhamnoides*, grown in soil from three different origins which was treated with different fungistatic or nematostatic compounds. Treatment codes are as in Fig. 1. Values are expressed as percentage of 25 incubated root pieces. Occasionally, multiple isolations were obtained from one root piece.

| SOIL A | CO | BE | PR | OX | OXBE | OXPR |
|-----------------------------------|----|----|----|----|------|------|
| <i>Cylindrocarpon destructans</i> | 28 | - | 36 | 60 | 4 | 36 |
| <i>Fusarium oxysporum</i> | 24 | - | 8 | 32 | - | 20 |
| <i>Verticillium</i> sp. | 12 | - | 12 | 4 | 4 | 8 |
| <i>Trichoderma</i> sp. | - | - | - | - | - | 4 |
| <i>Gliocladium roseum</i> | - | 4 | 4 | - | - | - |
| <i>Pythium</i> spp. | 4 | 12 | 4 | - | 60 | - |
| <i>Penicillium</i> spp. | 4 | 24 | 8 | 4 | 4 | - |
| <i>Doratomyces stemonitis</i> | - | - | - | - | - | - |
| <i>Coniothyrium</i> sp. | - | - | - | - | - | - |
| other | - | - | 4 | - | - | 4 |

| SOIL B | CO | BE | PR | OX | OXBE | OXPR |
|-----------------------------------|----|----|----|----|------|------|
| <i>Cylindrocarpon destructans</i> | 36 | - | 40 | 32 | - | 40 |
| <i>Fusarium oxysporum</i> | 8 | - | 24 | 32 | - | 20 |
| <i>Verticillium</i> sp. | - | - | - | 12 | - | 12 |
| <i>Trichoderma</i> sp. | 4 | - | 16 | 8 | - | - |
| <i>Gliocladium roseum</i> | 12 | 12 | 4 | 16 | - | - |
| <i>Pythium</i> spp. | 12 | 4 | - | - | - | - |
| <i>Penicillium</i> spp. | - | 16 | - | - | 20 | - |
| <i>Doratomyces stemonitis</i> | - | 4 | - | 4 | 8 | - |
| <i>Coniothyrium</i> sp. | - | - | - | - | - | - |
| other | 8 | 4 | 8 | - | - | - |

| SOIL C | CO | BE | PR | OX | OXBE | OXPR |
|-----------------------------------|----|----|----|----|------|------|
| <i>Cylindrocarpon destructans</i> | - | - | 4 | - | - | 8 |
| <i>Fusarium oxysporum</i> | - | - | 8 | 12 | 12 | 16 |
| <i>Verticillium</i> sp. | - | - | 8 | - | - | - |
| <i>Trichoderma</i> sp. | - | - | 4 | 4 | 4 | 4 |
| <i>Gliocladium roseum</i> | - | - | - | - | - | - |
| <i>Pythium</i> spp. | 48 | 36 | 28 | 60 | 56 | 20 |
| <i>Penicillium</i> spp. | - | 8 | 4 | - | - | - |
| <i>Doratomyces stemonitis</i> | - | - | - | - | - | - |
| <i>Coniothyrium</i> sp. | - | - | 4 | - | - | 4 |
| other | - | 4 | - | - | - | 28 |

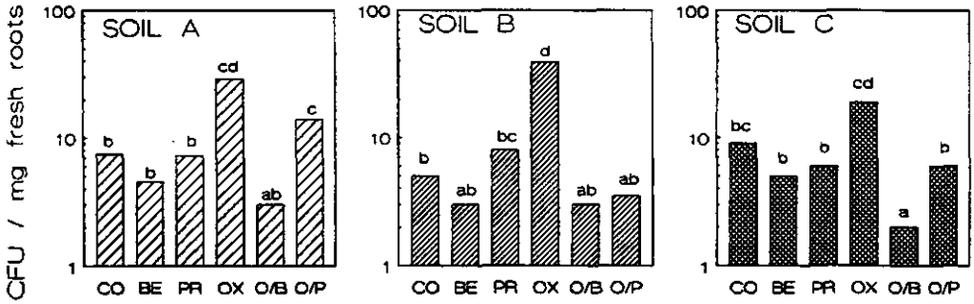


Fig. 4. The effect of treatments and soil origins on the fungal density on the rhizoplane. Sites and treatments as in Fig. 1. Different letters indicate significant differences ($p < 0.05$) in Tukey's test.

Rhizoplane mycoflora. The fungal density on the rhizoplane (Fig. 4) was only slightly reduced by benomyl, but increased strongly when oxamyl was applied. However, when oxamyl was combined with propamocarb, this effect was much less in soil A, and even absent in soils B and C. A similar interaction of propamocarb with oxamyl was found for root infection in soil A (Table 1). Combination of oxamyl with benomyl obliterates the stimulating effect of oxamyl on root infection (Fig. 3) and does the same in rhizoplane fungal populations (Fig. 4). There appears to be a positive correlation between the rhizoplane fungal density and the root infection percentage in soil A, but not in soils B and C.

Nematodes. Of the plant-feeding taxa, *Rotylenchus goodeyi* Loof and Oostenbrink was common in soils A and C, Criconematidae (mainly *Mesocriconema xenoplax* (Raski)) in soil A, Tylenchorhynchinae (mainly *Tylenchorhynchus microphasmis* Loof) in soil B and C, and *Longidorus dunensis* Brinkman, Loof and Barbez in soil B (Table 2). *Hemicycliophora conida* Thorne and Enchytraeid worms did not occur in soil C. Benomyl significantly reduced the final numbers of *R. goodeyi*, *L. dunensis*, predatory nematodes, omnivorous Dorylaimida and Enchytraeid worms (Table 3). Propamocarb did not influence nematode populations, but oxamyl reduced densities of all nematode groups. Combination of benomyl with oxamyl gave less reduction of *T. microphasmis* and omnivorous Dorylaimida than the sum of the effects of both treatments when applied alone.

Table 2. Effect of treatments on nematode taxa and trophic groups in soil from three origins. Mean numbers per 500 g soil are derived from $\log(x+1)$ -transformed counts. Treatment codes are as in Fig. 1.

| SOIL A | CO | BE | PR | OX | OXBE | OXPR |
|---|------------|------------|------------|-----------|-------------|-------------|
| <i>Rotylenchus goodeyi</i> | 104 | 58 | 107 | 22 | 15 | 21 |
| Criconematidae | 86 | 58 | 104 | 17 | 5 | 2 |
| <i>Hemicycliophora conida</i> | 12 | 23 | 12 | 3 | 1 | 3 |
| <i>Tylenchorhynchus microphasmis</i> | 2 | 0 | 0 | 0 | 0 | 0 |
| <i>Longidorus dunensis</i> | 0 | 0 | 0 | 0 | 0 | 0 |
| Other plant-feeding taxa | 2 | 3 | 4 | 1 | 0 | 0 |
| Plant-feeding nematodes | 212 | 149 | 233 | 45 | 22 | 26 |
| Fungivorous nematodes | 2 | 0 | 1 | 0 | 0 | 0 |
| Predatory nematodes | 58 | 3 | 23 | 7 | 0 | 0 |
| Bacterivorous nematodes | 246 | 26 | 209 | 24 | 6 | 118 |
| Omnivorous nematodes (<i>Dorylaimida</i>) | 94 | 4 | 24 | 8 | 2 | 5 |
| Enchytraeidae | 28 | 2 | 46 | 2 | 0 | 6 |
| SOIL B | CO | BE | PR | OX | OXBE | OXPR |
| <i>Rotylenchus goodeyi</i> | 1 | 0 | 2 | 0 | 0 | 1 |
| Criconematidae | 15 | 19 | 11 | 5 | 5 | 3 |
| <i>Hemicycliophora conida</i> | 25 | 18 | 19 | 3 | 1 | 3 |
| <i>Tylenchorhynchus microphasmis</i> | 248 | 43 | 216 | 7 | 31 | 1 |
| <i>Longidorus dunensis</i> | 36 | 2 | 23 | 4 | 2 | 1 |
| Other plant-feeding taxa | 1 | 2 | 0 | 1 | 0 | 0 |
| Plant-feeding nematodes | 334 | 85 | 279 | 24 | 42 | 9 |
| Fungivorous nematodes | 2 | 3 | 1 | 1 | 0 | 0 |
| Predatory nematodes | 16 | 1 | 13 | 0 | 2 | 0 |
| Bacterivorous nematodes | 110 | 37 | 184 | 20 | 97 | 19 |
| Omnivorous nematodes (<i>Dorylaimida</i>) | 99 | 2 | 76 | 13 | 8 | 0 |
| Enchytraeidae | 52 | 3 | 39 | 1 | 6 | 30 |
| SOIL C | CO | BE | PR | OX | OXBE | OXBE |
| <i>Rotylenchus goodeyi</i> | 57 | 13 | 25 | 10 | 4 | 6 |
| Criconematidae | 0 | 1 | 0 | 0 | 0 | 0 |
| <i>Hemicycliophora conida</i> | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Tylenchorhynchus microphasmis</i> | 79 | 14 | 58 | 15 | 5 | 4 |
| <i>Longidorus dunensis</i> | 6 | 0 | 2 | 2 | 0 | 2 |
| Other plant-feeding taxa | 5 | 5 | 6 | 4 | 3 | 1 |
| Plant-feeding nematodes | 154 | 37 | 96 | 36 | 15 | 18 |
| Fungivorous nematodes | 1 | 0 | 1 | 0 | 0 | 0 |
| Predatory nematodes | 3 | 0 | 3 | 0 | 0 | 1 |
| Bacterivorous nematodes | 160 | 13 | 108 | 18 | 4 | 3 |
| Omnivorous nematodes (<i>Dorylaimida</i>) | 158 | 6 | 150 | 28 | 2 | 6 |
| Enchytraeidae | 0 | 0 | 1 | 0 | 0 | 0 |

Table 3. Analysis of variance (ANOVA) on log(x+1)-transformed nematode data. TR1 is the treatment with or without oxamyl, TR2 is the treatment with none, benomyl, or propamocarb. Counting was done in duplicate for each object. Where necessary, soil origins with none or very low numbers of the group concerned were excluded from the analysis to obtain homogeneity of variance (Bartlett's test $p > 0.05$). Significance is indicated by *, **, *** for $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively.

| Taxon / Trophic group | Soil origin excluded | TR1 OX | TR2 BE/PR | ORIGIN OF SOIL | TR1 x TR2 | TR1 x ORIGIN |
|-------------------------------|----------------------|--------|-----------|----------------|-----------|--------------|
| <i>Rotylenchus goodeyi</i> | - | *** | ** | *** | ns | * |
| Criconematidae | C | *** | ns | ** | ns | * |
| <i>Hemicycliophora conida</i> | C | *** | ns | ns | ns | ns |
| <i>T. microphasmis</i> | A | *** | ns | ns | * | ns |
| <i>Longidorus dunensis</i> | A | * | * | * | ns | ns |
| Plant-feeding nematodes | - | *** | ns | ns | ns | ns |
| Fungivorous nematodes | C | ** | ns | ns | ns | ns |
| Predatory nematodes | - | *** | * | * | ns | ns |
| Bacterivorous nematodes | - | ** | ns | ns | ns | ns |
| Omnivorous nematodes | - | *** | *** | ns | ** | ns |
| Enchytraeidae | C | * | * | ns | ns | ns |

Table 4. Fungal populations at the start and at the end of the inoculation experiment, and mean relative growth rates (RGR) of fungus-inoculated and untreated *Hippophaë* seedlings. (* = significantly different from control with $p < 0.05$)

| Treatment species | isolate | initial density cfu.g ⁻¹ | final root infection % | rhizoplane density cfu.cm ⁻¹ | RGR (x 10 ⁻³) d ⁻¹ | SE |
|--------------------------|---------|-------------------------------------|------------------------|---|---|------|
| Control (sterile medium) | | 0 | 0 | | 4.47 | 0.16 |
| <i>C. destructans</i> | C09 | 540 | 56 | 715 | 4.30 | 0.08 |
| | C10 | 1080 | 32 | 509 | 3.44 * | 0.19 |
| | C25 | 2 | 24 | 214 | 3.96 | 0.23 |
| <i>F. oxysporum</i> | F02 | 110 | 28 | 290 | 4.75 | 0.24 |
| | F05 | 13600 | 8 | 90 | 4.06 | 0.23 |
| | F06 | 18200 | 12 | 220 | 4.32 | 0.34 |
| <i>Verticillium</i> sp. | V11 | 40 | 8 | 41 | 4.28 | 0.26 |

Fungus inoculation experiment

The initial density of colony forming units (CFU) differed strongly between the isolates used, probably due to differences in growth rate and sporulation. All inoculated fungi were amply recovered in the final re-isolation (Table 4), and in addition, a *Penicillium* sp. was found in all pots. *F. oxysporum* isolate F02 and *C. destructans* isolates were prominent within roots and *C. destructans* isolates C09 and C10 were also very numerous on the root surface. The cumulative water use followed an exponential curve for almost the entire period (data not shown), and thus calculation of a constant relative growth rate (RGR) was appropriate. Analysis of plant growth showed that *Cylindrocarpon destructans* isolate C10 was pathogenic (Table 4). The RGR of plants inoculated with other isolates was not different from the control. The transpiration coefficients (water uptake per unit biomass) of the various treatments were not significantly different (data not shown).

DISCUSSION

The goal of this study was to determine whether fungi and nematodes are involved in the decline of the nitrogen-fixing pioneer shrub *Hippophaë rhamnoides* L. in calcareous coastal sand dunes in the Netherlands, and whether there is any interaction between fungi and nematodes in their effects on growth and nodulation of this shrub. Oremus (1982) found no significant difference in fungal root infection between a site in the colonization stage and one with degenerating *H. rhamnoides*. In the present study, soils from three different stages were used to determine the effects of two fungicides and a nematicide. The dominant fungi on the rhizoplane and within roots of *H. rhamnoides* in untreated soil were *C. destructans* and *F. oxysporum*. These fungi tended to decrease, whereas *Pythium* spp. increased in dominance towards the oldest, acidified site (Table 1). The infection percentages of *C. destructans* and *F. oxysporum* strongly decreased in the benomyl treatment (Table 1). The plant-feeding nematodes *R. goodeyi* and *L. dunensis* were also significantly diminished by benomyl, but less than by oxamyl. Generally, free-living ectoparasitic nematodes such as those mentioned are not very sensitive to benomyl at the low rates used (Rodriguez-Kabana & Curl, 1980; Van der Putten, 1989). However, sedentary endoparasitic nematodes are much more sensitive, and effects in sand are much stronger than in soils containing organic matter or clay (Hoestra, 1976).

In natural *H. rhamnoides* soils the use of benomyl greatly enhanced *Frankia*

nodulation, but in sterilized soil it inhibited nodulation (Fig. 2a-d). The positive effect of benomyl in natural soil suggests that certain benomyl-sensitive soil organisms were antagonistic towards colonization or nodulation by the actinomycete *Frankia*. In analogy, a stimulating effect of benomyl on other soil actinomycetes was found by Van Faassen (1974). The decreased populations of certain plant-feeding nematodes are not the only cause of the large increase in the number of nodules in the benomyl treatment, because in soil C nodulation was not improved in the oxamyl treatment, despite the large nematofaunal similarity with the benomyl treatment. Furthermore, the combination of benomyl with oxamyl in soil A and B (Fig. 2fg) gave better nodulation than oxamyl alone, although nematode densities in the latter treatment were already low (Table 2). Thus, nodulation seems to benefit from the decrease in both nematodes and fungi. Also, Maas et. al. (1983) observed that mechanical killing of nematodes in natural soil, leaving fungal and microbial populations largely intact, did not improve nodulation. Our results (Table 1) indicate that *C. destructans* and *F. oxysporum* are potential candidates for the role of antagonists against *Frankia* nodulation.

Improved *Frankia* nodulation correlated with improved plant growth only in those soils where the natural level of nodulation was very low (sites B and C). Increases from about 20 nodules per plant in the control to 30 or more in the benomyl treatment seem to affect shoot growth much less than increases from less than 10, to 20 or more (Fig. 1 and 2).

The increase in the fungal population within roots and on the rhizoplane in the oxamyl treatment (Fig. 3 and 4) suggests that the fungivorous fauna is impeded. It is known that fungivorous nematodes and other soil mesofauna are able to suppress both beneficial and pathogenic soil fungi (Curl, 1979; Finlay, 1985; Zunke et al., 1986; Ingham, 1988) and thus, nematicides and insecticides may have substantial side-effects (Hofman, 1988). The fact that increased densities of fungi on the rhizoplane in the oxamyl treatment did not cause a reduction in growth or nodulation indicates that the rhizoplane fungi alone, or with very low nematode densities have little effect on the plant. This is corroborated by the results of the inoculation experiment (Table 4).

It seems that vesicular-arbuscular mycorrhizal (VAM) fungi were not very important in this experiment, because the expected negative effect of their reduction by benomyl on growth (Fitter, 1986) was amply outweighed by the positive effect of this fungicide, i.e. the inhibition of harmful fungi (Fig. 3) and nematodes (Table 2). In the field, VAM infection in *Hippophaë* roots increases together with long-term successional changes (Zoon et al., 1993). VAM infection percentages were not

assessed in the present study.

The positive effect of oxamyl on growth in soil B, and on nodulation in soils A and B, found after correction for phytotoxic effects (Fig. 1g and 2fg), is likely due to inhibition of plant parasitic nematodes (Table 2 and 3). The increased fungal populations in the oxamyl treatment, probably due to suppression of fungivorous fauna, appear not very harmful when plant-feeding nematode populations are reduced. Thus, plant-feeding nematodes seem a prerequisite for damage, including possible damage by fungi. These nematodes may either create ports of entrance for fungi or cause a physiological predisposition to fungal attack. However, an indirect decrease by fungi of plant tolerance to nematode damage can not be excluded (Sikora & Carter, 1987). Effects of the chemical treatments on the soil organisms studied are assumed to be direct, or caused by interaction between soil organisms. Little is known, however, about possible side effects of the chemicals due to changes in root physiology and exudation.

From previous experiments it was concluded that certain plant parasitic nematodes are more harmful to *H. rhamnoides* in the presence of unknown microbial factors, whereas these microbial factors alone are not very harmful (Maas *et al.*, 1983). From the present study growth-reducing effects of plant parasitic nematodes on *H. rhamnoides* and a synergic pathogenic interaction between these nematodes and fungi are concluded. The unknown microbial factors (Maas *et al.*, 1983) are probably mainly fungi. It is still unknown which fungi and which nematodes are the most important in this interaction.

REFERENCES

- Brown, J.C. 1958 Soil fungi of some British sand dunes in relation to soil type and succession. *J. Ecol.* 46: 641-664.
- Curl, E.A. 1979 Effects of mycophagous Collembola on *Rhizoctonia solani* and cotton-seedling disease. In: B. Schippers & W. Gams (eds.) *Soil-borne Plant Pathogens*. Academic Press, New York, pp. 253-269.
- Domsch, K.H., Gams, W. and Anderson, T.H. 1980 *Compendium of Soil Fungi*. Vol. I and II., London, Academic Press, 859 and 405 pp.
- Finlay, R.D. 1985 Interactions between soil micro-arthropods and endomycorrhizal associations of higher plants. In: A.H. Fitter (ed.), *Ecological Interactions in Soil*. Blackwell Sci. Publ. Oxford, p. 319-331.
- Fitter, A.H. 1986 Effects of benomyl on leaf phosphorus concentration in alpine grasslands: a test of mycorrhizal benefit. *New Phytol.* 103: 767-776.
- Hoestra, H. 1976 Effect of benomyl on the potato cyst nematode, *Heterodera*

- rostochiensis*. Neth. J. Pl. Pathol. 82: 17-23.
- Hofman, T.W. 1988 Effects of granular nematocides on the infection of potatoes by *Rhizoctonia solani*. Ph.D. Thesis, Agricultural University Wageningen, 125 pp.
- Ingham, R.E. 1988 Interactions between nematodes and vesicular arbuscular mycorrhizae. Agric. Ecosyst. Environ. 24: 169-182
- Maas, P.W.Th., Oremus, P.A.I. and Otten, H., 1983. Nematodes (*Longidorus* n. sp. and *Tylenchorhynchus microphasmis* Loof) in growth and nodulation of sea buckthorn (*Hippophaë rhamnoides* L.). Plant and Soil, 73: 141-147.
- Oremus, P.A.I., 1980. Occurrence and infective potential of the endophyte of *Hippophaë rhamnoides* L. ssp. *rhamnoides* in coastal sand-dune areas. Plant and Soil, 56: 123-139.
- Oremus, P.A.I., 1982. Growth and Nodulation of *Hippophaë rhamnoides* L. in the Coastal Sanddunes of the Netherlands. Ph.D. Thesis, Rijksuniversiteit Utrecht, 117 pp.
- Pearson, M.C. and Rogers, J.A. 1967 Flora of the British isles. *Hippophaë rhamnoides* L. J. Ecol. 50: 501-513.
- Rodriguez-Kabana R. and Curl E.A. 1980 Non-target effects of pesticides on soilborne pathogens and disease. Ann. Rev. Phytopathol. 18: 311-332.
- Sikora, R.A. and Carter, W.W. 1987 Nematode interactions with fungal and bacterial plant pathogens - fact or phantasy. In: Veech, J.A & Dickson, D.W. (eds.) Vistas on Nematology. Soc. of Nematologists, Hyattsville, Maryland USA, pp 307-312.
- Van Faassen, H.G. 1974 Effect of the fungicide benomyl on some metabolic processes, and on numbers of bacteria and actinomycetes in the soil. Soil Biol. Biochem. 6: 131-133.
- Van der Putten, W.H. 1989 Establishment, growth and degeneration of *Ammophila arenaria* in coastal sand dunes. Ph.D. Thesis, Wageningen Agricultural University, Wageningen NL, 152 pp.
- Van der Putten, W.H., Van Dijk C. and Peters B.A.M. 1993 Plant-specific soil-borne diseases contribute to succession in foredune vegetation. Nature 362: 53-55.
- Webley, D.M., Eastwood, D.J. and Gimingham, C.H. 1952 Development of soil microflora in relation to plant succession including rhizosphere flora associated with colonizing species. J. Ecol. 40: 168-178.
- Weste, G. 1981 Changes in the vegetation of sclerophyll shrubby woodland associated with invasion by *Phytophthora cinnamomi*. Austr. J. Bot. 29: 261-276.
- Zoon, F.C., Troelstra, S.R. and Maas, P.W.Th. 1993 Ecology of the plant-feeding nematode fauna associated with sea buckthorn (*Hippophaë rhamnoides* L. ssp. *rhamnoides*) in different stages of dune succession. Fundam. appl. Nematol. 16: 247-258.
- Zunke, U., Wyss, U. and Rössner, J. 1986 Parasitierungsverhalten von *Aphelenchoides hamatus* an fünf verschiedenen phytopathogenen Pilzen und an *Agaricus campestris*. Nematologica 32: 194-201.

CHAPTER 4

A device for automatic soil moisture control and registration of water use in pot experiments.

F.C. Zoon, F.J. Hage and A. de Zwart

Plant and Soil 125: 281-284 (1990)

SUMMARY

An apparatus was made for automatic replenishment of water lost by evapotranspiration in pot experiments. The system can handle 80 pots. The upper and lower weight limit for each pot, and the weighing frequency can be set. In this way, constant soil moisture levels as well as fluctuations can be arranged. The apparatus can be programmed for continuous cycling, thus minimizing position effects within an experiment. Cumulative daily water use per pot or transpiration per plant is recorded on tape and printed. Special applications, *e.g.* controlled nutrient dosage and determination of transpiration coefficients are discussed.

INTRODUCTION

Soil moisture regimes experienced by plants in pot experiments are often quite different from those in the field (De Vries, 1980). The compromise between the moisture regime required, and the labour and space available is often inadequate with respect to the purpose of the experiment. Water loss by evapotranspiration can cause more stress than was intended, especially in the case of soils with a low water holding capacity, or experiments requiring a low moisture level. Normally, large fluctuations of soil moisture content in pot experiments need to be avoided. Sometimes, however, controlled fluctuation is an essential experimental factor. Soil moisture control in pot experiments on soil organisms requires special attention, because the moisture regime can determine the activity of soil organisms and the damaging effect of root parasites and pathogens (Wallace, 1971; Van Hoof, 1976; Van Gundy, 1985; Duniway and Gordon, 1985; Schnürer *et al.*, 1986).

Different methods have been developed for semi-automatic regulation of soil

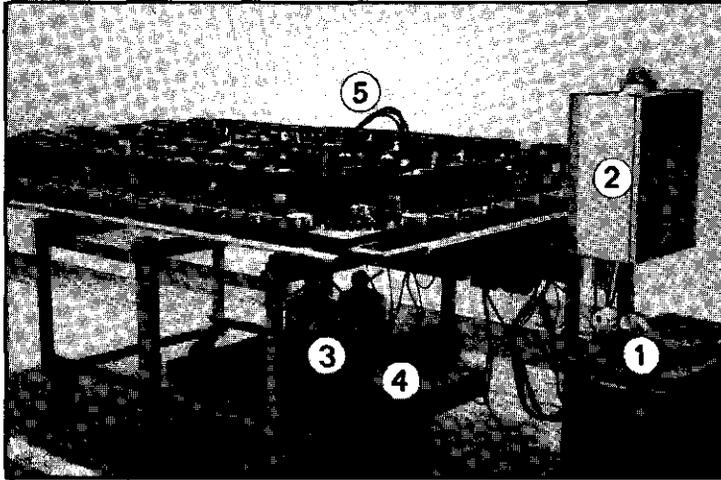


Fig. 1. Overview of the apparatus; 1. computer, 2. switch cupboard, 3. water reservoirs, 4. dosing pumps and 5. watering tube overhead the balance (not visible).

moisture in pots. For high soil moisture levels periodical flooding and draining methods (Hewitt, 1966) can be used. Papendick and Campbell (1975) described techniques for the control of low moisture levels, but these methods are not very suitable for a large number of pots. A computer-regulated watering device, such as the one described here, is able to conduct frequent watering and recording of water use, thus saving much repetitive work. The amount of water lost by transpiration can be estimated by correction for the evaporation, found in pots without plants. Using daily transpiration recordings, transpiration coefficients (TC) can be determined, or -given a near constant or known TC- growth can be monitored. Furthermore, it is possible to compensate for the plant's nutrient uptake by watering with a nutrient solution, based on previously obtained knowledge of plant nutrient contents, transpiration coefficient and evaporation. In this way, steady-state plant growth (Ingestadt, 1982) can to some extent be achieved if transpiration coefficients are about equal and fairly constant. In this paper the construction and functioning of an apparatus called 'AWARE' (Automatic WATERing and REgistration) is presented, and the performance and possibilities of the system are discussed.

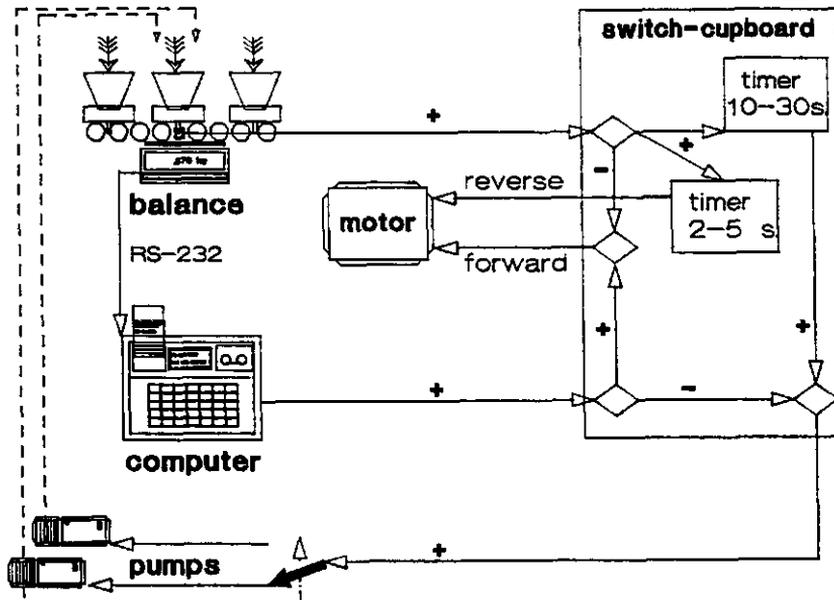


Fig. 2. Simplified diagram of functional links between system components. Relays are indicated by diamonds. Arrows with + have a switch-on effect, those with - have a switch-off effect.

MATERIALS AND METHOD

The main component of the system (Figs. 1 and 2) is a transport system by which a maximum of 80 pots are one after the other moved on to an electronic balance. The transport system consists of a meandering roller path and eight toothed belts driven by an electromotor. The belts have driver hooks that push the pots forward. Before weighing, the hooks are withdrawn from the pots. The pots (max. diameter 14 cm) are supported by trays, which have a small downwards extending shaft that activates a stop switch on the balance. The supporting tray of the last pot has a longer shaft that acts on a second stop switch shortly after releasing the first one. Thus, after being handled, the last pot does not leave the balance until a new cycle is started. The pots are covered with aluminum foil fitting around the plant base. In this way evaporation is reduced and a more homogeneous moisture distribution is provided. A few small holes in the lower part of the cover may be necessary to facilitate drainage of the added water.

Another essential component is a small portable computer (Epson HX-20) with a

built-in microprinter and a microcassette recorder (Fig. 1; 1). The computer program (in BASIC) activates a power relay -meant for an external cassette recorder- to start the transport as soon as a pot is treated or when a new cycle is begun. Transport speed, withdrawal of the driver hooks, and pump delay after pot arrival are regulated in the switch-cupboard (Fig. 1; 2). The computer reads out the electronic balance (Mettler PE6/RS-232C) after a pot has arrived. The actual pot weight is compared with the target weight and the maximum downward deviation, both stored in the computer memory (RAM). If the lower weight limit is exceeded, restart of the transport by the computer is postponed until the target weight is reached by watering.

Water or nutrient solution is added by an electronic magnet diaphragm dosing pump (Fig. 1; 4) (Prominent E 0407) starting 10 to 30 s. after pot arrival. Two different solutions can be added by separate pumps. One of the pumps is connected, depending on the position of a switch which is activated by detachable cams on one of the belts. The positions of the cams determine the fixed sequence of solution types. The computer program can either continuously cycle the pots for a certain period of the day, or perform separate cycles starting at certain times. If necessary, pots can be weighed every hour. Continuous cycling means minimal deviation from the intended moisture regime and elimination of position effects.

At the end of each day the total water supply for each pot is recorded on a microcassette tape. These data can later be transmitted (RS-232C) to another computer for further calculations and statistical analysis. Pots without plants can be incorporated in the experiment to allow correction of the water supply for evaporation from the pot. In this way, the amount of water used by the plant itself (transpiration) can be estimated. The correction for evaporation can be done automatically at the end of each day. Groups of pots, specified by different codes, can be distinguished and corrected with averaged data from corresponding check pots.

RESULTS AND DISCUSSION

In a number of experiments the system has shown to be accurate and reliable. Deviation due to errors of weighing and watering was less than one gram. The lower moisture limit had to be set at minimally 2 grams under the target weight to be sure that added water was drained from the cover into the pot. As a result, the weight of 'constant' moisture pots fluctuated between the target weight (± 0.5 g)

and 2 to 4 grams (± 0.5 g) under the target weight, when using continuous cycling. This means a variation of less than 1 % of soil moisture for pots containing 500 gram of dry soil.

A homogeneous moisture distribution could be achieved if the moisture content was kept above a minimum level for adequate conduction of added water to deeper layers. For dune sand this limit was about 2.5 % on a dry weight base (pF 3).

Evaporation from 9x9 cm (500 ml.) polypropylene pots with aluminum foil cover was on average 1.5 g.d^{-1} . Evaporation from pots with a dense rosette plant may be somewhat lower than from pots without a plant, because the pot microclimate is influenced by the plant. When this is the case, automatic estimation of daily transpiration is less precise, but compared to the substantial transpiration of such a plant the difference will only be a few per cent. Besides, rosette plants require support to leave free space for the watering tube.

During the course of an experiment, daily transpiration increases along with plant growth. Thus, the frequency of fluctuation of soil moisture content may also increase, until each weighing is followed by watering. If desired, a constant low frequency of fluctuation could be programmed by introducing slowly fluctuating target weights. Possibilities for high constant frequencies are limited by the desired magnitude of fluctuation and the rate of evapo-transpiration. Generally, it is not necessary to correct the target weights for plant growth every day. Many plants in pot experiments grow only a few grams (fresh weight) per week, which accounts for a deviation of the moisture content of less than one percent on a soil weight of 500 grams or more. A few adjustments of the target weights towards the end of an experiment, based on allometric estimation of plant fresh weight, are then sufficient. For fast growing crops automatic daily correction for plant growth can be programmed, either as a function of time, or as a function of cumulative water use per pot. The simulated course of soil moisture in pots as a function of increasing transpiration and the action threshold set is shown in Figure 3.

The electrical and mechanical components of the system appeared rather insensitive to disorders. Transport failures due to defective switches, fuses, or power supply are avoided through incorporation of trouble-solving routines in the program. The computer itself has an internal power reserve to bridge a period of about 4 hours. After longer power failure the program has to be restarted manually. The system could be further improved and simplified by providing a more direct communication between the computer and sensors and devices through a number of analogous input/output channels.

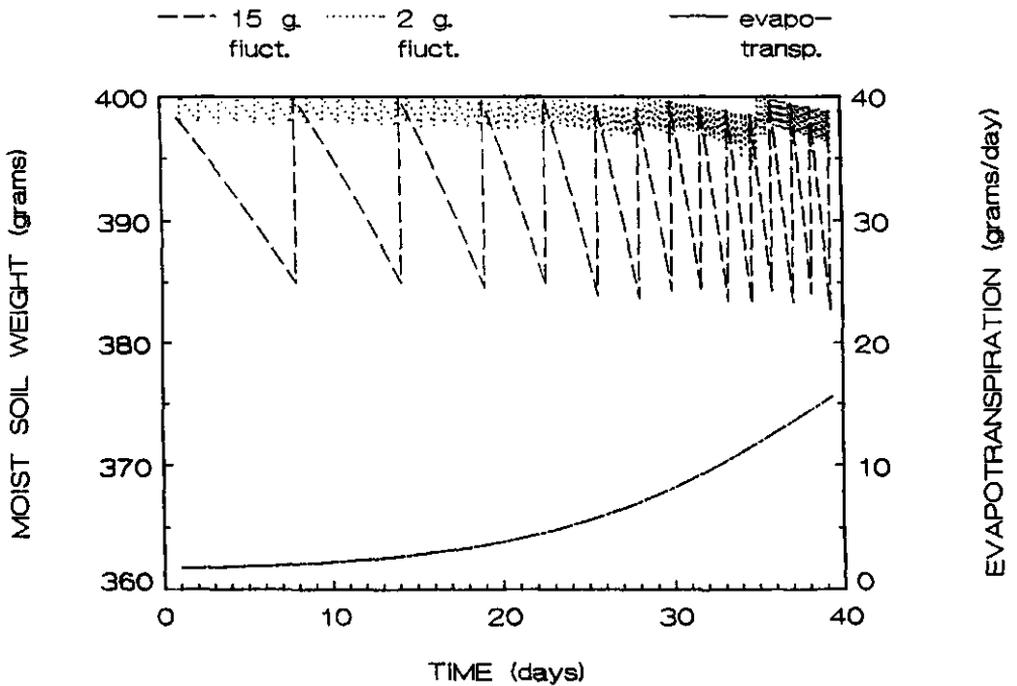


Fig. 3. Simulated fluctuations of soil moisture during a 40 day experiment as influenced by increasing evapo-transpiration (bottom curve). Set magnitude of fluctuation 2 (solid line) and 15 grams (dashed line). Weighing every second hour. Correction for plant weight every week.

The time saved by using this apparatus is considerable. At a required watering frequency of *e.g.* five times a day, a two minute routine check replaces 2.5 hours of hand-weighing and recording data for 80 pots. Besides, the entry of large data sets in a computer is circumvented by automatic data reduction and registration. The costs of materials, parts and components for the complete system (about 8000 US\$ in 1987) and the costs of labour involved in the construction are returned in about two years of use. The 'AWARE' system can be very helpful in maintaining low moisture levels, but also for studying effects of variation in level and fluctuation of soil moisture on transpiration and plant growth, *e.g.* for the development of mechanistic plant growth simulation programs. For the translation of effects to the field situation it is recommended to take into account not only climatic differences, but also the root length density (cm.cm^{-3}), as this is an important factor in determining the rate of depletion of water and nutrients from soil (De Willigen and Van Noordwijk, 1987). Preferably, root length densities in pots should not much exceed those found in the field.

REFERENCES

- De Vries M.P.C. 1980. How reliable are results of pot experiments? *Comm. Soil Sci. Plant Anal.* 11: 895-902.
- De Willigen P. and Van Noordwijk M. 1987. *Roots*. Dissertation, Agricultural University Wageningen, the Netherlands, 282 pp.
- Duniway J.M. and Gordon T.R. 1985. Water relations and pathogen activity in soil. *In: P.G. Ayres and L. Boddy (eds), Water, Fungi and Plants*. Cambridge Univ. Press.: 119-137.
- Hewitt E.J. 1966. *Sand and Water Culture Methods used in the Study of Plant Nutrition*. Farnham Royal, C.A.B.
- Ingestadt T. 1982. Relative addition rate and external concentration; driving variables used in plant nutrition research. *Plant, Cell and Environment* 5: 443-453.
- Papendick R.I. and Campbell G.S. 1975. Water potential in the rhizosphere and the plant, and methods of measurement and experimental control. *In: G.W. Bruehl (ed). Biology and Control of Soil-borne Plant Pathogens*. St. Paul, Minn., Am. Phytopathol. Soc. pp 39-49.
- Schnürer J., Clarholm M. Boström S. and Rosswall T. 1986. Effects of moisture on soil microorganisms and nematodes: a field experiment. *Microb. Ecol.* 12: 217-230.
- Van Gundy S.D. 1985. Ecology of *Meloidogyne* spp. -emphasis on environmental factors affecting survival and pathogenicity. *In: J.N. Sasser and C.C. Carter. An Advanced Treatise on Meloidogyne. Vol.I. Biology and Control*. N. Carolina State Univ. Graphics:177-182.
- Van Hoof H.A. 1976. The effect of soil moisture content on the activity of Trichodorid nematodes. *Nematologica* 22: 260-264.
- Wallace H.R. 1971. Abiotic influences in the soil environment. *In: B.M. Zuckerman et al. (eds) Plant Parasitic Nematodes. Vol.1*. New York, Academic Press: 257-280.

CHAPTER 5

A rapid quantitative measurement of root length and root branching by microcomputer image analysis.

F.C. Zoon & P.H. van Tienderen

Plant and Soil 126: 301-308 (1990)

SUMMARY

A computer program was made for fast and reliable measurement of root length, and estimation of the number of root tips and branching points. Image-processing procedures available in a program package for image analysis by means of a personal computer were used. The method is described in this paper and some results of tests on variance and systematic errors (bias) are discussed. The time required for analysis of an evenly spread root (sub-)sample with a total length of max. 300 cm was reduced to less than 20 seconds. Random deviations from the real length, determined by measuring known lengths of wire, did not exceed 5 %, after correction for length-density dependent bias. Counts of root tips appeared to be unreliable, but branching ratios could be determined fairly accurately, after correction for the length-density dependent number of pseudo-branches (e.g. crossings). Rhizotron root photographs were also analysed satisfactorily, after modification of a few steps in the program.

INTRODUCTION

Measurement of root length, and quantification of root branching is very laborious. In manual line-intersection methods (Newman, 1966, Marsh, 1971), both spreading the roots on a surface and counting intersections are time consuming. In addition, strict guidelines have to be followed to reduce inaccuracy, and to improve standardization (Tennant, 1975). In (semi-)automatic line-intersection methods (Rowse & Phillips, 1974, Richards et al., 1979, Wilhelm et al., 1983, Collins et al., 1987) spreading is also required, but the scanning of an image is usually much faster. Costigan et al. (1982) described a method for measuring length and number of

lateral and sublateral roots by tracing roots on a digitizing tablet. This method gives fairly accurate information on root length (variation 2.5%, mean overestimate 7%), and structure, but is too time consuming (8.5 minutes/m) to analyse large root samples. Böhm (1979) mentioned the use of an image analysing computer as the most sophisticated method for automatically measuring root length. Some tests of root length measurements using image analysis were published (Baldwin, 1971, Heringa et al., 1980, Voorhees et al., 1980, Ottman and Timm, 1984), but until now, the reliability of this quick way of measuring has not received much attention. Rhizotron root photographs or video recordings are increasingly used to study in situ root growth. Image analysis of these images (Smucker et al., 1987) is desirable, but requires procedures to eliminate the background soil structures. Since the introduction of the Quantimet (Fisher 1971) a number of general purpose image analysis packages have become available for use on micro-computers (PC). This has facilitated image analysis programming for a variety of purposes, making use of calculation procedures available in the package. The basic steps and procedures of a program for the estimation of root length and numbers of root tips and branching points are described in this paper. Moreover, results of tests concerning density dependent bias and variance of the measurements are presented.

METHODS

Principles of root image analysis

An image of a randomly spread root sample is digitized into a 256 x 256 pixel grid ('frame grabbing'). Root length is measured by counting the remaining 'root pixels' after first reducing the thickness of the roots to one pixel ('skeletonization'). The root length is subsequently calculated from the number of root pixels counted, and a conversion factor (cm/pixel) found by measuring a calibration line pattern of known total length. In fact, root pixel counting is equivalent to counting line intersections on a very dense 256 x 256 line grid. In a rectangular grid, a diagonally orientated line yields less pixels than a line of the same length in vertical or horizontal direction. Furthermore, with the 'frame grabber' (digitizing unit) used here, a rectangular image is put into a square pixel matrix, so that a pixel reflects a greater length in horizontal than in vertical direction. Both characteristics require mean neutrality of line direction in the image, to avoid inaccuracy of estimation. In theory, estimation of the number of root tips can be done by counting the pixels

with only one neighbouring pixel ('end pixels') in a skeletonized root image. However, this may not be feasible, as the root system often has to be cut into pieces to facilitate spreading. If the roots were cut to about standard length, counting of the number of end pixels might give some information on the occurrence of lateral roots. The number of root branches can be estimated by counting the number of pixels having three neighbouring pixels in a 3 x 3 pixel field ('branch pixels') in a skeletonized root image. Correction has to be made for the expected number of these points ('crossing pixels') that are due to cross-overs of roots, and to other factors, such as the frequency of adhesion of root pieces, and double counts at narrow angle crossings. The correction factor can be found empirically by analysis of images containing non-branching line objects.

Root image

Most methods described require a photograph, slide, or photocopy for scanning. This intermediary step costs time and may reduce image quality, but it may be desirable when root analyses cannot be finished shortly after the roots have been collected. The different methods for getting a root image all seem to have their drawbacks. The problem of dark or stained roots on a white background (Voorhees et al 1980, Wilhelm et al 1983, Ottman and Timm 1984) is unwanted organic debris. Measuring white roots on a black background (Collins et al 1987) means underestimation of the length of relatively dark coloured root pieces. In the present study a root image with high contrast was obtained by spreading the roots in a thin layer of water over a light box (2200/4400 lux). Root systems were cut into pieces of about 5 cm. to facilitate spreading. Close parallel positioning of root pieces was avoided but crossings were allowed. The size of the image field was such, that the thinnest roots were at least one pixel thick. The camera zoom-lens allows photographic slides (both positives and negatives) to be used instead of fresh root samples. As a preliminary experiment some minirhizotron root pictures were also analysed. These photographs were taken for manual counting purpose, and were probably not optimal for image analysis.

Frame grabbing.

Images were grabbed with a black and white video camera (Bosch T YK 9C/ Vidicon tube), having automatic light intensity correction, equipped with a macro-zoom lens (Tarcus 1:28/f 2.8). The image was digitized to 256 x 256 pixels with grey values between zero and 255, and processed following a command file made

within a general image analysis program package (TIM ^(R), Difa Measurements, Breda, Netherlands; Ekkers 1988). A standard IBM-compatible personal computer, equipped with a PC-Vision frame grabber unit is required to use this package .

Image analysis

The most essential steps in the computer program are:

1. Store a background image for the removal of camera- or light box induced light gradients. For rhizotron pictures a background image was made by blurring the image to be analysed (unifying the grey value of groups of pixels).
2. Grab an image of a line pattern of known length for calibration of the length per measured pixel. A pattern of circles is preferable, because of mean neutrality of direction.
3. Subtract the background image from the one with unknown root length to remove background light gradients. In this way structures with less contrast than roots are removed.
4. Make the image black and white according to a previously chosen threshold grey value. Procedures for threshold choosing were incorporated in the program. The optimal threshold value is such, that thin and translucent roots are not lost, but on the other hand clusters of roots still show small gaps.
5. Remove 'dust' smaller than a chosen number of pixels. Single pixel removal was fast and sufficient for the elimination of sand grains in rinsed root samples. However, when rhizotron root pictures were analysed, objects larger than one pixel also had to be removed from the image.
6. Remove the outer layers of the roots in the image, until the root is reduced to one pixel thickness ('skeletonizing').
7. Count the remaining root pixels, end pixels (one adjoining) and branch pixels (three adjoining). Calculate length and branching rate, using correction procedures when needed. Enter the output to the screen, file or printer.

Reliability tests

Repeated arrangements of different amounts of wire pieces were measured to simulate the effects of different root lengths per image on the precision and variance of the root length estimate. The effect of wire diameter was studied as well. The (real) root length is expressed as number of pixels. In this way the results apply to all 256 x 256 pixel images and a certain mean root thickness in pixels, irrespective of magnification. If the results are to be applied to grids of different

size, the length density has to be expressed as percentage of all pixels. The effect of light box intensity on the root length measurement was evaluated using half or full light (2200 or 4400 lux). Moreover, the influence of total root length in the image on the number of end pixels and crossing pixels (pseudo-branches) of non-branching objects was studied, using wire with a diameter of two pixels. This diameter is considered to be the most efficient, because it allows analysis in an image of a relatively large amount of roots with diameters ranging from 1 to 3 pixels.

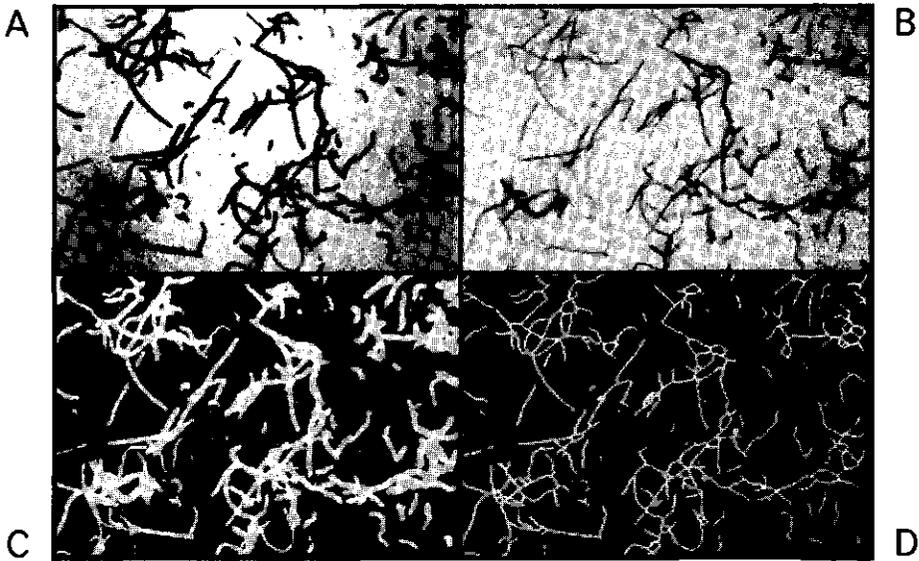


Fig.1. Stages in the processing of a root image: a. original image, b. after equalization of the background, c. after 'thresholding' with grey value 124, and d. after skeletonization.

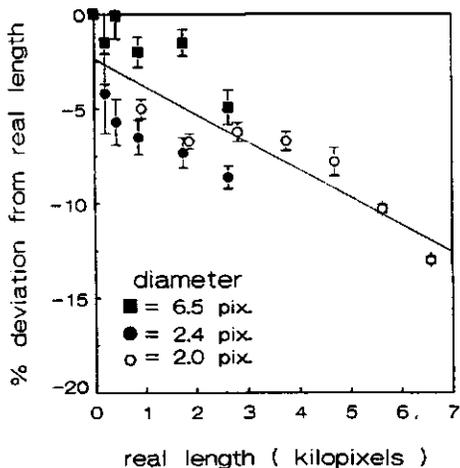


Fig.2. The effect of diameter and total length of wire in the image on the percentage deviation of the estimate from the real length. Length expressed as number of pixels in a 256 x 256 pixel grid. Line fit through all points $Y = -2.34 - 1.46 \times X/c$ ($R^2=0.67$; factor c is any total number of pixels in an image divided by 65536). Bars indicate standard errors, $n=10$.

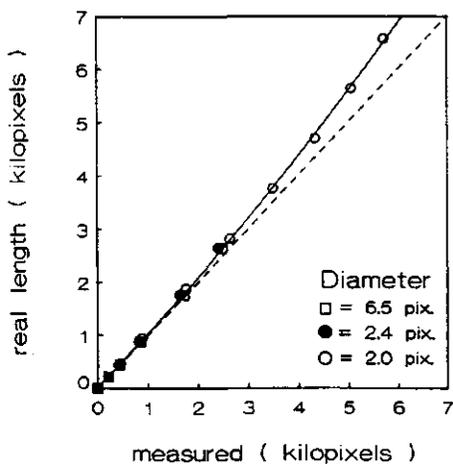


Fig.3. The relation between the estimated and the real length, of wire, expressed as number of pixels in a 256 x 256 pixel grid. Curve fit for all points ($Y/c = 0.021 + 0.97 \times X/c + 0.028 \times X^2/c^2$; $R^2=1.00$; conversion factor c is any total number of pixels in an image divided by 65536).

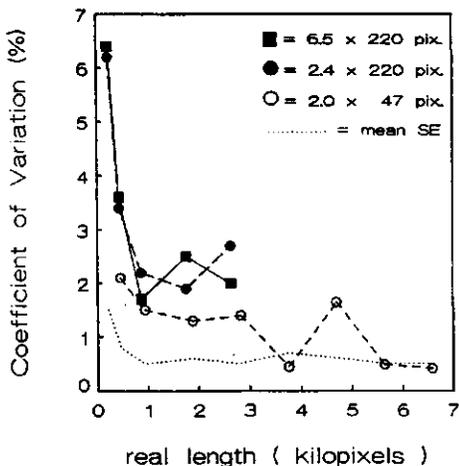


Fig.4. The effect of diameter and length of pieces, and the effect of total length of wire in the image on the coefficient of variation of the length estimate. Wire diameter and length are expressed as number of pixels. For each point, $n=10$. SE's were approximately equal for all segment sizes tested.

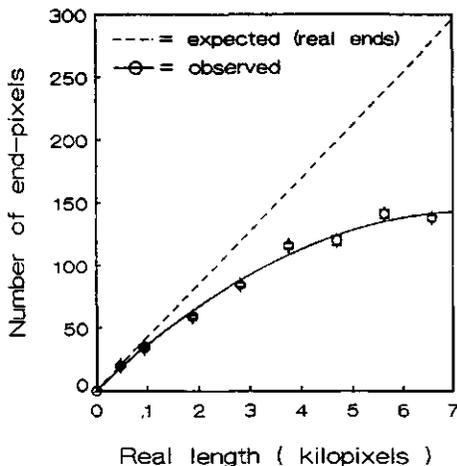


Fig.5. The influence of wire length in the image on the number of end pixels observed, compared to the real number of ends. Length is expressed as number of pixels in a 256 x 256 pixel grid. Wire pieces 47 pixels long, 2 pixels thick. Curve fit: $Y = 0.6 + 38.5 \times X/c - 2.60 \times X^2/c^2$ ($R^2=0.99$; factor c is any total number of pixels in an image divided by 65536). Bars indicate standard errors, $n=10$.

RESULTS

Image analysis

An example of the results of the subsequent steps in the processing of a root image is given in Figure 1. The resulting images from rhizotron root photographs were less clean than the ones from isolated roots, so that more and larger unwanted objects (up to 5 pixels in size) had to be removed. This gave satisfactory results with the clay soil. In the close up pictures from a sandy soil many strongly contrasting sand grains caused problems, as there is a software maximum of 256 objects larger than one pixel that can be automatically removed. The procedure from frame grabbing up to the output of the root length took about 20 seconds per image, while counting of end pixels and branch pixels took an additional four seconds. As the optimal root length per image is about 250 cm., this means an analysis time of less than 10 seconds per meter. However, most time is spent in arranging the roots. The mean total handling time for a large number of root samples was 2 minutes per meter.

Root length estimation

Figure 2 shows the influence of wire diameter (in pixels) and real wire length in the image, on the percentage deviation of the estimated length from the real length. It appears that as the length per image increases (more pieces), there is an increasing underestimation of length. Quadratic curve fitting in Figure 2 gave almost the same line as the linear curve fit used. Within the range of total length tested, there is little influence of the diameter on the deviation. Figure 3 shows that, in absolute terms, the influence of diameter on the deviation is insignificant. The variance of the repeated measurements is low over a wide range of total lengths in the image, except for images with very few pieces (Fig. 4). There was no difference in precision and variation of the measurement between light intensities of 2200 and 4400 lux. The automatic light intensity compensation by the camera means that in spite of different light intensities the image is grabbed with the same average grey value.

Estimation of number of root tips

The relation between real and estimated numbers of ends was studied using small pieces of wire (2 pix. thick, 47 pix. long). Estimates were too low, especially with a high density of pieces (Fig. 5). Presumably, this is a result of more frequent adhesion of ends to other pieces with increasing density. For these non-branching

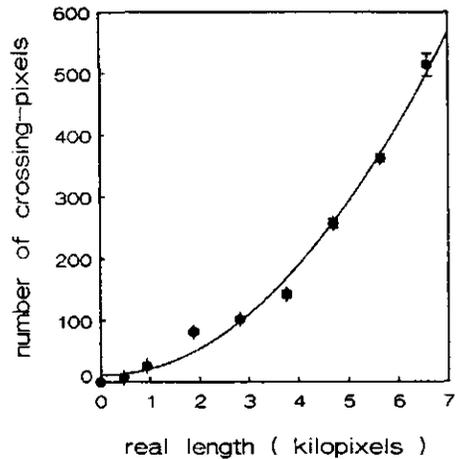
objects, it was found that the real number of ends is not only strongly related to the observed number of end pixels (EP), but also to the number of crossing pixels (CP). Multiple regression ($R^2=0.98$) revealed the relation:

$$\text{real ends} = -3.4 + 1.1 \times EP + 0.25 \times CP \quad (1)$$

Estimation of the number of crossing pixels.

For the assessment of branching from the number of 'branching pixels' (3 adjoining) it is necessary to compensate for the expected number of crossing pixels (branching pixels due to crossing and adjoining root pieces). Numbers of crossing pixels were estimated in the same images of non-branching wire objects as used for the counting of end pixels. The relation between the total length per image and the observed number of crossing pixels (Fig. 6) appears to be a quadratic function. About 25 % of the crossing pixels are likely to be caused by adhering ends (Eq. 1).

Fig.6. The influence of total length of (non-branching) wire in the image on the number of crossing pixels (pseudo-branches) observed, and after subtraction of the probable number of attached ends. Length expressed as number of pixels in a 256 x 256 pixel grid. Wire pieces 47 pixels long, 2 pixels thick. Curve fit for crossing pixels: $Y = 11.8 - 2.46 \times X/c + 11.7 \times X^2/c^2$ ($R^2=0.99$) factor c is any total number of pixels in an image divided by 65536). Bars indicate standard errors, n=10.



DISCUSSION

The results of the tests on the reliability of the length estimation indicate that underestimation increases as the length in the image increases, probably due to clustering and cross-overs. This deviation can automatically be corrected by using the calibration function found in Figure 3. The ordinate of Figure 3 should be converted to percentage or fraction of the total number of pixels (65536) to allow application to different grid sizes. When the root length in an image exceeds about

10 % of the total number of pixels in the matrix, the calibration function may no longer be valid.

Deviations for different diameters of wire in Figure 2 are only a few per cent different from the fitted line, and show the same dependence with regard to total length in the image. Thus, the proposed correction equation can be used for slightly different diameters. A diameter of 6.5 pixels is not efficient for root length measurements, because more images than necessary must be analysed to measure a certain amount of roots. The most efficient magnification of a root image is such, that the thinnest roots are one pixel thick, irrespective of the range of root diameter in the sample.

The effect of total length on the variance of the estimate indicates that measurement of few pieces, adding up to a very short total length, should be avoided. Presumably, insufficient randomization of orientation is the cause of the higher coefficients of variation when there are only a few pieces present. The same occurs in line intersect methods (Tennant, 1975), but the range for reliable measurement is wider in our method ($CV < 5\%$ if length $> 0.25 \times$ maximum (Tennant, 1975), vs. $0.05 \times$ maximum (Fig. 4)) Furthermore, relatively short pieces give slightly less variance of the measurement than the same total length of longer pieces (Fig. 4), probably because of ter randomization.

The automatic light intensity compensation of the Vidicon camera appeared to make the method insensitive to variation in background light. In this way the possible impact of lower light intensity during the warming up of the light box is eliminated. An additional benefit of this camera characteristic is that it reduces the sensitivity of the measurements to root length density in the image. At high densities, the roots are grabbed with higher grey values (lighter). As a result, clustered roots, which are numerous at high densities, are better separated after thresholding, thus reducing underestimation.

The estimation of the number of ends in the image was much too low at higher length densities in the image. It seems that many ends are interpreted as crossing pixels (Eq. 1.), probably because they are in contact with other pieces. Correction of the number of end pixels found in a root sample, by subtracting the number of end pixels estimated for the same length of non-branching objects, appeared unsuitable for estimating the number of root tips. This is due to the fact that the correction factor depends on a certain mean size of pieces, which may not be the same for a root sample. Moreover, the deviation of the measured numbers from the real numbers is substantial (up to 50%), and thus far it is not clear, whether or not

real root ends behave in the same way as ends of wire pieces.

The estimation of the number of branches, after correction for expected pseudo-branches (crossing pixels), appears to be a better way to quantify the branching rate. The expected number of crossing pixels can be estimated from calibration images containing different lengths of non-branched line segments (Fig. 6). As discussed above, attached ends give rise to higher numbers of crossing pixels. Although the fraction attached ends (0.25 in Eq. 1.) may vary for different root images (mean length of pieces, magnification), large differences are not expected, because variation in the measurements presented here was very low (Fig. 6), and normally, measurements are done according to standardized spreading and magnification. Moreover, touching ends form a smaller fraction of the number of crossing pixels than of the number of ends at higher length densities (Fig. 5 and 6), and thus, the number of crossing pixels is less sensitive to the phenomenon of touching segment ends. Another problem in the estimation of the number of crossing pixels might be the fact that often more than one pixel is counted at crossings with narrow angles. The proportion of crossing pixels associated with this remains unknown, but can be assumed to be equal for all images containing crossing line fragments of a certain diameter and with random orientation. Thick roots may more often yield two (instead of one) crossing pixels per crossing, than thin roots. Besides, there might be some influence of root architecture on the number of cross-overs, but this does not seem very important, provided the roots are cut and arranged properly.

Thus, the branching ratio of roots (branches/m) can with reasonable accuracy be described by the difference between the observed number of branch pixels (BP) and the number of crossing pixels (CP) estimated for the length measured, divided by this length:

$$BR = \frac{BP - \text{expected } CP}{\text{Root Length}} \quad (2)$$

Incidentally, a negative branching ratio may be found in roots with few branches. This is likely to be due to stochastic deviation of the number of crossing pixels found compared to the expected number.

From the results of this evaluation, it can be concluded that image analysis is a valuable tool for fast and accurate measurement of root length and for rapid, but

slightly less precise assessment of root branching. Rhizotron root images with more gradual grey value transitions on the boundaries of background structures than on the edge of roots can be analysed as well. A benefit of our system is its usefulness for many other purposes (e.g. area and shape measurements or counting, and the 'normal' personal computer functions) and the fairly reasonable price for the complete system including the computer (about \$10,000. in 1987). At present (1990), a 512 x 512 pixel frame grabber for this system is available, allowing four times as much root length to be analysed in one image as with the former 256 x 256 frame grabber.

REFERENCES

- Baldwin J.P., Tinker P.B. and Marriott F.H.C. 1971. The measurement of length and distribution of onion roots in the field and the laboratory. *J. appl. Ecol.* 8: 543-554.
- Böhm W. 1979. *Methods of studying Root Systems*. Springer Verlag, Berlin.
- Collins R.P., Gregory P.J., Rowse H.R., Morgan A. and Lancashire B. 1987. Improved methods of estimating root length using a photocopier, a light box and a bar-code reader. *Plant and Soil* 103: 277-280.
- Costigan P.A., Rose J.A. and McBurney T. 1982. A microcomputer based method for the rapid and detailed measurement of seedling root systems. *Plant and Soil* 69: 305-309.
- Ekkers R.J. 1988. Meten in beelden. *Natuur en Techniek* 56: 638-649.
- Fisher C. 1971. The new Quantimet 720. *Microscope* 19: 1-20.
- Heringa J.W., Groenwold J. and Schoonderbeek D. 1980. An improved method for the isolation and the quantitative measurement of crop roots. *Neth. J. Agric. Sci.* 28: 127-134.
- Marsh B. a'B. 1971. Measurement of length in random arrangements of lines. *J. appl. Ecol.* 8: 265-267.
- Newman E.I. 1966. A method of estimating the total length of root in a sample. *J. appl. Ecol.* 3: 139-145.
- Ottman M.J. and Timm H. 1984. Measurement of viable plant roots with the Image Analysis Computer. *Agron. J.* 76: 1018-1020.
- Richards D., Goubran F.H., Garwoly W.N. and Daly M.W. 1979. A machine for determining root length. *Plant and Soil* 52: 69-76.
- Rowse H.R. and Phillips D.A. 1974. An instrument for measuring the total length of root in a sample. *J. appl. Ecol* 11: 309-314.
- Smucker A.J.M., Ferguson J.C., DeBruyn W.P., Belford R.K. and Ritchie J.T. 1987. Image analysis of video-recorded plant root systems. *In: H.M. Taylor (ed.), Minirhizotron Observation Tubes: Methods and Applications for Measuring*

- Rhizosphere Dynamics. Spec. Publ. 50. Am. Soc. of Agronomy, Crop Sci. Soc. of America, and Soil Sci. Soc. of America, Madison, Wisconsin: 67-80.
- Tennant D. 1975. A test of a modified line intersect method for estimating root length. *J. Ecol.* 63: 995-1001.
- Voorhees W.B., Carlson V.A. and Hallauer E.A. 1980. Root length measurement with a computer-controlled digital scanning microdensitometer. *Agron. J.* 72: 847-851.
- Wilhelm W.W., Norman J.M. and Newell R.L. 1983. Semi-automated X-Y plotter based method for measuring root lengths. *Agron. J.* 75: 149-152.

CHAPTER 6

The influence of soil moisture content on the relation between *Tylenchorhynchus microphasmis* Loof and *Hippophaë rhamnoides* L.

F.C. Zoon

submitted to Plant and Soil

SUMMARY

The plant parasitic nematode *Tylenchorhynchus microphasmis* is commonly found in soil under sea buckthorn, *Hippophaë rhamnoides* L. ssp. *rhamnoides* in the calcareous coastal sand dune areas of the Netherlands. In order to study the effect of soil moisture on this pathosystem, inoculation experiments with *T. microphasmis* were conducted in dune sand under two different moisture regimes (dry and moderately moist) with or without seedlings of *H. rhamnoides*. Nematode survival and reproduction, and plant growth were monitored. Survival and multiplication of *T. microphasmis* were not affected by the soil moisture levels used. At the higher moisture level, *H. rhamnoides* grew better, and *T. microphasmis* appeared to be more pathogenic than in dry soil. The fact that this ectoparasitic nematode was found not to be harmful under the dry regime, may be due to the low growth rate of *H. rhamnoides* or to reduced mobility of *T. microphasmis*. Thus, the effects of drought and plant feeding nematodes were not additive or synergic as has often been found in other plant-nematode interactions.

INTRODUCTION

Hippophaë rhamnoides L. ssp. *rhamnoides* is a pioneer shrub species in the vegetation of calcareous coastal sand dunes in Western Europe. It can obtain atmospheric nitrogen from its root symbiosis with the actinomycete *Frankia* and it may, therefore, lead to accelerated N-enrichment of nutrient poor dune soils. After an initial period of vigorous growth, scrubs of *H. rhamnoides* tend to degenerate. Growth reduction was found to be caused by biotic soil factors (Oremus & Otten,

1981). Plant parasitic nematodes play an important role in the disease complex (Maas *et al.*, 1983). The ectoparasitic root nematode *Tylenchorhynchus microphasmis* Loof is very common and abundant under *Hippophaë* in the field (Zoon *et al.*, 1993) and was therefore chosen as a model organism in studies on effects of nematodes on *H. rhamnoides*.

Sand dune soils under *H. rhamnoides* in the field may be very dry (1-5 % w/w in the top 20 cm; F.C. Zoon, unpubl.res.). The level of soil moisture may influence survival and activity of nematodes (Vrain, 1986) and also host plant quality and tolerance. To assess the importance of these effects, the parasite-host combination of *T. microphasmis* with *H. rhamnoides* was studied in calcareous dune sand in a pot experiment under dry and moderately moist conditions, using special equipment for automatic regulation of the soil moisture content.

METHODS

The set up of the experiment comprised objects with or without *T. microphasmis* and with or without *H. rhamnoides*, at two moisture regimes. Dune sand was sterilized by 2.5 Mrad gamma irradiation and inoculated with *Frankia* sp. in the form of a suspension of homogenized, surface-sterilized nodules (about 0.4 g nodule tissue/kg soil). Plastic pots containing 500 g dry weight of this soil were inoculated with 350 individuals of *Tylenchorhynchus microphasmis*, half of the inoculum in the planting hole and the other half in two holes near the stem base after planting. Ten-week-old hydroponically cultured *Hippophaë* seedlings (Oremus 1980) were planted in half of the pots. To minimize evaporation, the soil was covered with aluminum foil. The pots were kept in a greenhouse at 22-25 °C with a day length of 16 hours. A light quantum flux between 150 and 350 $\mu\text{E/s/m}^2$ (PAR) was maintained during the day using additional illumination (Philips SON-T at 200 $\mu\text{E/s/m}^2$) and shutter screens. The soil water content was initially 8 per cent on a dry weight basis, and was further on maintained at 2.5 or 8.5 (± 1) per cent (-7.9 to -39.8, and -2.8 to -3.5 kPa, respectively) by replenishment of the water lost by transpiration with 1/2 strength modified Hoagland solution (Oremus, 1980). A special apparatus for automatic control of the soil moisture level (Zoon *et al.*, 1990) was used, since it proved extremely difficult to maintain these low levels manually. As all treatments started from 8 per cent soil moisture, it took almost 4 weeks before the dry treatment reached its set point (Fig. 1).

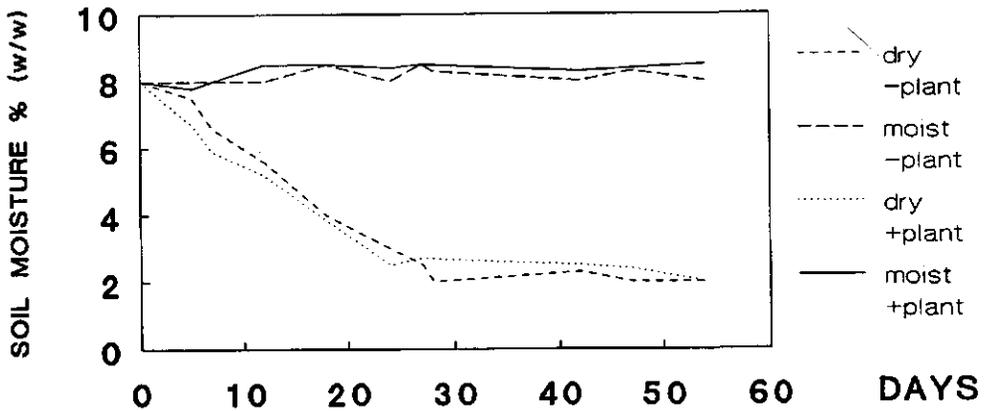


Fig. 1. Course of the soil moisture content (% water on dry soil weight) during the experiment.

The experiment was carried out using six replications. The plants were harvested, dried and weighed eight weeks after planting, nematodes were extracted by elutriation and filter extraction (Oostenbrink, 1960) and counted. Mid-term survival of *T. microphasmis* was estimated 18 days after inoculation by analysis of additional sets of pots with and without plants. Statistical analysis (ANOVA) was performed following a 1- or 2-factor randomised block design, for plant and nematode data, respectively. Bartlett's test for homogeneity of variance was run beforehand, and when necessary, ln-transformation of data was applied.

RESULTS

Growth of *Hippophaë rhamnoides*.

At harvest, the total weight and shoot weight of *H. rhamnoides* plants were significantly lower in the dry treatment than in the moist one (Table 1). Root weight was also lower in the dry treatment, but only when *T. microphasmis* was absent. Root growth in the inoculated treatments was not affected by the soil moisture level. The shoot/root ratio was significantly higher in the moist treatment than in the dry treatment, particularly when nematodes were present (Table 1).

Table 1. Biomass of *H. rhamnoides* (Hr) plants, and final populations of *T. microphasmis* (Tm) 54 days after inoculation (350 per pot) under two soil moisture regimes (M=moist; 8.5 % constant, D=drying from 8.0 to 2.5 % w/w of dry soil). Means in each column followed by the same letter are not significantly different in Tukey's HSD test ($p=0.05$). The degree of significance in the ANOVA is indicated by *= $p<0.05$, **= $p<0.01$ and ***= $p<0.001$.

| Treatment | Total dry wt mg | Shoot dry wt mg | Root dry wt mg | S/R ratio | Final nr of Tm per pot | Tm per mg root dry wt |
|---------------|-----------------|-----------------|----------------|-----------|------------------------|-----------------------|
| M Hr | 1166 a | 972 a | 194 a | 5.5 a | - | - |
| M Hr + Tm | 878 ab | 783 ab | 95 b | 8.3 b | 3075 a | 29.6 a |
| D Hr | 454 b | 375 b | 79 b | 4.9 a | - | - |
| D Hr + Tm | 548 b | 453 b | 95 b | 4.7 a | 3521 a | 44.2 b |
| ANOVA | 1) | | | | | |
| Moisture | ** | *** | ** | ** | ns | * |
| Tm | ns | ns | * | * | - | - |
| Moisture x Tm | ns | ns | ** | * | - | - |

1) analysis on $\ln(x)$ transformed data.

Nematode inoculation did not significantly influence shoot growth of *H. rhamnoides* and root growth was reduced only in the moist treatment. It is remarkable, however, that inoculated plants in the dry soil tended to have equal or slightly higher shoot and root weights than non-inoculated plants, whereas in the moist soil inoculated plants had lower weights than non-inoculated plants. In the moist soil, the shoot/root ratio was significantly higher after nematode inoculation (Table 1). All plants were nodulated, although to a varying extent (data not shown).

Population development of *T. microphasmis*.

Final populations of *T. microphasmis* at the two moisture levels in pots with *H. rhamnoides* were not significantly different (Table 1). When expressed per unit root dry weight, however, numbers were significantly higher in the dry treatment than in the moist treatment. The survival of *T. microphasmis* in soil without plants showed no difference between the dry and the moist treatment. Means were 50 and 49 individuals per pot, respectively, after 54 days. Eighteen days after inoculation there was no significant difference in numbers between pots with and without *H. rhamnoides* (Fig. 2), nor between the two soil moisture regimes (data not shown).

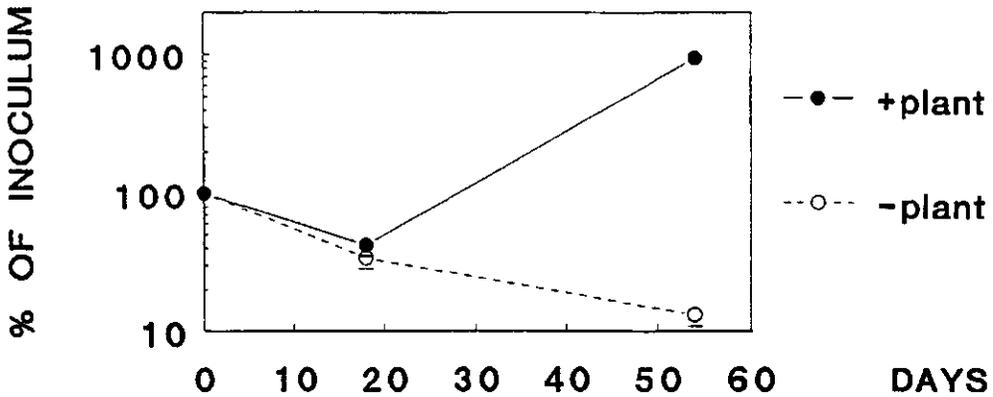


Fig. 2. Population dynamics of *T. microphasmis* in pots with and without plants. The dry and the moist treatment were taken together, because there was no significant difference between the two.

DISCUSSION

The results show that soil moisture is an important factor in the balance between *H. rhamnoides* and its parasite *T. microphasmis* in dune sand in a pot experiment. *H. rhamnoides* grew better in moderately moist soil than in dry soil, which can be explained by an improved uptake of water and nutrients. Soil water forms the pathway for diffusion and transport of plant nutrients. Especially the uptake of elements such as phosphorus, the availability of which is highly dependent on diffusion, benefits from the improvement of the transport at higher soil moisture levels and also from a larger root system (Marschner, 1986). Since plants would generally need a smaller uptake capacity for water and nutrients at a high soil moisture level than at a low moisture level, an increased tolerance to nematode damage might be expected at relatively high soil moisture levels. In the present experiment, however, the effect of the soil moisture content on the plant's tolerance to nematodes turned out to be the opposite of what was expected. *T. microphasmis* reduced plant growth only at the higher soil moisture level. A possible explanation for this is that *T. microphasmis* is much more mobile in the moist soil and thus better able to aggregate in large numbers on suitable root tips, whereas in dry soil their mobility may be hampered to such an extent that massive attacks on roots cannot take place. This does not contradict results with potato cyst nematodes

(PCN), where effects of nematodes and drought were additive (Haverkort *et al.*, 1992). PCN is an endo-parasitic nematode, which is sensitive to soil moisture effects only during the short period between emergence from the eggs and root invasion. Although she used the same range of soil suction pressures as in the present experiment, Den Toom (1988) found additive and synergic effects of drought and *T. dubius* on growth of *Lolium perenne*. Impeded mobility of the nematodes at the dry moisture level in her experiment was not likely, because the moisture content at 50 kPa in the soil used was not less than 9 % (w/w), compared to 1.5 % in dune sand.

Another reason for the absence of a nematode effect in the dry regime may be that *T. microphasmis* affects plant growth, at least in part, through other mechanisms than drought. The demand for resources of which the uptake rates are negatively affected by *T. microphasmis* may be reduced when plants grow slower due to direct growth-limiting effects of drought. Slower growth of the plants also delays depletion of available nutrients in pots (Fasan & Haverkort, 1991). Den Toom (1988) suggests that in dry soil plants may adapt to moisture stress and consequently they are affected less by similar stress due to nematode attack.

Maas *et al.* (1983) showed that in the same dune soil at 15% (w/w) soil moisture an inoculum of 400 *T. microphasmis* together with 80 *L. dunensis* Brinkman, Loof & Barbez did not cause any growth reduction within the first 60 days. There is no causal explanation for the absence of damage at this high soil moisture level. Probably, the larger pot size used (Maas *et al.*, 1983) has left the roots more room to escape nematode attack and to find more nutrients.

Final populations of *T. microphasmis* in the present experiment were not affected by soil moisture, but numbers per unit root weight were higher in the dry treatment than in the moist treatment (Table 1). The lower soil moisture level may have caused an increase in the quality of the roots as a food source for *T. microphasmis*. The lower shoot/root ratio in dry soil (Table 1) may imply an increased concentration of free amino-acids and sugars in roots, since the shoot demand for most nutrients and assimilates will be lower. Also, White (1984) found that stressed plants were better hosts for insect herbivores due to a higher concentration of free nitrogen compounds.

It is concluded that the soil moisture content in pot experiments using sand dune soil indeed affects the host-parasite relation between *T. microphasmis* and *H. rhamnoides*. However, effects of drought were not synergic or additive with the effects of *T. microphasmis* on growth of *H. rhamnoides*, as found in other

nematode host combinations or other soils (Den Toom, 1988; Haverkort *et al.*, 1992). Taking the moist treatment without nematodes as a reference, it is clear that growth of *H. rhamnoides* was affected more by the reduction of the soil moisture content than by the inoculum of 350 individuals of *T. microphasmis*. In the field, however, both stresses may not always act synchronously. When nematodes act prior to drought, a synergic interaction may be expected.

REFERENCES

- Brouwer R. 1962. Nutritive influences on the distribution of dry matter in the plant. *Neth. J. agr. Sci.* 10: 399-408.
- Den Toom A.L. 1988. Influence of temperature and soil moisture on the relation between *Tylenchorhynchus dubius* and *Lolium perenne*. *Neth. J. Pl. Path.* 94: 33-44.
- Fasan T. and Haverkort A.J. 1991. The influence of cyst nematodes and drought on potato growth. I. Effects on plant growth under semi-controlled conditions. *Neth. J. Pl. Path.* 97: 151-161.
- Haverkort A.J. Boerma M., Velema R. and Van de Waart M. 1992. The influence of drought and cyst nematodes on potato growth. 4. Effects on crop growth under field conditions of four cultivars differing in tolerance. *Neth. J. Pl. Path.* 98: 179-191.
- Maas P.W.Th., Oremus P.A.I. and Otten H. 1983. Nematodes (*Longidorus* n.sp. and *Tylenchorhynchus microphasmis* Loof) in growth and nodulation of sea buckthorn, *Hippophaë rhamnoides* L.). *Plant and Soil* 73: 141-147.
- Marschner H. 1986. Mineral Nutrition of Higher Plants. London, Academic Press, 674 pp.
- Oostenbrink M. 1960. Estimating nematode populations by some selected methods. *In: J.N. Sasser & W.R. Jenkins, Nematology.* Univ. N. Carolina Press, Chapel Hill: 85-102.
- Oremus P.A.I. 1980. Occurrence and infective potential of the endophyte of *Hippophaë rhamnoides* L. ssp. *rhamnoides* in coastal sand dune areas. *Plant and Soil* 56: 123-139.
- Oremus P.A.I. and Otten H. 1981. Factors affecting growth and nodulation of *Hippophaë rhamnoides* L. ssp. *rhamnoides* in soils from two successional stages of dune formation. *Plant and Soil* 63: 317-331.
- Vrain T.C. 1986. The role of soil water in population dynamics of nematodes. *In: Leonard K.J. and Fry W.R. (eds.) Plant Disease Epidemiology.* Macmillan, New York: 101-128.
- White T.C.R. 1984. The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia* 63: 90-105.

- Zoon F.C., Hage F. and de Zwart A. 1990. A device for automatic soil moisture control and registration of water use in pot experiments. *Plant and Soil* 125: 281-284.
- Zoon F.C., Troelstra S.R. and Maas P.W.Th. 1993. The ecology of the plant feeding nematode fauna associated with *Hippophaë rhamnoides* L. in different stages of dune succession. *Fundam. appl. Nematol.* 16: 247-258.

CHAPTER 7

Belowground herbivory of *Tylenchorhynchus microphasmis* Loof on actinorhizal and non-actinorhizal sea buckthorn (*Hippophaë rhamnoides* L. ssp. *rhamnoides*).

F.C. Zoon, A. van Herp & S.R. Troelstra
submitted to *Oecologia*

SUMMARY

The plant parasitic nematode *Tylenchorhynchus microphasmis* is associated with roots of Sea Buckthorn, *Hippophaë rhamnoides* L., in the calcareous coastal sand dune areas in the Netherlands. To study the effects of this nematode on growth and nodulation of this nitrogen-fixing pioneer shrub, inoculation experiments were conducted with seedlings in monoxenic agar culture and in dune sand in pots.

In vitro experiments showed a reduction of root hair density and quality when *T. microphasmis* was present. In pot experiments, the nematodes significantly reduced growth, shoot phosphorus concentration and the number of *Frankia* nodules per unit length of roots. Growth reduction appears to be caused mainly by the decreased root growth and by the consequently lower uptake of less available nutrients such as phosphate. The nematodes inhibited nodule formation and nitrogenase activity, directly or indirectly, but this did not result in growth-limiting nitrogen levels. It was concluded that the mechanisms of growth reduction as generated by *T. microphasmis* are likely to curtail the competitive ability of *H. rhamnoides* in mixed vegetation and affect the decline and succession of *Hippophaë* vegetation on nutrient-poor sand dune soils.

INTRODUCTION

Studies on the effects of soil-borne pathogens on pioneer plant species have recently shed new light on the mechanisms of succession in dune vegetation (Van der Putten *et al.*, 1993). Whereas soil diseases and pests are a major topic in agriculture, there are relatively few examples of their role in mixed vegetation and

natural ecosystems (Korteweg & s'Jacob, 1980; Weste, 1981; Van der Putten *et al.*, 1988; Brown & Gange, 1991). Soil-borne pathogens may reduce the competitive ability of a plant species in the vegetation (Crawley, 1993) and thereby affect the rate and direction of succession. However, the mechanisms of growth reduction are still largely unknown.

Hippophaë rhamnoides L. ssp. *rhamnoides* is a dominant shrub species in the early primary succession of the vegetation of calcareous coastal sand dunes in Western Europe (Pearson & Rogers, 1967). This shrub can acquire fixed atmospheric nitrogen from the root-symbiotic actinomycete *Frankia*. A few decades after colonization, *Hippophaë* scrub gradually starts to degenerate. When it disappears, *H. rhamnoides* may be succeeded by other woody species (Van der Maarel *et al.*, 1985), but often a retrogressive succession occurs, leading towards grass or moss vegetation. Unknown biotic soil factors play a role in this degeneration as was shown by gamma-sterilization of soil (Oremus & Otten, 1981; Oremus, 1982). Differences between two sites suggested that nematodes might be involved.

The occurrence of plant parasitic nematode species and other soil factors in different successional stages of vegetation with *H. rhamnoides* was surveyed in a number of dune areas along the Dutch coast (Zoon *et al.*, 1993). In these studies, *T. microphasmis* and *Mesocriconema xenoplax* (Raski) Loof & de Grisse were the most numerous and frequently isolated plant parasitic species under *Hippophaë*, whereas *L. dunensis* was found only in old calcareous dunes with degenerating scrubs. Maas *et al.* (1983) studied the effect of a mixed inoculum of the plant-feeding nematodes *Longidorus dunensis* Brinkman, Loof & Barbez and *Tylenchorhynchus microphasmis* Loof on growth of *H. rhamnoides*. Their results demonstrate that nematodes are an important part of the disease complex. The deteriorating effect of these nematodes on plant growth was less severe in sterilized soil than in natural soil (Maas *et al.*, 1983). Hence, it was concluded that other biotic factors act synergically with the nematodes in causing damage to the plant. Root fungi interact with nematodes in growth and nodulation of *H. rhamnoides* (Zoon, 1991).

The present study was set up to investigate the influence of *T. microphasmis* on growth, nodule formation and nutrient acquisition of *H. rhamnoides* *in vitro* and in soil. A pot experiment was conducted to explore whether *T. microphasmis* acts directly on the plant or, indirectly, via influence on the actinorhiza. To this end, part of the *Hippophaë* seedlings in this experiment were inoculated with *Frankia*.

METHODS

In vitro experiment.

Seedlings of *H. rhamnoides* were obtained as described by Oremus (1980). When the first pair of leaves had appeared, selected seedlings were surface-sterilized for one minute in 1% NaOCl and rinsed with sterile water. Weight and length were recorded and roots were mapped for each individual seedling. Subsequently, the seedlings were transferred to either 1/2 strength Hoagland agar, sterile quartz sand or glass beads (ϕ 0.2-0.5 mm) both with 20% v/v 1/2 Hoagland solution. After seven days, 400 individuals of *T. microphasmis* were inoculated close to the seedling. Before inoculation, the nematodes had been washed with one liter of sterile water on a millipore filter (diameter 2.5 cm, pore size 8 μ m). After 6, 10 and 13 days, seedlings were carefully removed from their substrate. Fresh weight, root and shoot length and number of lateral roots were determined. Roots were stained in a solution of 0.4% anilin-blue and 0.1% alkaline fuchsine in lactophenol (Noel, 1964) and root hairs were counted on two sides of 1.4 mm length of a lateral root which had been formed shortly before planting (according to the root map), so that the areas of counting had about the same age. As a measure of root hair damage, coagulation of cytoplasm was scored in three classes of frequency (0=0-5%, 1=5-50%, 2=more than 50% of root hairs with coagulation). Daily relative growth rates were calculated for fresh weight and length assuming exponential growth. Results were analysed statistically by general linear model procedures (GENLI) after calculating Kronecker products (KRON) for treatments (Overall & Klett, 1972).

Pot experiment.

Moist calcareous dune sand was sterilized by 2.5 Mrad gamma irradiation. Seedlings of *H. rhamnoides* were cultured hydroponically (Oremus, 1980). Ten-week old seedlings were planted in pots containing 500 ml of the soil, either mixed with a homogenised culture suspension of the root-symbiotic *Frankia* strain Hr1.1 (Burggraaf, 1984), or with the same amount of water. Different inoculation levels of *T. microphasmis* were used (0, 670 and 1340 individuals per pot). The nematodes were inoculated in 20 ml water in the planting hole. The soil moisture was kept at 5 (\pm 1) % of dry weight (3.9-6.3 kPa soil water potential; pF 1.6-1.8). Water lost by evapo-transpiration and nutrients taken up by the plant were replenished automatically with a nutrient solution, using an apparatus as described by Zoon *et al.* (1990). In the treatment without *Frankia*, 1/2 strength Hoagland solution with 6.5

Table 1. *In vitro* effects of *T. microphasmis* on growth of *H. rhamnoides* seedlings. Means of observations 6, 10 and 13 days after inoculation with approximately 400 nematodes per seedling. N= number of replications. Significance of inoculation effect is indicated by *=p<0.05, **=p<0.01, ***=p<0.001, and ns=not significant.

| Substrate and Treatment | N | Relative increase per day ($\times 10^{-3}$) | | | Number of root hairs per mm root | Index for root hair condition 1) |
|-------------------------|----|--|--------------|-----------------------|----------------------------------|----------------------------------|
| | | fresh weight | shoot length | length of root system | | |
| Quartz sand | | | | | | |
| non-inoculated | 15 | 27 | 17 | 40 | 116 | 0.73 |
| inoculated | 20 | 13 * | 13 ns | 27 ns | 40 *** | 1.67 ** |
| Glass beads | | | | | | |
| non-inoculated | 15 | 16 | 17 | 52 | 58 | 1.13 |
| inoculated | 20 | 10 ns | 16 ns | 27 ns | 36 * | 1.56 * |
| Agar | | | | | | |
| non-inoculated | 10 | -3 | 4 | 35 | 41 | 0.50 |
| inoculated | 15 | -4 ns | -2 ns | 35 ns | 35 ns | 1.80 ** |
| Overall effects: | | | | | | |
| substrate | | * | * | ns | * | ns |
| inoculation | | * | ns | ns | ** | ** |
| substr.x inoc. | | ns | ns | ns | * | ns |

1) Weighted mean of 3 classes: 0=0-5%, 1=5-50%, 2=more than 50% of root hairs with coagulated cytoplasm.

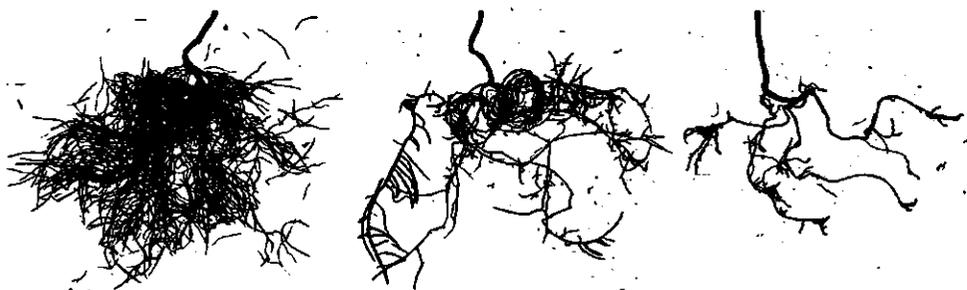


Fig 1. Silhouettes of root systems of *H. rhamnoides* 7 weeks after inoculation of seedlings with 0, 670 and 1340 individuals of *T. microphasmis* (from left to right).

meq/l NO_3^- , 1 meq/l H_2PO_4^- , Fe-citrate and trace elements according to Allen & Arnon (1955) was used throughout the experiment. In the *Frankia* treatment, a modified solution containing 20% of the original nitrogen concentration was used from 2 weeks onwards. After 7 and 13 weeks, the plants were harvested. Nematodes were extracted using the elutriation method of Oostenbrink (1960).

N_2 -fixation activity was estimated by acetylene reduction measurement of intact plants in soil incubated with 10% v/v C_2H_2 (Akkermans, 1971) in 7 l perspex cylinders. Subsequently, the same plants were washed from soil and acetylene reduction was measured in 250 ml bottles. Gas samples from the vessels were taken 1 and 3 hours after incubation at 25 °C to determine the acetylene reduction rate in the intermediate period. Ethylene concentrations in the samples were measured by gas chromatography using a column (110 cm long, 3.2 mm inner diameter) packed with Porapak R (100-120 mesh) maintained at 60° C and with 30 ml/min N_2 as carrier gas.

Root length and branching were estimated using image analysis (Zoon & Van Tienderen, 1990). Root nodules larger than about 2 mm were counted by eye. Weights of shoots, roots and nodules were determined after drying at 70° C. Specific root length was calculated as the ratio root length / root weight. The weight of the hypocotyl and woody roots was included in this 'root weight'. Concentrations of total N, P and K in the dried shoot material were determined as described by Troelstra (1983). Nutrient inflows were calculated following Williams (1948), using root length instead of root weight. Initial root length and nutrient pools of seedlings were estimated from fresh weight by means of an allometric calibration function based on a parallel series of seedlings.

Statistical analysis (ANOVA) was done following a two-factor randomised block design. Blocks coincided with classes of initial plant weight. Data were transformed prior to ANOVA if variances of treatment means were not homogeneous according to Bartlett's test.

RESULTS

In vitro experiment.

The relative increase in plant fresh weight was significantly reduced by *T. microphasmis* in quartz sand where the seedlings grew best, but not in glass beads or on agar (Table 1). The relative increase in the length of the shoot and of the root

Table 2. Effects of *T. microphasmis* (T= 0, 670 and 1340 per pot) and *Frankia* (F) on growth of *H. rhammoides* seedlings 7 weeks (A) and 13 weeks (B) after inoculation. Means followed by the same letter are not significantly different according to Tukey's test ($p < 0.05$). Significance in ANOVA is indicated by *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$ and ns=not significant. Positive and negative correlations with initial seedling weight (order in block effect) are indicated with + and -, respectively.

| A | | Shoot | Root | Shoot/Root | Root | Spec.root | Root |
|------------|------|---------|--------|------------|---------|-----------|----------|
| week 7 | | dry wt | dry wt | ratio | length | length | branches |
| | | mg | mg | g/g | cm | m/g | per cm |
| T | 0 | 339 a | 184 a | 1.9 a | 982 a | 55 a | not |
| T | 670 | 272 ab | 116 bc | 2.6 ab | 603 ab | 48 ab | deter- |
| T | 1340 | 231 bc | 84 cd | 3.2 b | 315 bc | 32 bc | mined |
| F T | 0 | 280 ab | 153 ab | 2.0 a | 773 a | 55 a | " " |
| F T | 670 | 194 cd | 75 cd | 2.7 ab | 345 bc | 45 abc | " " |
| F T | 1340 | 159 d | 50 d | 3.1 b | 142 c | 26 c | " " |
| ANOVA | | 1) | 1) | 1) | 2) | . | . |
| Frankia(F) | | ** | *** | ns | ** | ns | ns |
| Tyl (T) | | *** | *** | *** | *** | *** | *** |
| Block (B) | | ***+ | ***+ | ns | ***+ | ns | ns |
| F x T | | ns | ns | ns | ns | ns | ns |
| F x B | | ns | ns | ns | ns | ns | ns |
| T x B | | ns | ns | ns | * | ns | ns |
| B | | Shoot | Root | Shoot/Root | Root | Spec.root | Root |
| week 13 | | dry wt | dry wt | ratio | length | length | branches |
| | | mg | mg | g/g | cm | m/g | per cm |
| T | 0 | 700 a | 470 a | 1.4 a | 2253 a | 49 a | 2.1 ab |
| T | 670 | 436 ab | 298 ab | 1.6 a | 1367 ab | 42 a | 2.0 ab |
| T | 1340 | 255 bc | 101 c | 2.7 b | 243 cd | 24 b | 2.0 ab |
| F T | 0 | 500 ab | 366 a | 1.3 a | 1615 a | 43 a | 2.2 a |
| F T | 670 | 343 abc | 130 bc | 2.2 ab | 531 bc | 35 ab | 1.9 ab |
| F T | 1340 | 204 c | 102 c | 2.2 ab | 199 d | 21 b | 1.8 b |
| ANOVA | | 1) | 1) | . | 1) | . | . |
| Frankia(F) | | * | * | ns | ** | ns | ns |
| Tyl (T) | | *** | *** | *** | *** | *** | * |
| Block (B) | | * | ns | ns | ns | ns | ns |
| F x T | | ns | ns | ns | ns | ns | ns |
| F x B | | ns | ns | ns | ns | ns | ns |
| T x B | | * | ns | ns | ns | ns | ns |

1. ln(x)-transformed

2. sqrt(x)-transformed

system was not affected. The number of root hairs per mm young lateral root was significantly reduced by *T. microphasmis* in both quartz sand and glass beads, but not on agar (Table 1). In all three substrates tested, root hairs had more often coagulated cytoplasm in the inoculated objects than in the non-inoculated treatments. The mortality of the nematodes in this experiment was high. Only 5 to 10 per cent of the inoculum was recovered alive, irrespective of the time of incubation (6 to 13 days). Nematode mortality was stronger in quartz sand and glass beads than on agar (data not shown).

Pot experiment

Growth of shoots and roots was significantly reduced by inoculation with *T. microphasmis* or *Frankia* (Table 2). Initial seedling weight (blocks) was positively correlated with plant biomass after 7 weeks, but not after 13 weeks. *T. microphasmis* reduced root growth more than shoot growth and thus caused an increase in the shoot/root ratio (Table 2). Also, the specific root length and the root branching rate were lower. Side root formation was hampered and the few which were formed had a stout club-like appearance (Fig 1). At the highest inoculum level of *T. microphasmis*, growth between 7 and 13 weeks had almost completely stopped. The mean percentage of dry matter in shoot biomass was not significantly affected by the treatments, and varied between 35 and 38% in week 7 and between 37 and 40% in week 13.

The concentration of nitrogen in the shoot was significantly higher in the presence of *Frankia*, compared to the non-nodulated treatment (Table 3). When *Frankia* was not present, *T. microphasmis* tended to increase nitrogen and potassium contents (interaction FxT), probably due to insufficient N-fertilization for the fast growing seedlings without nematodes. Conversely, the nematodes reduced the concentrations of N and K in the shoots when *Frankia* was present. Shoot phosphorus was much lower in the presence of *T. microphasmis* than in the objects without nematodes after 7 weeks but less so after 13 weeks.

The inflow rates of nitrogen and potassium into the roots were enhanced in the nematode-inoculated treatments, and thus compensated for the reduced root length. However, the inflow rate of P per cm root did not increase (Table 3). Inflow rates were much higher during the first 7 weeks, than in the second period. Overall, the molar ratio total P : total N in shoot tissue was significantly reduced by *T. microphasmis* in weeks 7 and 13, and by *Frankia* in week 7 (Table 4). This reduction was stronger under mineral N supply than in the *Frankia* treatment.

Table 3. Effects of *T. microphasmis* inoculation (T= 0, 670 and 1340 per 500 ml pot) and *Frankia* (F) on nutrient acquisition by *H. rhamnoides* seedlings 7 weeks (A) and 13 weeks (B) after inoculation. Means followed by the same letter are not significantly different according to Tukey's test ($p < 0.05$). Significance in the ANOVA is indicated by *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$, and ns=not significant. Positive and negative correlations with initial seedling weight (order in block effect) are indicated with + and -, respectively.

| A | | Shoot N | Shoot P | Shoot K | N inflow | P inflow | K inflow |
|----------------|------|----------|---------|---------|----------------------|----------------------|----------------------|
| week 7 | | mmol/kg | mmol/kg | mmol/kg | nmol/cm/d wk 0-7 | nmol/cm/d wk 0-7 | nmol/cm/d wk 0-7 |
| T | 0 | 782 a | 56 a | 345 ab | 30 a | 2.2 a | 13 a |
| T | 670 | 887 ab | 31 bc | 435 a | 49 ab | 1.7 a | 26 ab |
| T | 1340 | 1011 ab | 25 c | 411 ab | 95 c | 1.2 a | 43 b |
| F T | 0 | 1548 c | 45 ab | 387 ab | 63 bc | 1.6 a | 15 ab |
| F T | 670 | 1145 bc | 23 c | 318 b | 61 bc | 0.7 a | 15 ab |
| F T | 1340 | 1058 b | 35 bc | 327 ab | 85 c | 1.8 a | 23 ab |
| ANOVA | | 1) | . | . | 1) | . | 2) |
| Frankia(F) | | *** | ns | ** | ** | ns | ns |
| Tyl (T) | | ns | ** | ns | *** | ns | ** |
| Block (B) | | ns | ns | ns | ns | ns | ns |
| F x T | | ** | ns | ** | * | * | ns |
| F x B | | ns | ns | ns | ns | ns | ns |
| T x B | | ns | ns | ns | ns | ns | ns |
| B | | Shoot N | Shoot P | Shoot K | N inflow | P inflow | K inflow |
| week 13 | | mmol/kg | mmol/kg | mmol/kg | nmol/cm/d wk 7-13 | nmol/cm/d wk 7-13 | nmol/cm/d wk 7-13 |
| T | 0 | 1063 a | 40 ab | 277 a | 5.2 a | .13 a | 0.8 a |
| T | 670 | 1152 ab | 30 b | 346 abc | 6.3 a | .14 a | 1.0 a |
| T | 1340 | 1130 ab | 41 ab | 380 bc | 6.7 a | .24 a | 1.1 ab |
| F T | 0 | 1434 c | 46 a | 334 abc | 5.6 a | .16 a | 0.9 a |
| F T | 670 | 1306 bc | 37 ab | 394 c | 7.4 a | .23 a | 1.7 b |
| F T | 1340 | 1246 abc | 44 ab | 296 ab | 6.5 a | .27 a | 1.4 ab |
| ANOVA | | . | .. | . | 1) | 1) | |
| Frankia(F) | | *** | ns | ns | ns | ns | * |
| Tyl (T) | | ns | ** | ** | ns | * | * |
| Block (B) | | ns | ns | ns | ***- | **- | ***- |
| F x T | | * | ns | ** | ns | ns | ns |
| F x B | | ns | ns | ns | ns | ns | ns |
| T x B | | ns | ns | ns | ns | ns | ns |

1) ln(x)-transformed; 2) arctg(x)-transformed

Table 4. Effects of *T. microphasmis* (T; 0, 670 and 1340 per 500 ml pot) and *Frankia* (F) on molar P:N ratios (total P/total N) in shoots of *H. rhamnoides* seedlings 7 and 13 weeks after inoculation. Means followed by the same letter are not significantly different according to Tukey's test ($p < 0.05$). The degree of significance in the ANOVA is indicated by *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$ and ns=not significant.

| treatment | | week 7 | week 13 |
|------------|------|---------|---------|
| T | 0 | 0.072 a | 0.038 a |
| T | 670 | 0.044 b | 0.037 a |
| T | 1340 | 0.032 b | 0.036 a |
| F T | 0 | 0.030 b | 0.032 a |
| F T | 670 | 0.025 b | 0.028 a |
| F T | 1340 | 0.021 b | 0.026 a |
| ANOVA | | | |
| Frankia(F) | | *** | ns |
| Tyl (T) | | ** | ** |
| Block (B) | | ns | ns |
| F x T | | ** | ns |
| F x B | | ns | ns |
| T x B | | ns | ns |

Table 5. Effects of *T. microphasmis* inoculation (T= 0, 670 and 1340 per 500 ml pot) on nodule formation and nitrogenase activity (acetylene reduction) of *Frankia actinorhiza* of *H. rhamnoides*, 7 weeks (A) and 13 weeks (B) after inoculation. Means followed by the same letter are not significantly different according to Tukey's test ($p < 0.05$). The degree of significance in the ANOVA is indicated by *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$ and ns=not significant. No interactions were found.

| A week 7 | Nodules per plant | Nodules per m root l. | Nodule d.w. mg/plant | % Nod.w./ shoot w. | Ac. red. nmol/hv/plant | Ac. red. nmol/hv/mg nod |
|--------------|----------------------|--------------------------|-------------------------|-------------------------|---------------------------|----------------------------|
| T 0 | 22.7 a | 2.5 a | 10.3 a | 3.5 a | not | not |
| T 670 | 3.3 b | 0.7 b | 1.9 b | 0.8 b | deter- | deter- |
| T 1340 | 1.0 b | 0.4 b | 0.2 b | 0.1 b | mined | mined |
| ANOVA | 1) | 1) | 1) | . | . | 1). |
| Tyl | *** | ** | *** | *** | * | ns |
| Block | ns | ns | * | ns | * | ns |
| B week 13 | Nodules per plant | Nodules per m root l. | Nodule d.w. mg/plant | % Nod.wt./ shoot wt. | Ac. red. nmol/hv/plant | Ac. red. nmol/hv/mg nod |
| T 0 | 23.0 a | 1.6 a | 30.3 a | 6.6 a | 619 a | 17 a |
| T 670 | 5.8 b | 1.2 a | 7.7 b | 2.1 b | 324 ab | 33 a |
| T 1340 | 0.5 c | 0.2 b | 2.3 b | 0.8 b | 67 b | 9 a |
| ANOVA | 1) | . | . | . | . | 1). |
| Tyl | *** | ** | *** | *** | * | ns |
| Block | ns | ns | ns | ns | * | ns |

1. $\ln(x+1)$ -transformed

Table 6. Effects of inoculation density of *T. microphasmis* (T= 670 or 1340 per pot) and *Frankia* (F) on the population density (Tyl) 7 and 13 weeks after inoculation. Means followed by the same letter are not significantly different according to Tukey's test ($p < 0.05$). The degree of significance in the ANOVA is indicated by *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$ and ns=not significant. Positive and negative correlations with initial seedling weight (order in block effect) are indicated with + and -, respectively. Non-inoculated treatments were excluded from the analysis.

| Treatments | week 7 | | week 13 | |
|------------|-----------|-----------------|-----------|-----------------|
| | Tyl x1000 | Tyl per cm root | Tyl x1000 | Tyl per cm root |
| T 670 | 5.5 a | 15.7 a | 10.4 a | 14 a |
| T 1340 | 5.2 a | 37.0 a | 9.3 a | 42 b |
| F T 670 | 6.0 a | 21.5 a | 11.3 a | 23 ab |
| F T 1340 | 4.5 a | 34.3 a | 7.1 a | 43 b |
| ANOVA | 1) | 1) | . | . |
| Frankia(F) | ns | ns | ns | ns |
| Tyl (T) | ns | * | * | ** |
| Block (B) | * | *- | **+ | ns |
| F x T | ns | ns | ns | ns |
| F x B | ns | ns | * | ns |
| T x B | ns | ns | ** | ns |

1. $\ln(x+1)$ -transformed

The number of actinorhizal nodules formed on the roots was strongly reduced by *T. microphasmis* at both observation dates, also when calculated on a unit root length or unit shoot weight basis (Table 5). The total nodule weight per plant increased significantly between week 7 and week 13, but the number of nodules did hardly change. Acetylene reduction activity measured using incubation of pots in large vessels did not differ from incubation of uprooted and washed plants in bottles, except for levels below 60 nmol C₂H₄/h where correlation was not apparent due to large variation. For further calculations the average activity of vessel and bottle between 1 and 3 hours after the start of the incubation was used. The nitrogenase activity per plant was significantly decreased by *T. microphasmis* (Table 5). Effects on the activity per unit nodule weight were not significant.

The population of *T. microphasmis* increased in such a manner that after 7 weeks the low-dosage objects had a slightly higher final population than the high-dosage objects. This difference was larger and significant in week 13 (Table 6). The average multiplication factor over the first 7 weeks was 8.2-9.0 and 3.4-3.9 for the

low and the high inoculum level, respectively. Over the second period it was 1.9 and 1.6-1.8, respectively. The block effect in week 13 indicates that higher initial seedling weights eventually led to higher population densities of *T. microphasmis*. Larger seedlings gave less difference between the nematode dosages in week 13 than smaller ones (interaction BxT; Table 6.)

DISCUSSION

Effects of *T. microphasmis* on plant growth.

The aim of the experiments presented here was two-fold: 1) to study the response of *H. rhamnoides* to different densities of the nematode *T. microphasmis*, and 2) to reveal mechanisms involved in the damage caused by this nematode species. *In vitro* observations showed that on the short term *T. microphasmis* reduces both the density and the condition of the root hairs (Table 1). This is in agreement with reports on feeding behaviour and cell response to feeding by other *Tylenchorhynchus* species (Klinkenberg, 1963; Wyss, 1973). However, other authors did not observe feeding by *Tylenchorhynchus* spp. on root hairs, but only on epidermal cells of *Lolium perenne* (Sharma, 1971; Bridge & Hague, 1974). Root hairs are important for the uptake of nutrients with steep diffusion gradients in soil, such as phosphate (Barber, 1984). Furthermore, the rate of increase in seedling fresh weight was reduced, but the increase in length was not significantly affected by *T. microphasmis* inoculation. Effects were mainly established during the first week after inoculation.

In the pot experiment *T. microphasmis* caused growth inhibition which was more severe in the case of roots than of shoots (Table 2). Thus, a reduced uptake capacity of the roots (length, root hairs, condition) is probably the primary cause of growth reduction. In shoots of both nodulated and non-nodulated plants, the concentration of phosphorus after 7 weeks was lower when *T. microphasmis* was added than in the control. This can not be explained by the inflow rate per unit root length, which was reduced only in non-nodulated plants (interaction FxT). However, there was a significant correlation between shoot P concentration and root length ($p < 0.01$). In week 13, phosphorus concentrations were slightly reduced at the low inoculum level, but unchanged at the high level. At the high inoculum level, growth was so little that uptake of P was probably not limiting. The concentration of N in shoots of the non-nodulated seedlings was lower in the absence than in the

presence of *T. microphasmis*, probably because the available N in soil was further depleted by the faster growing plants. On the contrary, in nodulated seedlings *T. microphasmis* caused a reduction in N concentration which can be explained from the reduced contribution of *Frankia* (F) in the acquisition of N (see below). The attempt to obtain a similar level of N uptake in -F and +F treatments was not completely successful, but the fact that the highest growth rate was associated with the lowest concentration and inflow rate of N indicates that the availability of this element was not the limiting factor for growth during most of the experiment.

Between weeks 7 and 13, the inflow rates of N, P and K per unit root length were much lower than before this period. Growth was also much slower in the second period, possibly due to limitations of the pot size. The nutrient inflow rates calculated for the second period (week 7 to 13) were negatively correlated with the initial seedling size (blocks; Table 3b), *i.e.* small seedlings could maintain their inflow rates for a longer period than large ones. This agrees with the observation that the block effect on biomass, which was present in week 7, disappeared afterwards (Table 2). In other words, small seedlings also maintained their growth rate longer than large ones, probably due to a larger remaining soil nutrient pool.

Nutrient ratios may indicate which element is limiting plant growth. In plant tissue the metabolism of P is coupled tightly to that of N. Therefore, the molar ratio of P to N is generally in the range between 0.02 and 0.07 (Penning de Vries, 1982). The molar ratio of total P : organic N is considered sufficient when it is higher than 0.05 (Dijkshoorn & Lampe, 1980). In shoots of *H. rhamnoides*, total N is essentially the same as organic N. Virtually no mineral N is present, because nitrate is reduced mainly in the roots (Troelstra *et al.*, 1987). In the pot experiment the ratio total P : total N in the shoots was always lower than the critical value mentioned, except in the control without nematodes and *Frankia* in week 7. At this stage, *T. microphasmis* strongly reduced the P:N ratio when mineral N was supplied, but less so when nitrogen-fixation was active. This confirms that P uptake is hampered more than mineral N uptake.

Effects on nodulation and N₂-fixation

After 7 weeks the nodule weight constituted on average 2.3 % of the plant dry weight (3.5 % of shoot dry weight) in the absence of *T. microphasmis*. During the first 7-week period, nodulated seedlings were more successful in acquiring nitrogen than non-nodulated seedlings (Table 3). The supply of mineral N to the latter was not quite sufficient. Comparison of the total amount of N acquired by nodulated and

non-nodulated plants without *T. microphasmis* gives an estimate of 167-194 nmol N per shoot obtained from N₂-fixation during the first 7 weeks. This amounts to about 40% of the total N acquired per shoot, which is fairly high for this nodule weight ratio when compared to estimates of Troelstra *et al.* (1987).

The formation of *Frankia* nodules was strongly inhibited by *T. microphasmis*. This is not a mere result of the reduction in root length, because also the number of nodules formed per unit root length was lower. At the low inoculum level there also seems some delay in nodulation. It is not clear whether these effects are direct and local, or due to nutritional or other systemic physiological changes in the plant which are caused by the nematodes. Lower root hair numbers and condition, as found in the *in vitro* study, are not likely to be a direct constraint to *Frankia* infection, because infection in Eleagnaceae can occur by intercellular epidermal penetration (Miller & Baker, 1985), but it may hinder the uptake of nutrients. A low P concentration inhibits nodule formation in actinorhizal plants (Reddell *et al.*, 1988), possibly by causing a decrease in photosynthesis, the energy surplus of which is essential for the establishment of root symbionts (e.g. Baas, 1989).

At 7 weeks, the nitrogenase activity was too low to be reliably measured. At 13 weeks, however, it appeared to be impeded in the nematode treatments. This may be related with the foregoing reduced internal P concentration in these objects. The specific activity per unit nodule weight was not significantly influenced by *T. microphasmis*, but variation in the results was fairly large. P deficiency in Leguminosae affects the amount and weight rather than the specific activity of nodules (O'Hara *et al.*, 1988). On the other hand, Rose & Youngberg (1981) found a decrease of nitrogenase activity per unit nodule weight in the absence of VA-mycorrhiza in actinorhizal *Ceanothus velutinus*. In general, the level of N within a nitrogen-fixing plant is biologically adjusted to the level of other limiting nutrients. The present results confirm that effects on N-fixation become important only when there is no P deficiency (Reddell *et al.*, 1988).

Population dynamics of *T. microphasmis*.

The multiplication rate of *T. microphasmis* was higher in the first period of the experiment than in the second period, and higher at the low inoculation level than at the high inoculation level. Both differences can be explained by changes in the relative growth rate of the host plant and its roots. The populations at the low inoculum level kept up with root growth between weeks 7 and 13, whereas those at the high level did not. It seems that the number of *T. microphasmis* per cm root

reached an equilibrium at the low inoculum level. Between the two harvests, the root length and the nematode population increased at the same rate. Conversely, the density of *T. microphasmis* per cm root at the high inoculum level was significantly higher than at the low inoculum level after 7 weeks, and even showed an increase towards the second harvest. This overpopulation explains the severe damage inflicted to the roots and the low multiplication rate at the high inoculum level. Obviously, the nematodes survive longer than the damaged rootlets.

Ecological implications

To evaluate the role of *T. microphasmis* in the degeneration of *H. rhamnoides* vegetation, the results should be translated to the field situation, i.e. nutrient deficient calcareous dune sands. Nitrogen and phosphorus are the major factors limiting plant growth in European sand dune soils (Willis & Yemm, 1961; Willis, 1963). P limitation may indeed occur in the degeneration stage of *H. rhamnoides* vegetation, because P concentrations in *H. rhamnoides* twigs were found to be lower in this stage than in earlier stages (Troelstra *et al.*, 1987), and infection of roots by vesicular-arbuscular mycorrhizal fungi was enhanced (Zoon *et al.*, 1993). The rhizosphere of *H. rhamnoides* seedlings planted at degeneration sites had higher concentrations of *T. microphasmis* compared to those planted amidst colonizing or optimal *H. rhamnoides* (Zoon *et al.*, 1993). It was suggested that the relative N surplus at the degeneration sites provides a better food source for the nematodes. The indication that more damage occurs in the later successional stages, where P limitation is pronounced, implies that *T. microphasmis* may accelerate succession in *Hippophaë* vegetation in calcareous sand dunes by aggravating P deficiency. Certain other plant parasitic nematodes may have similar effects. Further studies should consider the effect of plant parasitic nematodes on *H. rhamnoides* under different P fertilizer and VA-mycorrhiza regimes, as well as effects on the competition with associated and succeeding plant species in the vegetation.

REFERENCES

- Akkermans A.D.L. 1971. Nitrogen Fixation and Nodulation of *Alnus* and *Hippophaë* under natural Conditions. PhD Thesis, University of Leiden, NL, 85 pp.
- Allen M.B. and Arnon D.I. 1955. Studies on nitrogen-fixing blue green algae. I. Growth and nitrogen fixation of *Anabaena cylindrica* Lemm. Plant Physiol. Lancaster 30: 366.

- Baas R. 1989. Phosphorus nutrition and vesicular-arbuscular mycorrhizal infection in *Plantago major*. Ph.D. Thesis, Rijksuniversiteit Utrecht NL. 112 pp.
- Barber S.A. 1984. Soil Nutrient Bioavailability: a Mechanistic Approach. New York, John Wiley, 398 pp.
- Bridge J. and Hague N.G.M. 1974. The feeding behaviour of *Tylenchorhynchus* and *Merlinius* species and their effect on growth of perennial ryegrass. *Nematologica* 20: 119-130.
- Brown V.K. and Gange A.C. 1991. Effects of root herbivory on vegetation dynamics. In: Atkinson D (ed.) *Plant Root Growth: An Ecological Perspective*. Blackwell Sci. Publ.: 453-470.
- Burggraaf A.J.P. 1984. Isolation, Cultivation and Characterisation of *Frankia* Strains from Actinorhizal Root Nodules. Ph.D. Thesis, Rijksuniversiteit Leiden, NL, 179 pp.
- Crawley M J. 1993. Succeeding in the sand dunes. *Nature* 362: 17-18.
- Dijkshoorn W. and Lampe J.E.M. 1980. A plant test for sufficiency of phosphorus based on the phosphorus-nitrogen interaction in sunflower nutrition. *Neth. J. agric. Sci.* 28: 135-146.
- Klinkenberg C.H. 1963. Observations on the feeding habits of *Rotylenchus uniformis*, *Pratylenchus crenatus*, *P. penetrans*, *Tylenchorhynchus dubius* and *Hemicycliophora similis*. *Nematologica* 9: 502-506.
- Korteweg C.R. and 's Jacob J.J. 1980. Influence of *Heterodera trifolii* and nitrogen on white clover in a clover-grass mixture. *Meded. Fac. Landbouww. Rijksuniv. Gent* 45: 761-767.
- Maas P.W.Th., Oremus P.A.I. and Otten H. 1983. Nematodes (*Longidorus* n.sp. and *Tylenchorhynchus microphasmis* (Loof) in growth and nodulation of sea buckthorn, (*Hippophaë rhamnoides* L.). *Plant and Soil* 73: 141-147.
- Miller I.M. and Baker D.D. 1985. The initiation, development and structure of root nodules in *Eleagnus angustifolia* L. (Eleagnaceae). *Protoplasma* 128: 107-119.
- Noel J. 1964. A staining and mounting combination for sections of plant tissues. *Stain Technol.* 39: 324-325.
- O'Hara G.W., Boonkerd N. and Dilworth M.J. 1988. Mineral constraints to nitrogen fixation. *Plant and Soil* 108: 93-110.
- Oostenbrink M. 1960. Estimating nematode populations by some selected methods. In: J.N. Sasser and W.R. Jenkins, *Nematology*. Univ. N. Carolina Press, Chapel Hill: 85-102.
- Oremus P.A.I. 1980. Occurrence and infective potential of the endophyte of *Hippophaë rhamnoides* L. ssp. *rhamnoides* in coastal sand dune areas. *Plant and Soil* 56: 123-139.
- Oremus P.A.I. 1982. Growth and Nodulation of *Hippophaë rhamnoides* L. in the Coastal Sand Dunes of the Netherlands. Thesis, Rijksuniversiteit Utrecht. 120 pp.
- Oremus P.A.I. and Otten H. 1981. Factors affecting growth and nodulation of *Hippophaë rhamnoides* L. ssp. *rhamnoides* in soils from two successional stages of dune formation. *Plant and Soil* 63: 317-331.

- Overall J.E. and Klett C.J. 1972. Applied Multivariate Analysis. McGraw-Hill, New York, 500 pp.
- Pearson M.C. and Rogers J.A. 1967. Flora of the British isles. *Hippophaë rhamnoides* L. J. Ecol. 50: 501-513.
- Penning de Vries F.W.T. 1982. Crop production in relation to availability of nitrogen. In: F.W.T. Penning de Vries and H.H. Van Laar (Eds.) Simulation of Plant Growth and Crop Production. Wageningen, Pudoc: 213-221.
- Reddell P., Rosbrook P.A., Bowen G.D. and Gwaze D. 1988. Growth responses in *Casuarina cunninghamiana* plantings to inoculation with *Frankia*. Plant and Soil 108: 79-86.
- Rose S.L. and Youngberg C.T. 1981. Tripartite associations in snowbrush (*Ceanothus velutinus*): effect of vesicular-arbuscular mycorrhizae on growth, nodulation, and nitrogen fixation. Can. J. Bot. 59: 34-39.
- Sharma R.D. 1971. Studies on the plant parasitic nematode *Tylenchorhynchus dubius*. Meded. Landb.Hogesch. Wageningen 71-1: 1-154.
- Troelstra S.R. 1983. Growth of *Plantago lanceolata* and *Plantago major* on a $\text{NO}_3^-/\text{NH}_4^+$ medium and the estimation of the utilization of nitrate and ammonium from ionic-balance aspects. Plant and Soil 70: 183-197.
- Troelstra S.R., Blacquièrè T., Wagenaar R. and Van Dijk C. 1987. Ionic balance, proton efflux, nitrate reductase activity and growth of *Hippophaë rhamnoides* L. ssp. *rhamnoides* as influenced by combined-N nutrition or N_2 fixation. Plant and Soil 103: 169-183.
- Van der Maarel E., Boot R., Van Dorp D. and Rijntjes J. 1985. Vegetation succession on the dunes near Oostvoorne, The Netherlands; a comparison of the vegetation in 1959 and 1980. Vegetatio 58: 137-187.
- Van der Putten W.H., Van Dijk C. and Troelstra S.R. 1988. Biotic soil factors affecting the growth and development of *Ammophila arenaria*. Oecologia 76: 313-320.
- Van der Putten W.H., Van Dijk C. and Peters B.A.M., 1993. Plant-specific soil-borne diseases contribute to succession in foredune vegetation. Nature 362: 53-55.
- Weste G. 1981. Changes in the vegetation of sclerophyl shrubby woodland associated with invasion by *Phytophthora cinnamomi*. Austr. J. Bot. 29: 261-276
- Williams R.F. 1948. The effects of phosphorus supply on the rates of intake of phosphorus and nitrogen and upon certain aspects of phosphorus metabolism in gramineous plants. Austr. J. sci. Res. ser.B. 1: 333-359.
- Willis A.J. 1963. Braunton Burrows: the effect on the vegetation of the addition of mineral nutrients to the dune soils. J. Ecol. 51: 353-375.
- Willis A.J. and Yemm E.W. 1961. Braunton Burrows: mineral nutrient status of the dune soils. J. Ecol. 49: 377-390.
- Wyss U. 1973. Feeding of *Tylenchorhynchus dubius*. Nematologica 19: 125-136.
- Zoon F.C. 1991. Effects of rhizosphere fungi and nematodes on the actinorhiza and growth of *Hippophaë rhamnoides* L. In: A.B.R. Beemster et al. (eds.) Biotic

- Interactions and Soil-borne Diseases. Elsevier, Amsterdam: 47-52.
- Zoon F.C., Hage F. and De Zwart A. 1990. A device for automatic soil moisture control and registration of water use in pot experiments. *Plant and Soil* 125: 281-284.
- Zoon F.C. and Van Tienderen P.H. 1990. A rapid quantitative measurement of root length and branching by microcomputer image analysis. *Plant and Soil* 126: 301-308.
- Zoon F.C., Troelstra S.R. and Maas P.W.Th. 1993. The ecology of the plant-feeding nematode fauna associated with Sea Buckthorn (*Hippophaë rhamnoides* L.) in different stages of dune succession. *Fundam. appl. Nematol.* 16: 247-258.

CHAPTER 8

Interactions between the plant parasitic nematode *Tylenchorhynchus microphasmis* Loof, the root fungus *Cylindrocarpon destructans* Zinns. Scholten and *Frankia actinorhiza*, affecting nutrient acquisition and growth of sea buckthorn (*Hippophaë rhamnoides* L. ssp. *rhamnoides*).

F.C. Zoon, S.R. Troelstra & A.D.L. Akkermans

submitted to Appl. Soil Ecol.

SUMMARY

The plant parasitic nematode *Tylenchorhynchus microphasmis* and the root fungus *Cylindrocarpon destructans* are associated with sea buckthorn, *Hippophaë rhamnoides* L. ssp. *rhamnoides*, in calcareous coastal sand dune areas in the Netherlands. In a pot experiment, seedlings of *H. rhamnoides* were inoculated with the nematode, either alone or in combination with the fungus, to study their effects on plant growth and nodulation. All nematode and fungus treatments were done with *Frankia*-nodulated plants as well as non-nodulated plants to which an extra amount of mineral-N was added. Growth, shoot phosphorus concentration and the number of nodules per unit length of roots were significantly reduced by *T. microphasmis*. Growth reduction appears to be caused by decreased root growth and consequently lower uptake of less available nutrients, such as phosphorus.

C. destructans stimulated growth of *H. rhamnoides* during the first 6 weeks, probably due to increased N-mineralisation, but in the long run this fungus was harmful, and more so in the absence of *Frankia*. Multiplication of *T. microphasmis* was stimulated by *C. destructans*, but no interactions between the two organisms were found in their effect on growth or nodulation of *H. rhamnoides*.

In the pot experiment and in the field, reduced growth was correlated with a reduced concentration of phosphorus in shoot and leaves, respectively. *T. microphasmis*, and to a lesser extent also *C. destructans* are likely to decrease the vigour and competitive ability of *H. rhamnoides* in the field and may thus contribute to the succession of *Hippophaë* vegetation.

INTRODUCTION

Soil-borne diseases and pests may contribute to succession in dune vegetation (Oremus, 1982; Van der Putten *et al.*, 1993). However, the mechanisms by which soil organisms affect growth of plant species within the vegetation are largely unknown, especially when beneficial soil organisms play a role as well, as in the case of the non-leguminous nitrogen-fixing shrub *Hippophaë rhamnoides* L. ssp. *rhamnoides*. *H. rhamnoides* is a pioneer in the early primary succession of the vegetation of calcareous coastal sand dunes in Western Europe and it is able to accumulate nitrogen in symbiosis with the nitrogen-fixing actinomycete *Frankia*. Therefore, it has special importance for the N-enrichment of nutrient poor dune soils. A few decades after colonization, *Hippophaë* scrub tends to degenerate gradually, eventually leading to mixed scrub or grass vegetation (Van der Maarel *et al.*, 1985).

Biotic soil factors play a role in the degeneration of *H. rhamnoides* at certain locations (Oremus & Otten, 1981). The occurrence of plant parasitic nematode species in different successional stages of vegetation with *H. rhamnoides* was surveyed in dune areas along the Dutch coast (Zoon *et al.*, 1993; Chapter 2). *Tylenchorhynchus microphasmis* Loof and *Criconemella* (syn. *Mesocriconema*) *xenoplax* Raski were the most abundant and frequently isolated plant parasitic species under *Hippophaë*, whereas *Longidorus dunensis* Brinkman *et al.* was found only in older calcareous dunes. Maas *et al.* (1983) studied the effect of mixed inoculum of *L. dunensis* and *T. microphasmis* on growth of *H. rhamnoides* and demonstrated that nematodes form an important part of the disease complex. They also found indications that microbial factors act synergically with these nematodes in causing damage to the plant (Maas *et al.*, 1983). The fungus *Cylindrocarpon destructans* Zinns. Scholten is a dominant root fungus of *H. rhamnoides*. It is especially abundant on the rhizoplane, but only slightly pathogenic when inoculated alone (Zoon, 1991; Chapter 3).

To assess the importance of soil organisms in vegetational succession, more information is needed about the pathogenicity towards *H. rhamnoides* of organisms found in the field, about their interactions and about the underlying mechanisms. Therefore, the present study was set up to investigate the influence of *T. microphasmis* and *C. destructans*, and possible interactions between the two, on growth, nodule formation and nutrient acquisition of *H. rhamnoides* under a natural (*i.e.* fairly dry) soil moisture regime. Experiments were conducted with or without

the nitrogen-fixing root symbiont *Frankia* in order to investigate whether the added organisms act directly on the plant, or indirectly via influences on the actinorhiza.

MATERIALS AND METHODS

Calcareous dune sand was sterilized by 2.5 Mrad gamma irradiation and treated with *Cylindrocarpon destructans* Zinns. Scholten by mixing a composite of three isolates obtained from field soil (Zoon, 1991) through the soil as a homogenized sand-oats culture suspension. Control objects received an equivalent amount of sterile sand-oats medium. *Frankia* inoculum, consisting of a suspension of homogenized surface-sterilized nodules (collected in the seaward dunes of Oostvoorne), was mixed through the soil of the relevant objects prior to planting.

Ten-week-old hydroponically grown *Hippophaë* seedlings (Oremus, 1980) were classified according to fresh weight and planted in plastic pots containing 500 g dry weight of sand. Six replicates were treated as blocks representing seedling weight classes. *Tylenchorhynchus microphasmis* Loof was inoculated one day after planting in four holes around the seedling at zero, 460 and 920 individuals per pot in 20 ml water. The pots were covered with aluminum foil to minimize evaporation and kept in a greenhouse at 22-25 °C with a day length of 16 h. A light quantum flux between 150 and 350 $\mu\text{E/s/m}^2$ (PAR) was maintained during the day using automatic supplemental lighting (Philips SON-T 200 $\mu\text{E/s/m}^2$) and shutter screens. The soil moisture was kept at 5 (± 1) % of dry weight (-3.9 to -6.3 Kpa soil water potential; Pf 1.6-1.8) by automatic regulation (Zoon *et al.*, 1990). The amount of water lost by evapotranspiration was recorded and replenished automatically using a diluted Hoagland nutrient solution (Ph 5.6) containing 6.5 meq/l NO_3^- with Fe-citrate, and trace-elements according to Allen & Arnon (1955). In the *Frankia* treatment, the nitrogen in the Hoagland solution was replaced after two weeks by an equivalent amount of Cl. In the treatment without *Frankia* the regular solution was used during the first four weeks. A two-fold higher N-concentration (9.75 meq/l NO_3^- plus 3.25 meq/l NH_4^+) was applied from the fifth week onwards, because of symptoms of N-deficiency in the non-nodulated plants.

After 6 and 12 weeks plants were harvested and analyzed, and nematodes were extracted using an elutriation/sieving/cotton-wool filter method (Oostenbrink, 1960) and counted. At the first harvest, fungi were re-isolated from the rhizoplane by ultrasonic treatment of washed root pieces together with sterile glass beads in

Table 1. Effects of treatments *T. microphasma* (zero, 460 or 920 per pot), *C. destructans* (C) and *Frankia* (F) on plant growth, nodulation and nematode populations after 6 weeks. Means in each column followed by the same letter are not significantly different according to Tukey's HSD test ($p < 0.05$). Significance symbols in the ANOVA table are: ns=not significant, *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$. Positive correlation with initial seedling weight (Block) is indicated with +. Interactions without any significant effect are omitted.

| Treatment | shoot dry wt. g | root dry wt. g | shoot/ root ratio | fine root length m | specific root length m g ⁻¹ | - Frankia nodules - | | - T. microphasma - | |
|-------------|-----------------------|----------------------|-------------------------|--------------------------|--|---------------------|------------------|--------------------|-------------------|
| | | | | | | n. per plant | n. per m root | n.x1000 per pot | n. per cm root |
| .T 0 | .54 ab | .23 ab | 2.6 abc | 10.0 ab | 56 ns | 0 | 0.0 | 0.0 | 0.0 |
| .T 460 | .67 ab | .27 ab | 2.4 abc | 12.1 ab | 66 | 0 | 0.0 | 3.0 a | 2.9 a |
| .T 920 | .49 a | .18 a | 2.9 abc | 7.5 b | 63 | 0 | 0.0 | 3.2 ab | 5.3 ab |
| .C T 0 | .69 ab | .29 ab | 2.4 bc | 15.5 a | 70 | 0 | 0.0 | 0.0 | 0.0 |
| .C T 460 | .78 ab | .28 ab | 2.8 abc | 14.5 ab | 64 | 0 | 0.0 | 5.4 abc | 3.9 abc |
| .C T 920 | .59 ab | .19 a | 3.0 abc | 9.1 ab | 61 | 0 | 0.0 | 8.3 c | 10.6 c |
| F. T 0 | .79 ab | .38 b | 2.1 c | 18.4 a | 58 | 84 ns | 4.2 ns | 0.0 | 0.0 |
| F. T 460 | .77 ab | .27 ab | 3.0 abc | 13.0 ab | 60 | 78 | 5.1 | 5.8 bc | 5.8 bc |
| F. T 920 | .72 ab | .23 ab | 3.4 a | 9.9 ab | 58 | 56 | 4.6 | 8.8 c | 10.8 c |
| FCT 0 | .75 ab | .34 ab | 2.1 c | 19.4 a | 69 | 127 | 5.9 | 0.0 | 0.0 |
| FCT 460 | .85 b | .29 ab | 3.0 abc | 15.2 ab | 66 | 82 | 5.3 | 4.9 abc | 4.3 abc |
| FCT 920 | .68 ab | .22 ab | 3.1 ab | 10.2 ab | 58 | 58 | 5.9 | 7.1 c | 7.0 c |
| ANOVA table | ¹⁾ | ²⁾ | | ¹⁾ | | ^{1,2)} | ³⁾ | ^{1,2)} | ^{1,3)} |
| Frankia (F) | ** | * | ns | * | ns | - | - | ** | * |
| Cyl. (C) | * | ns | ns | *** | * | ns | * | ** | ns |
| Tyl. (T) | * | *** | *** | *** | ns | ns | ns | *** | *** |
| Block (B) | ***+ | ***+ | ** | ***+ | ***+ | ***+ | ns | ** | *** |
| FxC | ns | ns | ns | ns | ns | - | - | *** | *** |
| FxT | ns | ns | * | ns | ns | - | - | ns | ns |
| FxB | ns | ns | * | ns | ns | - | - | ns | ns |

¹⁾ =ln(x) transformed ; ²⁾ =sqrt(x) transformed ; ³⁾ =non-inoculated objects not included in ANOVA

Table 2. Effects of treatments *T. microphasma* (zero, 460 or 920 per pot), *C. destructans* (C) and *Frankia* (F) on plant growth, nodulation and nematode populations after 12 weeks. Means in each column followed by the same letter or none are not significantly different according to Tukey's HSD test ($p < 0.05$). Significance symbols in the ANOVA table are: ns=not significant, *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$. Positive correlation with initial seedling weight (Block) is indicated with +.

| Treatment | shoot | | root dry wt. g | shoot/root ratio | fine root length m | specific root length m.g-1 | Frankia nodules | | | <i>T. microphasma</i> | |
|-------------|---------------|---------------|----------------|------------------|--------------------|----------------------------|-----------------|------------------|-----------------|-----------------------|-----------------|
| | dry wt. g | root wt. g | | | | | n. per plant | dry wt. mg/plant | n. per m root | n.x 1000 per pot | n. per cm root |
| T 0 | 2.52 a | .82 a | 3.0 ab | 49.8 a | 82 abcd | 0 | 0 | 0.0 | 0.0 | 0.0 | 0.0 |
| T460 | 2.43 a | .80 a | 3.0 ab | 45.1 ab | 76 bcd | 0 | 0 | 0.0 | 12.5 | 3.0 | 3.0 |
| T920 | 2.10 ab | .78 a | 2.7 ab | 43.2 abcd | 72 cde | 0 | 0 | 0.0 | 12.2 | 2.9 | 2.9 |
| C T 0 | 2.05 ab | .73 a | 2.8 ab | 34.9 abcde | 67 de | 0 | 0 | 0.0 | 0.0 | 0.0 | 0.0 |
| C T460 | 1.60 b | .64 ab | 2.7 ab | 30.1 bcde | 65 de | 0 | 0 | 0.0 | 14.9 | 8.1 | 8.1 |
| C T920 | 1.54 b | .62 ab | 2.5 a | 26.8 e | 56 e | 0 | 0 | 0.0 | 13.6 | 7.6 | 7.6 |
| F T 0 | 2.38 a | .68 ab | 3.5 b | 45.0 abc | 93 ab | 86 a | 99 a | 2.0 ab | 0.0 | 0.0 | 0.0 |
| F T460 | 2.00 ab | .61 ab | 3.3 ab | 41.2 abcde | 88 abc | 57 ab | 74 ab | 1.4 ab | 12.9 | 3.3 | 3.3 |
| F T920 | 1.54 b | .56 ab | 2.8 ab | 27.7 cde | 71 cde | 29 b | 40 b | 1.1 b | 14.5 | 5.7 | 5.7 |
| FCT 0 | 2.09 ab | .59 ab | 3.5 b | 40.5 abcde | 97 a | 98 a | 89 a | 2.4 a | 0.0 | 0.0 | 0.0 |
| FCT460 | 1.82 ab | .61 ab | 3.0 ab | 34.4 abcde | 76 bcd | 76 ab | 78 ab | 2.1 ab | 12.3 | 4.6 | 4.6 |
| FCT920 | 1.47 b | .49 b | 3.0 ab | 27.3 de | 72 cde | 49 ab | 60 ab | 1.8 ab | 21.5 | 8.5 | 8.5 |
| ANOVA table | ¹⁾ | ²⁾ | | | | ^{1,2)} | ³⁾ | ³⁾ | ^{1,2)} | ^{1,3)} | ^{1,3)} |
| Frankia (F) | ns | *** | ns | ns | *** | - | - | - | ns | ns | ns |
| Cyl. (C) | *** | ** | ns | *** | *** | ns | ns | ** | ns | ns | * |
| Tyl. (T) | *** | * | *** | *** | *** | *** | *** | * | ns | ns | ns |
| Block (B) | ***+ | ***+ | ** | ***+ | ***+ | *** | ** | ** | ns | ns | ns |
| FxC | * | ns | ns | ** | ** | - | - | - | ns | ns | * |
| FxT | ns | ns | * | ns | * | - | - | - | ns | ns | ns |
| FxB | ns | ns | * | ns | ns | - | - | - | ns | ns | ns |

¹⁾ = ln(x) transformed ; ²⁾ = sqrt(x) transformed ; ³⁾ = non-inoculated objects not included in ANOVA

sterile water for 1 min, followed by plating out dilutions of the suspensions on malt agar. Similarly, dilutions of soil suspensions were plated out. Fungal colonies were counted after 4 days of incubation at 20°C. Root length and branching were estimated using image analysis (Zoon & Van Tienderen, 1990). Root nodules larger than about 2 mm were counted by eye. Weights of shoot, roots and nodules were determined after drying at 70° C for 48 h. Specific root length (SRL) was calculated as meters per gram dry weight of fine roots. Concentrations of total N, P, K, Ca, Mg and Fe in dried shoot material were determined as described by Troelstra (1983). The data were analyzed by three-way ANOVA of a completely randomized block design. The block numbers reflected the weight classes of the seedlings. Relations between biomass and nutrient concentrations were studied by simple correlation and multiple regression.

Field data on nutrient concentrations and biomass of current years twigs of *H. rhamnoides* were obtained at two dune locations in The Netherlands (*viz.* Voorne and Schouwen). At both locations two vigorous and two degenerating sites were sampled in July 1988. At each site, five twigs were taken from each of five shrubs. Bulk samples per site were analyzed as described above. Data were analyzed by two-way ANOVA for location and successional stage. Simple correlations between biomass and nutrient concentrations were calculated.

RESULTS

Plant growth and nodulation

During the first 6 weeks of the experiment both shoot and root growth were better in the *Frankia* treatment than in the non-nodulated treatment (Table 1). This was probably due to an insufficient nitrogen supply in the latter during the first 4 weeks, as could be judged from the slight chlorosis of the oldest leaves. The N-concentration of the nutrient solution in the treatment without *Frankia* was increased from week 5 onwards. The *Frankia* treatment also increased root length, but not by increasing the specific root length (Table 1). Growth and the number of nodules formed per plant were correlated to the seedling size at the start of the experiment (block effects with positive trend; Table 1).

After the second 6-week period, the *Frankia* treatment showed no significant effect on shoot weight any more, and root weight became even less compared to the non-nodulated control (Table 2). The initially lower shoot/root ratio in the *Frankia*

treatment without nematodes turned into a slightly higher one (interaction FxT; compare Tables 1 and 2). The specific root length in the *Frankia* treatment became significantly higher than in the non-nodulated treatment and thus the reduction in root weight did not affect root length (Table 2). The number of root nodules per plant remained constant after 6 weeks, but decreased on a per meter basis due to the extension of the root system. Newly-formed roots had very few nodules (Tables 1 and 2). Individual nodules were larger when fewer nodules were present, both in absolute sense as well as per unit root length (Table 2).

C. destructans initially stimulated shoot and root growth, especially in the treatment without *Frankia* (up to 28% in weight and 55% in root length; Table 1). During the second phase of the experiment, *C. destructans* impeded growth, and weight of shoots and roots fell 11-13% and 12-19% behind, respectively, compared to the treatments without the fungus (Table 2). The leading position in root length of the fungus treatment after the first period was reversed into a drawback of 10-30% at the end of the experiment. The fungus was especially harmful in the absence of *Frankia*. *C. destructans* slightly increased the number of actinorhizal nodules at both harvests, but this became only significant on a per meter basis at the end of the experiment. Isolation of fungi from roots and soil after 6 weeks revealed that in the fungus treatments *C. destructans* was abundant on the rhizoplane and in the soil (data not shown). In addition, a *Penicillium* sp. was isolated from soil in all treatments.

T. microphasmis reduced shoot growth at the high inoculum level, but stimulated it at the low level during the first 6 weeks (Table 1). In most cases, root weight (including hypocotyl) and root length were also reduced. Six weeks after inoculation the shoot/root ratio was increased, especially in the *Frankia* treatment (Table 1), but at the end of the experiment it was decreased (Table 2). After the first 6 weeks, the number of nodules per unit root length was not significantly affected by *T. microphasmis* (Table 1). At the end, however, nodulation became clearly negatively influenced in all respects with increasing numbers of nematodes inoculated (Table 2). No interactions between *T. microphasmis* and *C. destructans* on growth or nodulation of *Hippophaë* were found.

Acquisition of nutrients and water

After 6 weeks, shoot concentrations of N, P, Ca, and Mg were higher in the *Frankia*-inoculated plants than in the control plants (Table 3). For N and P, this effect was not found at the high nematode inoculum. At the second harvest, the

Table 3. Effects of treatments *T. microphasmis* (zero, 460 or 920 per pot), *C. destructans* (C) and *Frankia* (F) on nutrient concentrations (mmol/kg dry weight; Fe in mg/kg dry weight) in shoots after 6 weeks. Means in each column followed by the same letter are not significantly different according to Tukey's HSD test ($p < 0.05$). Significance symbols in the ANOVA table are: ns=not significant, *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$. Blocks represent classes of initial seedling weight.

| Treatment | N | P | K | Ca | Mg | Fe |
|--------------------|----------|-------|--------|----------|--------|--------|
| . . T 0 | 1386 ab | 67 ab | 484 ab | 119 a | 87 abc | 133 a |
| . . T460 | 1403 ab | 57 bc | 510 ab | 135 ab | 89 abc | 85 ab |
| . . T920 | 1475 abc | 48 bc | 543 b | 171 bcd | 87 abc | 109 ab |
| . C T 0 | 1427 abc | 50 bc | 439 a | 134 ab | 92 abc | 94 ab |
| . C T460 | 1321 a | 45 c | 478 ab | 137 ab | 76 c | 67 ab |
| . C T920 | 1372 a | 45 c | 497 ab | 157 abcd | 78 bc | 79 ab |
| F. T 0 | 1972 d | 85 a | 478 ab | 142 abc | 100 a | 121 a |
| F. T460 | 1708 bcd | 61 bc | 505 ab | 189 cd | 98 ab | 129 a |
| F. T920 | 1545 abc | 51 bc | 482 ab | 207 d | 92 abc | 96 ab |
| FCT 0 | 1735 cd | 83 a | 460 ab | 146 abc | 99 a | 45 b |
| FCT460 | 1575 abc | 61 bc | 484 ab | 183 bcd | 95 abc | 77 ab |
| FCT920 | 1370 a | 46 c | 498 ab | 206 d | 87 abc | 84 ab |
| ANOVA Table | | | | | | |
| Frankia (F) | *** | *** | ns | *** | *** | ns |
| Cyl. (C) | *** | * | * | ns | ns | ** |
| Tyl. (T) | *** | *** | * | *** | * | ns |
| Block (B) | *** | ** | ns | ns | ns | ns |
| FxC | * | ns | ns | ns | ns | ns |
| FxT | *** | ** | ns | ns | ns | ns |
| FxB | * | ns | ns | ns | ns | ns |
| FxCxB | * | ns | ns | ns | ns | ns |
| FxTxB | * | ns | ns | ns | ns | ns |
| CxTxB | * | ns | ns | ns | ns | ns |

level of N in the *Frankia* treatment had not changed, whereas in the plants relying solely on mineral-N the concentration was increased (compare Tables 3 and 4). The effect of *Frankia* on the shoot concentration of Ca was reversed from positive to negative between week 6 and 12 (compare Tables 3 and 4), and the same was found for Mg in the absence of nematodes (interaction FxT; Table 4). The initial seedling weight (expressed in block numbers) did not influence nutrient concentrations after 12 weeks.

C. destructans reduced shoot N concentrations in nodulated plants after 6 weeks, but less so in the non-nodulated objects (interaction FxC; Table 3). After 12 weeks,

Table 4. Effects of treatments *T. microphasmis* (zero, 460 or 920 per pot), *C. destructans* (C) and *Frankia* (F) on nutrient concentrations (mmol/kg dry weight; Fe in mg/kg dry weight) in the shoots after 12 weeks. Means in each column followed by the same letter are not significantly different according to Tukey's HSD test ($p < 0.05$). Significance symbols in the ANOVA table are: ns=not significant, *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$. Blocks coincide with classes of initial seedling weight.

| Treatment | N | P | K | Ca | Mg | Fe |
|-----------|----------|--------|----------|---------|-------|----------|
| . . T 0 | 2260 a | 76-ab | 639 cd | 155 cd | 102 a | 129 ab |
| . . T460 | 2045 abc | 56 cd | 716 bcd | 194 abc | 87 ab | 64 bcd |
| . . T920 | 2127 a | 62 bcd | 753 abcd | 241 a | 101 a | 127 abc |
| . C T 0 | 2099 ab | 66 abc | 593 d | 160 cd | 94 ab | 61 cd |
| . C T460 | 1983 abc | 49 d | 754 abcd | 247 a | 87 ab | 86 abcd |
| . C T920 | 1821 bcd | 46 d | 712 bcd | 236 a | 84 ab | 57 d |
| F. T 0 | 1984 abc | 78 a | 693 bcd | 128 d | 77 b | 104 abcd |
| F. T460 | 1757 cd | 66 abc | 824 ab | 170 bcd | 92 ab | 175 a |
| F. T920 | 1561 d | 53 cd | 912 a | 212 abc | 92 ab | 112 abcd |
| FCT 0 | 2096 ab | 79 a | 816 abc | 170 bcd | 85 ab | 105 abcd |
| FCT460 | 1800 bcd | 62 bcd | 841 ab | 200 abc | 87 ab | 100 abcd |
| FCT920 | 1658 d | 58 cd | 930 a | 224 ab | 102 a | 107 abcd |

ANOVA Table

| | | | | | | |
|-------------|-----|-----|-----|-----|-----|-----|
| Frankia (F) | *** | *** | *** | ** | ns | *** |
| Cyl. (C) | ns | ** | ns | ** | ns | ** |
| Tyl. (T) | *** | *** | ** | *** | ns | ns |
| Block (B) | ns | ns | ns | ns | ns | ns |
| FxC | *** | ** | ns | ns | ** | ns |
| FxT | * | ns | ns | ns | *** | ns |
| FxB | ns | ns | ns | ns | ns | ns |
| FxCxT | ns | ns | ns | ns | * | ** |

(other ns)

the effect had changed; a decrease in N concentration was only found in non-nodulated plants (interaction FxC; Table 4). *C. destructans* also reduced concentrations of P (especially in non-nodulated plants) and Fe at both harvests. It slightly reduced potassium concentrations after 6 weeks (Table 3) and increased Ca after 12 weeks (Table 4). At the end of the experiment, *C. destructans* increased the Mg concentration in the nodulated plants, but reduced it in the non-nodulated plants (interaction FxC; Table 4).

After 6 weeks, *T. microphasmis* significantly reduced shoot concentrations of N and P (less so in non-nodulated plants, where concentrations without nematodes were

Table 5. Simple linear correlations between shoot nutrient concentrations and growth parameters of nodulated and non-nodulated plants after 6 and 12 weeks. SDW= shoot dry weight, RDW= root dry weight, RL= root length, TDW= total dry weight. Correlation coefficients are shown for N=36. Critical values for $p<0.05$, $p<0.01$, and $p<0.001$ are (\pm) 0.32, 0.42 and 0.55, respectively.

| 6wks -F | SDW | RDW | RL | TDW |
|-----------------|------------|------------|-----------|------------|
| N | -0.35 * | -0.27 | -0.25 | -0.34 * |
| P | 0.15 | 0.26 | 0.28 | 0.18 |
| K | -0.31 | -0.30 | -0.39 * | -0.32 * |
| Ca | -0.57 *** | -0.61 *** | -0.57 *** | -0.60 *** |
| Mg | 0.04 | 0.23 | 0.15 | 0.10 |
| Fe | -0.28 | -0.09 | -0.18 | -0.23 |
| 6wks +F | SDW | RDW | RL | TDW |
| N | 0.48 ** | 0.65 *** | 0.65 *** | 0.54 ** |
| P | 0.41 * | 0.63 *** | 0.67 *** | 0.49 ** |
| K | 0.54 ** | 0.47 ** | 0.41 * | 0.53 ** |
| Ca | -0.25 | -0.40 * | -0.36 * | -0.30 |
| Mg | 0.17 | 0.30 | 0.38 * | 0.22 |
| Fe | -0.31 | -0.30 | -0.30 | -0.31 |
| 12wks -F | SDW | RDW | RL | TDW |
| N | 0.45 ** | 0.38 * | 0.55 *** | 0.44 ** |
| P | 0.53 ** | 0.56 *** | 0.67 *** | 0.55 *** |
| K | -0.41 * | -0.30 | -0.27 | -0.39 * |
| Ca | -0.61 *** | -0.42 ** | -0.39 * | -0.58 *** |
| Mg | 0.22 | 0.44 ** | 0.49 ** | 0.28 |
| Fe | 0.18 | 0.20 | 0.31 | 0.19 |
| 12wks +F | SDW | RDW | RL | TDW |
| N | 0.36 * | 0.13 | 0.27 | 0.32 * |
| P | 0.35 * | 0.17 | 0.37 * | 0.32 * |
| K | -0.23 | -0.10 | -0.26 | -0.20 |
| Ca | -0.65 *** | -0.46 ** | -0.59 ** | -0.63 *** |
| Mg | -0.37 * | -0.32 * | -0.29 | -0.37 * |
| Fe | -0.07 | -0.12 | 0.00 | -0.09 |

already lower) and Mg, and caused an increase in the concentrations of K and Ca (Table 3). Similar effects on N, P, K, Ca and Mg were observed after 12 weeks, but the effect on Mg was reversed in the nodulated plants (interaction FxT; Table 4). Mean transpiration coefficients (TC) per object, as calculated for the first six-week period, were between 206 and 286 g water per g dry matter formed. The TC was not significantly influenced by any of the treatments, but was significantly negatively correlated with the initial seedling size (data not shown).

Plant biomass parameters at the first harvest were positively correlated with shoot

concentrations of P and Mg, and negatively with Ca and Fe. At the second harvest, biomass parameters were positively correlated with N and P, and negatively with K and Ca (Table 5). The nutrients with positive correlations may have been limiting factors for growth. Root weight and length had the highest positive correlations with shoot P concentration. In the *-Frankia* treatment, plant biomass showed better positive correlations with P than with N, whereas in the *+Frankia* treatment there was no clear difference (Table 5).

Population development of *T. microphasmis*

Numbers of *T. microphasmis* per pot increased during the first 6 weeks by about 6- to 12-fold at the low inoculum level and by 3- to 9-fold at the high level (Table 1).

A difference between the two inoculum levels was still visible after 6 weeks, except in the treatments without *C. destructans* and *Frankia*. Nematode numbers per unit root length were also still higher at the high nematode inoculum level than at the low level, partly due to the more severe reduction of root length at the high inoculum level (Table 1). Between weeks 6 and 12, nematode multiplication (1.5- to 4-fold) was less than during the first period. The difference between the inoculum levels in numbers per plant and per unit root disappeared, especially in the treatments without *Frankia* (Table 2). Remarkably, *C. destructans* favoured multiplication of *T. microphasmis* in the treatment without *Frankia* during the first 6 weeks, whereas in the *+Frankia* treatment it was mildly negative (Table 1). The positive effect of *C. destructans* on the number of nematodes per unit root length in the *-Frankia* treatments was not due to reduction of the root length. After 12 weeks the effect on numbers per pot had disappeared. A positive effect of *C. destructans* on numbers of *T. microphasmis* per unit root was found, especially in the *-Frankia* treatments, but this could partly be explained from a reduction of the root system.

Field material

The leaf/total weight ratio of current years twigs was higher in degenerating *H. rhamnoides*, and the total weight was smaller compared to vigorous shrubs (Table 6). Leaf concentrations of P, Ca and Fe were lower in degenerating shrubs (Table 6) and only the concentration of P in the leaves was positively correlated ($p < 0.05$) with dry weight of leaves and stems (data not shown).

Table 6. Leaf nutrient concentrations in *Hippophaë rhamnoides* in the field in July 1988, as related to successional stage and location. Means of two sites per object. Per site 5 shrubs were sampled (5 twigs per shrub). Values are expressed as mmol.(kg dry weight)⁻¹ for N, P, K, Ca and Mg, and as mg.(kg dry weight)⁻¹ for Fe. Significance (p<0.05) in ANOVA is indicated by *.

| location | stage | N _{org} | H ₂ PO ₄ ⁻ | K ⁺ | Mg ²⁺ | Ca ²⁺ | Fe ³⁺ |
|------------------|----------|------------------|---|----------------|------------------|------------------|------------------|
| Voorne | vigorous | 1858 | 57 | 151 | 90 | 288 | 477 |
| | decline | 1805 | 46 | 174 | 63 | 196 | 137 |
| Schouwen | vigorous | 1910 | 61 | 146 | 81 | 250 | 217 |
| | decline | 1648 | 54 | 99 | 93 | 211 | 174 |
| ANOVA | | | | 1) | | | |
| location | | ns | ns | ns | ns | ns | ns |
| stage | | ns | * | ns | ns | * | * |
| location x stage | | ns | ns | ns | ns | ns | * |

1) = ln(x) transformed

DISCUSSION

The present experiment was set up to study the effects of *T. microphasmis*, alone and in combination with the fungus *C. destructans*, on *H. rhamnoides*. The mechanism of reduction of plant growth by *T. microphasmis* seems not a simple and direct one. Growth inhibition due to this nematode was initially stronger in roots than in shoots (Table 1). From *in vitro* observations (Zoon *et al.*, Chapter 7) it was concluded that *T. microphasmis* reduces the density and the condition of root hairs. This is in agreement with reports on the feeding sites (epidermal cells and root hairs) and feeding behaviour of members of this genus (Klinkenberg, 1963; Wyss, 1973; 1981). Therefore, it is likely that the reduced uptake capacity of the roots (length, hairs, condition) is the primary cause of growth reduction. The concentration of P in the shoot was consistently positively correlated with root weight and length (Table 5). The uptake of phosphate, in particular, is highly dependent on an adequate root distribution (Marschner, 1986). The initial increase in shoot/root ratio (Table 1) and its subsequent decrease (Table 2) may indicate that initially root growth is severely impeded and that the plant needs some time to re-establish the functional equilibrium between shoot and roots. Water relations were not significantly affected by *T. microphasmis*.

Effects of *T. microphasmis* may occur shortly after inoculation, as was concluded

from *in vitro* studies (Zoon *et al.*, Chapter 7). Effects in pot experiments seem more severe when inoculation occurred in the planting hole (Zoon *et al.*, Chapter 7) compared to inoculation after planting and a few centimetres away from the test plant, as in the present study. In the former situation *T. microphasmis* reduced nodulation already during the first 6 weeks, whereas in the present study this occurred only later on. Inoculum densities of 400 or more nematodes per seedling may seem high, but in similar experiments 50 to 75 % mortality of *T. microphasmis* occurs during the first three weeks (Maas *et al.*, 1983; Zoon, Chapter 6). Moreover, nematode numbers per unit root length increased between the first and the second observation at the lower inoculum level, which indicates that the carrying capacity of the plant was not reached here. In the field, on average 174 *T. microphasmis* individuals per 500 ml soil (10-50 cm depth) were found under *H. rhamnoides* vegetation (Zoon *et al.*, 1993), whereas occasionally up to 1500 individuals per 500 ml soil (0-50 cm depth) were found (Zoon, unpublished). Thus, at least the lower nematode density in this experiment corresponds to those occurring in the field.

C. destructans initially stimulated growth in the non-nodulated plants, possibly by increasing mineralisation of the soil organic-N pool. In the nitrogen-fixing plants, and also later on in the non-nodulated plants, the fungus was harmful, but less so than *T. microphasmis*. Reversal of the effect of *C. destructans* on growth and shoot-N concentrations of non-nodulated plants in the second period may have been induced by the addition of extra N in the nutrient solution during this period. No interactions between the nematode and the fungus related to growth of *H. rhamnoides* were found. A synergic action, for which indirect proof was found by Maas *et al.* (1983), may well be caused by other nematode-fungus combinations.

C. destructans remarkably stimulated the multiplication of *T. microphasmis* on non-nodulated plants (Table 1). This may be related to an initial positive effect of this fungus on the nutrition of the plant, which may have improved the quality of the roots as a food source for *T. microphasmis*. However, such an effect on nutrient concentrations was not found any more after 6 weeks. Positive effects of increased N-acquisition of host plants on reproduction of small herbivores have been described earlier for plant feeding nematodes (Yeates, 1987) and insects (White, 1984).

To assess the importance of *T. microphasmis* and *C. destructans* in the degeneration of *H. rhamnoides* the results have to be translated to the field situation, viz. nutrient-deficient calcareous sandy dune soils. *T. microphasmis* and *C. destructans* and also the actinorhizal symbiont *Frankia* are commonly present on

roots of *H. rhamnoides* in these soils, and concentrations of *T. microphasmis* on the roots tend to increase towards the degeneration stage of this shrub (Zoon *et al.*, 1993). Other plant parasitic nematodes, such as *Criconebella* (syn. *Mesocriconema*) *xenoplax* Raski, occur in the same soils (Zoon *et al.*, 1993) and may have similar effects as *T. microphasmis*. Twigs of degenerating *H. rhamnoides* shrubs in the field had lower dry weights and lower concentrations of phosphorus, calcium and iron than those of vigorous shrubs (Table 6). Troelstra *et al.* (1987) obtained similar results for phosphorus. The nutrient concentrations in field material were comparable to those found in shoots of nematode-inoculated plants in the pot experiment. As in the pot experiment, the concentration of phosphorus in leaves of field material was positively correlated with the dry weight of leaves and stem. No other nutrient concentrations were correlated to biomass parameters. This suggests that reduced P acquisition is a key factor in the decline in the field, as well as in the effect of nematodes in the pot experiment. VA-mycorrhiza, which was found especially in the degeneration stage of *H. rhamnoides* (Zoon *et al.*, 1993), is expected to alleviate the reduction of P uptake in the field, but apparently this is not enough to compensate for the harmful effect of plant parasitic nematodes. It is concluded that *T. microphasmis*, and to a lesser extent *C. destructans*, reduce the vigour of *H. rhamnoides* in calcareous dune sands, especially by reducing the nutrient uptake capacity of the roots. Hence, the resistance to stress and the belowground competitive ability of *H. rhamnoides* is decreased, giving way to other (*e.g.* gramineous) plant species in the vegetation.

REFERENCES

- Allen M.B. and Arnon D.I. 1955. Studies on nitrogen-fixing blue green algae.
I. Growth and nitrogen fixation of *Anabaena cylindrica* Lemm. *Plant Physiol.*
Lancaster 30: 366.
- Klinkenberg C.H. 1963. Observations on the feeding habits of *Rotylenchus uniformis*, *Pratylenchus crenatus*, *P. penetrans*, *Tylenchorhynchus dubius* and *Hemicycliophora similis*. *Nematologica* 9: 502-506.
- Marschner H. 1986. *Mineral Nutrition of Higher Plants*. Academic Press, London, 674 pp.
- Maas P.W.Th., Oremus P.A.I. and Otten H. 1983. Nematodes (*Longidorus* n.sp. and *Tylenchorhynchus microphasmis* Loof) in growth and nodulation of sea buckthorn, *Hippophaë rhamnoides* L.). *Plant and Soil* 73: 141-147.

- Oostenbrink M. 1960. Estimating nematode populations by some selected methods. In: J.N. Sasser and W.R. Jenkins (Editors), *Nematology*. Univ. of N. Carolina Press, Chapel Hill, pp. 85-102.
- Oremus P.A.I. 1980. Occurrence and infective potential of the endophyte of *Hippophaë rhamnoides* L. ssp. *rhamnoides* in coastal sand-dune areas. *Plant and Soil* 56: 123-139.
- Oremus P.A.I. 1982. Growth and Nodulation of *Hippophaë rhamnoides* L. in the Coastal Sand Dunes of The Netherlands. Ph.D. Thesis, University of Utrecht, The Netherlands, 120 pp.
- Oremus P.A.I. and Otten H. 1981. Factors affecting growth and nodulation of *Hippophaë rhamnoides* L. ssp. *rhamnoides* in soils from two successional stages of dune formation. *Plant and Soil* 63: 317-331.
- Troelstra S.R. 1983. Growth of *Plantago lanceolata* and *Plantago major* on a $\text{NO}_3^-/\text{NH}_4^+$ medium and the estimation of the utilization of nitrate and ammonium from ionic-balance aspects. *Plant and Soil* 70: 183-197.
- Troelstra S.R., Blacquièrè T., Wagenaar R. and van Dijk C. 1987. Ionic balance, proton efflux, nitrate reductase activity and growth of *Hippophaë rhamnoides* L. ssp. *rhamnoides* as influenced by combined-N nutrition or N_2 -fixation. *Plant and Soil* 103: 169-183.
- van der Maarel E., Boot R., van Dorp D. and Rijntjes J. 1985. Vegetation succession on the dunes near Oostvoorne, The Netherlands: A comparison of the vegetation in 1959 and 1980. *Vegetatio* 58: 137-187.
- van der Putten W.H., van Dijk C. and Peters, B.A.M. 1993. Plant-specific soil-borne diseases contribute to succession in foredune vegetation. *Nature* 362: 53-56.
- White T.C.R. 1984. The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia* 63: 90-105.
- Wyss U. 1973. Feeding of *Tylenchorhynchus dubius*. *Nematologica* 19: 125-136.
- Wyss U. 1981. Ectoparasitic root nematodes: feeding behavior and plant cell responses. In: B.M. Zuckerman and R.A. Rohde (Editors), *Plant-Parasitic Nematodes*. Vol. 3. Academic Press, New York, pp. 325-351.
- Yeates G. 1987. How plants affect nematodes. *Adv. Ecol. Res.* 17: 61-113.
- Zoon F.C., Hage F.J. and de Zwart A. 1990. A device for automatic soil moisture control and registration of water use in pot experiments. *Plant and Soil* 125: 281-284.
- Zoon F.C. and Van Tienderen P.H. 1990. A rapid quantitative measurement of root length and branching by microcomputer image analysis. *Plant and Soil* 126: 301-308.
- Zoon F.C. 1991. Effects of rhizosphere fungi and nematodes on the actinorhiza and growth of *Hippophaë rhamnoides* L. In: A.B.R. Beemster *et al.* (Editors), *Biotic Interactions and Soil-Borne Diseases* (Proc. 1st Int. Symp. EFPP, Wageningen 1990). Elsevier, Amsterdam, pp. 47-52.

- Zoon F.C., Troelstra S.R. and Maas P.W.Th. 1993. The ecology of the plant-feeding nematode fauna associated with Sea Buckthorn (*Hippophaë rhamnoides* L.) in different stages of dune succession. *Fund. appl. Nematol.* 16: 247-258.
- Zoon F.C. 199*. The influence of soil moisture content on the relation between the nematode *Tylenchorhynchus microphasmis* Loof and its host plant *Hippophaë rhamnoides* L. ssp. *rhamnoides*. (submitted).
- Zoon F.C., Van Herp A. and Troelstra S.R. 199*. Belowground herbivory of *Tylenchorhynchus microphasmis* Loof on actinorrhizal and non-actinorrhizal Sea Buckthorn (*Hippophaë rhamnoides* L.). (submitted).

CHAPTER 9

General discussion

Potential factors in the decline and succession of *H. rhamnoides*.

The impact of changes in the complex of abiotic soil factors and interacting soil biota on succession of *H. rhamnoides* was the subject of the present study. The possible role of nematodes and other soil organisms in the succession of *H. rhamnoides*, and the mechanisms of growth inhibition and scrub degeneration were addressed first by studying the field situation. Soil samples from vigorous and degenerating *H. rhamnoides* scrubs in different dune areas along the Dutch coast were analysed physico-chemically and biologically (chapter 2 and 3). In young coastal sand dunes various stages of succession can be found in a spatial sequence which can be considered to represent the temporal sequence of succession (Olson, 1958; Crawley, 1993). Canonical correspondence analysis and ordination of environmental and nematofaunal variables (chapter 2) showed that the soil develops from low to higher contents of nitrogen and organic matter. This is a general feature of primary succession (Vitousek & Walker, 1987). The increase in soil N concentration was accompanied by a reduction of the vigour of *H.*

rhamnoides and an increase of VA mycorrhizal infection of roots (Chapter 2).

Comparison of two representative sites from early and late successional stages with *H. rhamnoides* indicated that available P in the soil may decrease, although the concentration of total P is unaltered (appendix chapter 2). Other authors found similar concentrations of total P in calcareous dune soil without change during the presence of *H. rhamnoides* (Troelstra et al., 1987; Olf, 1992). Troelstra et al. (1987) found no change in Olsen-P, whereas labile organic P increased towards the degeneration stage. Probably, the available P is more efficiently taken up by the grasses and mycorrhiza present at the late successional stage of *H. rhamnoides* vegetation, than by *H. rhamnoides* itself. Over a period of ten thousands of years total P in dune soils may decrease (Walker & Syers, 1976). In the present study, however, a much shorter period of maximally a few hundreds of years is considered and the soils have a large initial pool of inavailable P, mainly present as calcium apatite.

Total soil nitrogen tends to double from early to late *H. rhamnoides* stages

(Boerboom, 1963; Oremus, 1980; Troelstra *et al.*, 1987). This was confirmed in the present study (appendix chapter 2). Available N (mainly NO_3^-) was a factor two higher on a site of the late *H. rhamnoides* stage compared to an early stage site within the same area, whereas total N differed by 39 per cent (an increase of 98 mg/kg soil; appendix chapter 2). In the more extensive survey of chapter 2 an average difference in total N between early and late *H. rhamnoides* stages of 81 per cent (an increase of 114 mg/kg soil) was found. The change in the N/P ratio of the soil suggests that the role of nitrogen fixation becomes less important. Therefore, the advantage of *H. rhamnoides*, compared to plant species without N_2 -fixing abilities, is probably diminishing as dune soils are enriched with nitrogen.

The percentage of *H. rhamnoides* feeder roots infected by VA mycorrhizal fungi remarkably increased during succession (chapter 2). This is probably related to a deficiency in phosphorus, which stimulates VAM infection (Menge *et al.*, 1978). Such a higher need for VAM may be due to a reduction of the root system by pathogens as discussed below. An increase in the percentage of VAM infection along with dune age and fixation was also observed in a series of earlier successional stages with *Ammophila arenaria* (L.) Link (Nicolson & Johnston, 1979). The efficacy of VA-mycorrhizal symbioses is, however, related more to the extent of extramatrical hyphae, than to the percentage of root length infected (Wright & Millner, 1994). The dominant VAM fungal species of *H. rhamnoides* in the present study, *Glomus fasciculatum* Gerd. & Trappe, was also predominant in other dune soils in north-western Europe (Koske & Halvorson, 1981; Nicolson & Johnston, 1979) and was reported to increase phosphorus uptake, nitrogen-fixation and growth of *H. rhamnoides* (Gardner *et al.*, 1984). However, this fungal species did not stimulate nutrient uptake or growth of certain dune grasses (Ernst *et al.*, 1984), probably due to the high uptake capacity of gramineous roots. Two other species of VAM fungi were reported from *H. rhamnoides* in England by Rose (1980). Incomplete colonization by VAM fungi in early successional stages was found by others to be due to instability of the soil (Walker *et al.*, 1981; Nicolson & Johnston, 1979), but this is not likely the case in the present study on *H. rhamnoides*, because the scrubs studied were older than 10 years.

A decrease in soil pH, which was formerly mentioned as a cause of succession in coastal sand dunes (Boerboom, 1960), seems to be of limited importance for *H. rhamnoides*. To obtain a pH lower than 7, almost complete decalcification is required, which, depending on the initial lime content, occurs only after a long period of time, mainly in wet slacks where organic matter accumulates (Salisbury,

1925; Olson, 1958; Rozema *et al.*, 1985). In dune areas with initially high concentrations of lime, *H. rhamnoides* generally disappears long before the pH drops below 7 (appendix of chapter 2; Boot & Van Dorp, 1986). Moreover, hydroponical growth of nodulated *H. rhamnoides* was not affected by a low pH (Troelstra *et al.*, 1987) and thus this plant is fairly acid tolerant. However, the formation of new nodules may be inhibited in acid soils (Bond, 1957). According to Troelstra *et al.* (1987), the contribution of N₂-fixation to N-acquisition of *H. rhamnoides* in later successional stages is small.

In the ordination study (chapter 2), sites with a recent history of disturbance (early secondary succession) closely resembled the sites with recent colonization in a primary succession sere with respect to soil factors and plant-feeding nematode fauna. This suggests that disturbance acts as a renewal of the soil, both biologically and chemically. In fact, often fresh sand is deposited, or the old top layer is blown away by wind. The plant-feeding nematode fauna showed marked shifts between successional stages (chapter 2). *Tylenchorhynchus microphasmis* and *Mesocriconema xenoplax* (syn. *Macroposthonia xenoplax*; syn. *Criconemella xenoplax*) were most dominantly associated with *H. rhamnoides* throughout the successive stages of *Hippophaë* vegetation. *Longidorus dunensis* was found only under degenerating vegetation, which agrees with earlier observations (Oremus & Otten, 1981). All of these nematode species, and additionally *Paratrichodorus pachydermus* and *Pratylenchus* spp., were able to multiply on *H. rhamnoides* and were present primarily in the rhizosphere of this plant (chapter 2). There was no obvious change in the number of plant-feeding nematodes in bulk soil along with succession. However, the density of *Tylenchorhynchus microphasmis* on a per unit root basis tended to be higher in later successional stages. From these results, however, it is hard to decide which of these nematode species are the most harmful to *H. rhamnoides*. In further experimental studies (chapters 6, 7 and 8), *T. microphasmis* was taken as a model species for the various ectoparasitic root nematodes found. This species was non-deliberately involved in a previous experiment of Maas *et al.* (1983) concerning the effect of *Longidorus dunensis* on growth and nodulation of *H. rhamnoides*. Sterilization of part of the soil in that experiment showed that other soil organisms interacted with the added plant-feeding nematodes.

The question which soil organisms are involved in the disease complex of *H. rhamnoides* was approached also by selective inhibition of groups of fungi and nematodes in natural soil from different successional stages (chapter 3). The

fungicide benomyl stimulated *Frankia* nodulation and growth of *H. rhamnoides*, suggesting that fungi are harmful. The nematostaticum oxamyl reduced populations of plant feeding nematodes, but also stimulated root infection by fungi, possibly by immobilization of fungivorous soil mesofauna. However, the net effect of oxamyl was positive, which indicates that either nematodes are much more harmful than fungi, or fungi are not harmful if nematodes are absent. The dominant fungi on roots of *H. rhamnoides* were *Cylindrocarpon destructans* and *Fusarium oxysporum*. One isolate of *C. destructans* was mildly pathogenic when inoculated in sterilized soil. These results led to the hypothesis that fungi may be harmful only in the presence of plant-feeding nematodes (chapter 3). The two fungal species mentioned are known for their interaction with plant-feeding nematodes (Mauza & Webster, 1982; Evans & Haydock, 1993). Thus, amongst the 'unknown' soil biotic factors which interacted with nematodes in the experiment of Maas *et al.* (1983) are at least fungal candidates. In conclusion, potential factors in the decline of *H. rhamnoides* are the enrichment of soil with N, the effects of plant-parasitic nematodes and possible interactions between nematodes and root-rot fungi. Soil acidification and subsequent inhibition of *Frankia* growth and nodulation seems of limited importance only. It is not clear to what extent VA mycorrhiza may cause any relief from soil-borne stress factors.

Interactions between nematodes, fungi, *Frankia* and *H. rhamnoides*; experimental studies.

A study on the effect of the soil moisture regime on the interaction between *T. microphasmis* and *H. rhamnoides* (chapter 6) illustrated the importance of maintaining a regime, which is representative for the natural situation. At a higher soil moisture level *T. microphasmis* was more harmful than at a low level, probably because of a lower mobility of the nematodes in dry soil (Wallace, 1956). Dune sand is often rather dry, due to the low water holding capacity and the limited capillary rise of freatic water (Wösten *et al.*, 1987). The maintenance of a natural moisture level in pot experiments with dune sand requires frequent and accurate watering. An apparatus for automatic watering and registration of water use was developed (chapter 4) which allowed us to fulfill this requirement.

The effects of *T. microphasmis* on *H. rhamnoides* in sterilized soil in pot experiments were reduced root and shoot growth, reduced *Frankia*-nodulation, and

reduced tissue concentrations of P (chapter 7 and 8). Reduction of root biomass and P concentration are considered to be the main effects, because they occurred early, and were stronger than for other growth or nutrient parameters. Moreover, of the shoot concentrations of various nutrients in the pot experiments, that of P showed the strongest correlation with biomass production. The inflow rate of phosphate per unit root length, unlike that of nitrogen, was not increased when the root length was reduced (chapter 7). The rate of phosphorus uptake by plants is generally dependent on the ion concentration of P and on the root length density (cm.cm^{-3}) in soil (Marschner, 1986). The latter is probably of key importance here. From selective inhibition experiments (chapter 3) the hypothesis was inferred that fungi may be harmful when nematodes are numerous. However, no interactions were found between *T. microphasmis* and the fungus *C. destructans* with respect to growth of *H. rhamnoides* in a pot experiment (chapter 8). It is possible that other nematode-fungus combinations present on *H. rhamnoides* show synergic pathogenic interactions. In this respect, *Mesocriconema xenoplax* and *Pratylenchus penetrans*, which were both found under *H. rhamnoides* and the latter predominantly in later successional stages, were reported as synergists with root-rot fungi in various plant species (Evans & Haydock, 1993). Furthermore, infection by the root pathogen *Fusarium oxysporum* (chapter 2) may be largely dependent on nematodes.

In pot experiments, the nematode *T. microphasmis* also caused a reduction of the number of actinorhizal nodules per unit root length and a decrease in the nitrogenase activity per plant, but not per unit nodule weight (chapter 7 and 8). However, in these experiments N was not limiting, because the highest N concentrations at harvest corresponded with the lowest growth rates of *H. rhamnoides* (chapter 7). Nematodes might also reduce the number or lifespan of nodules in the field, but no observations were made with respect to this. Only in field soils with a very low infection potential of *Frankia* and a low nitrogen availability, may reduced nodulation have consequences for nitrogen acquisition and growth of *H.*

rhamnoides (chapter 3). These results support the hypothesis that P-limitation, rather than N-limitation occurs due to plant-parasitic nematodes. The importance of P nutrition for nitrogen nutrition in *Frankia*-nodulated *H. rhamnoides* as stressed by Oremus (1982) could not be confirmed. It seems that phosphorus has a more direct effect on plant growth.

Synthesis: ecological implications for the field situation.

The results of pot experiments indicate that especially P uptake of *H. rhamnoides* is hampered due to reduction of root growth by plant feeding nematodes. In subsequent successional stages in the field, shoot and leaf nutrient concentrations showed a decreasing trend in potassium, phosphate and nitrate, whereas organic N did not change (appendix chapter 2; Troelstra *et al.*, 1987). Furthermore, leaf P concentration in July was the only nutrient correlated with current twig biomass (F.C. Zoon, unpublished results). The soil concentration of available P may decrease during succession, whereas available N concentrations (mainly NO_3^-) tend to double (appendix chapter 2). These observations, and the increased VAM infection percentage (chapter 2) strongly support the idea that in later stages of *H. rhamnoides* vegetation P becomes limiting, especially due to plant-feeding nematodes. Numbers of plant-feeding nematodes per unit soil do not increase dramatically during the period between colonization and succession of *H. rhamnoides* vegetations (chapter 2). On a per unit root basis, however, the number of certain nematodes (such as *T. microphasmis*) increased, and thus the damage caused by these nematodes is most likely increased. It is hypothesized that the increased number of nematodes per unit root is caused by an increased food quality of plant cell contents (more proteins and amino-acids), possibly due to the increased availability of N in the soil. Such effects are known for herbivorous insects (White, 1984), but for nematodes this has hardly been studied (Yeates, 1987). As *T. microphasmis* may reduce *Frankia* nodulation and N_2 -fixation (chapter 2), it may cause limitation of N acquisition in colonization stages of *H. rhamnoides* and thereby delay the accumulation of N due to this shrub. This interesting possibility of delay of succession (Crawley, 1989) deserves further study.

It seems most likely that the increased ratio of available N/P in the dune soil ecosystem is the ultimate force behind the succession of *H. rhamnoides* vegetation. Plant species with nitrogen-fixing capacity will gradually be replaced by species with a superior capacity of phosphate uptake (Grime, 1977; Tilman, 1985). In fact this is what we observe where grasses fill the open spaces between degenerating shrubs of *H. rhamnoides*. The type of succession is of the 'facilitation model' (Connell & Slatyer, 1977). Plant-feeding nematodes can be seen as a proximate factor. They have a catalysing function, which accelerates the replacement process which otherwise would take much more time. Interactions with root fungi may aggravate the harmful effects of nematodes (chapter 3). Many of the plant-feeding

nematode species found do not seem to be very specific with respect to their host plants. Some were also found on *Ammophila arenaria* L., a preceding plant species in the succession of sand dune vegetation (Van der Putten *et al.* 1989; chapter 2). However, the preference for, or the sensitivity of, different plant species may differ and thus specific effects are possible when plant species occur together. The rhizosphere fungi *F. oxysporum* and *C. destructans* are not specific to *H. rhamnoides*, either, but the former may have specific races and the latter was not found on *A. arenaria*. Specificity of effects of soil-borne pathogens and pests may be important for the direction of succession (Van der Putten, 1993). The main inference from the present study is that interactions of soil biota with changing availability of nutrient resources are important for the way soil pathogens affect the succession of sand dune vegetation.

REFERENCES

- Boerboom J.H.A. 1960. De plantengemeenschappen van de Wassenaarse duinen. Meded. Landb.hogesch., Wageningen 60(10): 1-135.
- Boerboom J.H.A. 1963. Het verband tussen bodem en vegetatie in de Wassenaarse duinen. Boor en Spade 13: 121-155.
- Boot R.G.A. & Van Dorp D. 1986 De Plantengroei van de Duinen van Oostvoorne in 1980 en Veranderingen sinds 1934. Rotterdam, Stichting Het Zuidhollands Landschap, 120 pp.
- Connell J.H. & Slatyer R.O., 1977. Mechanisms of succession in natural communities and their role in community stability and organisation. Am. Nat. 111: 1119-1144.
- Crawley M.J. 1989. The relative importance of vertebrate and invertebrate herbivores in plant population dynamics. In: Bernays E.A. (ed.), Insect-Plant Interactions, CRC Press, Boca Raton, 45-71.
- Crawley M.J. 1993. Succeeding in the sand dunes. Nature 362: 17-18.
- Ernst W.H.O., Van Duin W.E. & Oolbekking G.T. 1984. Vesicular-Arbuscular Mycorrhiza in dune vegetation. Acta Bot. Neerl. 33: 151-160.
- Evans K. & Haydock P.P.J. 1993. Interactions of nematodes with root-rot fungi. In: M.W. Kahn (ed.) Nematode Interactions. Chapman & Hall, London, 104-133.
- Gardner I.C., Clelland D.M. & Scott A. 1984. Mycorrhizal improvement in non-leguminous nitrogen-fixing associations with particular reference to *Hippophaë rhamnoides* L. Plant and Soil 78: 189-201.
- Grime J.P., 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. Am. Nat. 111: 1169-1194.

- Koske R.E & Halvorson W.L. 1981 Ecological studies of vesicular-arbuscular mycorrhizae in a barrier sand dune. *Can. J. Bot.* 59: 1413-1422.
- Maas P.W.Th., Oremus P.A.I. & Otten H. 1983. Nematodes (*Longidorus* n.sp. and *Tylenchorhynchus microphasmis* Loof) in growth and nodulation of sea buckthorn (*Hippophaë rhamnoides* L.). *Plant and Soil* 73: 141-147.
- Mauza B.E. & Webster J.M., 1982. Suppression of alfalfa growth by concomitant populations of *Pratylenchus penetrans* and two *Fusarium* species. *J. Nematol.* 14, 364-367
- Menge J.A., Steirle D., Bagyaraj D.J., Johnson E.L. & Leonard R.T. 1978. Phosphorus concentrations in plants responsible for inhibition of mycorrhizal infection. *New Phytol.* 80: 575-578.
- Nicolson T.H. & Johnston C. 1979 Mycorrhiza in the gramineae. III. *Glomus fasciculatus* as the endophyte of pioneer grasses in a maritime sand dune. *Trans Br. mycol. Soc.* 72: 261-268.
- Olf H. 1992 On the Mechanisms of Vegetation Succession. PhD. Thesis, Rijksuniversiteit Groningen, The Netherlands. 211 pp.
- Olson J.S. 1958. Rates of succession and soil changes on southern Lake Michigan sand dunes. *Bot. Gaz.* 119: 125-170.
- Oremus P.A.I. 1980. Occurrence and infective potential of the endophyte of *H. rhamnoides* ssp. *rhamnoides*. *Plant & Soil* 56: 123-139.
- Oremus P.A.I. 1982. Growth and Nodulation of *Hippophaë rhamnoides* L. in the coastal Sanddunes of The Netherlands. Ph.D. Thesis, Univ. of Utrecht NL, 118 p.
- Oremus P.A.I. & Otten H. 1981. Factors affecting growth and nodulation of *Hippophaë rhamnoides* L. ssp. *rhamnoides* in soils from two successional stages of dune formation. *Plant & Soil* 63: 316-331.
- Rose S.L. 1980. Mycorrhizal associations of some actinomycete nodulated nitrogen-fixing plants. *Can.J.Bot.* 58: 1449-1454.
- Rozema J., Laan P., Broekman R., Ernst W.H.O. & Appelo C.A.J. 1985. On the lime transition and decalcification in the coastal dunes of the province North Holland and the island Schiermonnikoog. *Acta Bot. Neerl.* 34: 393-411.
- Salisbury E.J. 1925. Note on the edaphic succession in some dune soils with special reference to the lime factor. *J. Ecol.* 13: 322-328.
- Tilman D, 1985. The resource-ratio hypothesis of plant succession. *Am. Nat.* 125, 827-852
- Troelstra S.R., Blacquièrre T., Wagenaar R. and Van Dijk C. 1987. Ionic balance, proton efflux, nitrate reductase activity and growth of *Hippophaë rhamnoides* L. ssp. *rhamnoides* as influenced by combined-N nutrition or N₂-fixation. *Plant and Soil* 103: 169-183.
- Van der Putten W.H. 1989 Establishment, growth and degeneration of *Ammophila arenaria* in coastal sand dunes. PhD. Thesis Wageningen Agricultural University, Wageningen, 152 pp.

- Van der Putten W.H., Van Dijk C. & Peters B.A.M. 1993. Plant-specific soil-borne diseases contribute to succession in foredune vegetation. *Nature* 362: 53-55.
- Vitousek P.M. & Walker L.R., 1987 Colonization, succession and resource availability: ecosystem-level interactions. In: Gray A.J., Crawley M.J. & Edwards P.J. (eds) 1987. *Colonization, Succession and Stability*. Oxford, Blackwell Sci. Publ.: 207-223.
- Walker J., Thompson C.H., Fergus I.F. & Tunstall B.R., 1981. Plant succession and soil development in coastal sand dunes of subtropical eastern Australia. In: D.C. West, H.H. Shugart & D.B. Botkin (eds) *Forest Succession, Concepts and Application*. New York, Springer Verlag, pp 107-131.
- Walker T.W. & Syers J.K., 1976 The fate of phosphorus during pedogenesis. *Geoderma* 15: 1-19.
- White T.C.R. 1984. The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia* 63: 90-105.
- Wösten J.H.M., Bannink M.H. & Beuving J. 1987 *Waterretentie- en Doorlatendheidskarakteristieken van Boven- en Ondergronden in Nederland: De Staringreeks*. Instituut voor Cultuurtechniek en Waterhuishouding, Rapport 18 (STIBOKA-rapport 1932), Wageningen, 75 pp.
- Wright S.F. & Millner P.D. 1994. Dynamic processes of vesicular-arbuscular mycorrhizae: a mycorrhizosystem within the agroecosystem. In: Hatfield J.L & Stewart B.A. (eds.) *Soil Biology: Effects on Soil Quality*. Lewis, Boca Raton, USA, 29-59.
- Yeates G. 1987. How plants affect nematodes. *Adv. Ecol. Res.* 17: 61-113.

SUMMARY

Succession in the vegetation is an intriguing natural process mainly governed by plant dispersal characteristics, inter-species competition and soil development. Development of abiotic soil factors involves changes in nutrient availability and pH. The role of biotic soil factors is relatively obscure. Beneficial organisms, such as nitrogen-fixing root symbionts and mycorrhiza, may aid the establishment and survival of plant species on nutrient-poor soils. Moreover, harmful soil organisms may reduce the competitive ability of plant species in the vegetation and thereby facilitate succession. Sand dune systems are recognized subjects for succession studies, because of the clear vegetation patterns and soil development. The topographic sequence in the vegetation of dune systems often illustrates the chronologic sequence of primary vegetation succession. Sea buckthorn, *Hippophaë rhamnoides* L. ssp. *rhamnoides*, is a dominant plant species in primary succession of dune vegetation in the Netherlands. It colonizes dynamic seaward dune ridges with *Ammophila arenaria* L. and young dune slacks and degenerates after decades, leading to mixed scrub or grass vegetation. The degeneration of *H. rhamnoides* scrub has previously been shown to be associated with presence of plant-parasitic nematodes and other (unknown) biotic soil factors. The organisms and mechanisms involved needed further attention.

In the present study, a field survey was made of the occurrence of nematodes, VA-mycorrhiza and *Frankia*-actinorhiza on the roots of *H. rhamnoides* in different stages of succession in coastal sand dunes in the Netherlands, in relation to soil physical and chemical factors and plant vigour. Ordination of samples according to nematode fauna, plant factors and abiotic soil factors revealed a succession in the plant-feeding nematode fauna along with the development of soil and vegetation.

The average degeneration of *H. rhamnoides* vegetation proceeded with increasing soil nitrogen and organic matter concentration and increasing VA-mycorrhizal infection percentage of the roots. Current year's twig weight was correlated to leaf concentrations of phosphate. Most sites which had recently developed after disturbance closely resembled those of first colonization by *H. rhamnoides*. The maturity index of the obligate plant-feeding nematode fauna and the mean number of species increased significantly. The plant-feeding nematodes *Tylenchorhynchus microphasmis* Loof and *Mesocriconema xenoplax* (Raski) were common in all stages, whereas *Longidorus dunensis* Brinkman *et al.* was found only under degenerating *Hippophaë*. The abundance of plant-feeding nematodes in the rhizosphere of *Hippophaë* increased towards later stages on a succession gradient,

whereas population densities in the bulk soil remained virtually constant.

Results of subsequent pot experiments with selective chemical inhibition of groups of soil organisms suggested that plant parasitic nematodes, apart from being harmful by themselves, may predispose the plants to damage by fungi. In natural *H. rhamnoides* soils, the fungicide benomyl markedly increased actinorhizal nodulation, whereas it did the opposite in sterilized soil. The dominant fungi isolated from roots and rhizoplane of *H. rhamnoides* in field soils were *Cylindrocarpon destructans* Zinns. Scholten and *Fusarium oxysporum* Schlecht, emend. Sny.& Hansen. Both were strongly decreased by benomyl and are thus potential antagonists for *Frankia* nodulation. Inoculation in sterilized sand with *H. rhamnoides* without *Frankia* showed that only one *Cylindrocarpon* isolate was slightly pathogenic, when present alone. Increased fungal colonization of roots and rhizoplane due to the nematostaticum oxamyl did not hamper but rather stimulated nodulation and shoot growth, probably due to the simultaneous decrease of plant-parasitic nematodes. The results indicate that plant-parasitic nematodes alone, or in combination with soil fungi may decrease the vigour of *H. rhamnoides* in the field.

The impact of various densities of *T. microphasmis*, either alone or in combination with the nitrogen-fixing symbiont *Frankia* or the fungus *Cylindrocarpon destructans*, was assessed in pot experiments with controlled soil moisture conditions. An apparatus for automatic recording and replenishing of water loss from pots, and methods for measuring root length and branching rate by image analysis were developed. Results of the pot experiments showed inhibitory effects of the nematode on *Hippophaë* growth and on the acquisition of certain nutrients, except in very dry soil. Root length was more severely suppressed than shoot weight and the former was positively correlated with shoot P concentration. Shoot growth was positively correlated to P concentration and not, or negatively, to other nutrients. Thus, reduction of root growth by the nematodes is likely the key mechanism. Although root nodulation and nitrogen fixation by *Frankia* was also reduced, this appeared not the primary cause of growth reduction. *C. destructans* did not affect nodulation and was only slightly pathogenic to *H. rhamnoides*. No synergic interaction was found between *T. microphasmis* and *C. destructans* in their effect on growth of *H. rhamnoides*. The results of this study support the hypothesis that plant-feeding nematodes, possibly stimulated by increased N availability, accelerate the decline and succession of *H. rhamnoides* by reducing root growth and acquisition of phosphate. This deteriorates the competitive ability of this shrub and gives way to other plant species in the vegetation which are less sensitive to root damage by nematodes.

SAMENVATTING

Successie in de vegetatie is een intrigerend natuurlijk proces dat voornamelijk wordt gestuurd door verbreiding van plantesoorten, competitie tussen soorten en bodemontwikkeling. De ontwikkeling van abiotische bodemfactoren omvat ondermeer veranderingen in de zuurgraad (pH) en in de beschikbaarheid van voedingsstoffen. De rol van biotische bodemfactoren (bodemorganismen) is nog weinig onderzocht. Organismen die met de plantewortels samenleven (symbionten), zoals stikstofbinders en mycorrhiza kunnen van nut zijn bij vestiging en overleving van planten op voedselarme standplaatsen. Schadelijke bodemorganismen kunnen de concurrentiekracht van plantesoorten in de vegetatie verminderen en daarmee de weg effenen voor successie. Duinsystemen dienen dikwijls als onderwerp van successieonderzoek, omdat er duidelijke vegetatiepatronen en bodemontwikkelingen te vinden zijn. De topografische opeenvolging van vegetatietypen weerspiegelt hier vaak de tijdreeks van successie. De duindoorn, *Hippophaë rhamnoides* L. ssp. *rhamnoides*, is een dominante soort in de primaire successie van de duinvegetatie in Nederland. Deze soort koloniseert mobiele helmduinen en jonge duinvalleien en degenereert na verloop van decennia, waarbij de vegetatie overgaat in gemengd struweel of duingrasland. De aftakeling van duindoornstruweel is in verband gebracht met het optreden van plantparasitaire nematoden en andere (onbekende) bodemorganismen. De aard en effecten van deze organismen vroegen om nadere studie.

In het hier gepresenteerde onderzoek werd een inventarisatie gemaakt van nematoden, VA-mycorrhiza en stikstofknolletjes (*Frankia-actinorhiza*) in en rond de wortels van *H. rhamnoides* in verschillende stadia van successie in Nederlandse duingebieden, in relatie met abiotische bodemfactoren en duindoornvitaliteit. Ordening van plekken op grond van de nematodenfauna, plantfactoren en abiotische bodemfactoren gaf aan dat er een successie in de nematodenfauna optreedt die gelijke tred houdt met de ontwikkeling van bodem en vegetatie. De gemiddelde aftakeling van *H. rhamnoides* verliep in de richting van toenemende concentraties van stikstof en organische stof in de bodem en een toenemende infectie van wortels met mycorrhizaschimmels. Het gewicht van nieuwgevormde duindoornstwijgen was gecorreleerd met de fosfaatconcentratie in de blaadjes. De meeste locaties die zich recent hadden ontwikkeld vanuit een verstoorde situatie hadden dezelfde karakteristieken als plekken waar voor het eerst een duindoornvegetatie voorkwam. De 'Maturity index' van de obligaat plantparasitaire nematodenfauna en het aantal soorten daarin nam toe in de successie.

De nematoden *Tylenchorhynchus microphasmis* Loof en *Mesocriconema xenoplax* (Raski) kwamen in alle stadia algemeen voor, terwijl *Longidorus dunensis* Brinkman *et al.* alleen onder degenererende struwelen werd gevonden. Naarmate de aftakeling vorderde kwamen hogere concentraties van plantparasitaire nematoden voor rond de wortels van duindoorn, terwijl de concentraties in de 'bulk'-grond nagenoeg constant bleven.

De resultaten van potexperimenten met selectieve chemische onderdrukking van groepen bodemorganismen gaven aan dat plantparasitaire nematoden, naast hun eigen schadelijkheid, de plant vatbaarder kunnen maken voor pathogene bodemschimmels. In veldgrond werd de wortelknolvorming (*Frankia*-nodulatie) bevorderd door het fungicide benomyl, maar in gesteriliseerde grond was het effect omgekeerd. De belangrijkste schimmels die werden geïsoleerd uit wortels en van het worteloppervlak in veldgrond waren *Cylindrocarpon destructans* Zinns. Scholten en *Fusarium oxysporum* Schlecht emend. Sny. & Hansen. Beide werden sterk onderdrukt door benomyl en lijken dus potentiële antagonisten van *Frankia*. Bij inoculatie in steriel zand met *H. rhamnoides* zonder *Frankia* bleek slechts één van de *C. destructans* isolaten enigzins schadelijk. Een toename van schimmelinfectie in wortels door het nematodenremmende oxamyl was niet nadelig voor de nodulatie, vermoedelijk door de gelijktijdige onderdrukking van plantparasitaire nematoden. Deze resultaten geven aan dat plantparasitaire nematoden, eventueel in combinatie met bodemschimmels, in staat zijn de vitaliteit van *H. rhamnoides* te verminderen. De invloed van verschillende dichtheden van het aaltje *T. microphasmis*, alleen of in combinatie met de stikstofbindende symbiont *Frankia* of de schimmel *C. destructans*, werd nagegaan in potexperimenten met gecontroleerde vochtvoorziening. Een installatie voor automatische registratie en regulatie van de vochtigheid in potten en beeldanalysemethoden voor het meten van wortellengte en -vertakking werden hiervoor ontwikkeld. Uit deze potproeven bleek een remmend effect van *T. microphasmis* op de groei van *H. rhamnoides* en op de opname van bepaalde nutriënten, behalve in zeer droge grond. De wortellengte werd sterker verlaagd dan het gewicht van de spruit en was positief gecorreleerd met de fosfaatconcentratie in de spruit. Het spruitgewicht was positief gecorreleerd met het fosfaatgehalte en niet, of negatief, met gehalten van andere nutriënten. Onderdrukking van de wortelgroei door nematoden lijkt daarom het belangrijkste mechanisme. Hoewel wortelknolvorming en stikstofbinding door *Frankia* eveneens werden onderdrukt is dit niet de hoofdoorzaak van de groeiremming. *C. destructans* had geen invloed op de nodulatie en was slechts zwak pathogeen. Er werd geen synergistische interactie gevonden tussen *T. microphasmis* en *C. destructans* in

hun effect op de groei van *H. rhamnoides*. De resultaten van dit promotieonderzoek ondersteunen de stelling dat plantparasitaire nematoden, mogelijk gestimuleerd door verhoogde stikstofbeschikbaarheid, de aftakeling en successie van de duindoorn bevorderen via de onderdrukking van wortelgroei en fosfaatopname. Hierdoor wordt de concurrentiekracht van deze soort ondermijnd en ontstaan er mogelijkheden voor andere plantesoorten die minder last hebben van wortelschade door nematoden.

CURRICULUM VITAE

Frans Zoon werd op 8 april 1959 geboren te Heiloo. In 1977 behaalde hij het diploma Gymnasium B aan het Petrus Canisius College te Alkmaar en begon hij met de studie Planteziektenkunde aan de toenmalige Landbouwhogeschool te Wageningen. Hij behaalde het kandidaatsexamen in 1981 met een oecologisch getint vakkenpakket. In de doctoraalfase deed hij onderzoek bij de vakgroepen Fytopathologie en Nematologie aan eiparasieten van het bietecysteaaltje, bij Theoretische Teeltkunde aan modellering van penetratie en schade door ditzelfde aaltje en bij Tuinbouwplantenteelt aan aspecten van kunstmatige vermeerdering van bloembollen. Stages werden vervuld aan de Universiteit van Guelph (Canada) met onderzoek aan *Botrytis* op aardbei en bij het PAGV te Lelystad met onderzoek aan de epidemiologie en bestrijding van voetziekten en valse meeldauw in erwten. Het ingenieursdiploma behaalde hij in 1985. Van 1985 tot 1989 werkte hij bij het Instituut voor Oecologisch Onderzoek (IOO-KNAW) te Oostvoorne en Heteren aan een promotie-onderzoek naar de rol van bodemorganismen in de successie van de duindoorn. De resultaten hiervan zijn weergegeven in dit proefschrift. Voordat het zover kwam voerde hij op het Instituut voor Bodemvruchtbaarheid (IB-DLO) te Wageningen van november 1989 tot maart 1990 een programmeringsstudie uit naar de perspectieven voor mycorrhiza-onderzoek en inventariseerde hij als freelancer de bodemmesofauna van natuurterreinen. Sinds september 1990 werkt hij bij het Instituut voor Planteziektenkundig Onderzoek (IPO-DLO) te Wageningen aan de oecologie van virus-overdragende nematoden, gericht op vermindering van chemische grondontsmetting in de bloembollenteelt.