SOIL-BORNE PLANT PATHOGENS OF *AMMOPHILA ARENARIA* IN COASTAL FOREDUNES

P.C.E.M. de Rooij-van der Goes

Promotoren: dr. L. Brussaard
hoogleraar in de bodembiologie

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

Co-promotor: dr. ir. W.H. van der Putten
senior onderzoeker NIOO-CTO

P.C.E.M. de Rooij-van der Goes

SOIL-BORNE PLANT PATHOGENS OF *AMMOPHILA ARENARIA* IN COASTAL FOREDUNES

Proefschrift
ter verkrijging van de graad van doctor
in de landbouw- en milieuwetenschappen
op gezag van rector magnificus
dr. C.M. Karssen
in het openbaar te verdedigen
op dinsdag 6 februari 1996
des namiddags te vier uur in de Aula
van de Landbouwuniversiteit te Wageningen

ISBN 921952

CIP-DATA KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Rooij-van der Goes, P.C.E.M., de

Soil-borne plant pathogens of *Ammophila arenaria* in coastal foredunes / P.C.E.M. de Rooij-van der Goes. - [S.I. : s.n.]
Thesis Landbouw Universiteit Wageningen. - With ref. - With summary in Dutch.
ISBN 90-5485-478-2
Subject headings: soil biology / *Ammophila arenaria* / plant-pathogenic organisms.

omslagontwerp: Jelle de Rooij

This thesis contains results of a research project of the Netherlands Institute of Ecology, Centre of Terrestrial Ecology, P.O. Box 40, 6666 ZG Heteren, The Netherlands. The study was financed by the Hydraulic Engineering Division of the Ministry of Transport, Public Works and Water Management (Rijkswaterstaat, Dienst Weg- en Waterbouwkunde, Delft) and the Water- and Civil Boards concerned with coastal management.

Stellingen

1 De vitaliteit van helm na zandaanstuiving wordt niet alleen bepaald door fysiologische veranderingen in het planteweefsel, maar ook doordat planten ontsnappen aan infecties van bodemorganismen.
Van der Putten et al. 1988. Oecologia 76: 313-320; Yuan et al. 1993. Functional Ecology 7: 676-682; dit proefschrift.

2 De hoeveelheid instuivend zand en het moment waarop dit gebeurt zijn cruciale factoren voor de ontsnapping van helm aan infecties door bodemorganismen.
Dit proefschrift.

3 Door het beter begrijpen van de groei van helmplanten in relatie tot aantastingen door bodemorganismen kunnen effectievere beheersmaatregelen voor de instandhouding van de begroeiing van zeewerende duinen worden ontwikkeld.
Dit proefschrift.

4 De ontwikkeling van stuifkuilen resulteert in een verbetering van de vitaliteit van gedegenereerde helm, een verbetering van de zeewerende funktie en een meer natuurlijk uiterlijk van de Nederlandse duinen. Een dergelijke invulling van het begrip "dynamisch zeereepbeheer" kan polarisering van duinbeheerders en natuurbeheerders voorkomen.
Dit proefschrift.

5 De angst voor overmatige verstuivingen in stuifkuilen tijdens zuidwester stormen is niet gegrond.
Dit proefschrift.

6 In landbouwkundig opzicht onbeduidende aantallen plantenparasitaire nematoden kunnen in natuurlijke vegetaties aanzienlijke gevolgen hebben.
Dit proefschrift.

7 De rol van mycorrhiza's in de fosfaatvoorziening van duinplanten wordt overschat.
Allen, M.F. 1991, The Ecology of Mycorrhizae, Cambridge University Press, Cambridge.

8 Schimmels zijn te mooi om door de gootsteen te spoelen.
Loesje 1994.

9 Ons geheugen vaccineert ons niet tegen fouten die we in de toekomst zullen maken.
Mario Vargas Llosa, 1995.

10 Ons verwachtingspatroon van natuurwetten ligt niet in de wetmatigheden zelf maar in ons bewustzijn besloten.
David Hume, filosoof, 1711-1776.

11 Statistiek is ooit begonnen als afleiding en vrije tijdsbesteding.
Oosterhuis, T. 1991, De Pijl van Zeno, Fontein, Baarn.

12 Een mooie kaft is leuk, maar een goede band is noodzakelijk.

Stellingen behorende bij het proefschrift van Petra de Rooij-van der Goes: 'Soil-borne plant pathogens of *Ammophila arenaria* in coastal foredunes.'
Wageningen, 6 februari 1996.

CONTENTS

Abstract		7
1.	General introduction.	9
2.	Analysis of nematodes and soil-borne fungi from *Ammophila arenaria* (Marram grass) in Dutch coastal foredunes by multivariate techniques. *with*: W.H. van der Putten & C. van Dijk European Journal of Plant Pathology 101 (1995): 149-162	15
3.	The role of plant-parasitic nematodes and soil-borne fungi in the decline of *Ammophila arenaria* (L.) Link. New Phytologist 129 (1995): 661-669	39
4.	Effects of sand deposition on the interaction between *Ammophila arenaria*, plant-parasitic nematodes and pathogenic fungi. *with*: W.H. van der Putten & B.A.M. Peters Canadian Journal of Botany 73 (1995): 1141-1150	57
5	Vertical migration of nematodes and soil-borne fungi to developing roots of *Ammophila arenaria* (L.) Link after sand accretion. *with*: B.A.M. Peters & W.H. van der Putten Submitted to Oikos	77
6.	The effects of sand movement by wind in coastal foredunes on nematodes and soil-borne fungi. *with*: C. van Dijk, W.H. van der Putten & P.D. Jungerius	97
7.	General discussion.	117
References.		125
Summary.		133
Samenvatting.		137
Nawoord.		141
Curriculum vitae.		143

ABSTRACT

De Rooij-van der Goes, P.C.E.M. 1996. Soil-borne plant pathogens of *Ammophila arenaria* in coastal foredunes. Ph.D Thesis, Wageningen Agricultural University, Wageningen, The Netherlands. 143 pp.

Ammophila arenaria (Marram grass) is the most dominant sand-fixing plant species in the Dutch coastal foredunes. This species has a natural ability to emerge from being buried and is therefore used to stabilize the coastal foredunes. On seaward slopes where plants are buried regularly with windblown sand, plants retain their vigour, but start to degenerate when sand accumulation diminishes. One of the factors that may cause degeneration at stabilized sites is the infection of roots by nematodes and fungi. Burial by fresh windblown sand may enable the plants to overcome these harmful soil organisms. In the present study, the nature of the soil-borne disease and its relationship with sand deposition is investigated. In a field survey, a wide range of nematodes and fungi were isolated from the root zone of *A. arenaria*. Subsequent inoculation-experiments showed that adding single fungal species did not reduce the growth of seedlings whereas combining all commonly found fungi together did, thus indicating synergistic effects. Adding 80 times more individuals of the semi-endoparasitic nematode *Telotylenchus ventralis* than present in natural soil reduced the growth of seedlings to the same extent as in natural soil. Several groups of soil organisms, especially those groups that include plant-parasitic nematodes, have shown to affect the growth of *A. arenaria*. Burial with unsterilized root zone sand was less beneficial for plant growth than burial with sterilized or beach sand. This implies that plants are able to escape infection by soil organisms through upward growth following sand accumulation. Fungi colonized the freshly deposited layer of sand faster than plant-parasitic nematodes. Furthermore, it could be shown that in windblown soil numbers of fungal propagules and nematodes were reduced. Rejuvenation of stands along the accumulating edges of blowouts can, therefore, be explained by the reduced inoculum pressure of plant-pathogenic organisms in the deposited soil. The amount of sand and the time when sand is deposited are important components in the chances of *A. arenaria* to escape infection by soil organisms.

Keywords: *Ammophila arenaria*, plant-parasitic nematodes, soil-borne pathogens, sand dune vegetation, sand movement, blowouts, synergism, upward growth, clonal growth, migration, *Telotylenchus ventralis*

CHAPTER 1
GENERAL INTRODUCTION

Coastal foredunes: function, morphogenesis and stabilization

In a country below sea-level such as the Netherlands, the value of the dunes as defence against the sea is evident. In addition to this function, dunes have both ecological and recreational values (Boorman 1978, Anonymous 1990). The main prerequisite for the function as sea-defence is a high continuous dune ridge with an adequate degree of stability. Any factor that could affect its stability is a threat to its value as a sea-wall (Boorman 1978, Anonymous 1990, Carter et al. 1990). Generally, dune sand originates from intertidal beach sand that has been blown inland (Boorman 1978, Jungerius and Van der Meulen 1988, Pye 1990). The morphology of the coastal dunes, including both the shape of the individual dunes and the spatial arrangement of dune complexes, is governed by four main factors: beach morphology and shoreline dynamics, which regulate the amount and rate of sand supply, wind characteristics, vegetation cover and human activities (Pye 1990). Coasts are prograding when they receive abundant sand supply or are eroding when sand supply is limited. In the Netherlands, most coastal foredunes are stable or regressive (Anonymous 1990, Arens 1994).

After sand has settled, the initial stabilization of windblown sand is usually due to the action of soil organisms (Webley et al. 1952) that aggregate sand grains. Fungi are the most important in this respect (Webley et al. 1952, Forster 1979), but bacteria and algae may also play a role (Pluis and De Winder 1994). The presence of such aggregates reduces wind erosion, increases soil moisture levels and increases the nutrient status of the soil (Webley et al. 1952, Forster 1979). Subsequently, higher plants establish that further stabilize the soil surface.

The vegetation reduces wind speed allowing sand particles to settle, resulting in sand accumulation. For dune managers, the major advantage of using dune vegetation above fences, which can also be used to accumulate sand, is that dune plants may emerge from sand burial so that the developing dune remains to be covered by vegetation (Boorman 1978, Maun and Lapierre 1984, Maun and Baye 1988). The two species *Ammophila arenaria* (L.) Link (Marram grass) and *A. breviligulata* Fern. (American Beach grass) are among the most important sand-fixing and dune-forming species in the world (Knutson 1978, Huiskes 1979, Marshall 1965, Willis 1989, Disraeli 1984, Maun and Baye 1988, Baye 1990). *Ammophila* species readily regenerate from rhizome fragments (Gemmel et al.

1953, Maun and Baye 1988, Van der Putten 1990) so that eroded *Ammophila* dune faces may become recolonized naturally as soon as erosion is stopped. In coastal dune management, it is common practice to plant *A. arenaria* in order to control sand erosion of the foredunes.

Sand accumulation and the ecological response of Ammophila arenaria

It is well documented that *Ammophila* species only can thrive in a situation where there is continuous accretion of fresh windblown sand. When sand accretion ceases, plants will lose their vigour (Marshall 1965, Hope-Simpson and Jefferies 1966, Huiskes 1979, Willis 1989, Disraeli 1984, Maun and Baye 1989, Maun and Lapierre 1984, Baye 1990). If sand is deposited in the vegetation, *A. arenaria* emerges by stem-elongation and node formation (Huiskes 1979, Baye 1990). In the fresh sand layers, new white healthy roots are formed. But when no sand is accumulated, new roots are formed in the layer of sand that has already been colonized by old ones. These new roots remain short, are dark coloured, deformed and contain little or no root hairs. Degenerated stands are characterized above ground by shorter culms and increasing numbers of dead shoots (Huiskes 1979, Maun and Baye 1989, Van der Putten et al. 1989). Coastal foredunes with degenerated stands of *Ammophila* spp. are susceptible to erosion which may threaten their role as a defence against the sea.

Many explanations have been suggested for the widely-reported decline in vigour of *A. arenaria* and *A. breviligulata* (generally summarized by Marshall (1965) and Laing (1967)). According to one hypothesis, the decline is caused by nutrient deficiency, and soil accretion is considered to provide nutrients. However, the plant vigour is less stimulated by fertilizers than by burial with sand, in spite of the low amounts of nutrients in the new sand layers (Salisbury 1952, Willis 1965, 1989). According to a second hypothesis, *Ammophila* species are outcompeted after dune stabilization (Huiskes 1979). However, degeneration also occurs in the absence of competing plant species (Hope-Simpson and Jefferies 1966, Disraeli 1984). According to a third hypothesis, nodal root production and the uptake of nutrients and water is inhibited when dunes become stabilized. The efficiency of old roots declines with age and in stabilized dunes old roots cannot be replaced by new ones due to morphological constraints (Marshall 1965, Willis 1965, Wallén 1980). Formation of new roots was found to be inhibited when dead leaves accumulate at the base of the shoots (Laing 1967). However, the positive response of shoots to sand accretion can not be explained by the replacement of degenerating roots or by the impaired uptake capacity of the root system, since vigorous

growth was evident directly after emergence from deep burial even before new nodal root systems had developed (Baye 1990). Recently, these findings led to a fourth hypothesis: when plants are buried by sand, their physiological activity increases (Yuan et al. 1993), which, in turn, leads to vigorous growth (Baye 1990). Finally, a fifth hypothesis has been put forward by Van der Putten et al. (1988), who concluded that the decline of *A. arenaria* may be due to the occurrence of harmful soil organisms in its root zone. In beach sand, the natural source of the deposited sand, no harmful soil organisms were found (Van der Putten and Troelstra 1990). Nevertheless, within one growing season, harmful soil organisms could be detected in the newly formed root layer. Apparently by the formation of new roots following emergence of the plants from burial with beach sand, the plants may temporarily escape from harmful soil organisms in their root zone by upward growth (Van der Putten et al. 1989). When no sand accumulates, new roots become immediately infected by the soil organisms, so that plant growth becomes inhibited. In the inner dunes degenerated stands regain their vigour when they are subjected to burial by fresh windblown sand, as occurs along the accumulating edges of blowouts (Van Dieren 1934, Willis 1989). In these cases, the windblown sand does not originate from the beach, but from the soil profile of existing dunes. This sand has already been colonized by *A. arenaria* roots. It is not understood why *A. arenaria* under these conditions regains its vigour, as this sand once or still contained harmful soil organisms prior to becoming windborne.

The nature of the harmful soil organisms in the root-zone of *A. arenaria* has been studied by experiments with nematicides and fungicides. Both nematodes and fungi may be involved in the degeneration process (Van der Putten et al. 1990). A large number of fungi (Brown 1958, Dennis 1983, Moreau and Moreau 1941) and nematodes (Bussau 1990, Yeates 1968, Zoon et al. 1993) has been isolated from coastal foredunes with a vegetation of *A. arenaria*. Thus far, it has not been established which soil organisms contribute to the pathosystem.

Pathogenic soil-organisms in natural ecosystems

Plant roots are associated with different types of soil organisms, such as nematodes, fungi, bacteria and insects. Associations with soil organisms can be either beneficial or harmful to plants. The fitness-reducing effects of soil organisms on wild plants have, untill now, not received much attention (Newman 1978, Sewell 1981, Alexander 1990, Harper 1990). Although the presence of soil-borne diseases in natural vegetations is rather inconspicuous, their impact on plant

populations is not (Sewell 1981, Bever 1994, Dobson and Crawley 1994). Infections by soil-borne diseases may affect the rate of growth of plants and their ability to withstand competition (Van der Putten et al. 1993, Bever 1994, Dobson and Crawley 1994). The extent to which soil-borne diseases reduce the plants' fitness and their interaction with other biotic and abiotic factors are, however, poorly understood (Alexander 1990).

In natural ecosystems, soil-borne diseases can, occasionally, be lethal to plants (e.g. Augspurger 1988), but more often they are important regulators of plant vigour (Sewell 1981, Alexander 1990). By comparing plant growth in sterilized and unsterilized soil (Van der Putten et al. 1988, 1993, Bever 1994) or by comparing plant growth when groups of soil-organisms were eliminated using biocides (Brown 1990, Van der Putten et al. 1990), the detrimental role of soil organisms could be demonstrated. The actual species reducing growth of *A. arenaria* have, thus far, not been identified. Although the decline of *A. breviligulata* is most likely caused by plant-parasitic nematodes (Seliskar and Huettel 1993), the pathogenicity of the various species of nematodes has not been established. The sole example in which soil organisms causing detrimental effects have been specified concerns species involved in the decline of Sea buckthorn (*Hippophaë rhamnoides* L.), particularly the plant-parasitic nematodes *Tylenchorhynchus microphasmis* and *Longidorus dunensis* (Oremus 1982, Maas et al. 1983, Zoon et al. 1993, Zoon 1995). Additionally, also abiotic soil factors, in case of *H. rhamnoides* the phosphate availability, may contribute to plant degeneration (Zoon 1995). Likewise, the decline of *A. arenaria* (Van der Putten et al. 1988, 1989, 1990) and *A. breviligulata* (Seliskar and Huettel 1993) seemed to be caused by a combination of biotic and abiotic soil factors. Both biotic soil factors, i.e. the involvement of harmful soil organisms, and abiotic soil factors, i.e. a reduced supply of windblown sand, are within the scope of this thesis.

Objective of the present study

The objective of this thesis is to elucidate which nematodes and fungi are involved in the disease complex causing decline of *A. arenaria* in Dutch coastal foredunes. Furthermore, the relationship between plant vigour, sand accretion and pathogenic soil organisms is studied. The response of plants to sand deposition in relation to soil-borne diseases is studied of plants that are buried with windblown beach sand and of plants along the accumulation edges of blowouts where they are buried with sand from existing dunes. The implications of the presence of soil-borne diseases for management practices are discussed.

Outline of the thesis

A survey was carried out at various locations in the coastal foredunes to identify potentially harmful nematodes and soil-fungi in the root-zone of *A. arenaria* (chapter 2). The data were analysed by TWINSPAN and CANOCO to generate groups of simultanously occurring fungi and nematodes.

In chapter 3, experiments are described in which these isolated fungi and some nematode species were added singly or in groups, assembled after cluster analysis and ordination, to healthy seedlings of *A. arenaria* in order to establish their pathogenicity.

A degenerated stand of *A. arenaria* was subjected to burial with 20 cm of beach sand, sterilized root zone sand or non-sterile root zone sand. Growth of these plants was compared with growth of non-buried plants. In addition, potted plants were buried with sterilized root zone soil and growth was compared with that of plants buried with non-sterile root zone soil. These experiments, described in chapter 4, were done in order to verify the hypothesis that *A. arenaria* plants escape infection by soil organisms by means of upward growth after sand deposition. Non-sterile sand was used to test whether burial in itself had a positive effect on plant growth.

Migration of soil organisms towards the newly formed roots after the plants had been buried was studied during the elongation phase of plant growth. Plants were grown in non-sterile soil and buried with sterilized root-zone soil or sand from the beach. The plants were harvested at intervals throughout the growing season. The results are presented in chapter 5.

Rejuvenation of *A. arenaria* occurs along the accumulation edges of blowouts, suggesting that sand relatively free of pathogens and parasites is deposited. In chapter 6, the potentially reducing effects of wind-driven sand movement on numbers of soil organisms is experimentally tested in two blowout areas in a windtunnel as well as by experimentally stirring of soil. The pathogenicity of soil-organisms in nonblown and windblown sand was quantified in bioassays.

In the general discussion, the results are reviewed with respect to the vigorous response of *A. arenaria* to sand accretion. The impact of harmful soil organisms on the ecology of *A. arenaria* and vegetation succession is discussed.

ANALYSIS OF NEMATODES AND SOIL-BORNE FUNGI FROM *AMMOPHILA ARENARIA* (MARRAM GRASS) IN DUTCH COASTAL FOREDUNES BY MULTIVARIATE TECHNIQUES

P.C.E.M. de Rooij van der Goes, W.H. van der Putten and C. van Dijk

European Journal of Plant Pathology 101 (1995): 149-162.

CHAPTER 2
ANALYSIS OF NEMATODES AND SOIL-BORNE FUNGI FROM *AMMOPHILA ARENARIA* (MARRAM GRASS) IN DUTCH COASTAL FOREDUNES BY MULTIVARIATE TECHNIQUES

SUMMARY

A survey was carried out at nine locations in the Dutch coastal foredunes to identify the species of soil borne fungi and nematodes associated with *Ammophila arenaria* (Marram grass). *Ammophila arenaria* is a sand binding grass that is very important for the stabilization of coastal foredunes. Degeneration of the plants occurs at stabilized sites and is supposed to be caused by a combination of soil-borne fungi and nematodes.

Canonical correspondence analysis (CCA) and two-way indicator species analysis (TWINSPAN) were used to examine which fungal and nematode species usually coexist in the rhizosphere of vigorous and early declining stands of *A. arenaria*. In total, 47 species of fungi and 10 genera of plant-parasitic nematodes were found. According to CCA, the community of soil organisms of stands that were more than 10 years old was significantly different from recently established stands of 3 years old. Also, the community of soil organisms isolated from calcareous locations differed significantly from that of lime-poor locations. No relationship between the vigour of the plants (vigorous vs. early declining) and the soil-borne species composition was found, although in roots of vigorous stands, the number of nematodes was higher than that of early declining stands. A relatively large group of soil organisms occurred generally. This group possibly contains an ubiquitous pathocomplex that cause the growth reducing effects of biotic origin which generally occur in *A. arenaria*. Analysis of this group of nematodes and fungi by TWINSPAN resulted in 9 different combinations of concurring soil organisms of which 5 combinations were present at all investigated locations. Two of the latter combinations contained both nematodes and fungi. The first contained three endoparasitic nematodes (*Meloidogyne maritima*, *Heterodera* spp. and *Pratylenchus* sp.) that concurred with the fungus *Mucor hiemalis*. The second group contained *Heterodera* spp., *Telotylenchus ventralis*, *Filenchus* sp. together with the potentially plant-pathogenic fungi *Microdochium bolleyi* and *Fusarium culmorum*, as well as the fungi *Mortierella* sp. and *Trichoderma harzianum*, all in

relatively high numbers.
It is concluded that both CCA and TWINSPAN are valuable exploratory techniques, especially when used in combination, to detect possible combinations of soil organisms which may be involved in the degeneration of *A. arenaria*. Further identifications of harmful organisms should be obtained from experiments.

INTRODUCTION

Soil-borne diseases appear to be common in vegetation succession of coastal foredunes. For instance, the nematodes *Longidorus dunensis* and *Tylenchorhynchus microphasmis* play an important role in the degeneration of *Hippophaë rhamnoides* (Sea buckthorn), a shrub species that occurs in a later successional stage in coastal foredunes (Maas et al. 1983, Zoon et al. 1993). Such soil-borne diseases are supposed to have a high degree of specificity (Van der Putten et al. 1993). It is, however, as yet unknown which soil organisms are involved in the decline of the different plant species that succeed each other.
Ammophila arenaria (L.) Link (Marram grass) is a sand-binding grass that naturally dominates European coastal foredunes (Huiskes 1979). The vigour of *A. arenaria* is highest on sea-facing slopes where it is buried regularly by fresh windblown sand. When sand accumulation decreases, the plants degenerate (Huiskes 1979, Willis 1989). The above-ground symptoms are a decline in flowering, a less dense vegetation with shorter culms and increasing amounts of dead shoots and leaves. The newly formed roots remain short, lack root hairs and are deformed (Eldred and Maun 1982, Willis 1989, Van der Putten et al. 1990).
In North-America and Canada, *Ammophila breviligulata* Fern., American Beach grass, occurs under conditions comparable to those of *A. arenaria* in Europe. Both plant species grow vigorously in response to sand deposition and both plants degenerate when sand accumulation diminishes (Eldred and Maun 1982, Maun and Baye 1988, Baye 1990).
Van der Putten et al. (1989) showed that degeneration of *A. arenaria* is strongly correlated with the presence of harmful soil organisms in its root zone. The effects of these soil-organisms were already present in vigorous *A. arenaria* stands. Upward growth upon sand accumulation is supposed to allow the plants to escape from harmful soil organisms. This upward growth has to happen each year, as newly formed roots are colonized by the soil organisms within one growing season (Van der Putten et al. 1989).

A recent study showed that several nematode species may be involved in the decline of *A. breviligulata* (Seliskar and Huettel 1993). As with *A. arenaria* (Van der Putten and Troelstra 1990), only low and variable numbers of nematodes were present in *A. breviligulata*'s root zone. Soil treatments with nematicides enhanced the growth of *A. arenaria* seedlings in spite of the low numbers of nematodes that were present. Soil treatments with the fungicide benomyl also resulted in increased plant growth, but this may have been due to a nematicidal effect of the fungicide applied. Nevertheless, it was concluded that, apart from nematodes, fungi may also be involved in the degeneration of *A. arenaria* (Van der Putten et al. 1990). In the present study, the composition of the communities of soil organisms (nematodes and fungi) in the rhizosphere of vigorous and early declining *A. arenaria* stands was investigated. Furthermore, the community of soil organisms of *A. arenaria*, previously planted in 'virgin' dredged sea sand, was compared to that of established dunes. Samples were taken from May till September to study potential changes in the community of soil organisms during the growing season. The concurrence of fungi and nematodes and the degeneration of *A. arenaria* was analysed using ordination techniques (Canonical Correspondence Analysis; CCA) and clustering (Two-Way INdicator Species ANalysis; TWINSPAN). The combinations of soil-borne fungi and (plant-parasitic) nematodes thus generated were used to compare calcareous versus lime-poor foredunes and recently established versus existing stands of *A. arenaria*. It is discussed how these results may be used for further studies on the identification of the species involved in the degeneration of *A. arenaria*.

MATERIALS AND METHODS

Study sites

Samples were collected from vigorous and early declining stands of *A. arenaria* at nine locations along the Dutch coastal foredunes: Voorne (5 locations), Goeree and Schouwen, which are located in the calcareous dune area in the southern part of the Netherlands, and Callantsoog and Texel, located in the north western lime-poor area (Rozema et al. 1985) (Table 2.1). In 1986, the locations 1, 2, 3 and 5 had been raised artificially with dredged sea sand that did not contain the specific rhizosphere organisms of *A. arenaria* (Van der Putten 1990). Early 1987, the sand depot was reshaped and stands of *A. arenaria* were established from culms (location 1), rhizomes (location 2), seeds (location 3) or a combination of culms

and rhizomes (location 5). The different methods are described by Van der Putten (1990). Voorne location 4 is a foredune that existed already for more than 10 years. At all the other locations, the examined stands were more than 10 years old and were regarded as naturally developing foredunes (Table 2.1). Vigorous *A. arenaria* stands were subject to annual burial by 10 to 30 cm of windblown sand from the beach. Early declining stands were those stands where sand accumulation was less than 5 cm at least during the last year.

General survey

In March 1990, soil samples were collected from the foredunes of Voorne (locations 1 to 4; Table 2.1), Goeree, Schouwen, Callantsoog and Texel (Table 2.1). At each location, two sites of 250 m long and 10 m wide (parallel with the coastline) were chosen: one in vigorous and one in early declining stands of *A. arenaria*. At each site, 12 random samples with a total of 20 kg were collected with a small shovel. The samples were taken close to tussocks of *A. arenaria* from the layer of sand between 5 to 40 cm below the sand surface containing roots. Per site, the 12 samples were sieved through a sieve with a mesh size of 1.5 cm and mixed gently. A subsample of 500 ml of soil, as well as 20 g roots were taken from each composit sample for the analysis of nematodes and fungi.

Table 2.1. Characteristics of the locations used to study nematodes and fungi possibly involved in the degeneration of *Ammophila arenaria*. The vegetation of *A. arenaria* was established 3 years ago starting from culms, rhizomes, seeds or culms and rhizomes or was a vegetation that existed for more than 10 years. Lime-content was high when the $CaCO_3$ content was up to 10% and low when less than 0.01% (Rozema et al. 1985). NL = Northern latitude, EL = Eastern longitude. At each location, samples were taken from vigorous and early declining stands.

Location	Sample-numbers	Establishment	Lime-content	Coordinates (NL*EL)
1 Voorne	1, 2	recent, culms	high	51°52' 4°04'
2 Voorne	3, 4	recent, rhizomes	high	idem
3 Voorne	5, 6	recent, seeds	high	idem
4 Voorne	7, 8	existing vegetation	high	idem
5 Voorne		recent, culms + rhizomes	high	idem
6 Goeree	9, 10	existing vegetation	high	51°45' 3°50'
7 Schouwen	11, 12	existing vegetation	high	51°35' 3°32'
8 Callantsoog	13, 14	existing vegetation	low	52°50' 4°42'
9 Texel	15, 16	existing vegetation	low	53°07' 4°45'

Seasonal and within-location variation
Seasonal sampling was conducted in May, June and September 1990 at Voorne-locations 3, 4 and 5 (Table 2.1). For logistic reasons, only vigorous stands of *A. arenaria* were sampled. Four composit samples, each made up of 12 random samples, were collected from each location in order to study the variation throughout the season. For the analysis of within-location variation only the samples of May were considered. Each of the 4 composit samples was sieved and homogenized. Of each composit sample, both roots (± 20 g) and soil (± 500 ml) were used for identification and quantification of fungi and nematodes.

Soil organisms
Free-living nematodes were isolated from a subsample of 300 ml by elutriation (Oostenbrink 1960). Endoparasitic nematodes were isolated by cutting the collected roots into 1 cm pieces and extracting these in Baermann-funnels ('s Jacob and Van Bezooijen 1984). Subsequently, the nematodes were counted and identified to at least genus level according to Bongers (1988).
Soil-fungi were isolated by plating soil dilutions (10^{-2} - 10^{-5}) in triplicate on malt extract agar (20 g malt extract (Oxoid), 3 g peptone (Oxoid), 15 g agar (Merck), 100 ppm validamycine (Solacol, Aagrunol (Gams and Van Laar 1982)) and 50 ppm oxytetracyclin. Root-fungi were isolated from 0.5 cm pieces of root. Per sample, 15 root pieces were washed three times in sterile demineralized water and placed on malt extract agar. After incubating at 23°C for 4 to 7 days, the fungi were subcultured on potato dextrose agar (Oxoid), counted and identified. Nomenclature according to Domsch et al. (1980) was used for the fungi other than *Fusarium* throughout this study. *Fusarium* species were identified according to Nelson et al. (1983).

Statistical analysis
All isolations from roots and soil of fungi and nematodes were analyzed by means of two-way indicator species analysis (TWINSPAN) (Hill 1979) and canonical correspondence analysis (CCA). Both multivariate techniques are described in detail by Jongman et al. (1987).
After identifying the species and assessing their densities, numbers of fungi and nematodes were standardized by dividing the number of a species in a sample (n_i) by the largest number (n_{max}) of that species found in any of the samples according to $100^* n_i/n_{max}$ (Jongman et al. 1987). By this standardization the data set is liberated from variation caused by the intrinsic differences between species of

different taxonomical and functional groups, without introducing negative numbers, since they are not allowed for CCA and TWINSPAN.
With CCA, the samples are arranged according to species composition with environment as constraint in a canonical optimization process (direct gradient analysis). Forcing the theoretical variables to be linear combinations of environmental variables, relative numbers of fungi and nematodes can be related to these variables. In the ordination diagram, samples with similar soil-inhabiting species are close to each other, whereas samples with a different assemblages of soil organisms are far apart. The analyses were performed using the CANOCO programme developed by Ter Braak (1988). The standardized data were sqrt-transformed to obtain non-skewed distributions.
The environmental variables used (presented as zeros and ones) were derived from Table 2.1:

recently established (RE) = 1	versus	existing vegetation (EV) = 0
calcareous (LIM+) = 1	versus	lime-poor (LIM-) = 0
vigorous stands (VIG+) = 1	versus	early declining (VIG-) = 0

Relationships between the composition of the community of soil organisms and environmental data was investigated by using forward selection of environmental variables in CCA. Environmental variables were added as long as the significance level of the Monte Carlo permutation test of the eigenvalue of the first four canonical axes was below 0.05 (Ter Braak 1988).
By TWINSPAN, a two-step analysis of the data is made. First the samples are clustered on the basis of their species composition. To account for differences in abundance of the species, pseudospecies are defined, based on so-called cut-levels for the abundances. We applied cut-levels 0, 5, 10, 20 and 50 to the standardized data ($100*n_i/n_{max}$), resulting in a maximum of 5 pseudospecies per species. Secondly, the species are grouped based on their steadfastness to sample clusters. The samples within each cluster have a comparable combination of soil organisms and can be characterized by the occurrence of species clusters. The TWINSPAN analysis in this study was performed by making a dichotomy of 4 levels without using indicator species (Jongman et al. 1987), because we were not interested in the dominance of species within the clusters. The standardized data ($100* n_i/n_{max}$) of the general survey and the sampling of May were analysed with the 5 cut-levels as presented above.

The data of the seasonal sampling were analyzed with TWINSPAN as presence/-absence data. The latter was chosen instead of the standardized data due to infections of bacteria in the isolations of fungi. Calculations with numbers of fungi would, therefore, not be reliable. Divisions were made when the Eigenvalue was ≥ 0.25.

RESULTS

General survey

In total, 34 species of fungi were isolated from the various samples in the general survey (Table 2.2). Thirteen additional species were found in the seasonal sampling. The genus *Penicillium* was not split-up into species. The genus *Phoma* was represented by *P. exigua* and *P. leveillei*. There seems to be a higher amount of CFU's of fungi (especially *Penicillium*) in samples from existing vegetation compared to those from recently established vegetation (Table 2.2). However, the number of colonies per 15 root pieces (Table 2.2) appears to be rather independent of the age of the vegetation.

Ten genera of nematodes were isolated (Table 2.3). Saprobiotic nematodes consisted of Rhabditida (e.g. *Acrobeles* sp.) and Dorylaimida (e.g. *Eudorylaimus* sp., *Aporcelaimellus* sp., *Mylonchulus* sp.). Approximately 30 plant-parasitic nematodes per 100 ml of soil (bulk density = 1.4 g/cm^3) were extracted from each sample. In existing stands, higher numbers of plant-parasitic nematodes were collected from roots of vigorous *A. arenaria* (about 20 per g fresh root) than from early declining stands (about 6 per g fresh root). This difference was not found in recently established stands.

Statistical analysis of the number of fungal CFU and the number of nematodes in soil could not be done due to the large variation and the relatively low number of samples.

Table 2.2. Colony forming units (CFU) per g dry soil of fungi isolated from soil (average of 3 replicates per sample) and from roots (number of colonies growing from 15 root pieces). The fungi were isolated from the root zone of vigorous (VIG+) and early declining (VIG-) stands of *Ammophila arenaria* on calcareous or lime-poor foredunes. The *A. arenaria* stands which were sampled, were established 3 years ago (recent) or more than 10 years existing. Saprophagous fungi and pathogenic fungi are listed separately according to Domsch et al. (1980).

	Calcareous				Lime-poor	
	Recent		Existing		Existing	
	VIG+	VIG-	VIG+	VIG-	VIG+	VIG-
SOIL						
Saprophagous fungi						
Absidia corymbifera	0	0	0	110	0	20
Acremonium furcatum	70	0	0	0	0	0
A. murorum	0	0	0	0	0	170
A. rutilum	0	10	0	0	0	0
A. strictum	60	0	0	0	0	0
Apiospora montagnei	70	0	0	560	0	330
Aspergillus sydowii	0	0	110	0	0	0
Chrysosporum pannorum	0	0	110	0	0	0
Humicola grisea	0	10	0	0	0	0
Mortierella alpina	0	170	1910	110	0	0
Mucor hiemalis	0	80	1690	0	20	30
Nectria inventa	0	0	0	0	0	330
Penicillium spp.	170	180	1690	14560	10670	14670
Pleospora sp.	0	0	0	330	0	0
Stachybotrys chartarum	0	60	0	110	0	0
Scopulariopsis sp.	30	90	0	0	0	0
Trichoderma harzianum	130	0	110	670	20	1680
Trichothecium roseum	0	0	10	0	0	0
Verticillium lecanii	10	0	0	0	1330	0
Pathogenic fungi						
Alternaria alternata	10	0	0	0	0	0
Arthirinium phaeospermum	0	0	0	0	330	330
Chaetomidium fimeti	10	100	440	220	3670	830
Chaetomium funicola	0	0	0	0	170	0
C. globosum	0	0	0	0	330	0
Fusarium culmorum	20	120	20	330	170	80
F. nivale	0	0	1110	1110	330	0
Harzia acremonioides	30	0	0	0	0	0
Microdochium bolleyi	0	0	110	0	0	0
Phoma spp.	30	0	220	1780	0	0
Pyrenochaeta sp.	90	0	890	440	0	330
Ulocladium sp.	0	0	0	110	0	330
Total	730	820	8420	20440	17040	19130

Table 2.2, continued

	Calcareous Recent		Existing		Lime-poor Existing	
	VIG+	VIG-	VIG+	VIG-	VIG+	VIG-
ROOTS						
Saprophagous fungi						
Acremonium furcatum	0.7	0	0	0.3	0	0
Apiospora montagnei	0	0	0	0.7	5.5	0
Mortierella alpina	+	+	+	+	+	+
Mucor hiemalis	3	5.3	0.3	0	0	0
Penicillium spp.	6.3	3.7	2.3	11.3	6.5	7.5
Plectosphearella cucumerina	0	0	0	0.3	0	0
Trichoderma harzianum	4.7	0.7	4	6	0	1
Pathogenic fungi						
Chaetomidium fimeti	0.7	0	0	0	0	0
Chaetomium globosum	0	0	0	0	1	2.5
Cladosporium cladosporioides	0.7	0	0.7	0	0	0
Fusarium culmorum	0	1	0.7	0	0.5	0.5
F. chlamydosporium	0	0	0	0.3	0	0
F. equiseti	0.3	5	0	0	0	0
Microdochium bolleyi	2.3	5.7	0.3	0.7	2.5	1
Phoma spp.	0	0	2	0.3	0	0
Ulocladium sp.	0.3	0	1	1.7	0	2.5
Mean number of colonies per 0.5 cm root	1.3	1.4	0.8	1.4	1.1	1.0

Table 2.3. Nematodes isolated from soil (number per 100 ml soil) and from roots (number per g fresh roots. The nematodes were isolated from the root zone of vigorous (VIG+) and early declining (VIG-) stands of *Ammophila arenaria* at calcareous and lime-poor foredunes. The *A. arenaria* stands which were sampled, were established 3 years ago (recent) or more than 10 years.

	Calcareous				Lime-poor	
	Recent		Existing		Existing	
	VIG+	VIG-	VIG+	VIG-	VIG+	VIG-
SOIL						
Pratylenchus sp.	3.3	3.9	4.1	0	10.7	1.5
Paratylenchus sp.	2.1	3.1	1	1	1.5	0
Rotylenchus goodeyi	0	0	0	0	3.1	1.5
Helicotylenchus sp.	0	0	0	0	0	4.6
Filenchus sp.	2.9	5.1	10.2	11.2	7.6	6.1
Heterodera juveniles	2.1	3.6	6.1	1	0	0
Meloidogyne juveniles	3.9	4.5	14.3	4.1	0	1.5
Heteroderidae males	0	0.1	2	0	0	1.5
Aphelenchus sp.	1.3	0.5	1	2	0	0
Telotylenchus ventralis	9.6	12	4.1	3.1	0	0
Total plant-parasites	25.2	32.8	42.8	22.4	22.9	16.7
Saprobiotic nematodes	239.6	362.7	394.3	354.5	414.2	507.4
ROOTS						
Pratylenchus sp.	0.25	0.76	4.32	0.42	12.68	0.85
Paratylenchus sp.	0.29	0.31	0.59	0.33	0.51	0
Rotylenchus goodeyi	0.07	0	0.07	0.39	0	0
Helicotylenchus sp.	0	0	0.17	0.07	0.18	0
Filenchus sp.	1.11	1.38	1.06	0.75	3.33	2.29
Heterodera juveniles	1.03	0.2	4.53	0.13	0	2.84
Meloidogyne juveniles	0.63	0.2	16.05	0.46	2.02	1.69
Heteroderidae males	0.23	0	0.46	0	0.37	0
Aphelenchus sp.	0.13	0.34	0.07	0.57	0	0
Telotylenchus ventralis	1.17	1.67	0.57	1.32	1.1	0.85
Total plant-parasites	4.91	4.86	27.89	4.44	20.19	8.52
Saprobiotic nematodes	50.2	61	110.8	164.6	287.8	125.9

The canonical correspondence analysis revealed some differences in species composition (Fig. 2.1). Three groups of species could be distinguished which were clearly related to the environmental variables (Fig. 2.1). A clear contrast was found between the community composition in calcareous soils and that at locations poor on lime, as is indicated by the centroids called LIM+ and LIM-, respectively. Recently established stands (RE) clearly differed from the existing stands (EV) in their rhizosphere species. Species groups related to these contrasts are indicated in Fig. 2.1 and listed in Table 2.4. Both contrasts were significant at the 0.05 level in the Monte-Carlo permutation test. In addition to the contributions of the other variables, the vigour of the various *A. arenaria* stands contributed little to the fit of the species data, as is also indicated by the short distance between the centroids of vigorous (VIG+) and non-vigorous (VIG-) stands (Fig. 2.1).

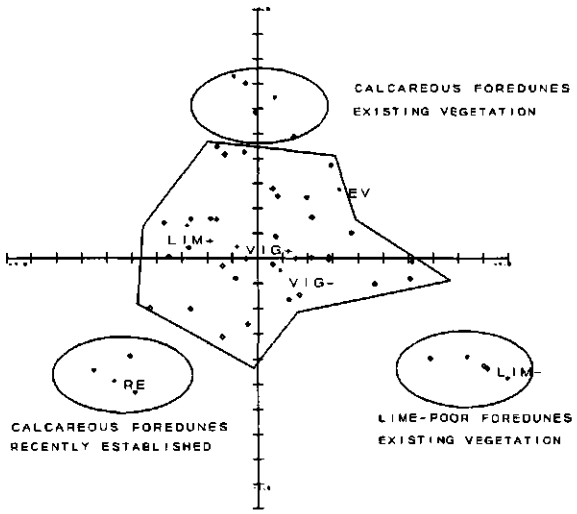


Figure 2.1. The arrangement of species and environmental variables as generated by canonical correspondence analysis. The samples were collected from the root zones of vigorous (VIG+) and early declining (VIG-) *Ammophila arenaria* stands of calcareous (LIM+) or lime-poor (LIM-) foredunes. The vegetation was recently established (RE) or already existing for more than 10 years (EV). Each dot represents one or two species. The 4 different assemblages of soil organisms are separated. The individual species from the 3 separate groups are presented in Table 2.4.

A group of 15 species of fungi and 10 genera of nematodes (excluding the non-plant-feeding nematodes and the Dorylaimids) occurred at all locations independent of the environmental factors. These species are represented by symbols in the enclosure around the origin (Fig. 2.1). In total, 36.2% of the variation was explained by the four axes calculated in CCA. The first two axes,

related to lime-content and age of the foredune, explained 10.9 and 7.1% of the variation, respectively. The remaining axes 3 and 4 enlarged the distance between the centroids of vigorous (VIG+) and non-vigorous (VIG-) stands, but did not result in a further separation of soil organisms within the central cluster.

Table 2.4. Groups of soil organisms that were significantly related to environmental variables when analysed with canonical correspondence analysis (CCA) (Fig. 2.1). The root zone of *Ammophila arenaria* stands on lime-poor or calcareous foredunes was sampled. On calcareous foredunes, samples were taken from recently established stands and existing stands of more than 10 years old. The nematode names are in capitals.

Calcareous foredunes Recently established	Calcareous foredunes Existing vegetation	Lime-poor foredunes Existing vegetation
Acremonium furcatum	*Pleospora* sp.	*Nectria inventa*
A.rutilum	*Fusarium chlamydosporium*	*Acremonium murorum*
A.strictum	*Aspergillus sydowii*	*Arthirinium phaeospermum*
Harzia acremonioides	*Chrysosporium pannorum*	*Verticillium lecanii*
Humicola grisea	*Trichothecium roseum*	*Chaetomium globosum*
Scopulariopsis sp.	*Phoma* spp.	*HELICOTYLENCHUS* sp.
Alternaria alternata	*Microdochium bolleyi*	*ROTYLENCHUS GOODEYI*

When the same data were analyzed by TWINSPAN, 5 clusters of samples occurred when the threshold Eigenvalue was set at 0.25 (Fig. 2.2). Unlike CCA, TWINSPAN did not separate samples from the calcareous foredunes from those of lime-poor samples. Also samples taken from an existing vegetation were not separated from those from a recently established vegetation. In accordance with the CCA results, no divisions were made between samples of vigorous or early declining stands of *A. arenaria*. Neither did various types of planting methods result in a division between the sampled locations of Voorne (Fig. 2.2).
According to the created clusters of samples (Fig. 2.2), the soil organisms were assembled into 6 clusters (Fig. 2.3). A relatively large group of 18 species of fungi and 5 genera of nematodes isolated from soil, and 10 species of fungi and 8 genera of nematodes isolated from roots (r) (cluster 1; Fig. 2.3) were separated from the other soil organisms. The species of cluster 1 occurred in most soil and root samples. Most of these soil organisms were grouped in the centre of the axes according to CCA (Fig. 2.1). In TWINSPAN, however, no correlation between

the grouping of samples with similar environmental parameters and the subsequent grouping of species was found.

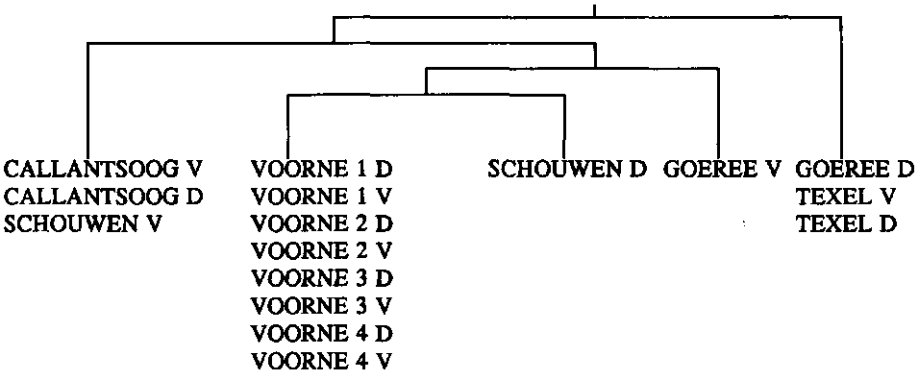
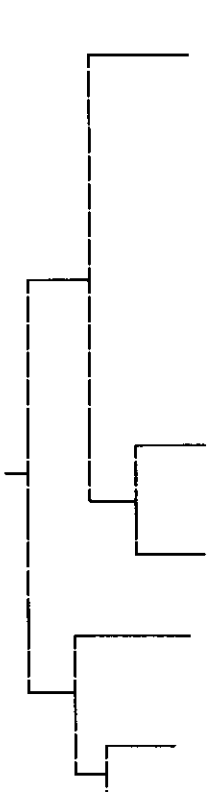


Figure 2.2. Dendrogram of the locations. The divisions were made with the aid of TWINSPAN when Eigenvalue ≥ 0.25. Locations with the largest differences in numbers of species are split off first. V = vigorous and D = early declining stands, Voorne 1, 2, 3 and 4 represent the sampled locations of Voorne with a vegetation recently established from culms (1), rhizomes (2) or seeds (3) or a vegetation existing for more than 10 years (4). Voorne, Goeree and Schouwen are located in the calcareous area, Texel and Callantsoog in the lime-poor foredunes (Table 2.1).

The more or less specific groups of soil organisms which were generated by CCA (Table 2.4) were henceforth omitted from the data set. The remaining group in the centre of the axes (Fig. 2.1) was subsequently analyzed by TWINSPAN. In total, 9 clusters were formed (Table 2.5). The first 4 clusters consisted of plant parasitic nematodes and fungi. Cluster 2 contained three endoparasitic nematodes: *Heterodera* spp., *Meloidogyne maritima*, and *Pratylenchus* sp. together with the fungus *Mucor hiemalis*. The organisms in cluster 4 occurred at almost all locations and often in relatively high numbers.

In this cluster the plant-parasitic nematodes *Heterodera* spp., *Telotylenchus ventralis* and *Filenchus* sp. were combined with the potentially plant-pathogenic fungi *Fusarium culmorum* and *Microdochium bolleyi*, as well as the fungi *Mortierella* sp. and *Trichoderma harzianum*. Furthermore, 4 clusters were formed consisting only of fungi of which two (clusters 5 and 7) occurred at all locations (Table 2.5). Cluster 9 only contained the root/fungus feeding nematode *Filenchus* sp. and non-plant-feeding nematodes.



Isolations from soil	Isolations from roots
Aspergillus sp.	*Acremonium furcatum*
Acremonium furcatum	*Apiospora montagnei*
A. rutilum	*Chaetomidium fimeti*
A. strictum	*Chaetomium globosum*
Alternaria alternata	*Cladosporium cladosporioides*
Chaetomium funicola	*Fusarium chlamydosporium*
Chrysosporium pannorum	*F. equiseti*
Harzia acremonioides	*Mortierella alpina*
Humicola grisea	*Mucor hiemalis*
Microdochium bolleyi	*Plectosphaerella cucumerina*
Mortierella alpina	*Ulocladium* sp.
Mucor hiemalis	*HELICOTYLENCHUS* SP.
Pleospora sp.	*MELOIDOGYNE MARITIMA*
Pyrenochaeta sp.	*HETERODERA* SPP.
Stachybotrys chartarum	*TELOTYLENCHUS VENTRALIS*
Scopulariopsis sp.	*APHELENCHUS* SP.
Trichothecium roseum	*ROTYLENCHUS GOODEYI*
Verticillium lecanii	HETERODERIDAE M
PRATYLENCHUS SP.	*PRATYLENCHUS* SP.
HETERODERIDAE M	
MELOIDOGYNE MARITIMA	
TELOTYLENCHUS VENTRALIS	
APHELENCHUS SP.	
Phoma spp.	*Trichoderma harzianum*
PARATYLENCHUS SP.	*PARATYLENCHUS* SP.
Fusarium culmorum	*Fusarium culmorum*
	Penicillium spp.
	Phoma spp.
Chaetomidium fimeti	*Microdochium bolleyi*
HETERODERA SPP.	*FILENCHUS* SP.
Apiospora montagnei	SAPROBIOTIC NEMATODES
FILENCHUS SP.	
SAPROBIOTIC NEMATODES	
Absidia corymbifera	
Acremonium murorum	
Arthirinium phaeospermum	
Chaetomium globosum	
Fusarium nivale	
Nectria inventa	
Penicillium spp.	
Trichoderma harzianum	
Ulocladium sp.	
ROTYLENCHUS GOODEYI	
HELICOTYLENCHUS SP.	

Seasonal and within-location variation

Almost all soil organisms isolated by sampling throughout the season were also found in the general survey. A slight shift from May to June and September was observed, which was, however, not significant (data not presented). The fungi *Phoma* spp., *Trichoderma harzianum*, *Fusarium culmorum* and the nematodes, *Hemicriconemoides* sp., Dorylaimidae together with the saprobiotic nematodes were isolated throughout the season.

The analysis with TWINSPAN of the samples collected in May at Voorne locations 3, 4 and 5, calculated with the abundance of species, revealed that the communities of soil organisms from the location with an existing vegetation differed from those that were recently established (Table 2.6). The organisms were arranged into 7 clusters. In the first cluster, soil organisms were grouped that mostly occurred in samples from recently established stands. Cluster 2 contained organisms that were present in relatively high numbers at almost all investigated locations. Clusters 5, 6 and 7 contained soil organisms specific to location 4 (existing vegetation). In recently established stands originating from seeds, the community of soil organisms was similar to those in stands grown from culms and rhizomes. *Microdochium bolleyi* and the non-plant-feeding nematodes were found in each of the samples. *Penicillium* spp. were abundant in samples of site 4 (Table 2.6).

An analysis by TWINSPAN of the soil organisms of the samples collected in May showed that the replicates were generally grouped into the same cluster, indicating that the within-location variation was small, whereas the between-location variation was large (Table 2.6).

Figure 2.3. Dendrogram of the nematode and fungal species from the root zone of *Ammophila arenaria*. The divisions, based on the groups of samples according to locations, were made by TWINSPAN when Eigenvalue $\geq$ 0.25. Groups of species with large differences in species composition are separated first. Species marked with r were isolated from roots. The nematodes are in capitals.

Table 2.5. TWINSPAN-analysis of soil fungi and nematodes that occurred in the root zone of *Ammophila arenaria* independently of the environmental data. The numbers represent the abundance classes made in TWINSPAN (see materials and methods for further explanation). The samples are presented by numbers (1 to 16; see Table 2.1). The even numbers are samples taken from early declining stands. The isolations from roots are marked with r. The abbreviations consist of the first 3 characters of genus-name and 3 of species-name. The nematodes are in capitals. Blank columns and rows represent divisions made by TWINSPAN when Eigenvalue > 0.25. - = not isolated.

cluster	samples	7	11	2	3	4	6	8	1	5	9	10	15	12	13	14	16
	PARA	5	-	5	5	5	5	5	4	4	-	-	-	-	5	-	-
	r *PARA*	5	-	4	4	4	-	5	2	3	-	-	-	-	5	-	-
	Har acr	-	-	-	-	-	-	-	-	5	-	-	-	-	-	-	-
1	*Sta cha*	-	-	-	-	-	5	5	-	-	-	-	-	-	-	-	-
	TEL VEN	5	-	5	5	5	5	5	5	5	-	-	-	-	-	-	-
	r *Acr fur*	-	-	-	-	-	-	5	5	-	-	-	-	-	-	-	-
	r *Cha fim*	-	-	-	-	-	-	-	-	5	-	-	-	-	-	-	-
	r *Cla* sp.	5	5	-	-	-	-	-	5	5	-	-	-	-	-	-	-
	r *Mucor*	2	-	2	5	5	4	-	-	2	-	-	-	-	-	-	-
	r *ROT GOO*	3	-	-	3	-	-	5	-	-	-	-	-	-	-	-	-
	Mucor	5	5	-	-	2	1	-	-	-	1	-	1	-	-	1	-
2	*MEL* jv	5	5	4	4	4	3	4	2	1	-	3	4	-	-	-	3
	r *PRAT*	1	1	2	1	-	1	1	-	1	5	2	4	-	-	-	1
	r *HET* jv	5	1	-	4	1	-	1	1	-	-	4	1	-	-	1	-
	PRAT	-	-	3	4	4	3	-	3	2	5	3	5	-	-	-	-
3	*HET* m	-	5	3	-	-	-	-	-	-	-	5	5	-	-	-	-
	r *Ulo* sp.	-	-	-	-	-	-	5	4	-	-	5	5	-	-	-	-
	r *HELICO*	-	-	-	-	-	-	4	-	-	5	-	5	-	-	-	-
	r *MEL* jv	5	1	-	1	1	-	1	1	-	2	2	1	-	-	-	-
	r *HET* m	5	-	-	4	-	-	-	4	-	5	-	-	-	-	-	-

Table 2.5, continued

		HET jv	5	-	1	4	5	2	-	4	1	-	-	4	4	-	-	-
		APH	-	-	5	5	-	-	5	4	3	-	-	5	-	-	-	5
	r	*Mic bol*	-	-	5	-	4	4	-	3	5	-	4	3	-	5	-	4
	r	*FIL*	5	-	4	4	4	4	4	3	3	5	5	4	-	5	4	4
4	r	*TEL VEN*	5	3	4	5	5	5	5	4	5	5	5	-	5	-	-	4
		Mort	5	5	2	-	1	1	-	-	-	-	-	3	-	-	-	3
	r	*Fus cul*	5	-	-	-	5	-	-	-	-	-	4	-	-	4	-	-
	r	*Tri har*	5	-	2	5	2	-	-	3	3	-	3	-	5	-	-	5
	r	*APH*	3	-	-	4	3	4	-	-	-	-	-	-	-	-	-	5
5		*Fus cul*	-	-	-	2	2	5	5	-	2	-	-	3	5	5	4	-
	r	*Pen* spp.	2	4	4	-	4	2	5	5	5	-	-	3	5	5	5	5
		Abs cor	-	-	-	-	-	-	-	-	-	-	-	-	5	-	3	-
6		*Fus niv*	-	-	-	-	-	-	-	-	-	-	-	5	5	4	-	-
		Ulo	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	5
		Api mon	-	-	-	3	-	-	-	1	-	-	4	-	-	-	4	5
7		*Pen* spp.	1	1	-	1	1	1	1	1	-	1	2	3	5	5	5	4
		Tri har	-	-	-	1	-	-	-	1	3	-	-	3	5	1	5	-
8		*Pyreno*	-	-	-	-	-	-	-	2	2	-	4	5	-	-	-	5
	r	*Api mon*	-	-	-	-	-	-	-	-	-	5	-	-	-	-	-	3
		FIL	3	5	4	3	4	4	3	3	3	4	3	5	5	5	5	5
9		SAPRO	4	5	4	4	5	4	4	3	4	5	4	5	4	5	5	5
	r	SAPRO	4	3	2	4	4	4	3	2	3	5	5	5	5	5	4	5

DISCUSSION

In this survey, 47 species of fungi and 10 genera of plant-parasitic nematodes were isolated from the root zone of *A. arenaria* (Tables 2.2, 2.3 and 2.6). Among the fungi 18 plant-pathogens, 2 pathogens of seeds (*Chaetomium* spp.) and 2 secondary pathogens were found, whereas the remaining 25 species have been described as saprophytes (Domsch et al. 1980). A large number of these species of fungi was also isolated from the root zone of *A. arenaria* by Brown (1958), Dennis (1983) and Moreau and Moreau (1941) and from the root zone of *A. breviligulata* by Wohlrab et al. (1963). These studies only dealt with isolations of fungi from soil. Brown (1958) isolated *Fusarium culmorum* more frequently from sand collected from the alkaline than from acid dunes. In this study, *Microdochium bolleyi* was usually isolated from roots, which is where it is also usually found for other plant species (Murray and Gadd 1981). Bussau (1990a), Yeates (1968) and Zoon et al. (1993) isolated nematodes from dunes in Germany, Denmark, New Zealand and the Netherlands. Unlike Van der Putten et al. (1989), they did not detect *Meloidogyne* and *Heterodera* in the rhizosphere of *A. arenaria*. There seem to be two species of *Heterodera* present in the rhizosphere of *A. arenaria*: *H. avenae* and a *Heterodera* from the trifolii-group (H. Brinkman, pers. comm.), however, further taxonomic studies are required to confirm these observations. *Meloidogyne maritima* was found to be parasitising roots of *A. arenaria* in the dunes of Wales (Jepson 1987). Some of the nematode species occurring in the root zone of *A. breviligulata* (Seliskar and Huettel 1993) are different from those occurring in the root zone at of *A. arenaria*.

In both CCA and TWINSPAN-analyses no relation between groups of soil organisms and the vigour of the plants was found. There are two possible explanations. First, the between-location variation in the general survey was so large that it would require more locations (or better more locations within a few separate soil and vegetation types) to detect vigour effects. As repeated samples within a site gave similar results (Table 2.6), it is concluded that sampling is not the major source of variation. Secondly, in vigorous stands potentially harmful soil organisms were already present, as was shown by Van der Putten et al. (1989). In that case no differences between communities of soil organisms from vigorous and early declining stands of *A. arenaria* could be expected. According to Van der Putten et al. (1989), roots of vigorous stands, which received 10 to 30 cm of windblown sand annually, are supposed to be colonized by soil organisms after they migrated towards the newly formed root layer. Since throughout the growing

season only a slight shift in the composition of the community of soil organisms was found, soil organisms may already have migrated towards the newly formed root layer as early as March. It is concluded that both vigorous and early declining stands of *A. arenaria* contained similar communities of soil organisms.

The rhizosphere communities of *A. arenaria* from calcareous versus lime-poor stands and from dredged sea sand versus established stands foredunes were significantly different according to CCA. The differences were more significant for fungi than for nematodes. All nematode species, except *Rotylenchus goodeyi* and *Helicotylenchus* sp. from soil, were clustered in the centre of the axis together with only 6 species of fungi that have been classified as plant-pathogenic (Domsch et al. 1980). In TWINSPAN, most of the soil organisms which occurred independent of environmental variables, were grouped in cluster 1 (Table 2.5).

The 'pathogenicity' of the soil collected from each location has also been tested in pot-experiments (Van der Goes and Van der Putten 1992). The growth reduction of *A. arenaria* seedlings in non-sterilized sand from vigorous stands was similar to that in sand obtained from early declining stands. Besides this, growth reduction of *A. arenaria* seedlings was observed in soil from each location (Van der Goes and Van der Putten 1992). It was therefore concluded that at all locations degeneration could be related to soil-borne organisms. It may be that at all locations the same pathosystem is responsible for the degeneration. In that case, the potentially harmful soil organisms to *A. arenaria* will be found in the ubiquitous group. It may also be that differences in the community of soil organisms between locations contribute to the degree of degeneration of *A. arenaria* on a local scale. Then all possible combinations of harmful soil organisms may be involved in the degeneration.

The additional analysis with TWINSPAN of the community of soil organisms which occurred indiscriminately in CCA and in cluster 1 by TWINSPAN, resulted in several clusters of concurring soil organisms (Table 2.5). If an omnipresent pathosystem is assumed as one of the possibilities discussed above, then 5 clusters of soil organisms can be involved: i.e. clusters 2, 4, 5, 7 and 9 (Table 2.5). Both nematodes and fungi are supposed to be involved in the degeneration of *A. arenaria*, although the role of fungi only has not been clearly assessed yet (Van der Putten et al. 1990).

If fungi are not involved, then clusters 2, 4 and 9 are possible (Table 2.5), because the presence of plant-parasitic nematodes within these clusters is sufficient. In cluster 2, three endoparasitic nematodes (*Meloidogyne maritima*, *Heterodera* spp. and *Pratylenchus* sp.) occur in combination with *Mucor hiemalis*.

Table 2.6. TWINSPAN-analysis of soil-organisms collected from Voorne in May 1990. The vegetation of the different locations of Voorne originates from 1987 and has been established from seeds (location 3) or culms and rhizomes (location 5). Location 4 was an existing vegetation of more than 10 years old. The replicates were analysed separately with TWINSPAN. The numbers represent the classes made in TWINSPAN (see materials and methods). The isolations from roots are marked with r. The nematode-genera are in CAPITALS. Blank columns and rows represent divisions made by TWINSPAN with an Eigenvalue > 0.25. - = not isolated.

location	3	5	5	5	5	3	3	3	4	4	4	4
replicate number	2	1	2	3	4	3	1	4	1	2	3	4
r *Acremonium furcatum*	3	-	-	-	-	5	5	5	-	-	-	-
A. furcatum	-	-	-	-	-	-	5	5	-	-	-	-
Aspergillus sydowii	-	-	-	-	-	2	5	-	-	-	-	-
Pyrenochaeta sp.	4	-	-	-	-	5	-	5	-	-	-	-
Stachybotrys chartarum	-	-	-	-	5	5	-	-	-	-	-	-
r *Cladosporium cladosporioides*	-	5	-	-	-	-	-	-	-	-	-	-
r *Stachybotrys chartarum*	5	-	-	-	-	-	-	-	-	-	-	-
r *Thielavia heterothallica*	-	-	-	-	5	-	-	-	-	-	-	-
Acremonium fusidioides	-	-	-	-	5	-	-	-	-	-	-	-
A. strictum	-	-	-	-	5	-	-	-	-	-	-	-
Chaetomium funicola	-	-	-	-	5	-	-	-	-	-	-	-
Fusarium culmorum	5	-	-	-	5	-	-	-	-	-	-	-
Phoma spp.	-	-	-	-	5	-	-	-	-	-	-	-
Trichoderma harzianum	5	-	-	-	-	-	-	-	-	-	-	-
Ulocladium sp.	3	5	5	-	-	-	-	-	-	-	-	-
PRATYLENCHUS sp.	-	-	5	-	-	-	-	-	-	-	-	-
TYLENCHORHYNCHUS sp.	-	-	-	-	5	-	-	-	-	-	-	-
FILENCHUS sp.	-	5	-	5	5	5	5	-	-	-	5	-
HETERODERIDAE males	-	-	5	-	-	-	-	-	-	-	-	-
TELOTYLENCHUS VENTRALIS	3	3	5	5	4	5	-	-	-	-	-	-
HETERODERA juveniles	4	5	5	4	5	-	-	-	-	-	-	4

Table 2.6, continued

r *Microdochium bolleyi*	4	5	4	4	5	4	4	4	4	-	-	5
M. bolleyi	-	-	4	-	5	5	-	-	-	-	5	-
SAPROBIOTIC NEMATODES	5	4	4	4	4	5	5	5	3	3	3	4
r *Trichoderma harzianum*	-	-	-	5	-	-	5	-	-	-	-	5
Mucor hiemalis	5	-	-	-	-	-	5	-	5	-	-	-
r *Ulocladium* sp.	-	-	5	4	-	-	-	-	-	4	-	-
Acremonium murorum	-	-	4	-	-	-	-	-	-	-	5	-
Cladosporium cladosporioides	5	-	-	-	3	-	-	-	-	-	5	-
Thielavia heterothallica	5	-	-	-	-	-	-	-	5	-	5	-
HEMICRICONEMOIDES sp.	-	5	-	-	-	-	-	-	-	-	-	5
Fusarium dimerum	-	-	-	-	-	-	5	-	2	2	2	-
r *Fusarium culmorum*	-	-	-	-	-	-	-	-	5	-	-	-
r *Penicillium* spp.	-	-	-	-	-	-	-	-	5	5	5	4
Alternaria tenuissima	-	-	-	-	-	-	-	-	-	-	-	5
Penicillium spp.	-	-	2	-	-	1	-	-	5	5	5	5
ROTYLENCHUS GOODEYI	-	-	-	-	-	-	-	-	5	-	5	5
HELICOTYLENCHUS sp.	-	-	-	-	-	-	-	-	-	5	-	-
MELOIDOGYNE MARITIMA	-	-	-	-	-	-	-	-	5	-	-	-
APHELENCHUS sp.	-	-	-	-	-	-	-	-	-	-	5	-

Endoparasitic nematodes are known to cause severe damage to many crops (Yeates 1987), but hardly anything is known about their role in natural vegetations. Cluster 4 was formed by the nematodes *Heterodera* spp., *Filenchus* sp. and *Telotylenchus ventralis* with the potential plant-pathogenic fungi *Fusarium culmorum* and *Microdochium bolleyi* and the fungi *Mortierella* sp. and *Trichoderma harzianum*. Cluster 9 contained the root/fungal feeding *Filenchus* and the non-plant-feeding nematodes.
If fungi are involved, then cluster 2 (with *Mucor hiemalis*) and 4 are possible. As the fungus *M. hiemalis* (in cluster 2) is not known to infect plants (Domsch et al. 1980), only cluster 4 seems to be relevant.
Combinations of nematodes and fungi as disease-complexes in agricultural crops, especially combinations of root-knot nematodes and *Fusarium* diseases, are well documented (e.g. Powell et al. 1971, Mai and Abawi 1987). Usually, saprophytic fungi do not contribute to degeneration. However, species which may enhance plant growth like *Trichoderma* (Windham et al. 1986) can, in combination with other soil organisms, inflict damage to crops (Powell et al. 1971). Therefore, saprophytic fungi that were combined with pathogenic fungi or nematodes should be included in tests for pathogenicity as well.

Both CCA and TWINSPAN created lower dimensionality in a rather complex set of data and led to a higher interpretability. CCA arranged sites and/or species along environmental gradients. The most important limitation of CCA is that the environmental variables are assumed to be measured without error and to be constant within a site (Palmer 1993). In this study, the environmental variables are presented as zero's and one's and thus are constant within one site. The canonical analysis showed that these discrete environmental 'gradients' could be used to explain the complex set of data. Furthermore, the Monte Carlo permutation test does not depend on the type of distribution of the environmental gradients and, therefore, the significance of environmental variables to clusters of species are reliable.
TWINSPAN arranged sites and species into groups and demonstrated to be useful for creating subsets of soil organisms. Although each analysis made with TWINSPAN resulted in different combinations of soil organisms, and results of TWINSPAN are therefore hard to interpret, several combinations of pathogenic and parasitic soil organisms of possible interest could be derived from the combined application of CCA and a subsequent TWINSPAN-analysis. Thus, both CCA and TWINSPAN have shown to be valuable exploratory techniques and may

be used to detect possible combinations of soil organisms which may be involved in the degeneration of *A. arenaria*. However, subsequent inoculation-experiments will be needed to elucidate the disease complex further on.

THE ROLE OF PLANT-PARASITIC NEMATODES AND SOIL-BORNE FUNGI IN THE DECLINE OF *AMMOPHILA ARENARIA* (L.) LINK

P.C.E.M. de Rooij-van der Goes

New Phytologist 129 (1995): 661-669

CHAPTER 3
THE ROLE OF PLANT-PARASITIC NEMATODES AND SOIL-BORNE FUNGI IN THE DECLINE OF *AMMOHPILA ARENARIA* (L.) LINK

SUMMARY

In coastal foredunes, *Ammophila arenaria* grows vigorously when it is buried regularly by windblown sand and degenerates at stabilized sites. Nematodes and soil-borne fungi were found to be involved in its decline. In order to establish their role in the disease complex, seedlings of *A. arenaria* were inoculated with several groups of potentially harmful soil organisms that were isolated from its root zone. Inoculation of single species of fungi did not reduce the growth of the seedlings, but combining all fungi that were commonly found in the Dutch coastal foredunes significantly reduced growth to about 80% of that in sterilized soil. This indicates synergistic effects between the commonly found plant-pathogenic fungi. The addition of large numbers of the nematode *Telotylenchus ventralis*, the only species that could be successfully grown on *A. arenaria* in the laboratory, reduced plant growth to the same level as in non-sterile soil, but numbers needed to be 80 times greater in the latter soil. The combined inoculation with relatively large numbers of *T. ventralis* in combination with the commonly occurring fungi reduced plant growth in sterilized soil a similar level to that in non-sterile soil.

Thus far, only *T. ventralis* was tested with or without different combinations of fungi and there was more than one assemblage that could inhibit plant growth. The involvement of other species of nematodes, such as *Heterodera* spp. or *Meloidogyne maritima*, in the decline of *A. arenaria* in non-sterile soil could not be proven in inoculation experiments with sterile soil, but it is likely that these species may also be involved. It is, therefore, concluded that several different combinations of soil organisms can be harmful to *A. arenaria*, so that the decline is not caused by one simple well defined pathosystem.

INTRODUCTION

In West-European coastal foredunes, *Ammophila arenaria* (L.) Link is the most important naturally occurring sand-fixing plant species. The vigour of this species is strongly correlated with the supply of fresh windblown sand from the beach. Degeneration occurs at stabilized sites (Willis 1965, 1989, Huiskes 1979). Similar relationships between plant vigour and sand burial are also known to occur in the North American species *Ammophila breviligulata* (Laing 1967, Eldred and Maun 1982). Above ground, degenerating stands are characterized by a less dense vegetation with shorter culms, a decline in flowering and increasing amounts of dead shoots and leaves. New roots, which are formed each spring, remain short, lack root hairs and are deformed (Eldred and Maun 1982, Willis 1989, Van der Putten, Van der Werf-Klein Breteler and Van Dijk 1989).

The decreased vigour of the plants, when the supply of fresh windblown sand ceases, has been considered to be caused by a diminished supply of nutrients, a changing physiological status of the plants or malfunctioning of old roots (Marshall 1965, Laing 1967, Willis 1965, 1989, Huiskes 1979, Disraeli 1984). Recently, it was demonstrated that the root zone of *A. arenaria* contains harmful organisms which may be involved in the degeneration at stabilized sites (Van der Putten and Troelstra 1990). The addition of nematicides or fungicides to non-sterile sand stimulated the growth of *A. arenaria*, whereas bactericides had no effect. Application of nematicides or fungicides did not improve growth to the same extent as could be found when soil was gamma-radiated. Hence, both nematodes and fungi are involved in the disease complex causing decline of *A. arenaria* (Van der Putten, Maas and Brinkman 1990).

From various coastal foredunes dominantly covered by either *A. arenaria* or *A. breviligulata*, species of fungi (Moreau and Moreau 1941, Brown 1958, Wohlrab, Tuveson and Olmsted 1963) and nematodes (Yeates 1968, Bussau 1990b) have been isolated. Fungal hyphae and bacteria, observed near the root tip of *A. arenaria*, were assumed to play a part in the extensive breakdown of root cells (Marchant 1970). Although degeneration of *A. breviligulata* has been related to the presence of plant-parasitic nematode species in its root zone (Seliskar and Heuttel 1993), in these studies relationships between plant-pathogens and plant growth were not considered. In Dutch coastal foredunes, several species of potentially plant-pathogenic fungi and parasitic nematodes have been isolated from the root zone of *A. arenaria* (chapter 2). The numbers of nematodes were few and variable. There was no correlation between the numbers of plant-parasitic

nematodes and the vigour of *A. arenaria*. However, seedlings grown in non-sterile soil with few nematodes exhibited a significantly reduced growth (Van der Goes and Van der Putten 1992). To analyse possible relationships between plant vigour and the presence of nematodes and fungi collected from the root zone, multivariate techniques (CANOCO and TWINSPAN) were used (chapter 2). These analyses generated several clusters of concurring fungi and nematodes. In the present study, healthy *A. arenaria* seedlings growing in sterilized soil were inoculated with these groups to examine their pathogenicity.

The purpose of this study was to elucidate which nematodes and fungi may be involved in the decline of *A. arenaria* by testing the involvement of nematodes and fungi separately and in different combinations, the latter to detect potential interactions between the two groups of soil organisms. Regrettably *Telotylenchus ventralis* was the only nematode available in large quantities. To check the infections of *T. ventralis* to *A. arenaria*, the feeding habits and the pathogenicity of this semi-endoparasitic species were studied.

MATERIALS AND METHODS

Plants and soil

At the foredunes of Voorne, the Netherlands, at a site north of the Haringvlietdam (51°52' NL 4°04' EL), soil was collected from the root zone of vigorous *A. arenaria* stands. The soil was sieved (mesh size: 2 cm) and thoroughly mixed. Sifted roots were chopped into 2-5 cm pieces and mixed through the soil. Most of the soil was sterilized by means of gamma-radiation (4 Mrad). Non-sterile soil contained about $1.1*10^4$ colony forming units per gram of dry soil. The nematode community consisted of about 27 plant parasites, 27 Dorylaimids and 380 saprobiotic nematodes per 100 ml of soil (chapter 2).

Seeds, obtained from *A. arenaria* at Voorne, were germinated on glass beads at an 8/16 hour dark/light regime of 10/30°C. Pots of 1.5 l were filled with 1500 g of soil containing 10% soil moisture (w w^{-1}). Each pot was covered with tinfoil to protect the soil from desiccation and was planted with six two-week-old and 3 to 5 cm tall *A. arenaria* seedlings. The pots were placed in a growth chamber (experiments 1 and 3) or in the greenhouse (experiments 2 and 4) at 23°C (± 2°C) in a complete randomized design. A day length of 16 hours was achieved by additional illumination (250 μmol m^{-2} h^{-1}).

Once a week, the soil moisture content was set at 10% (w w^{-1}) with demineralized

water. To avoid nutrient deficiency during plant growth, increasing amounts of nutrients were added to all pots: week 1 to 4: 15 ml, week 5 to 6: 25 ml and week 7 to 8: 50 ml of full strength Hoagland nutrient solution.
Some plants did not establish. Therefore, two weeks after planting, the plants were randomly thinned to 4 plants per pot. The pots were harvested 6 to 8 weeks after planting. Fresh weights of roots were assessed, and, after taking a subsample for the analysis of soil-organisms in the root tissue, dried for 48 hours at 70 °C. The biomass of shoots was determined after drying at 70°C for 48 hours.

Inoculum preparation
Fungi. The fungi used in the present experiments were isolated from soil and roots of *A. arenaria*. Fungi grown for two weeks on Potato Dextrose Agar (Oxoid). After one week, the Petri-dishes were checked for spore production. If spore production was not apparent, the dishes were placed under near UV to stimulate sporulation. The spores of the fungi were washed from the culture with sterile demineralized water. The suspension was filtered through glass wool to discard hyphal material and spores were counted to assess the concentration.
If sporulation remained insufficient, a mycelial inoculum was prepared by mixing 20 ml of sterile demineralized water with cultures of two completely colonized Petri-dishes (ϕ 9 cm) in a blender for 10 seconds. Dilutions of this suspension were plated out in order to check the concentration of colony forming units added as an inoculum.
Fungal inoculum was added to the pots directly after planting. The inoculum was added in the planting hole at a minimum concentration of 10^5 spores/plant or ¼ ml mycelial suspension per plant containing $\geq 10^5$ cfu per ml.
Nematodes. *T. ventralis* was collected from a field population and cultured for several months on *A. arenaria* seedlings that were planted in sterilized root zone soil. Prior to inoculation, nematodes were collected from whole pots by decanting (Hooper 1986) and, subsequently, they were counted. Only the nematode *Telotylenchus ventralis* could be successfully cultured on roots of *A. arenaria* in the greenhouse.
The feeding habits of *T. ventralis* were observed on two-week-old roots of *A. arenaria* seedlings placed on a thin layer of water-agar in a Petri-dish. When, after one week, the roots had grown, 20 individuals of *T. ventralis* were added. The movement and feeding habits of the nematodes were observed microscopically.
Cultivation of the cyst-nematode *Heterodera* and the root-knot nematode *Meloidogyne maritima* failed. Therefore, mature cysts and egg masses were

collected from one-year old roots originating from a vigorous *A. arenaria* stand at the site where soil was collected. The cysts were crushed gently. Crushed cysts and egg masses were suspended in 50-100 ml tap-water and placed in extraction dishes. After 24 hours, the nematodes were collected and their numbers were counted. The suspension was washed several times with tap-water and stored in a cold room until use (usually the next day). Directly after planting, nematodes were inoculated with a dispenser in the planting hole near the *A. arenaria* seedling. In case of *T. ventralis*, the whole population, containing adults and different stages of juveniles, was added.

Experiment 1.
Healthy seedlings were inoculated with the most frequently isolated fungi from *A. arenaria* from 9 locations of the Dutch coastal foredunes (chapter 2) at a concentration of 10^6 spores per plant. Each isolated fungus was added to two pots. After 6 weeks the plants were harvested. Dry weights of the treated plants were compared with those in sterilized and non-sterilized soil.

Experiment 2.
To establish the relationship between dry weight, growth reduction of *A. arenaria* and the number of nematodes, seedlings planted in sterilized soil were inoculated with different concentrations of *T. ventralis*. The numbers of *T. ventralis* added were: 17, 33, 67, 100, 133 or 267 individuals per 100 g, respectively. Dry weights of plants inoculated with the nematodes were compared with those of plants in sterilized as well as non-sterilized soil. After five days the initial population of *T. ventralis* was determined by decanting three replicate pots. After growing periods of 25 and 54 days, respectively, five replicate pots were harvested. The density of the nematode population and the biomass production of *A. arenaria* were assessed.

Experiment 3.
In a previous study soil-borne fungi of *A. arenaria* were surveyed (chapter 2). The fungi, excluding the infrequent isolations, were manually assembled into three groups. Groups 1 and 3 represented the fungi commonly found in calcareous and lime-poor foredunes, respectively. The fungi in group 2 were commonly found at each location. Group 2 was split into the potential plant-pathogenic fungi and saprophytic fungi (Table 3.1). Each of these groups was inoculated with or without *T. ventralis* to study possible interactions between infections of fungi and

Table 3.1. Groups of fungi assembled from the most frequently found fungi in roots and soil of *Ammophila arenaria* used in inoculation experiment 3. Groups 1 and 3 contain the species more or less specific for the calcareous and lime-poor foredunes, respectively. The fungi in group 2 were commonly found at all *Ammophila arenaria* sites. Group 2 was added as a whole or plant-pathogenic (2a) or non-plant-pathogenic (2b) fungi. The fungi were added either as spores (s) in a concentration of 10^5 spores of each species per plant or as a suspension of mycelial fragments (m) that contained about 10^5 colony forming units per ml (¼ ml per plant).

no fungi	group 1	group 2	group 3
	Alternaria alternata (s)	a	*Arthirinium phaeospermum* (s)
	Acremonium murorum (s)	*Phoma* spp. (s)	*Doratomyces stemonites* (s)
	A. furcatum (m)	*Microdochium bolleyi* (s)	*Nectria inventa* (s)
	A. fusidioides (m)	*Cladosporium cladosporioides* (s)	*Paecilomyces* sp. (s)
	Fusarium dimerum (s)	*Fusarium culmorum* (s)	*Cylindrocarpon magnusianum* (s)
	F. equiseti (s)	*Ulocladium* sp. (s)	
	Stachybotrys chartarum (m)	*Harzia acremonioides* (m)	
		b	
		Trichothecium roseum (s)	
		Mucor hiemalis (s)	
		Apiospora montagnei (s)	
		Mortierella alpina (m)	
		Penicillium sp. (s)	
		Trichoderma harzianum (s)	
		Chaetomium funicola (m)	
		C. globosum (m)	
		Chaetomidium fimeti (m)	

nematodes on plant growth.
The groups of fungi were inoculated at densities of 10^5 spores per fungal species per plant or ¼ ml mycelial fragments of 10^5 cfu ml^{-1} with or without 144 individuals of *T. ventralis* per 100 g of soil. Of the 144 individuals of *T. ventralis* that were inoculated, only 47.4 ± 2.6 per 100 g of soil (N= 3) were recovered after 4 days. Treatments 'no fungi' with or without nematodes were used as controls. The growth of inoculated *A. arenaria* seedlings was compared to that in non-sterile root zone soil. Eight replicate pots were harvested after a growing period of 6 weeks.

Experiment 4.
The fungi and nematodes isolated from 9 locations along the Dutch coastal foredunes were assembled into 11 groups with the aid of CCA and TWINSPAN (Table 3.2) (chapter 2). Healthy seedlings of *A. arenaria* were inoculated with these groups. The fungi were added at a concentration of 10^6 spores per fungal species per plant. The numbers of nematodes that were added were for *T. ventralis*: 33, *Heterodera* sp.: 15 and *Meloidogyne maritima*: 51 individuals per 100 g soil. After five days, the initial number of nematodes was established by decanting three replicate pots. The growth of *A. arenaria* in sterilized soil inoculated with the different combinations of fungi and nematodes was compared with that in sterilized or non-sterile soil. After eight weeks, six replicate pots of each treatment were harvested and dry weights of the plants were established.

Analysis of the soil-organisms
At the end of the experiments, the numbers of nematodes in soil were established by decanting the pots. Nematodes from roots were assessed by the Baermann-funnel-method (Hooper 1986). Individuals were identified to at least genus level according to Bongers (1988). Fungi were assessed on root pieces. Of each replicate 5 pieces of about 1 cm roots were washed three times in sterile demineralized water and were placed on malt-extract agar to re-isolate the inoculated fungi (chapter 2). After incubation at 23°C for 7 days, the fungi were subcultured on potato dextrose agar (Oxoid), counted and identified according to Domsch, Gams and Anderson (1980) and for *Fusarium* according to Nelson, Toussoun and Marasas (1983).

Table 3.2. Experiment 4: composition of the inoculated groups of fungi and nematodes isolated from roots and soil of *Ammophila arenaria* that were assembled with the aid of CANOCO and TWINSPAN (chapter 2). Groups 5 to 10 only contain fungi.

Treatment number	Nematode	Fungi
1	*Telotylenchus ventralis*	*Stachybotrys chartarum, Acremomium furcatum, Chaetomidium fimeti, Mucor hiemalis, Cladosporium cladosporioides*
2	*Heterodera* spp. *Meloidogyne maritima*	*Mucor hiemalis*
3	*Heterodera* spp. *Meloidogyne maritima*	*Ulocladium* sp.
4	*Heterodera* spp. *Meloidogyne maritima* *Telotylenchus ventralis*	*Fusarium culmorum, Trichoderma harzianum, Microdochium bolleyi, Mortierella alpina*
5	-	*Fusarium culmorum, Penicillium* spp.,
6	-	*Microdochium nivale, Absidia corymbifera, Ulocladium* sp.
7	-	*Apiospora montagnei, Penicillium* spp., *Trichoderma harzianum*
8	-	*Pyrenochaeta* sp., *Apiospora montagnei*
9	-	*Acremonium furcatum, A. rutilum, A. strictum, Harzia acremonioides, Humicola* sp., *Scopulariopsis* sp., *Alternaria alternata*
10	-	*Pleospora* sp., *Aspergillus sydowii, Phoma* spp., *Microdochium bolleyi*
11	*Telotylenchus ventralis*	*Acremonium murorum, Arthirinium phaeospermum, Verticillium lecanii, Chaetomium globosum*

Data analysis

The data of plant biomass were statistically analysed by one-way analysis of variance after testing for homogeneity of variances by means of Cochran's Q. If required, data were ln-transformed to obtain homogeneity. Treatment means were compared by Tukey's test or by least significant difference ($P < 0.05$).

RESULTS

Experiment 1.

Inoculation of *A. arenaria* grown in sterilized soil with single species of fungi did not reduce the growth of the plants (data not shown). Within 6 weeks, plants that were grown in sterilized soil weighed about 185 mg each. The biomass of inoculated plants ranged from 160 to 270 mg per plant, whereas in non-sterile soil a biomass of only 54 mg per plant was produced in 6 weeks (data not shown). Thus, all plants inoculated with fungi produced more biomass than those planted in non-sterile soil.

Most fungi, that were described as potential plant pathogens (Domsch, Gams and Anderson 1980), were isolated from roots of seedlings at harvest of the pot-experiment. Also a few saprophytes, such as *Trichoderma harzianum, Acremonium furcatum, Mortierella* spp. and *Mucor* spp., were found on roots (data not shown).

Experiment 2.

In separate laboratory tests, *T. ventralis* fed on the epidermal cells of the root near the root hair region. Grazing on root hairs was not observed. The nematodes first explored the root region by probing it with their stylets. When a favourable site was found, the stylet was thrust at the cell wall, until it had been perforated. The median bulb then contracted and continued to steadily pump indicating the ingestion of the cell content. When the nematodes stopped feeding the whole nematode withdrew and moved towards a new feeding site along the root, where the process of exploration started again. Sometimes, however, *T. ventralis* did not withdraw and continued feeding on the next root cell. *Telotylenchus ventralis* was able to invade the root laterally over a length of about four cells and centrally to a depth of two cells with, at most, half of its body length inside the root. Similar experiments to study the feeding habits of *Heterodera* sp. and *Meloidogyne maritima* failed.

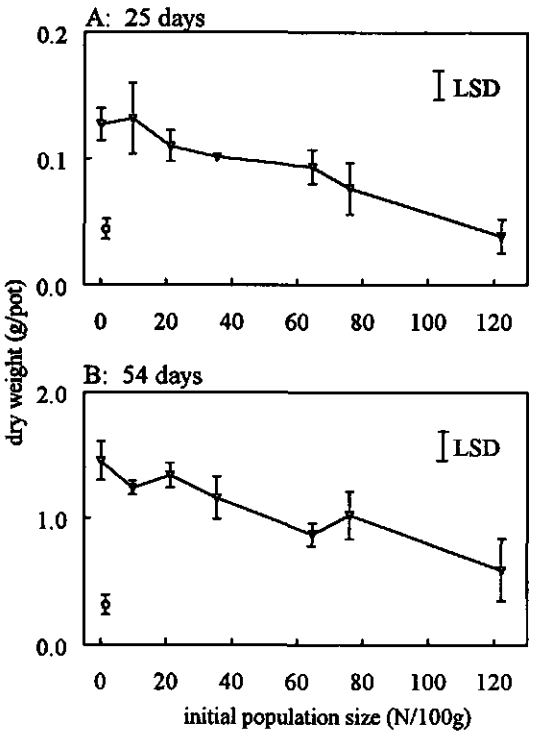


Figure 3.1. Dry weight of *Ammophila arenaria* in sterilized soil inoculated with different numbers of the nematode *Telotylenchus ventralis* after a growing period of (A) 25 and (B) 54 d (▽). Growth of inoculated plants was compared with that in non-sterile root zone sand (○).

When *T. ventralis* was added in increasing numbers to plants in sterilized soil, approximately half of the nematode survived inoculation (data not shown). In non-sterile soil, initially only 1.6 ± 0.21 (N = 3) individuals of *T. ventralis* per 100 g were found, whereas the initial total number of plant parasitic nematodes in these pots was 43.4 ± 13.7 per 100 g (N = 3). The dominant species among these were *Heterodera* sp. (22.6 ± 3.0), *Meloidogyne maritima* (10.1 ± 8.7), *Pratylenchus* sp. (1.7 ± 0.6) and *Rotylenchus goodeyi* (5.2 ± 1.7).

After growing periods of 25 and 54 days, the biomass of *A. arenaria* was inversely related to the initial number of *T. ventralis* (Fig. 3.1). After a growing period of 25 days, the biomass production of plants inoculated with the largest number of *T. ventralis* (122 per 100 g dry soil) was not significantly different from the biomass in non-sterile soil. However, initial populations smaller than 63 per 100 g dry soil did not inhibit plant growth significantly (Fig. 3.1A). After a

growing period of 54 days, the severity of the growth reduction seemed to decrease, which coincided with the smaller numbers of nematodes after 54 days, mainly at the higher inoculation densities (Fig. 3.1B and Fig. 3.2).

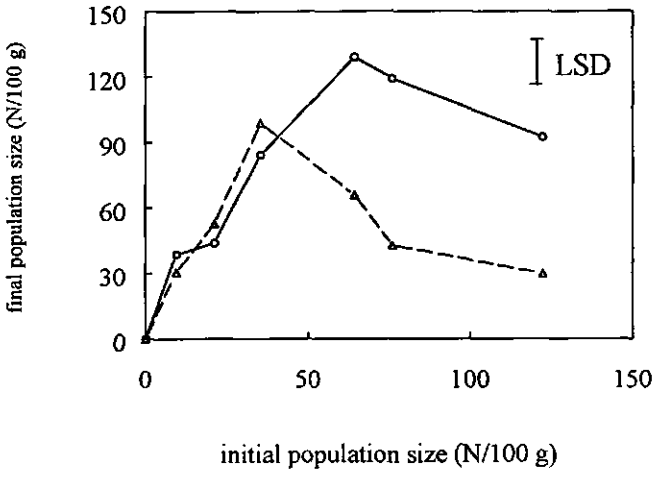


Figure 3.2. Population size of *Telotylenchus ventralis* after a growing period of 25 (○) and 54 d (△) in relation to the size of the initial population (measured 5 d after inoculation) added to healthy *Ammophila arenaria* seedlings.

After 25 days, roots of plants inoculated with the highest number of *T. ventralis* were short, brownish and deformed, but these symptoms were less pronounced than those of roots of plants in non-sterile soil. After 54 days, plants inoculated with the largest initial number of nematodes had developed new roots, which appeared to be uninfected (personal observation).

The multiplication of *T. ventralis* depended on the initial number of nematodes (Fig. 3.2). When few were added, the population increased. The population size after 25 or 54 days became maximally 130 individuals per 100 gram of soil. At the larger initial numbers the resulting population size after 25 or 54 days had decreased.

Experiment 3.

The biomass production of seedlings inoculated with fungi from groups 2 and 2a was significantly less than when grown in sterilized soil (Fig. 3.3). However, this reduction was small as compared with that in non-sterile soil. Adding only *T. ventralis*, the dry weight of plants was significantly smaller than that in sterilized soil, but the biomass production was still greater than in non-sterile soil (Fig. 3.3).

In all cases the addition of both nematodes and fungi caused more growth reduction than the addition of fungi only. Inoculations with group 1, 2 and 3 with *T. ventralis* all inhibited growth of the seedlings more than inoculations with only nematodes. This was not the case when groups 2a and 2b were added separately. Inoculations of fungi from groups 1, 2 and 3 with *T. ventralis* reduced plant growth close to the level of that in non-sterile soil, group 2 with *T. ventralis* was the only inoculation that did not differ significantly from non-sterile soil.

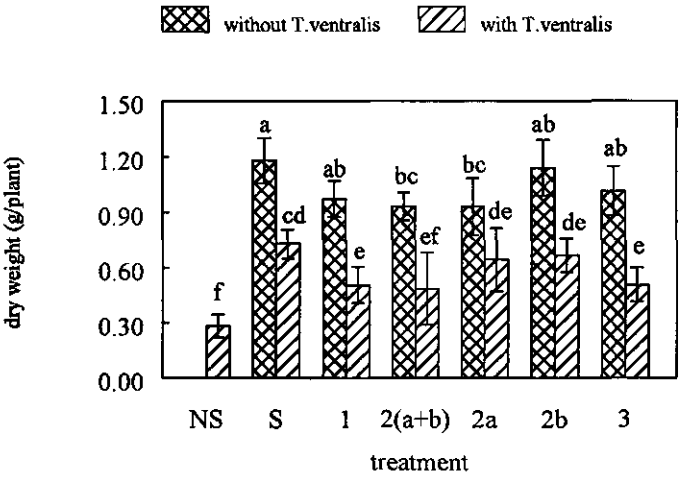


Figure 3.3. Dry weight of *Ammophila arenaria* grown in sterilized sand inoculated with different groups of fungi with or without the nematode *Telotylenchus ventralis*. S, sterilized soil; NS, non-sterile root zone sand. For explanations of the other abbreviations see Table 3.1. Bars bearing different letters are significantly different at $P = 0.05$.

At the end of the experiment, the final population size of *T. ventralis* was about 10 times greater than the initial 47.4 individuals per 100 g of soil. Almost all fungi of group 2 could be recovered. This included the potential plant pathogens as well as the saprophytic fungi. In the other groups the recovery of the inoculated fungi was less. In all treatments, roots were infected by *Chaetomium* spp. (data not shown), although they were only inoculated with groups 2 and 2b. These fungi had also been isolated from the caryopses and were probably introduced in the soil when seedlings were planted.

Experiment 4.

The initial numbers of nematodes five days after inoculation are presented in Table 3.3. In contrast to *T. ventralis*, most *Heterodera* and *Meloidogyne* juveniles did not survive the step of inoculation.

The biomass of plants inoculated with groups 1, 4 and 11 was so much reduced that it did not differ from that in non-sterile soil (Fig. 3.4). All these groups contained the nematode *T. ventralis*. The biomass of plants inoculated with groups 2, 3 or 5 to 10 was significantly greater than that of plants in non-sterile soil. As the dry weight was not significantly different from that in sterilized soil, it was concluded that these groups of fungi and nematodes did not inhibit the growth of *A. arenaria*.

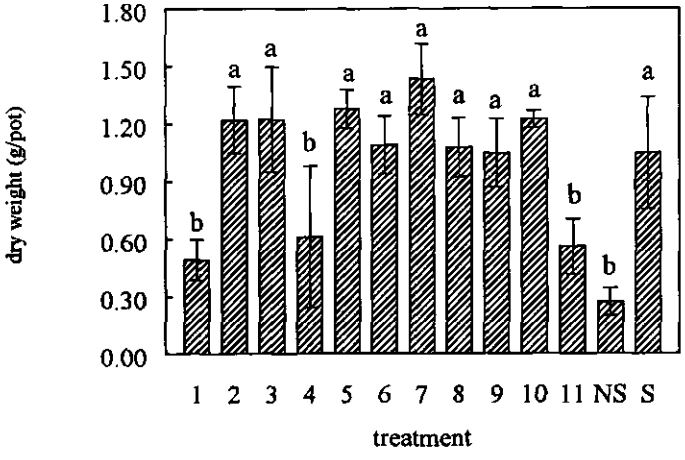


Figure 3.4. Growth of *Ammophila arenaria* inoculated with different groups of fungi and/or nematodes. The groups were assembled with the aid of CANOCO and TWINSPAN. S, growth in sterilized; NS, growth in non-sterile root zone sand. For explanations of other treatments see Table 3.2. Bars bearing different letters are significantly different at $P = 0.05$.

The numbers of the nematode *T. ventralis* increased during the pot-experiment (Table 3.3), whereas the numbers of *Heterodera* spp. and *Meloidogyne maritima* decreased. Some nearly mature cysts of *Heterodera* were found on the roots. This group of nematodes was, therefore, able to infect the roots of *A. arenaria*. Both endoparasitic species could not be isolated from roots at harvest of the pot-experiment (data not shown). In non-sterile soil, substantial numbers of many different species were found (e.g. *Heterodera* sp., *Meloidogyne maritima*, *Rotylenchus goodeyi* and *Filenchus* sp.).

Of the 44 inoculated fungi, 11 were not recovered from roots of *A. arenaria* at the end of the experiment (data not shown). Most of them infected the roots successfully although this did not lead to growth reduction.

Table 3.3. Number of *Telotylenchus ventralis*, *Heterodera* sp. and *Meloidogyne maritima* (N/100 g dry soil) at inoculation and after 5 days (i.e. initially). Other plant-parasites, Dorylaimids and non-plant-feeders were present in the added suspensions as contaminations. The final population size (for explanations of the treatment codes see Table 3.2) were also expressed as N per 100 g dry soil. An average number of < 0.1 is expressed by +.

	T. ventralis	*Heterodera* sp.	*M. maritima*	Other plant parasites	Dorylaimids	Non-plant feeders
Added number	33.3	14.8	51.1			
Initial number (after 5 days)	22.0	4.4	6.4	1.0	0.5	51.6
Final population size of treatment:						
1	479.9				0.1	123.3
2	1.4	0.2	0.2	0.6	0.3	456.2
3	1.3	0.5	0.1	+	0.1	355.2
4	572.7	0.6	0.1	0.6	0.1	104.4
11	729.4				+	12.3
Non-sterile soil	0.7	3.2	0.1	6.0	22.7	121.0

DISCUSSION

In the present paper it was tried to elucidate which species of fungi and nematodes were actually involved in the decline of *A. arenaria* occurring at sites along the coasts where the plant species is used in dune protection.

When only fungi were added, adding single species of fungi (experiment 1) did not result in a significant growth reduction, although most of the added plant pathogens were isolated from the roots at harvest of the pot-experiment (data not shown). Certain combinations of fungi tested in experiment 3 (groups 2 and 2a) reduced the growth significantly by about 20%, whereas the other combinations of fungi did not (Fig. 3.3). The species of groups 2 and 2a (consisting of the plant-pathogens of group 2) were commonly found in the root zone of *A. arenaria* (chapter 2). Thus the general conclusion is that growth reduction only occurred when the plant pathogenic fungi were combined.

In natural, non-sterile soil about $1.1*10^4$ colony forming units (CFU) per g dry soil was found (chapter 2), which meant that the 1.5 l pots used in the present experiments contained about $15*10^6$ CFU's. In experiment 1, the fungi were applied in a concentration of 10^6 spores per plant (= $6*10^6$ per pot), whereas in experiment 3, the fungi were added in a concentration of 10^5 spores per fungal species per plant. In group 2a, the total colony forming units of fungi per pot was less than that of experiment 1. Adding the fungi of group 2a reduced the growth of the seedlings significantly, whereas adding single species of this group did not. This indicated synergistic interactions between plant-pathogenic fungi. The growth inhibition of fungi alone, in case of, for instance, *Fusarium culmorum*, may be even more severe when plants suffer from abiotic stress (Papendick and Cook 1974).

The nematode *T. ventralis* reduced the growth of *A. arenaria* seedlings. The number of individuals needed for a similar reduction of biomass of *A. arenaria* as in non-sterile soil was, however, 80 times greater than that in non-sterile soil (Fig. 3.2). Observations on the feeding behaviour of this nematode showed that it can feed on roots of *A. arenaria*. Except for the semi-endoparasitic features (Yeates et al. 1993), the feeding habit resembled that of other plant-parasitic species such as *Tylenchorhynchus dubius* (Wyss 1973).

The growth reduction of seedlings was more severe when young roots were rapidly invaded by large numbers of *T. ventralis* than when roots were infected by only a few numbers. The population of *T. ventralis* that resulted from a range of inoculation densities seemed, therefore, to be determined by the number of

suitable feeding sites. At the highest inoculation level, roots remained short and were poorly developed, but when the plants were grown for a longer period, the newly formed roots appeared to be uninfected. In this case the population size of *T. ventralis* had decreased to such a low level that the nematodes could not infect all roots. The highest initial inoculation level (122 per 100 g dry soil) reduced plant growth to the same extent as in non-sterile soil, but the size of the population decreased dramatically, most likely due to a decrease in available feeding sites. The low initial population size of 21 per 100 g of soil, the nematodes increased to 53 individuals (Fig. 3.2). Although in natural soil similar amounts of plant parasitic nematodes were found (chapter 2), these small initial numbers did not inhibit plant growth (Fig. 3.1).

In non-sterile soil, the dry weights of the plants were about 25% of those in sterilized soil (experiment 3). Adding *T. ventralis* reduced the growth by about 40%, whereas the fungi of group 2 reduced it by about 20%. Inoculations with both nematodes and fungi reduced the growth of plants in sterilized soil with about 60%. Thus this indicated that, under the present condition, the interactions between *T. ventralis* and fungi were additive rather than synergistic. However, at high inoculation densities used in the experiment 3, there is little room for synergistic effects. Therefore, synergistic effects between nematode and fungal damage might be established when smaller numbers of *T. ventralis* are added. Old and Nicolson (1975) found that fungi and bacteria invaded the root cells of *A. arenaria* through pre-existing pits. These pits might have been made by nematodes probing into the root cells. Predisposition of roots by nematodes has been demonstrated for several plant species (Labruyère 1979, Mai and Abawi 1987).

In the inoculation experiments, only a few *Heterodera* individuals successfully infected the roots but this did not inhibit the growth of the inoculated seedlings. Inoculations with *Meloidogyne maritima* failed. Culture trials of the two separate species were also unsuccessful. Inoculation of *Heterodera* sp. to seedlings of *A. arenaria* planted in sterilized root zone soil resulted only once in successful infections of roots (P.C.E.M. de Rooij-Van der Goes, unpublished results). Instead of cysts, only males developed from the roots. This indicates that growth conditions in the sterilized soil were not optimal (Yeates 1987). However, on roots of seedlings planted in non-sterile soil that were placed in the same greenhouse, mature cysts developed within 8 weeks. Both species of endoparasitic nematodes were also frequently found in natural soil in the root zone and on roots of *A. arenaria*. Because under natural conditions, both nematodes are able to infect plants, their role in the degeneration can be misjudged when only effective

inoculations in pot trials are considered.

Besides abiotic factors such as decreasing pH, decreasing amounts of sand accretion, changing nutrient status of the soil (Willis 1989) and biotic factors such as competition, also soil-borne diseases seem to contribute to vegetation succession in coastal foredunes (Van der Putten, Van Dijk and Peters 1993). In the latter paper, a considerable specificity of soil-borne diseases in different successional stages in coastal foredunes was demonstrated. These results were obtained by comparing plants grown in sterilized and non-sterile soil without identifying the soil organisms involved (Van der Putten, Van Dijk and Peters 1993). Based on the results of the experiments in this paper, it is plausible that plants can be infected by more than one combination of plant pathogens and parasites. For *A. arenaria*, infections with *T. ventralis* and different combinations of fungi were demonstrated to be harmful, but *Heterodera* sp. and *Meloidogyne maritima* may also either alone or in combination with fungi or other nematodes, contribute to the disease complex. The number of pathosystems may, therefore, even increase. The occurrence of more than one pathosystem is a well known phenomenon in agriculture. The search for unique disease complexes may be inadequate to study the role of plant-pathogens and parasites in the succession of plants in coastal foredunes.

EFFECTS OF SAND DEPOSITION ON THE INTERACTION BETWEEN *AMMOPHILA ARENARIA*, PLANT-PARASITIC NEMATODES AND PATHOGENIC FUNGI

P.C.E.M. de Rooij-van der Goes, W.H. van der Putten and B.A.M. Peters

Canadian Journal of Botany 73 (1995): 1141-1150.

CHAPTER 4
EFFECTS OF SAND DEPOSITION ON THE INTERACTION BETWEEN AMMOPHILA ARENARIA, PLANT-PARASITIC NEMATODES AND PATHOGENIC FUNGI

SUMMARY

Ammophila arenaria is a dominant sand-fixing plant species of the European coastal foredunes. It remains vigorous under regular burial conditions on seaward slopes, but starts to degenerate when sand accumulation diminishes. Several hypotheses have been put forward to explain this degeneration. In this study, we are testing the hypothesis that upward growth of plants following sand burial enables them to escape harmful soil organisms.

Plants in a degenerating field stand of *A. arenaria* and potted plants grown in sterilized sand (outdoor pot-experiment) were buried with sterilized or non-sterile sand. Both burial in sterilized and non-sterilized sand resulted in stem elongation, increased numbers of living shoots, and shoot and root biomass. However, when plants were grown in sterilized sand and buried with sterilized sand, the numbers of shoots were significantly higher than those buried with non-sterile sand. The new root zone of buried plants was colonized by pathogenic soil organisms (nematodes and fungi) during the same growing season. It is concluded that by upward growth through pathogen-free sand, the plants benefit, at least temporarily, from escaping its pathogens and parasites.

INTRODUCTION

In coastal foredunes, windblown sand is trapped by the vegetation. *Ammophila arenaria* (L.) Link is the most important sand-fixing species in coastal foredunes of north-western Europe, the Mediterranean, and (introduced) in Australia and the west coast of the USA (Knutson 1978, Huiskes 1979). The vigour of *A. arenaria* is strongly correlated with the accretion of windblown sand (Marshall 1965, Hope-Simpson and Jefferies 1966, Huiskes 1979, Willis 1989). *A. breviligulata* Fern., the American beach grass, shows a similar response (Disraeli 1984, Maun and Lapierre 1984, Maun and Baye 1989, Baye 1990).

Several hypotheses have been advanced to explain the effects of sand deposition

on the vigour of both *A. arenaria* and *A. breviligulata*; generally summarized by Marshall (1965) and Laing (1967), but no experimental evidence has become available over the years for any of these hypotheses. The decline of *Ammophila* spp. would be caused by nutrient deficiency, and sand accretion was considered to provide nutrients. However, the vigour of *A. arenaria* was less stimulated by fertilizers than by burial with sand in spite of the low amounts of nutrients in the new sand layers (Willis 1965, 1989). Recently, a new hypothesis was put forward by Baye (1990), who showed that the physiological activity of the plants increases in response to burial thus producing vigorously growing plants. Earlier work reported by our group (Van der Putten et al. 1988) showed that the decline of *A. arenaria* is due to the occurrence of harmful soil organisms in its root zone. Sand brought up by waves and deposited on the plants by wind does not contain harmful soil organisms (Van der Putten and Troelstra 1990). However, within one growing season, harmful soil organisms were present in the newly formed root layer, suggesting that by upward growth and the formation of roots in the deposited layer of windblown sand, the plants escape harmful soil organisms (Van der Putten et al. 1989). This would explain why *A. arenaria* regularly has to receive windblown sand to remain vigorous.

Both nematodes and fungi were involved in the degeneration process (Van der Putten et al. 1990). Subsequent surveys on nematodes and fungi in the root zone of *A. arenaria* stands (chapter 2) revealed several clusters of potentially plant-pathogenic and parasitic soil organisms. Subsequent addition of several of these clusters to seedlings of *A. arenaria* grown in sterilized sand affected plant growth (chapter 3). Nevertheless, the disease-complex of *A. arenaria* is specific and differs from complexes found for other plant species from dry outer dunes such as *Hippohaë rhamnoides* (Maas et al. 1983, Zoon et al. 1993), *Carex arenaria* and *Festuca rubra* (Van der Putten et al. 1993).

To test the hypothesis that by colonizing windblown beach sand *A. arenaria* remains vigorously through escape, the effect of sand deposition on the growth and vigour of *A. arenaria* was studied. Plants were buried with sand containing the whole rhizosphere microflora and -fauna (including the soil-borne pathogens) and compared to plants buried with sand not containing these organisms.

MATERIALS AND METHODS

Source of sand

Sand used for these experiments was collected from a vigorous natural stand of *A. arenaria*, located at the foredunes at Voorne, the Netherlands (51°52' NL 4°04' EL) at a site immediately north of the Haringvlietdam. The stand naturally received annual burial of approximately 20 cm of windblown beach sand. Random samples were taken from the layer between 5 to 40 cm below the sand surface containing one- and two-year-old roots of *A. arenaria*. The sand was sieved (mesh size: 2 cm) and homogenized. Sifted roots were chopped into 3 to 5 cm pieces and mixed with the sand. Part of the collected sand was sterilized by means of gamma-radiation (4 Mrad).

Field experiment

The study site was located close to the location from where the sand samples were collected. Here, the *A. arenaria* stand had not received any windblown sand for several years and the plants were degenerating. In October 1990, 16 plots of 1 by 1 m were laid-out in a grid pattern. Four treatments, with four replicates each, were applied according to a completely randomized design: burial with 20 cm of windblown beach sand (B); burial with 20 cm of sand from the root zone of *A. arenaria* (R); burial with 20 cm of sterilized sand from the root zone of *A. arenaria* (RS) and no burial (N).

Each plot was fenced with a 20 cm high wooden frame (except for the treatment 'no burial'). Windblown beach sand was collected near the experimental site in front of the seaward slope and was used for the burial treatments. The different types of sand were manually and carefully deposited around the plants, so that the leaf tops emerged from the deposited layer.

The numbers of viable (green) shoots were counted each month throughout the experimental period. At the end of the experiment, October 1991, the fresh weight of roots, below-ground shoots of the deposited layer and above-ground shoots was assessed. Dry weights were determined after drying at 70°C for 48 hours.

In October 1990, April, June and October 1991, 4 cores of approximately 160 cm^3 of sand were taken from a depth of 0 to 20 cm below soil surface in each plot. Per plot the four cores were combined and mixed gently. The numbers of nematodes and fungi were assessed from the mixed sample as described below. Because only very few roots were present in the collected samples, numbers of nematodes and fungi were only assessed from sand and not from roots.

Outdoor pot-experiment

Two-weeks-old seedlings were planted in 23.5 cm high plastic pots with 4.5 kg sterilized sand containing 10% moisture content (w/w). In April 1991, 10 weeks after planting the seedlings, a 23.5 cm long PVC-tube (ϕ 13 cm) was placed on top of each pot. The connection between the pot and tube was sealed with tape and the tube was filled manually to 20 cm depth with approximately 4.5 kg of either sterilized or non-sterile root-zone sand (5% moisture). The leaf tops remained above the new soil surface.

The pots were placed outdoors and were buried within a wooden frame filled with river sand, so that soil temperatures approximated natural conditions. After 8, 16 and 25 weeks of burial, 6 replicate pots of each treatment were harvested. The deposited layer (PVC-tube) and the original core (the pot) were separated. In both layers, the biomass of the above-ground and below-ground shoots as well as that of roots was assessed. Also the number of nematodes in sand and the number of fungi inside the roots were assessed. Dry weight of plant samples was determined after drying for 48 hours at 70°C.

Isolation of soil organisms

Nematodes. Nematodes were isolated from the sand samples by elutriation (experiment 1) (Oostenbrink 1960) or by decantation (experiment 2) ('s Jacob and Van Bezooijen 1984). Individuals of plant parasitic nematodes were identified to genus level according to Bongers (1988).

Fungi. In experiment 1, the fungi from the sand were isolated by diluting sand (10^{-2} - 10^{-5}) in triplicate on malt extract agar (20 g malt extract (Oxoid), 3 g pepton (Oxoid), 15 g agar (Merck) and 2.5 g bile (Sigma) per liter) with $50*10^{-6}$ g per ml oxytetracyclin. In experiment 2, the fungi were assessed on pieces of roots of approximately 0.5 cm in length. In total, 20 root pieces per sample (per layer per pot) were washed three times in sterile demineralized water and placed on malt-extract agar. After incubation at 23°C for 4 to 7 days, the fungi were subcultured on potato dextrose agar (Oxoid), counted and identified according to Domsch et al. (1980). The *Fusarium* isolates were identified to species level according to Nelson et al. (1983).

Data analyses

Field experiment. Repeated measurements in time of the numbers of living shoots were analysed by multivariate ANOVA (SPSS procedure MANOVA) with time as a within-subject factor and the initial number of shoots as covariate in the

analysis. Significant combinations that occurred within each of the subjects were tested with the averaged F-test. The means were compared by single degree of freedom contrasts ($P < 0.05$). The above-ground shoot biomass, the shoot biomass in the deposited layer, the total shoot biomass and root biomass were analysed by one-way ANOVA. The means were compared by Tukey's test ($P < 0.05$). The numbers of plant-parasitic nematodes and the numbers of fungi were analysed by two-way ANOVA: The numbers of fungi (expressed as colony forming units (CFU)) were log-transformed in order to normalize the data. Means of the various treatments were compared by means of Least-Significant-Difference ($P < 0.05$).

Outdoor pot-experiment. The means of numbers of shoots were compared by Least-Significant-Differences ($P < 0.05$). The biomass data were analysed by one-way and two-way ANOVA. The treatment means were compared by Tukey's test ($P < 0.05$).

The presence of individual species of plant parasitic nematodes and of fungi was analysed by fitting a loglinear model to the corresponding likelihood tables. A full factorial design including time (T) (harvesting date: a growing period of 8, 16 and 25 weeks), sterilization (S) (burial by sterilized or non-sterile sand) and layer (L) (deposited layer and original core) was used for the different species of nematodes and fungi. A Chi-square goodness of fit was given for the model. The analysis of data with loglinear models is based on independent variables. In this study the variable 'layer' is assumed to be an independent variable.

RESULTS

Field experiment

Number of shoots. For all treatments numbers of living shoots were lowest in April 1991 but increased significantly until the end of the experiment (Fig. 4.1, Table 4.1). The variability in the initial number of shoots at the start of the experiment (October 1990), the overall mean ('regression') and main effect ('treatment') were not significantly different between the treatments (Table 4.1). The numbers of living shoots in the non-buried plants were significantly lower than of the buried plants. Burial by sterilized sand (RS) did not result in a significantly higher number of living shoots than burial by unsterilized sand (R). However, when covered with sand from the beach (B) the numbers were significantly higher than those buried with non-sterile sand (R). The number of living

shoots of plants buried with RS was not significantly different from plants buried with B (Table 4.1, Fig. 4.1).

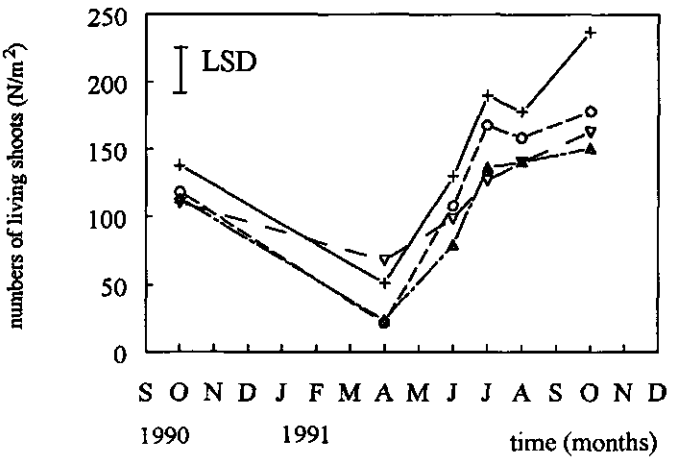


Figure 4.1. Numbers of living shoots per m^2 during one growing season of a degenerating *Ammophila arenaria* stand at Voorne that was buried with 20 cm of root zone sand (▲), sterilized root zone sand (○) or sand from the beach (+) or was not buried (▽).

Table 4.1. Field experiment: F-ratios and significance from an analysis of variance for the numbers of shoots of *Ammophila arenaria* that was buried with 20 cm of root zone soil, sterilized soil or sand from the beach or was not buried. Time is a within plot repeated factor, for which the averaged F-test and significance for repeated measurements is presented. The means of Treatment by Time were compared by single degree of freedom contrasts. N= no sand, B= buried with sand from the beach, R= buried with root zone soil and RS= buried with sterilized root zone soil.

	number of shoots		
	df	F	P
Regression	1	2.14	0.175
Constant	1	4.28	0.065
Treatment	3	3.52	0.057
Time	4	140.30	<0.001
Treatment by Time	12	3.14	0.003
N vs R	4	3.22	0.021
N vs RS	4	5.06	0.002
N vs B	4	5.16	0.002
R vs RS	4	0.93	0.453
R vs B	4	2.89	0.033
RS vs B	4	1.33	0.274

Plant biomass. In the deposited layer of root-zone sand (R), the biomass of living shoots was significantly lower than in sand from the beach (B) (Fig. 4.2A). The aboveground shoot biomass of the treatment 'no burial' (N) was not significantly different from that of the treatment R or RS, but was significantly lower than in the treatment B. The total (above- and below-ground) shoot biomass was significantly lower when plants were not buried than when plants were buried with the different types of sand. There was no difference in the root biomasses from different sand deposits (Fig. 4.2B).

The dry weight per shoot (= above ground shoot biomass divided by the numbers of shoots at the end of the experiment) in non-buried plots (0.78 ± 0.20) was significantly lower than in plots buried with the different sand types. However, there were no differences between all three burial treatments (B, 1.04 ± 0.13; RS, 1.08 ± 0.15; R, 1.01 ± 0.06).

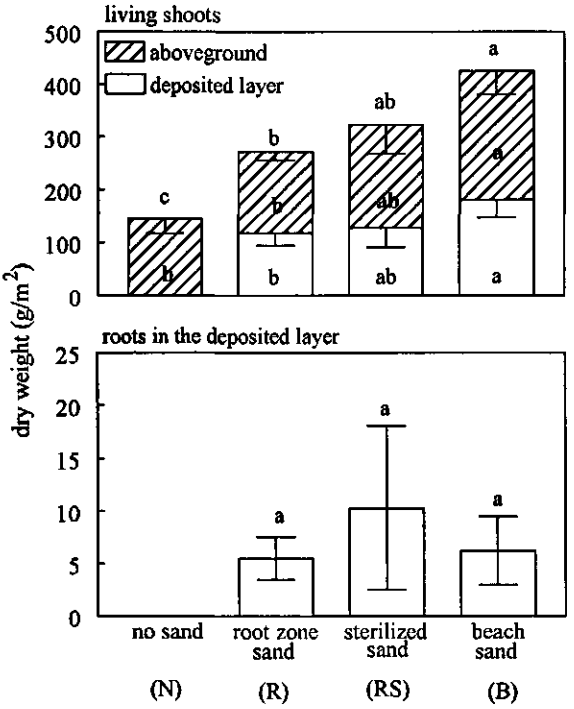


Figure 4.2. Shoot and root biomass of a degenerating *Ammophila arenaria* stand at Voorne buried with 20 cm of root zone sand (R), sterilized root zone sand (RS) or sand from the beach (B) or was not buried (N). Bars (also within bars) bearing different letters are significantly different at $P = 0.05$.

Nematodes. No nematodes were found directly after burial in the collected beach sand and in sterilized root-zone sand (Fig. 4.3). There was a significant interaction between different treatments and harvest time for the numbers of plant parasitic nematodes (Table 4.2). In root-zone sand, the numbers of plant-parasitic nematodes decreased between October and April ($P < 0.05$) and increased in the following period, whereas the number of plant parasitic nematodes in beach sand gradually increased in time (Fig. 4.3). At the end of the experiment, the numbers of plant parasitic nematodes for the three sand types were not different (Fig. 4.3).

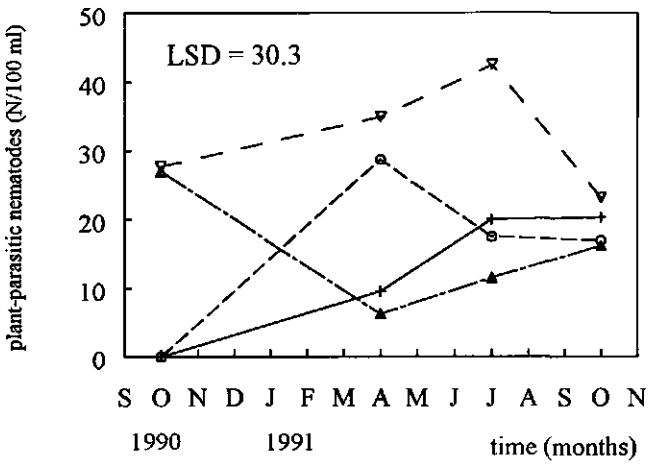


Figure 4.3. Numbers of plant-parasitic nematodes per 100 ml of soil collected from a degenerating *Ammophila arenaria* stand at Voorne that was buried with 20 cm of root zone sand (▲), sterilized root zone sand (○) or sand from the beach (+) or was not buried (▽). The numbers were assessed at the start of the experiment in October 1990 and in April, June and October 1991.

The taxa of plant-parasitic nematodes were: *Pratylenchus* sp., *Rotylenchus goodeyi*, *Helicotylenchus* sp., *Filenchus* sp., *Telotylenchus ventralis*, *Hemicriconemoides* sp., *Heterodera* sp., *Meloidogyne maritima* and *Aphelenchus* sp.. Also several non-plant-feeders (saprophagous nematodes) as well as a few individuals of *Longidorus kuiperi* were found. There was no relation between the type of sand and nematode-taxa found (data not shown).

Table 4.2. Field experiment: ANOVA results (F-values) of the number of plant-parasitic nematodes and fungi isolated from soil collected from an *Ammophila arenaria* stand which was buried with: one of 20 cm of root zone soil, sterilized root zone soil, sand from the beach, or was not buried (factor treatment). The nematodes and fungi were isolated in October 1990, April, June and October 1991 (factor harvest time). MSE = mean square error.

	Plant-parasites			Fungi		
	df	F	P	df	F	P
Treatment (T)	3	2.99	0.043	3	53.19	<0.001
Harvest time (H)	3	2.69	0.060	3	15.81	<0.001
T*H	9	3.77	0.002	9	4.56	0.001
MSE	37	102.39		33	0.245	

Fungi. Directly after burial, the number of fungi was significantly lower in the treatments burial with beach sand (B) and sterilized root zone sand (RS) than in root zone sand (R) (Fig. 4.4). The number of fungi found in the control plots was significantly higher than in the buried plots. The number of fungi increased from October 1990 to June 1991 in the plots buried with beach sand and RS, whereas

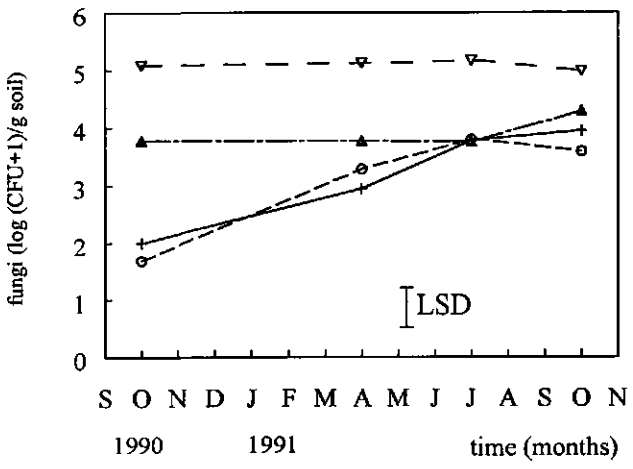


Figure 4.4. Log N+1 of number of colony forming units (CFU) of fungi per gram dry soil. The fungi were collected from a degenerating *Ammophila arenaria* stand at Voorne buried with 20 cm of root zone sand (▲), sterilized root zone sand (○) or sand from the beach (+) or was not buried (▽). The numbers were assessed at the start of the experiment in October 1990 and in April, June and October 1991.

it remained constant during the experiment in the control plots (N) and R-plots (Fig. 4.4). This interaction between time and treatment was also confirmed by the results of ANOVA (Table 4.2). In October 1991, at the end of the growing season the numbers of fungi in the plots that were buried with the different types of sand did not differ from each other (Fig. 4.4).

The high numbers of fungi in the control plots were due to the dominance of *Penicillium* spp. and plant-pathogenic *Phoma* spp. (data not shown). Together with the possible plant-pathogenic *Cladosporium* sp., these fungi dominated the isolations in all plots. In October 1990 only yeasts were isolated from sterilized root zone sand. The number of *Penicillium* and *Phoma* in both sand from the beach and sterilized root-zone sand increased with time.

Outdoor pot-experiment

Numbers of shoots. After 8 weeks of burial, seedlings buried with sterilized or non-sterile sand already showed stem elongation. Between sterilized and unsterilized sand, the numbers of emerged shoots did not differ significantly until week 14. From 16 weeks until the end of the experiment, the plants that were buried with sterilized sand produced significantly more shoots than plants that were buried with non-sterile sand (Fig. 4.5).

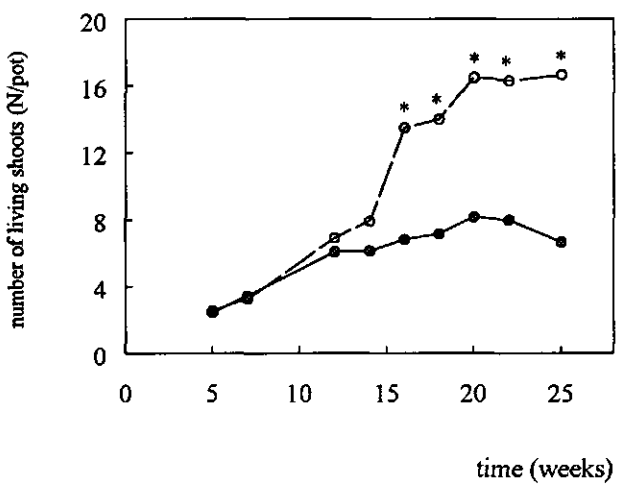


Figure 4.5. Number of living shoots of *Ammophila arenaria* during a growth period of 25 weeks in a pot-experiment with seedlings grown in sterilized root zone soil that were covered with 20 cm of sterilized (○) or non-sterile soil (●). * = significant difference between ○ and ●.

Plant biomass. On average, the biomass of plants covered with sterilized sand was higher than those buried with non-sterile sand (total biomass: P= 0.012, statistical analysis not shown). However, these differences were not significant at each separate harvest times (Fig. 4.6). The biomass of aboveground shoots, shoots and roots in the deposited layer and the roots in the original core increased significantly with time (Table 4.3). Between 8 and 16 weeks after burial, the first roots were formed in the deposited layer. The majority of roots was formed between 8 and 16 weeks after burial. The shoot biomass in the deposited layer and the above-ground shoot biomass also increased significantly between 8 and 16

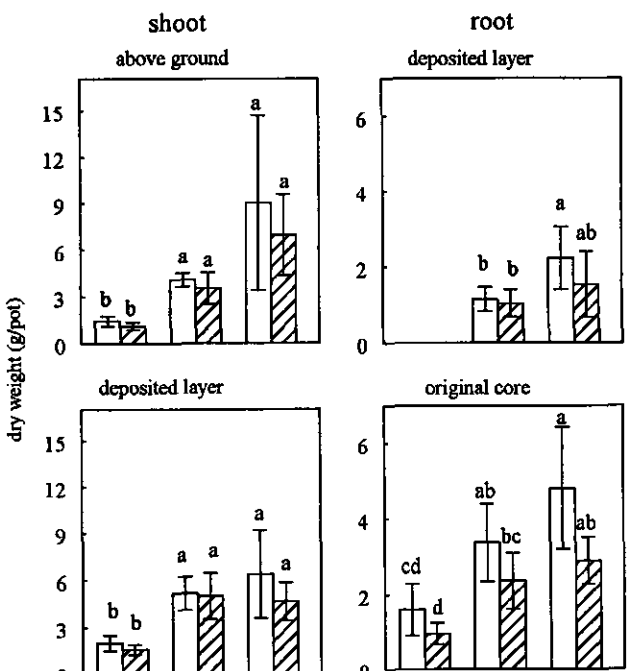


Figure 4.6. Shoot and root biomass of *Ammophila arenaria* that were buried with sterilized or non-sterile soil after a growing period of 8, 16 or 25 weeks after burial. Seedlings were grown in sterilized soil. The shoot biomass was assessed in the deposited layer and above ground. The root biomass was assessed in the original core and in the deposited layer. Bars bearing different letters are significantly different at $P = 0.05$.

weeks. Dry weights of aboveground shoots tended to increase between weeks 16 and 25. The increase in shoot biomass of plants buried with non-sterile sand tended to be less than those buried with sterilized sand (Fig. 4.6). It should be noted that in the latter case also root production in the original core tended to be stimulated.

Table 4.3. Outdoor pot-experiment: ANOVA results (degrees of freedom, F- and P-values) of above ground shoot biomass, biomass of roots and shoots in the deposited layer of sand and in the original core of seedlings of *Ammophila arenaria* that were buried with non-sterile or sterilized soil (factor sterilization) and harvested after 8, 16 or 25 weeks (factor harvest time). MSE = mean square error.

above ground		shoots				
	df	F	P			
Sterilization (S)	1	1.44	0.239			
Harvest time (H)	2	50.74	<0.001			
S*H	2	0.11	0.899			
MSE	29	0.174				
deposited layer		shoots			roots	
	df	F	P	df	F	P
Sterilization (S)	1	2.30	0.141	1	2.37	0.140
Harvest time (H)	2	40.75	<0.001	1	9.28	0.007
S*H	2	0.23	0.796	1	1.30	0.269
MSE	29	0.112		19	0.404	
original core		roots				
	df	F	P			
Sterilization (S)	1	16.65	<0.001			
Harvest time (H)	2	38.26	<0.001			
S*H	2	0.14	0.869			
MSE	30					

Nematodes. Low numbers of plant-parasitic nematodes were isolated from pots that were buried with sterilized sand. Non-plant-feeding nematodes were recovered from this sand frequently in relatively large numbers. These nematodes must have spontaneously infected the pots. Except for *Tylenchorhynchus* sp., Heteroderidae males, *Aphelenchus* sp. and *Telotylenchus ventralis*, all other taxa of nematodes

were significantly more frequently found in pots buried with non-sterile sand than in those with sterilized sand (Table 4.4; statistical analysis not shown). Harvest time significantly influenced the frequency of recovery of the nematodes *Paratylenchus* sp., *Rotylenchus goodeyi*, *Hemicriconemoides* sp. and juveniles of *Heterodera* and *Meloidogyne*. *Paratylenchus* sp. was isolated 8 and 16 weeks after the start of the experiment, but not after 25 weeks. *Heterodera* and *Meloidogyne* were not found in the sand 16 weeks after burying the plants. Apparently, they all had infected the roots of *A. arenaria* at this time, so that no free-living specimens of these species could be found. After 25 weeks, free-living Heteroderidae were found again in the original core as well as in the deposited layer of non-sterile sand (Table 4.4).
In pots buried with non-sterile sand, roots in the original core were infected by plant parasitic nematodes that had migrated downwards from the deposited layer towards the original core. In the original core nematodes were found already after 8 weeks, although some individuals remained in the deposited layer (Table 4.4). *Hemicriconemoides* sp. was significantly more frequently recovered from the deposited layer than from the original core. They apparently could not migrate downwards to the original core (Table 4.4; statistical analysis not shown).

Fungi. The number of fungal species isolated was higher after a growing period of 16 or 25 weeks than after a period of 8 weeks, although the total number of colonies per root piece was roughly the same (Table 4.5). Fungal species isolated from roots in the original core were generally also isolated from the roots in the deposited layer (Table 4.5). All species of fungi except *Cladosporium* sp. were isolated equally frequent from both layers, implying that most fungi grew downwards and, possibly multiplied there as well (Table 4.5; statistical analysis not shown).
The frequency of only a few species of fungi was affected by time of harvest. For example *Microdochium bolleyi* was frequently isolated from roots after 8 weeks, but was rarely isolated at week 16 or 25, whereas *Chaetomium globosum* was more frequently isolated at weeks 16 and 25 than at week 8 (Table 4.5). The numbers of isolates of *Fusarium dimerum*, *Trichoderma harzianum* and the total group of *Fusarium* spp. were significantly higher in roots of plants buried with non-sterile sand than in those buried with sterilized sand. For *Nectria radicicola* and the Basidiomycetes the opposite was the case. The frequency of *Ulocladium* sp. was significantly higher in the original layer of plants buried with sterilized sand than in the same layer of plants buried with non-sterile sand (Table 4.5).

Table 4.4. Average total number of nematodes in the original core (ORI) and in the deposited (DEP) layer of sterilized (S) and non-sterile (NS) soil of pots with seedlings of *Ammophila arenaria* (6 replicates). The nematodes were assessed after 8, 16 and 25 weeks of burial. The frequency of recovery is presented between brackets. + = present in average numbers lower than 1. m = not assessed, jv= juveniles.

	8 weeks				16 weeks				25 weeks			
	ns		s		ns		s		ns		s	
	ori	dep	ori	dep	ori	dep	ori	dep	ori	dep	ori	dep
semi-endoparasitic												
Rotylenchus goodeyi	3 (2)	7 (3)	-	2 (2)	17 (1)	-	-	-	-	13 (4)	-	-
ectoparasitic												
Paratylenchus sp.		12 (4)	2 (1)	-	-	33 (1)	2 (1)	-	-	-	-	-
Tylenchorhynchus sp.	2 (1)	-	-	-	-	-	-	-	-	-	-	-
Telotylenchus ventralis	7 (2)	3 (1)	5 (1)	2 (1)	-	33 (2)	-	-	-	3 (2)	5 (1)	-
Hemicriconemoides sp.	2 (1)	28 (6)	-	-	-	2 (1)	4 (1)	-	13 (3)	17 (6)	-	-
endoparasitic												
Pratylenchus sp.	3 (2)	-	-	-	17 (1)	-	-	-	4 (1)	8 (3)	-	-
Heterodera spp. (jv)	103 (5)	18 (5)	+ (2)	-	-	-	-	-	363 (5)	115 (6)	3 (1)	-
Meloidogyne maritima (jv)	37 (6)	5 (3)	-	-	-	3 (1)	-	-	283 (5)	105 (6)	2 (1)	-
Heteroderidae males	-	-	+ (1)	-	-	2 (1)	-	-	-	-	-	-
root/fungal feeding												
Filenchus sp.	35 (5)	5 (3)	-	-	350 (5)	285 (6)	-	3 (1)	42 (5)	20 (4)	10(1)	-
Aphelenchus sp.	-	-	-	-	167 (2)	50 (2)	-	-	13 (1)	-	3 (1)	2 (1)
Dorylaimidae	m	m	m	m	867	578	65	13	246	133	55	3
Non-plant feeders	5565	2668	2523	1355	18250	14480	13380	5901	12310	5062	9710	5363

DISCUSSION

Ammophila arenaria buried with beach sand and sterilized sand, produced more new above-ground shoots and shoot biomass than plants buried with unsterilized sand containing fungi and nematodes. This implies that by upward growth through pathogen-free sand, *A. arenaria* benefits, at least temporarily, from escaping its soil-borne pathogens and parasites. Both the degenerating *A. arenaria* in the field experiment and the vigorously growing seedlings of *A. arenaria* in the outdoor pot-experiment showed a similar reaction to burial by sand with and without pathogens.

The hypothesis that the decline of *A. arenaria* in stabilized dunes is caused by a diminished uptake of nutrients, as proposed by Marshall (1965) and Laing (1967), did not take the role of pathogens into account. As the functioning of roots decreases rapidly when they are infected by pathogens, pathogens will increase the nutrient stress for *A. arenaria* when sand deposition stops. In this study, when pathogens were present in the deposited layer of sterile sand (i.e. non-sterile sand), roots of *A. arenaria* became infected as soon as they started to develop. This may have resulted in a reduced root formation and consequently in a reduced uptake capacity which, in turn, inhibited shoot development. Root biomass in the original core of the pot-experiment was significantly lower when plants were buried with non-sterile sand (Table 4.4). The root biomasses in the field experiment were not significantly different between the different treatments.

According to Baye (1990), the effects of sand accretion on tillering rates was strongly related to changes in the ability of buds to escape inhibition caused by apical dominance. He showed that high vigour was already evident immediately following emergence from deep burial, while new nodal root systems were only beginning to develop. Therefore, Baye (1990) concluded that the rapid reaction of shoot vigour to sand accretion was not the result of the replacement of degenerating roots or of the uptake capacity of the root system alone (Marshall 1965) but to changes in the physiology of the buried plants as well. This indicates that burial in itself has a positive effect on the growth of plants, which is also confirmed by the present results.

Roots, especially root tips, are the major source of cytokinins for plants. A direct relationship between the supply of ample cytokinins and shoot growth has been established (Carmi and Heuer 1981). Consequently, constraints in rooting space or new root development will reduce plant growth when not buried by sand (Baye 1990). Nevertheless, our results show that maximum vigour cannot be maintained

Table 4.5. Average number of colonies per 20 root pieces collected from roots of the original, sterilized core (ORI) and the deposited layer (DEP) of non-sterile (NS) or sterilized (S) soil of pots with seedlings of *Ammophila arenaria* (6 replicates). The pots were harvested at 8, 16 and 25 weeks after burial. The frequency of recovery is presented between brackets only when significant differences were found.

	8 weeks				16 weeks				25 weeks			
	ns		s		ns		s		ns		s	
	ori	dep	ori	dep	ori	dep	ori	dep	ori	dep	ori	dep
Basidiomycetes	-	-	-	-	5.5 (2)	2.5 (1)	5.3 (3)	7.2 (5)	-	-	-	-
Absidia corymbifera	-	-	-	0.3	-	-	-	-	-	-	-	-
Acremonium fusidioides		-	-	-	-	-	-	-	0.2	-	-	-
A. murorum	-	-	-	-	-	2.1	-	-	-	-	-	-
Alternaria alternata	-	-	-	-	0.2	-	1.2	0.5	-	-	0.2	0.2
Apiospora montagnei	-	-	-	-	-	-	-	-	-	-	0.2	0.8
Chaetomidium fimeti	1.0	0.7	-	-	-	-	0.2	1.7	-	-	-	0.2
Chaetomium funicola	10.2	5.7	9.5	10.7	2.3	1.8	0.2	-	3.3	0.5	2.2	0.7
C. globosum	0.3 (1)	3.0 (2)	-	0.7 (1)	3.1 (3)	2.5 (2)	7.8 (5)	10.7 (6)	3.7 (5)	6.8 (4)	4.8 (5)	7.2 (4)
Cladosporium sp.		-	-	-	-	0.2 (1)	-	0.2 (1)	-	0.3 (2)	-	-
Cylindrocarpon sp.	-	-	-	-	0.3	-	-	-	-	-	-	-
Epicoccum purpurascens	-	-	-	-	0.2 (1)	-	0.2 (1)	-	0.2 (1)	0.5 (1)	0.5 (2)	1.0 (2)
Fusarium culmorum	-	-	-	-	-	-	-	-	-	2.8	-	0.2
F. dimerum	0.8 (1)	-	-	-	2.5 (2)	1.2 (2)	-	-	-	1.0 (2)	-	0.2 (1)
F. nivale	-	-	-	-	0.2	-	-	-	-	0.5	-	-
F. poae	-	0.7	-	-	-	-	-	-	-	-	-	-
F. oxysporum	-	-	-	-	-	0.3	0.3	1.8	-	-	-	-
Gliocladium catenulatum	-	-	-	-	-	-	-	-	0.3	-	-	0.2
Glomerella sp.	-	-	-	-	0.8	1.0	-	-	-	-	-	-
Microdochium bolleyi	3.8 (3)	9.0 (3)	0.5 (1)	1.0 (2)	1.3 (2)	-	-	0.3 (2)	0.3 (2)	0.5 (2)	-	-
Minimedusa sp.	-	-	-	-	-	0.3	-	-	-	-	-	-

Table 4.5, continued

	8 weeks				16 weeks				25 weeks			
	ns		s		ns		s		ns		s	
	ori	dep	ori	dep	ori	dep	ori	dep	ori	dep	ori	dep
Mortierella alpina	-	2.3	1.8	-	-	-	0.2	-	-	-	-	-
Mucor hiemalis	-	-	-	-	-	-	-	-	-	0.3	-	2.0
Nectria radicicola	-	-	2.0 (2)	1.0 (2)	-	-	0.2 (1)	-	-	-	-	1.7 (3)
Penicillium spp.	0.5 (2)	4.0 (2)	-	1.7 (2)	0.2 (1)	0.2 (1)	0.2 (1)	0.2 (1)	5.0 (3)	1.5 (3)	6.3 (4)	4.7 (6)
Peziza ostracoderma	0.5	-	0.5	-	-	0.2	0.7	-	-	-	-	-
Phoma spp.	0.5 (2)	-	0.8 (2)	-	-	-	0.7 (2)	-	-	0.5 (1)	-	0.2 (1)
Plectoshaerella cucumerina	-	0.3	-	-	0.3	-	-	-	-	0.5	-	-
Pleospora sp.	-	-	-	-	0.2	-	0.2	-	-	-	-	0.2
Thielavia heterothallica	-	-	-	-	0.5 (1)	0.2 (1)	0.5 (1)	1.5 (2)	-	-	-	0.7 (1)
Trichoderma harzianum	-	0.7 (1)	-	0.3 (1)	2.2 (2)	2.0 (2)	0.8 (1)	0.8 (1)	1.2 (2)	0.5 (2)	-	-
T. koningii	-	-	-	-	-	0.3	-	-	-	-	-	-
Ulocladium sp.	1.5 (3)	1.0 (2)	-	-	0.3 (2)	-	2.3 (2)	1.3 (1)	0.2 (1)	0.3 (1)	0.7 (1)	0.2 (5)
Not identified	1.0	1.3	7.3	3.7	0.2	8.7	2.7	1.7	1.7	0.2	1.5	0.3
number of colonies per root	1.00	1.46	1.09	0.96	1.03	1.17	1.19	1.39	0.84	0.94	0.81	0.83

when the deposited sand contains pathogens and parasites. The escape hypothesis (Van der Putten et al. 1989) seems therefore to be valid, although there is also a physiological mechanism involved due to activation of plant hormones when plants become buried (Baye 1990, Yuan et al. 1993).

Plant-parasitic nematodes commonly infect the roots near root tips at the cell-elongation sites. These infections usually result in modifications of roots (Prot 1980). For example, the endoparasitic nematodes of the genus *Meloidogyne* modify roots by inducing an extensive feeding site within the root tissue and by the formation of new rootlets near the feeding site (Prot 1980, Eisenback and Griffin 1987). If only a few nematodes infect the roots, the development of extra rootlets can enhance plant growth as more root tips produce more cytokinins. However, nematodes also consume root cell contents. Thus, increasing numbers of nematodes will finally suppress plant growth. Therefore, the reduced formation of new shoots when plants were buried with non-sterile sand was most likely the result of infections of roots by soil organisms. In the outdoor pot-experiment roots in the original core of plants buried with non-sterile sand (Fig. 4.6) were also infected by soil organisms (Tables 4.4 and 4.5). The reduced production of biomass will, therefore, not only have been the result of reduced production of new roots in the deposited layer, but also of reduced root functioning in the original core. Downward migration of soil organisms in the field experiment was not observed. It seems unlikely that in the field the soil organisms will have migrated downwards, because the buried plants were degenerated and had few healthy roots prior to burial.

Nematode-numbers in the field experiment were very variable and a significant increase in numbers could not be detected. However, it was obvious that nematodes migrated upwards to the newly formed root layer. In the pot-experiment, all nematode species, except *Hemicriconemoides* sp., first migrated downwards from the deposited layer of non-sterile sand towards the original core containing roots of *A. arenaria* (Table 4.4). When roots were being formed in the deposited layer, the nematodes migrated upwards again. Migration of nematodes in soil towards their hosts, even over relatively large distances, has been reported. For example, *Globodera rostochiensis*, the potato cyst-nematode is able to migrate over 45 cm in order to reach the host plant and *Meloidogyne* spp. can even reach host roots over a vertical distance of 125 cm (Prot 1980). *Rotylenchus* spp. are more frequently found in deep soil layers where the soil moisture content is relatively high (Nombela et al. 1993). In this study, *Rotylenchus goodeyi* was frequently isolated in the original core at weeks 8 and 16. At week 25 it was

found in the deposited layer (Table 4.4). Like most plant-parasitic nematodes, the occurrence of this species seemed to be associated with (healthy) roots rather than with abiotic soil conditions.

Migration or growth of fungi through sand was also found to occur in the present experiments. The slower colonization of beach sand by fungi compared to sterilized sand may be due to a smaller amount of organic material in beach sand which can be used by fungi as a food source. When potted plants were buried with non sterile sand, fungi seemed to have spread downwards to the roots in the original core as they were equally frequently recovered from roots in the deposited layer and original core. The growth of fungi through soils depends largely on the species involved. *Mucor* spp. and *Trichoderma* spp. are able to spread over great distances, whereas *Penicillium* spp. only colonize small pieces of substrate and hardly spread (Pugh 1980).

Field observations showed that sand deposition mainly takes place in autumn and winter and that new roots are formed as early as February. Colonization of the new layer of sand by soil organisms was already evident in April, early in the growing season. The majority of new shoots is produced in June. Timing of migration of soil organisms and the subsequent effect of colonization of the root zone seem, therefore, important factors in the "escape" of *A. arenaria* from its soil-borne pathogens.

In conclusion, the vigour of *A. arenaria* in response to sand accretion is the result of upward growth through the deposited sand by means of which the plants becomes temporarily free of soil organisms, so that new healthy roots can be formed. The present experiments have established that plant vigour is primarily determined by the presence of such healthy roots. From the field experiment, the impression was obtained that nematodes migrate upwards only when new roots are formed (i.e. in spring), while the number of fungi increases more gradually, and already before new roots had been formed. However, in the present experiments no samples were taken between October and April. Therefore, the timing of upward growth of buried stands of *A. arenaria*, the formation of new roots followed by migration of soil organisms and subsequent reduction in tillering under field conditions remains to be investigated.

VERTICAL MIGRATION OF NEMATODES AND SOIL-BORNE FUNGI TO DEVELOPING ROOTS OF *AMMOPHILA ARENARIA* (L.) LINK AFTER SAND ACCRETION.

P.C.E.M. de Rooij-van der Goes, B.A.M. Peters and W.H. van der Putten

Submitted to Oikos.

CHAPTER 5
VERTICAL MIGRATION OF NEMATODES AND SOIL-BORNE FUNGI TO DEVELOPING ROOTS OF *AMMOPHILA ARENARIA* (L.) LINK AFTER SAND ACCRETION.

SUMMARY

Ammophila arenaria benefits from regular burial of windblown beach sand as it allows escape from soil-borne pathogens (nematodes and fungi). Within one growing season, the newly formed roots become colonized by pathogens. This study was done to obtain more insight in the mode and timing of migration of these soil-organisms towards the newly formed roots. Plants were grown in non-sterile soil, buried with sterilized root-zone sand or sand from the beach and harvested frequently.

The fungi were already present in the deposited layer as early as Februari. At this time, significantly higher amounts of colony forming units were present in sterilized root-zone soil than in sand from the beach. During the growing season a shift in the community of fungi in soil and shoots was found. In spite of differences in the species composition of fungi in soil and on shoots between the two soil types, the species composition of root-inhabiting fungi was similar. Upward migration of the majority of plant-parasitic nematodes only occurred when roots were formed in the deposited sand. Then, roots were immediately infected. No differences were found in the species composition of plant-parasitic nematodes isolated from new roots between plants buried by sterilized sand or beach sand. Sand burial did not allow *A. arenaria* to escape from fungal infection, but infections by nematodes was avoided during early stages of root formation. Thus, the succes of escape of *A. arenaria* from its soil-borne pathogens by colonizing windblown sand largely depends on the formation of roots prior to migration of and infection by nematodes.

INTRODUCTION

Ammophila arenaria (L.) Link is a natural sand-fixing plant species that is dominant in European coastal foredunes (Huiskes 1979). On seaward slopes where plants are regularly buried by windblown beach sand, the plants remain vigorous, whereas they start to degenerate as soon as sand accumulation diminishes (Marshall 1965, Hope-Simpson and Jefferies 1966, Huiskes 1979, Willis 1989). *Ammophila breviligulata* Fern., the American beach grass, shows a similar response (Disraeli 1984, Maun and Lapierre 1984, Maun and Baye 1989, Baye 1990). Soil-borne fungi and nematodes are involved in the degeneration of *A. arenaria* (Van der Putten and Troelstra 1990, chapter 3 of this thesis), whereas Seliskar and Huettel (1993) showed that nematodes may also be involved in the degeneration of *A. breviligulata*.

The colonization of fresh windblown sand enables the plants to escape from its pathogens (chapter 4). Burial by windblown sand changes the physiology of the plants (Baye 1990, Yuan et al. 1993, Seliskar 1994). As a result of burial, the length of the internodes increases, and both new nodes and new roots are formed (Disraeli 1984, Maun and Lapierre 1984, Baye 1990). The emergence of *A. arenaria* from accreted sand appeared to be independent of the presence or absence of soil organisms in the deposited sand (chapter 4). However, if buried by unsterilized root zone sand, new roots became immediately infected by the soil pathogens, leading to a reduced formation of new above ground shoots (chapter 4). It was concluded that burial by sand followed by upward growth enables the plants, at least temporarily, to escape from soil-borne pathogens and parasites.

Under natural conditions, within one growing season following sand deposition and emergence of the plants, newly formed roots became gradually colonized by soil organisms (Van der Putten et al. 1989, chapter 4 of this thesis). This is presumably due to vertical migration from the former to the new root layer. Vertical migration of nematodes (Prot 1980) and of fungi (Bollen 1974) has been reported from other systems as well. In coastal foredunes, fungi seem to colonize the deposited sand slowly, whereas plant-parasitic nematodes migrate and infecte roots soon after being formed (chapter 4). The mode and timing of vertical migration and infection of the newly formed roots by fungi and nematodes is still unclear.

The present study aims at clarifying the mode and timing of migration of soil-borne fungi and nematodes as well as to determine when newly formed roots become infected. *A. arenaria* was grown experimentally in non-sterile root zone

sand (containing fungi and nematodes) and buried by sand. After burial by sand, the migration of soil organisms was studied during one growing season under outdoor conditions. Two types of sand were used for burial: beach sand and sterilized root zone sand. Because beach sand may contain less organic matter and higher amounts of salt than root zone sand collected from the foredunes (Van der Putten and Troelstra 1990), differences in colonization and infection by fungi and nematodes of the deposited sand can be expected.

MATERIALS AND METHODS

Origin of soil

In June and October 1993, soil was collected from the foredunes of Voorne, the Netherlands (51°52' NL 4°04' EL) at a site immediately north of Haringvlietdam. Approximately 550 kg of soil was collected from a vigorous stand of *A. arenaria* that was annually subjected to burial by 10 to 20 cm of windblown beach sand. This sample was taken from in total 30 sites randomly chosen over an area of 800 m^2. The soil was collected from the layer between 5-40 cm below the sand surface containing one- and two-years-old roots of *A. arenaria*. Approximately 250 kg of beach sand was collected from 10 sites at the base of the outer seaward foredune slope over a length of 250 m where the windblown sand was deposited in front of the *A. arenaria* vegetation.

The soil was sieved (mesh size: 1.5 cm) and gently mixed. Sifted roots were chopped into 3-5 cm pieces and mixed through the soil. Part of the collected root zone sand was sterilized by means of gamma-radiation (4 Mrad).

Outdoor pot-experiment

In August 1992, *A. arenaria* seedlings were obtained as described in the preceding chapters and grown in 23.5 cm high plastic pots (diameter of 15 cm) with 3.5 kg non-sterile soil containing 10% moisture (w/w) (one seedling per pot). The pots were placed in the greenhouse at 23 $\pm$ 2°C. Once a week, the soil moisture content was set at 10%. In November 1992, after a growing period of 19 weeks, the plants had minimally 2 shoots and the length of the longest shoot was approximately 66 cm. Then, a PVC-tube (length: 23.5 cm, diameter: 15 cm) was placed on top of each pot. The connection with the pot was sealed and the tube was filled with approximately 5 kg of sand corresponding with 20 cm of sand burial. Forty tubes were filled either with sand from the beach (4.4% moisture)

or with sterilized sand collected from the root zone (3.3% moisture). The pots were at random placed outdoors in 80 cm high wooden frames upon a layer of 10 cm of gravel and 10 cm of river sand. This was done to prevent the bottom layer from saturation. Then, river sand was placed between the pots, so that soil temperatures approximated natural conditions. Besides natural rainfall (approximately 105 mm during the growing period) no additional water was given. At each harvest date, 5 random pots (i.e. replicates) of each treatment were harvested. The harvest dates are presented in Table 5.1.

Table 5.1. Growth period and harvest dates. The experimental burial was done at 24 November 1992 (= week 0).

harvest number	growth period (number of weeks)	harvest date (1993)
1	11	3 February
2	17	15 March
3	20	5 April
4	23	26 April
5	26	17 May
6	29	7 June
7	39	17 August
8	49	26 October

Plant parameters

The deposited layer (PVC-tubes) and the original core (the pots) were separated at each harvest date. The fresh weights of the above-ground and below-ground shoots and of roots in the deposited and original layer were assessed. Dry weights of all plant samples were determined after drying for 48 hours at 70°C.

Soil organisms

The deposited layer of sand of the harvested pots was split into 4 layers: 0-5 cm (A), 5-10 cm (B), 10-15 cm (C) and 15-20 cm (D) from the top of the deposited layer downwards. The layer 20-35 cm (E) is the original core. The remaining 8.5 cm was not analysed. From each layer a subsample of 100 ml of soil was taken for collecting nematodes and approximately 20 g of soil for collecting fungi. From each layer (A-D), fungi were isolated from the buried shoots of *A. arenaria*. For the analysis of fungi and nematodes from roots, the layers A-D were not separated

because roots in the deposited layer could not be traced back to a certain layer. Therefore, the only distinction between root layers was between the whole deposited layer and the original core. At harvest 8, the layers A-D were not split at all.

Isolation of nematodes. Nematodes were isolated from the 100 ml soil samples by means of elutriation (Oostenbrink 1960) and from roots using Baermann-funnels (Hooper 1986). Individuals were identified to the genus level.

Isolation of fungi. To isolate the fungi from soil, a small amount (0.03 $\pm$ 0.002 g) of soil was spread in triplicate on malt-extract agar (Warcup 1960), containing 20 g malt extract (Oxoid), 3 g pepton (Oxoid), 15 g agar (Merck) and 2,5 g bile (Sigma) with 100 ppm oxytetracyclin per liter of tap water. To isolate fungi from buried shoots, 5 pieces of $\pm$ 1 cm length were collected from each layer. Root fungi were determined on 5 pieces of approximately 0.5 cm length each. These root and shoot pieces were washed three times with sterile demi-water and placed on malt-extract agar. After incubation at 23°C during 4 to 7 days, the fungi were counted, subcultured on potato dextrose agar (Oxoid) and identified. Nomenclature according to Domsch et al. (1980) was used throughout this study for all fungal isolates except those of the genus *Fusarium*. The latter were identified to species level according to Nelson et al. (1983).

To investigate where infection by fungi takes place (i.e. inside or at the surface of the buried shoots), an additional sample of shoot pieces of each layer was studied in more detail at harvest 2. The shoots were stained with lactophenol cotton blue at 80°C and destained with lactophenol (Goodey 1937). The infections of fungi of the outer and inner leaves of the buried shoots were observed microscopically.

Data analyses

The biomass data were analysed by one-way and two-way ANOVA. Three-way ANOVA was used for the number of colony forming units of fungi isolated from soil (harvests 1 to 7). If required, the data were ln-transformed to obtain homogeneity as is tested by means of Cochran's Q test. The treatment means were compared by Tukey's test ($P \leq 0.05$).

In each of the 5 replicates, the presence of individual species of nematodes and fungi was calculated per layer. The frequencies of individual species were analysed by fitting a loglinear model to the corresponding likelihood tables. A full factorial design including data of harvest (H), treatment (T) (burial with sterilized root-zone sand or sand from the beach) and layer (L) (deposited layer (A-D) and

original core (E)) was used for the different species of nematodes and fungi. A Chi-square goodness of fit was given for the model. The analysis of data using loglinear models is based on independent variables. In this study the variable 'layer' is assumed to be an independent variable.

RESULTS

Plant biomass

In February, 11 weeks after the plants had been buried, no morphological development had appeared. In early spring (from harvest 2 onwards), the stems elongated and new nodes were formed. The growing tip of the plants was brought up by stem elongation onto a few centimeters below the sand surface. After 23

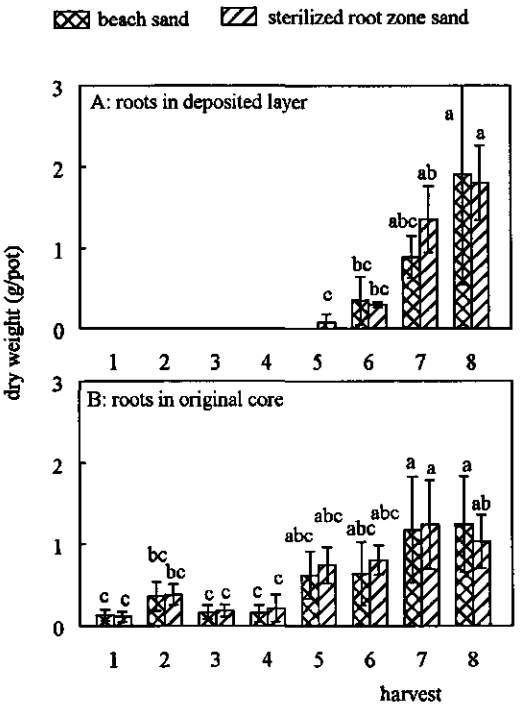


Figure 5.1. Dry weights of roots at different harvest dates (see Table 5.1) in the deposited layer (A) and in the original core (B). The plants were buried with sand from the beach or with sterilized root zone sand. Bars bearing different letters are significantly different at $P = 0.05$.

weeks (from April onwards; harvest 5), roots developed from the new nodes. Root biomass of plants buried by sterilized soil initially developed faster than those of plants buried with sand from the beach, however, one harvest later no significant difference remained (Fig. 5.1A). At the same time as when new roots in the deposited layer were formed, root biomass in the original core started to increase (Fig. 5.1B). No significant differences in root dry weight occurred between harvest 6 and 7 (Fig. 5.1).

After a period of 39 weeks (harvest 7), the above ground shoot biomass had increased significantly as compared to the above ground shoot biomass directly after sand deposition (Fig. 5.2A). Dry weights of shoots in the deposited layer also increased from harvest 6 onwards. During the experiment, no significant differences in shoot dry weights were found between plants buried with beach sand or sterilized soil (Fig. 5.2).

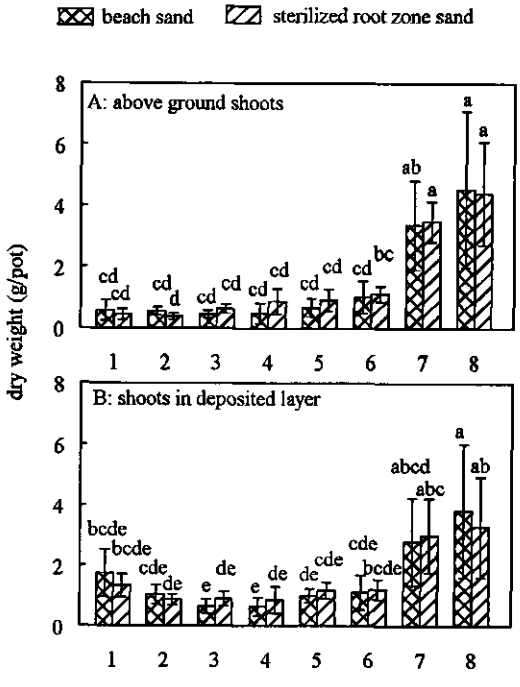


Figure 5.2. Dry weights of the above ground shoots (A) and shoots in the deposited layer of sand (B) at different harvest dates (see Table 5.1). The plants were buried with sand from the beach or sterilized root zone sand. Bars bearing different letters are significantly different at $P = 0.05$.

The increase in shoot biomass coincided with an increase in the number of shoots. The majority of new above ground shoots developed from June until September (data not shown), to be starting one harvest later then new root development in the deposited layer had started (Fig. 5.1A). Also the number of shoots in the deposited layer increased and individual shoots became thicker (P.C.E.M. de Rooij-van der Goes, personal observation), thus leading to an increased biomass of the buried shoots.

Fungi

Before burial, sand from the beach only contained a few yeast colonies and colonies of *Penicillium* species. In the sterilized soil used for burying *A. arenaria* no fungi were found (data not shown).

Fungi in soil. Three-way ANOVA of the number of colony forming units (CFU) showed a significant interaction between harvest time and treatment (Table 5.2). In the deposited beach sand, the number of fungi in soil significantly increased between harvest 1 and 5, after which they slowly declined. In sterilized soil, however, the numbers of CFU were initially high (at least twice as high as in sand from the beach) but these decreased drastically between harvest 1 and 2. After the second harvest, the numbers of CFU slowly increased during the rest of the experiment (data not shown). The initial difference in soil colonization by fungi

Table 5.2. Three-way analysis of variance of the number of colony forming units of fungi isolated from soil. *Ammophila arenaria* plants were grown in non-sterile root zone sand and buried with sterilized root-zone sand or sand from the beach (TREATMENT; T). The plants were harvested at different dates (HARVEST; H) (see Table 5.1). The soil was split into 5 layers: 0-5, 5-10, 10-15, 15-20 (deposited layer) and 20-35 cm (original core) (LAYER; L). MSE = Mean Square Error. The CFU was ln-transformed and analysed only for harvest 1 to 7.

	df	F	P
HARVEST (H)	6	4.58	0.0002
TREATMENT (T)	1	15.45	0.0001
LAYER (L)	4	1.17	0.3234
H*T	6	3.87	0.0011
H*L	24	0.61	0.9234
T*L	4	1.83	0.1217
H*T*L	24	0.93	0.5601
MSE	270	1.283	

was mainly the result of a significantly higher colonization of the sterilized soil by *Chaetomium* spp. (3920 CFU per gram of soil) than of the beach sand (6.5 CFU per gram of soil). *Penicillium* spp., *Chaetomium* spp., *Phoma* spp., *Cladosporium* spp. and the Mucorales were dominant in both sand types (data not shown).

Fungi on buried shoots.

The number of CFU on the buried shoots, i.e. leaves and stems, showed that already as early as February (at harvest 1), shoots pieces of all layers were infected by fungi (Fig. 5.3). There were no significant interactions between layer and harvests indicating that fungi were equally frequent recovered from all layers at all harvest times during the experiment (Table 5.3). There seemed to be a slight decline between the first and the final harvest in the number of pathogenic fungi in pots buried with sterilized sand but this difference was not statistically significant. There were only a few two-way interactions between layer and treatment or layer and harvest and three-way interactions were absent (Table 5.3).

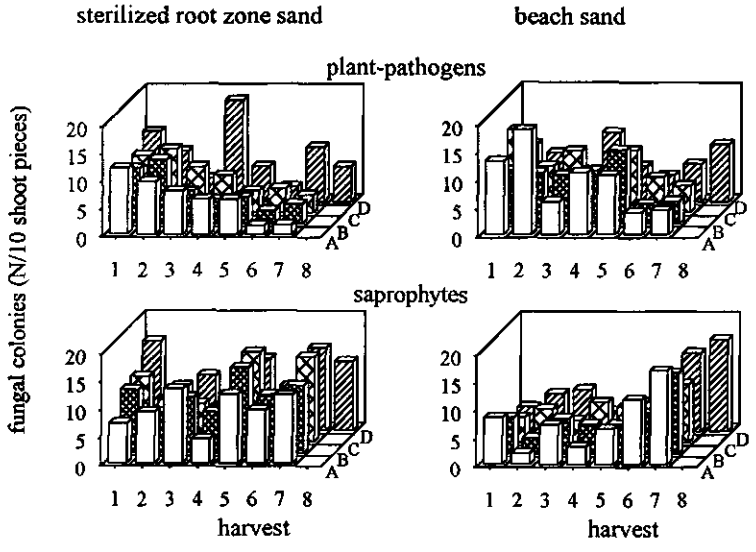


Figure 5.3. Fungi isolated from buried shoots at different harvest dates (see Table 5.1). The fungi were divided into plant-pathogenic and saprophytic species (Domsch et al. 1980). Plants were buried with sand from the beach or with sterilized root zone sand. The fungi were isolated from plant pieces of 0-5 (A), 5-10 (B), 10-15 (C) and 15-20 cm (D) in the deposited layer. At harvest 8, the deposited layer was not split (A-D).

Table 5.3. Analysis of the presence or absence by loglinear models of species of fungi isolated from the buried shoots. The effects of harvest (H) (harvests 1 to 7), treatment (T) (burial by sterilized soil or sand from the beach), layer (L) (0-5, 5-10, 10-15 and 15-20 cm) and their interactions were tested. NS= not significant, * P< 0.05, ** P< 0.01 and *** P< 0.001. All *Fusarium* species were combined and analyzed as one group (= *FUSARIUM* spp.). No third order interactions were significant. The pathogenicity (p) or saprophagous nature (s) of the fungi after Domsch, Gams and Anderson (1980) are presented between brackets.

	Layer	Treatment	Harvest	L*T	L*H	T*H
Acremonium spp. (s)	ns	ns	*	ns	ns	ns
Alternaria spp. (p)	ns	*	***	ns	ns	ns
Apiospora montagnei (s)	ns	ns	***	ns	ns	ns
Aspergillus sp. (s)	ns	ns	ns	ns	ns	ns
Botrytis cinerea (p)	ns	ns	*	ns	ns	ns
Chaetomium spp. (s)	ns	***	***	ns	ns	**
Cladosporium spp. (p)	*	ns	ns	ns	ns	ns
Cylindrocarpon sp. (p)	ns	ns	***	ns	ns	ns
Epicoccum purpurascens (p)	ns	ns	***	ns	ns	*
Fusarium culmorum (p)	ns	***	***	ns	ns	*
F. dimerum (s)	ns	***	***	*	ns	ns
F. equiseti (p)	ns	*	*	ns	ns	ns
F. nivale (p)	ns	ns	***	*	ns	ns
F. poae (p)	ns	ns	***	ns	ns	ns
F. oxysporum (p)	ns	ns	ns	ns	ns	ns
F. sambucinum (p)	ns	***	***	ns	ns	ns
F. sporotrichoides (s)	ns	ns	ns	ns	ns	ns
Fusarium sp.	ns	***	***	ns	ns	ns
FUSARIUM spp.	ns	***	**	ns	ns	ns
Microdochium bolleyi (p)	ns	ns	*	ns	ns	ns
Mortierella spp. (s)	ns	***	**	ns	ns	ns
Mucor spp. (s)	ns	ns	ns	ns	ns	ns
Myrothecium roridum (s)	ns	ns	***	ns	ns	ns
Penicillium spp. (s)	ns	ns	***	ns	ns	*
Phoma spp. (p)	ns	ns	**	ns	ns	ns
Plectosphearella sp. (s)	ns	ns	ns	ns	ns	ns
Pleospora sp. (p)	ns	ns	ns	ns	ns	ns
Trichoderma harzianum (s)	ns	ns	ns	ns	ns	ns
Stachybotrys chartarum (s)	ns	ns	ns	ns	ns	ns
Ulocladium sp. (p)	ns	**	ns	ns	ns	ns

All fungi (except *Cladosporium* spp.) were regularly isolated from all layers (Table 5.3). The number of isolations of *Fusarium culmorum*, *F. dimerum*, *Fusarium* spp. and *Mortierella* spp. were significantly higher in beach sand than in the sterilized soil, whereas *Alternaria* spp., *Chaetomium* spp. and *Ulocladium* sp. were more frequently found in sterilized soil (Table 5.3; data of treatment means not shown). Recovery of most fungi differed significantly at the various harvest times. The species *Alternaria* spp., *Epicoccum purpurascens*, *Fusarium culmorum*, *Microdochium bolleyi* and *Mortierella* spp. were more frequently isolated at the beginning of the experiment, whereas *Apiospora montagnei*, *Fusarium dimerum*, *Penicillium* spp., *Myrothecium roridum*, *Acremonium* spp., *Botrytis cinerea* and *Cylindrocarpon didymum* were more numerous at later harvests. The latter group seemed to be better competitors than the first, who are fast colonizers. Other fungal isolates were more or less uniquely found at the individual harvests (Table 5.3; data of treatment means not shown).

Microscopic observations showed that fungi regularly infected shoots. This was, however, mostly superficial. The buried shoots mainly consisted of leaves. The outer leaves were most heavily infected by fungi. In some cases, fungal spores of *Fusarium*, *Epicoccum* and *Apiospora* were found at the surface of the outer leaves. At the inner leaves only a few infections were observed.

Fungi in roots. Most fungi isolated from roots colonizing the deposited layer were also isolated from roots in the original core (Table 5.4). There were only a few significant differences between the fungal isolates from roots of plants buried with sand from the beach and those of plants buried with sterilized soil.

Only a few two-way interactions between treatment, harvest or layer regarding the frequency of isolations had occurred, while three-way interactions were not found (Table 5.4). The frequency of isolations of *Microdochium bolleyi* and *Epicoccum purpurascens* were affected by both treatment and harvest. Both fungal species were regularly isolated from roots colonizing the beach sand whereas in the roots growing into the sterilized soil, they were only found during the first part of the experiment (Table 5.4; data of treatment means not shown). The number of isolated *Fusarium* spp. was higher in the deposited layer of beach sand than in the deposited layer of sterilized sand.

Chaetomium spp., *Cochliobolus* sp. and *Apiospora montagnei* were more frequently isolated at the end of the experiment, whereas the frequency of recovery of *Phoma* spp., *Penicillium* spp., *Cladosporium* spp. and *Stachybotrys chartarum* was significantly higher at harvest 5 than in the autumn (harvest 8) (Table 5.4; data of treatment means not shown).

Table 5.4. Analysis of the presence or absence by loglinear models of species of fungi isolated from roots. The effects of harvest (H) (harvests 5 to 8), treatment (T) (burial by sterilized soil or sand from the beach), layer (L) (deposited layer and original core) and their interactions were tested. NS= not significant, * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. All *Fusarium* species were combined and analyzed as one group (= *FUSARIUM* spp.). No third order interactions were significant. The pathogenicity (p) or saprophagous nature (s) of the fungi after Domsch, Gams and Anderson (1980) are presented between brackets.

	Layer	Treatment	Harvest	L*T	L*H	T*H
Acremonium spp. (s)	ns	ns	ns	ns	ns	ns
Alternaria spp. (p)	ns	ns	ns	ns	ns	ns
Apiospora montagnei (s)	ns	ns	*	ns	ns	ns
Arthrinium phaeospermum (p)	ns	ns	ns	ns	ns	ns
Aspergillus sp. (s)	ns	ns	ns	ns	ns	ns
Botrytis cinerea (p)	ns	ns	ns	ns	ns	ns
Chaetomium spp. (s)	ns	*	*	ns	ns	ns
Cladosporium spp. (p)	ns	ns	**	ns	ns	ns
Cochliobolus spp. (p)	ns	ns	*	ns	ns	ns
Cylindrocarpon spp. (p)	ns	ns	ns	ns	ns	ns
Doratomyces stemonites (s)	ns	ns	ns	ns	ns	ns
Epicoccum purpurascens (p)	ns	ns	ns	ns	ns	**
Fusarium culmorum (p)	ns	ns	ns	ns	ns	ns
F. dimerum (s)	ns	ns	ns	ns	ns	ns
F. nivale (p)	ns	*	ns	ns	ns	ns
F. oxysporum (p)	ns	ns	ns	ns	ns	ns
F. sambucinum (p)	ns	ns	ns	ns	ns	ns
F. solani (p)	ns	ns	ns	ns	ns	ns
Fusarium sp.	ns	*	*	ns	ns	ns
FUSARIUM spp.	ns	ns	ns	*	ns	ns
Humicola grisea (s)	ns	ns	ns	ns	ns	ns
Microdochium bolleyi (p)	ns	ns	ns	ns	*	*
Mortierella spp. (s)	ns	ns	ns	ns	ns	ns
Mucor spp. (s)	ns	ns	ns	ns	ns	ns
Myrothecium roridum (s)	ns	ns	ns	ns	ns	ns
Nectria inventa (p)	ns	ns	ns	ns	ns	ns
Paecilomyces sp. (s)	ns	ns	ns	ns	ns	ns
Penicillium spp. (s)	ns	ns	***	ns	ns	ns
Phoma spp. (p)	ns	ns	**	ns	ns	ns
Plectosphaerella sp. (s)	ns	ns	ns	ns	ns	ns
Rhizopus sp. (p)	ns	ns	ns	ns	ns	ns
Stachybotrys chartarum (s)	ns	ns	**	ns	ns	ns
Thanetophorus sp. (p)	ns	ns	ns	ns	ns	ns
Trichoderma harzianum (s)	ns	ns	ns	ns	ns	ns
Ulocladium sp. (p)	ns	ns	ns	ns	ns	ns
Verticillium sp. (s)	ns	ns	ns	ns	ns	ns

Nematodes

There were no nematodes present in sand from the beach and sterilized soil prior to burial (data not shown).

Nematodes in soil. Compared to saprophytes, only a few plant-parasitic nematodes had migrated towards the deposited layer prior to root growth (at harvest 5). At the final harvest date (after 49 weeks), the number of plant parasites had increased considerably, which was mainly due to *Heterodera* juveniles. Mature cysts were observed on the roots in both layers and the juveniles were apparently released from the cysts. The dorylaimids slowly increased from harvest 5 onwards in both the original core and the deposited layer. The non-plant-feeders in sterilized root zone sand were strongly influenced by the occurrence of new roots in the deposited layer as there was a pronounced increase after harvest 4. The numbers in sand from the beach were, however, not as high as in sterilized soil (data not shown). The layer-effect expresses that *Rotylenchus goodeyi*, the *Heterodera* juveniles, *Filenchus* sp., *Telotylenchus ventralis*, *Heterodera* males and the dorylaimids were more frequently recovered from the original core than from the deposited layer (Table 5.5; data of treatment means not shown). The frequencies of only a few species were affected by differences in the type of sand used for

Table 5.5. Analysis by loglinear models of the presence or absence of nematodes isolated from soil. The effects of harvest (H) (harvests 1 to 7), treatment (T) (sterilized soil or sand from the beach), layer (L) (deposited layer: 0-5, 5-10, 10-15 and 15-20 cm and original core: 20-35 cm) and their interactions were tested. NS= not significant, * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. No third order interactions were significant.

	Layer	Treatment	Harvest	L*T	L*H	T*H
Pratylenchus sp.	ns	ns	ns	ns	ns	ns
Rotylenchus goodeyi	***	ns	**	ns	ns	*
Helicotylenchus sp.	ns	ns	ns	ns	ns	ns
Filenchus sp.	**	ns	ns	ns	ns	ns
Telotylenchus ventralis	*	***	*	*	ns	**
Hemicriconemoides sp.	ns	ns	ns	ns	ns	ns
Heterodera sp. (jv)	***	*	***	ns	**	ns
Meloidogyne maritima (jv)	ns	ns	**	ns	ns	ns
Heteroderidae (males)	***	ns	***	ns	ns	ns
Aphelenchus sp.	ns	ns	**	ns	ns	ns
Dorylaimidae	***	**	***	ns	ns	*

burying the plants (Table 5.5).
The numbers of isolated *T. ventralis* and *Heterodera* juveniles were more numerous in sand from the beach than in sterilized soil, whereas the opposite accounts for isolated of the dorylaimids (Table 5.5; data of treatment means not shown). Almost all species of nematodes were affected by time of harvest (Table 5.5). *Rotylenchus goodeyi*, *T. ventralis* and *Aphelenchus* spp. were the most frequently isolated in June (harvest 5), whereas the numbers of *Heterodera* and *Meloidogyne* juveniles, the *Heteroderidae* males and dorylaimids were more abundant in autumn (harvest 8). The interaction between harvest time and layer was significant for the *Heterodera* juveniles (Table 5.5). In early spring, they were only isolated from the original core, whereas in autumn they were also found in the deposited layer.

Nematodes from roots. Plant-parasitic nematodes were isolated from roots in the deposited layer as soon as they had been formed (Table 5.6). This indicated a rapid migration and infection at the time of root formation in the deposited layer (Table 5.6). In roots of the original core, low numbers of plant parasitic nematodes were present during the whole experiment. *Heterodera* dominated at the final harvest in both layers. Cysts had developed, not only on roots of the deposited layer, but also on the roots of the original core (numbers not assessed).

DISCUSSION

Fungi. The first colonizers of the deposited layer of sterilized soil were mainly saprophytic fungi, whereas in sand from the beach all types of fungi were present. The saprophytic fungi in the sterilized sand were dominated by *Chaetomium* species. They were assumed to consume the organic matter (Domsch et al. 1980) that was initially available as a result of sterilization. Their numbers declined and other fungi, including plant-pathogenic species, subsequently colonized the deposited layer of sterilized soil. Although the free organic matter in beach sand (0.27%) will not differ much from that of the foredunes (0.21-0.28%) (Van der Putten and Troelstra 1990), the sampled soil also contained root- and shoot-pieces. These were sterilized along with the soil and presumably raised the quality of available organic compounds.
Pathogenic and saprophytic fungi were abundant on buried shoots. These shoots consisted mainly of leaves. The most outer, older leaves were heavily infected,

whereas in the inner, younger, leaves hardly any infection occurred. The fungi may already have been present on the surface of the leaves prior to burial. For example, *Coniothyrium* sp., *Cladosporium* spp., *Phoma* spp. and *Alternaria* spp. have been isolated from the phyllosphere of *A. arenaria* and were supposed to be involved in the decomposition of leaf tissue (Dennis 1983). In our experiment, some of these fungi were also isolated from the buried shoots at the first harvest. The other fungi that were isolated from the buried shoots may already have been present on the shoots or may have colonized the shoots coming from the soil in the original core.

The potentially plant-pathogenic fungi *Phoma* spp. and *Cladosporium* spp. were more frequently isolated from roots at harvest 5 than in the autumn. They were presumably replaced by the potential plant-pathogen *Cochliobolus* sp. and saprophytic fungi, such as *Chaetomium* spp. and *Apiospora montagnei*. The frequency of recovery of *Cochliobolus* sp. in roots was, however, low (data not shown). The more abundantly isolated *Chaetomium* spp. in sterilized soil are known as saprophytes (Domsch et al. 1980) and may therefore not be of importance for the plant's vigour. Still, combined inoculation of plant-pathogens, saprophytes and plant-parasitic nematodes to seedlings proved to be more damaging then this combination but without the saprophytic fungi (chapter 3).

The buried shoots covered by sand from the beach were predominantly infected by *Fusarium* species. The inoculum density of these species was higher in beach sand than in sterilized soil. At harvest 5, when roots were formed, there were 45.8 CFU of *F. culmorum* per g of beach sand, whereas in sterilized soil only 0.4 CFU per g of the same species was found. At higher inoculum densities of the plant-pathogenic *Fusarium* an increased number of root segments infected by the fungus may be expected (Beckman 1987). In the present study, however, a high inoculum density of *F. culmorum* did not result in higher numbers of infected root segments when buried by beach sand. The colonization of shoots or the differences in the community of soil fungi between the two types of sand did not affect the composition of fungi collected from the roots.

Nematodes. The number of non-plant-feeding nematodes, dominated by bacteria-feeders, was higher in sterilized soil than in sand from the beach. As a result of recolonization following sterilization, numbers of bacteria will increase (Coleno et al. 1965) and this, in turn, will increase the numbers of bacteria-feeders (Yeates 1987). Also as a result of root growth, higher bacterial numbers will be found in the deposited layer. This leads again to increasing numbers of nematodes that feed

Table 5.6. Mean number of nematodes per 10 gram fresh root weight (5 replicates). No roots were analysed in the deposited layer at harvest 2 to 4 in case of plants buried with sterilized sand and at harvest 2 to 5 in plants buried with sand from the beach. jv= juveniles.

original core				beach sand							sterilized soil			
harvest	2	3	4	5	6	7	8	2	3	4	5	6	7	8
Pratylenchus sp.	0	0	0	0	0	0	3	0	0	0	0	0	0	1.5
Rotylenchus goodeyi	0	0	2.6	0	0	0	0	0	0	6.6	1.2	0	0	0
Helicotylenchus sp.	0	0	0	0	5.9	0	0	0	0	1.6	0	0.5	0	0
Filenchus sp.	4.3	0	0	1.1	0	0	1.8	0	0	0	0.4	1.8	0	1.7
Telotylenchus ventralis	13.8	0	0	0.6	0.6	0	0	9	0	0	0	0.8	0	0
Heterodera sp. (jv)	0	0	1.6	5	0	0.5	36.3	0	0	9.2	0	0	8.7	91.7
Meloidogyne maritima (jv)	0	0	0	0	0	0.8	0	0	0	0	0.9	0	0	0
Heteroderidae (males)	0	0	0	2.4	17.6	0	1.1	0	0	0	2.9	21.6	1.7	0.4
Aphelenchus sp.	14.1	0	0	0	0	0	0.7	7.8	0	0	0	3.2	0	0.4
Longidorus sp.	0	0	0	0.6	0	0	0	0	0	0	0	0	0	0
Dorylaimidae	9.5	0	1.9	2.4	10.9	3.2	34.6	50.6	0	0	3.3	22.2	1.9	172.9
Non-plant-feeders	684	58	135	135	136	79	72	884	49	84	76	228	82	330

deposited layer			beach sand							sterilized soil				
harvest	2	3	4	5	6	7	8	2	3	4	5	6	7	8
Rotylenchus goodeyi					0	0	0				0	0	0	0.3
Filenchus sp.					0	0	0				7.9	2.2	0	16.7
Telotylenchus ventralis					0	0	0				0	0	0	3.1
Heterodera sp. (jv)					0	3.4	169.3				7	0	0.5	189.7
Meloidogyne maritima (jv)					0	0	0.8				4	0	0	0
Heteroderidae (males)					3.4	0	4.9				4	3.4	4	5.8
Aphelenchus sp.					1.1	0	4.2				0	58.4	0	6.8
Dorylaimidae					16.1	2.1	38.1				13.3	9.2	3.1	104.2
Non-plant-feeders					33.4	51.9	772				175	202	79	1586

on them.
An increase in the numbers of dorylaimids after root formation may have been the result of an increase in plant-feeding dorylaimids (e.g. *Eudorylaimus* sp.) or an increase in nematode-feeding dorylaimids (e.g. *Aporcelaimus* sp.) (Yeates 1987, Yeates et al. 1993). Both groups of species were isolated from the root zone of *A. arenaria* (chapter 2) and in this experiment.
When new roots were formed, plant-parasitic nematodes migrated upwards where they immediately infected the roots. Before roots had been formed, only a few individuals migrated upwards. Such fast migration in time and space as a reaction on root development have also been described earlier (Prot 1980). There were no differences between beach sand and sterilized soil in rate of migration and species composition of plant-parasitic nematodes neither in soil nor in roots.

Plant performance. Plants buried with sand from the beach responded similarly as those buried with sterilized soil in spite of the differences of especially the species composition and inocula densities of soil and shoot fungi.
In a previous study, it was concluded that *A. arenaria* can temporarily escape from harmful soil organisms by colonizing the deposited layer of fresh windblown sand (chapter 4). In the present study, the same fungal species, however, showed to be present in the deposited layer before roots had been formed, whereas nematodes migrated upwards after the formation of roots. Plants that rapidly form roots in the deposited layer, will benefit the most by upward growth. In our experiment, plants started to develop new roots in May (harvest 5), whereas in coastal foredunes, the development of new roots may be as early as February (P.C.E.M. de Rooij-Van der Goes, personal observation). This delayed root development in the outdoor experiment as compared to the field may have been the result of the use of seedlings in the present experiment instead of well established tussocks. In contrast to tussocks, the seedlings had no rhizomes and could not mobilize reserve assimilates for the initial growth (Steen and Larssen 1986, Baye 1990, Seliskar 1994). In February, soil temperatures are usually low. As a result of such low soil temperatures, most fungi and nematodes are supposed to remain inactive. For example, infections by *Tylenchorhynchus dubius* were less severe for *Lolium perenne*, when temperatures were low enough to be favourable for plant growth but not for nematode activity (Den Toom 1988). Consequently, *A. arenaria* plants will benefit more when the plants are buried in autumn, so that roots may already be formed on the buried shoots in winter than when this takes place at higher soil temperatures, such as in spring after late winter burial.

As nematodes infected the plants only after roots had been formed, the amount of sand deposited on the vegetation may be an important factor in the ability of plants to escape infections. High amounts of sand deposition may result in a delay of infection of roots by nematodes. *A. breviligulata* proved to be vigorous when buried annually by minimally 7 cm of sand (Eldred and Maun 1982, Disraeli 1984). Whether this is needed for optimal root formation or a minimum for escape from damaging numbers of plant-parasitic nematodes and pathogenic fungi is not known. During the summer, the plants continue to form new roots as root biomass had increased during the experiment. Because these new formed roots provide fresh substrate, numbers of plant-parasitic nematodes and pathogenic fungi will increase. Without sand deposition, the population of pathogens will build up to damaging levels, which, eventually, leads to degeneration of the plants. A renewed burial of the plants, which rejuvenate stands of *A. arenaria* (Willis 1989), allows the plants to escape from these soil organisms. Thus, sand accretion provides the plants with new rooting space and lower numbers of soil organisms. Consequently, it will also prevent an increase in the population-size of the harmful soil organisms to damaging thresholds.

It can be concluded that in case of *A. arenaria*, the presence of pathogens and parasites has a negative effect on the plants' vigour, but the succes of escape will largely depend on the ability of plants to form roots prior to nematode migration and infection and, consequently, will depend on the time and amount of sand deposition.

THE EFFECTS OF SAND MOVEMENT BY WIND IN COASTAL FOREDUNES ON NEMATODES AND SOIL-BORNE FUNGI

P.C.E.M. de Rooij-van der Goes, C. van Dijk, W.H. van der Putten and P.D. Jungerius

CHAPTER 6
THE EFFECTS OF SAND MOVEMENT BY WIND IN COASTAL FOREDUNES ON NEMATODES AND SOIL-BORNE FUNGI

SUMMARY

In stabilized dunes *Ammophila arenaria* degenerates due to a process involving soil-borne pathogens and parasites. This exposes the sand surface and wind erosion creates blowouts. *A. arenaria* rejuvenates on the edges of the blowouts, where the sand has accumulated. We assume this to be related to a reduction of the plant-parasitic nematodes and fungal propagules at these sites.

The indication, derived from field measurements during storm that the numbers of harmful soil organisms were reduced in windblown sand from the blowouts, was confirmed in wind-tunnel experiments. Most fungi were attached to the sand particles. Nevertheless, the inoculum densities of some fungal species were negatively affected by the wind-borne sand movement. The numbers of nematodes were mainly reduced at high wind velocities (22 m s^{-1}, 9 Beaufort). Sand that had been deposited by wind was made up of a larger proportion of large-sized particles. The relatively small particles were lost in transport.

Stirring the soil scoured the soil particles and reduced the numbers of nematodes and fungi significantly. Both sand movement in the wind-tunnel at high wind velocities and intensive stirring of the sand enhanced the growth of *A. arenaria* test plants in a bioassay. It was, therefore, concluded that in windblown sand the pathogen inoculum potential is reduced by sifting as well as by death of the pathogens. Therefore, serious consideration should be given to allowing controlled re-activation of blowouts to rejuvenate the declining *A. arenaria* in stabilized foredunes.

INTRODUCTION

Wind-driven sand is one of the most conspicuous characteristics of coastal foredunes. The vegetation plays an important role in trapping the sand (Willis 1989). In European coastal foredunes, Marram grass (*Ammophila arenaria* (L.) Link) is the dominant sand-catching plant species (Huiskes 1979). In North-America and Canada the American Beachgrass, *A. breviligulata* Fern., is one of

the most important sand-fixing species (Eldred and Maun 1982, Baye 1990). The vigour of both plant species is highest on slopes where fresh windblown beach sand regularly accumulates. Both species degenerate when sand accumulation diminishes (Marshall 1965, Hope-Simpson and Jefferies 1966, Huiskes 1979, Disraeli 1984, Maun and Lapierre 1984, Willis 1989, Baye 1990). A number of factors have been reported to be involved in the decline of *A. arenaria* at stabilized sites. Among them are infections by plant-pathogenic soil organisms, particularly nematodes and fungi (Van der Putten et al. 1990, chapter 3 of this thesis).

Severely degenerated stands are vulnerable to erosion. This may endanger the stability of the dunes that serve as natural sea-walls. Rejuvenation of declining *A. arenaria* is known to occur when the vegetation is buried deeply by fresh windblown sand originating from the beach (Hope-Simpson and Jefferies 1966, Willis 1989). Rejuvenation also occurs in the inner dunes along the edges of blowouts (i.e. erosion hollows within the dune complex (Carter et al. 1990)) where sand transported by wind accumulates (Van Dieren 1934, Willis 1989). Blowouts usually start at sites where the vegetation is nearly absent (Jungerius and Van der Meulen 1988). The soil surface of sand dunes may then still be fixed by algae, mosses or microbial mats (Pluis and De Winder 1990), but after disturbance of the soil surface e.g. by animal activity or rainfall, superficial sand deflation can occur which may start the erosion process. Further deflation increases the size of a blowout, usually in the opposite direction to prevailing winds (Jungerius and Van der Meulen 1988, Carter et al. 1990). Aeolian transport may start when the wind-velocity exceeds a threshold value, which is about 6 m s^{-1} in the case of dry sand (Bagnold 1954, Arens 1994).

Previous studies showed that burial with sand containing plant-pathogens and parasites is less beneficial for the plants than burial with sand free of these soil organisms (chapter 4). We hypothesize that the restored vigour along the edges of blowouts will be partly due to either disappearance or death of soil-borne pathogens and parasites during sand drift. The same was also suggested in the case of the regeneration of Sea buckthorn (*Hippophaë rhamnoides*) after soil disturbance (Zoon et al. 1993).

Sand can be transported by wind in three ways: in suspension in dusts (usually smaller than sand-sized particles), by bouncing/saltation, or by rolling (Brown and McLachlan 1990). Therefore during long distance transport of the material in which the sand is suspended, the nematodes and fungi may either be physically separated from the sand or die when scoured between sand grains. Thus, sand that

is deposited on the vegetation is assumed to be less infested by pathogens and parasites than the original sand from the deflation site.
In this study, the hypothesis that numbers of pathogenic soil organisms are reduced as a result of sand movement by wind, has been tested by examining windblown sand from blowouts and by experiments in a wind-tunnel. Furthermore, the effects of a component of aeolian transport (scouring of sand particles) on numbers of pathogenic soil organisms was simulated and tested under laboratory conditions. Nonblown soil is used for *in situ* soil.

MATERIALS AND METHODS

Drifting sand in blowouts
On January 18th 1994, drifting sand was trapped in a blowout in the calcareous foredunes of Goeree (the Netherlands) at km-landmark 18.75 (51°48'N, 3°52'E). The wind direction was that day dominantly south-west with a velocity of 7.5-13 m s^{-1} (weather report of the Royal Netherlands Meteorological Institute). On January 20th 1994, the same procedure was repeated in a blowout in the lime-poor foredunes near Julianadorp (the Netherlands) at km-landmark 6.78 (52°54'N, 4°43'E), with a south-western wind of 5-10 m s^{-1}.
The sand traps used consisted of cotton bags with an opening of 5 by 25 cm and were 25 cm deep (after Draga 1983). The bags were placed at 5, 15, 25, 35 and 45 cm above the sand surface in a metal frame which was anchored in the soil. To collect drifting sand, all traps were directed with openings facing the prevailing wind. In each blowout, they were placed in a fixed pattern: at the centre of the blowout, halfway up the deposition slope and on top of the deposition lobe (Fig. 6.1). Sand was trapped during a period of 6 hours and the contents of each bag was weighed. Nematodes and fungi in the trapped sand were analyzed.
Additional soil samples from the top 10 cm layer were taken near the sand traps. Each soil sample consisted of three subsamples of approximately 150 ml collected using a scoop, and was analyzed.

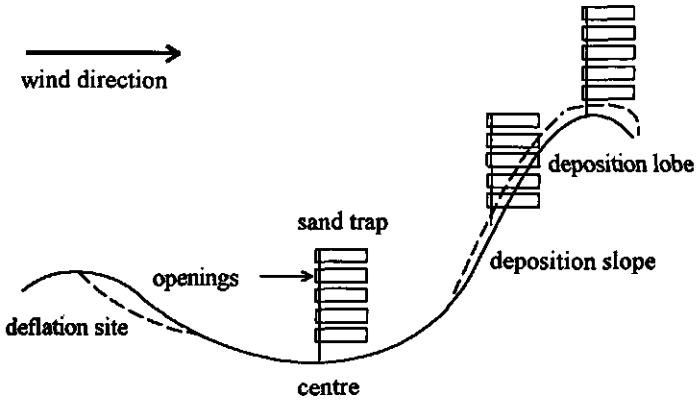


Figure 6.1. Schematic view of a blowout with the sand traps. The development of the blowout is presented by the dotted line.

The fungi were isolated according to Warcup (1960) on malt extract agar, containing 20 g malt extract (Oxoid), 3 g pepton (Oxoid), 15 g agar (Merck) and 2.5 g bile (Sigma) with 100 ppm oxytetracycline per liter of tap water. In contrast to the additional samples taken from the soil surface of the blowout, the amount of sand in the cotton bags was too small to allow analysis of nematodes by elutriation (Oostenbrink 1960). Therefore, 10 g of soil from each bag was spread over an extraction dish and placed in 100 ml of tap-water during 48 hours (Hooper 1986) after which the nematodes were identified and counted. Nematodes in the samples collected from the upper 10 cm soil layer were analyzed in triplicate by elutriation (Oostenbrink 1960), identified and counted.

In the blowout at Goeree, direct observations of windblown sand and adhering soil organisms were made. Petridishes, containing 2% water-agar (Merck), were held up to the wind for a few minutes. After incubation for 3 days at 23°C, the centre square of 2 x 2 cm^2 was examined using a low magnification microscope. The total number of soil particles as well as the numbers of fungal and/or bacterial propagules were counted.

Soil samples

In experiments 2 and 3, soil was collected from the root zone of vigorous *A. arenaria* stands in the foredunes of Voorne, the Netherlands, at a site immediately north of the Haringvlietdam (51°52'N, 4°04'E). The samples were taken from 15 random sites in an area of 600 m^2 and originated from the layer between 5 to 40 cm below the surface containing one- and two-years old roots of *A. arenaria*. The sand was gently sieved (mesh size: 2 cm) and homogenized. Sifted roots were chopped into 2-5 cm pieces and mixed through the soil. Part of the collected soil was sterilized by gamma-radiation (4 Mrad).

Sand movement by wind in a wind-tunnel

The effects of sand movement by wind on numbers of nematodes and fungal propagules was experimentally tested in a wind-tunnel. The tunnel was 75 cm wide, 75 cm high and 15 m long. The wind speed could be set between 0 and 28 m s^{-1} (0 to 10 Beaufort). In the wind-tunnel, two experiments were performed: soil was blown at a velocity of 22 m s^{-1} (9 Beaufort; soil sample V1) and 12 m s^{-1} (6 Beaufort; soil sample V2), respectively.

In case of soil sample V1, 50 kg of soil was collected in August 1993, half of which was blown in the wind-tunnel. The initial moisture content of the blown sand was 4% (w/w) (field humidity). It was not dried prior to the experimental treatment.

For soil sample V2, a total of 85 kg of soil was collected in October 1993. To facilitate wind movement, soil was gradually dried at room temperature from 4 to 1.5% (w/w), which took two weeks. An amount of 60 kg of dried soil was used in the wind-tunnel.

For each experiment, soil was spread on a plate of 1 x 0.75 m^2 in a 2 cm thick layer. The plate was positioned on the floor at the beginning of the wind-tunnel. The wind speed was slowly increased to the desired level. The windblown soil was caught in traps of 6 cotton bags each (similar to those used in experiment 1). The outer frames of the traps were 60 cm wide and 40 high thus covering about half the wind-tunnel cross section. Three traps were placed at 4, 8 and 12 m distance from the source. After all the soil had been blown from the plate, the wind speed was slowly reduced to zero. The windblown soil was collected from the bags in the traps and from the windtunnel floor near the trap. The soil was lumped over all traps and analyzed.

Particle size distribution: air-dried windblown and nonblown soil, discarded from roots, was sieved through mesh sizes of 600, 425, 300, 212, 150 and 75 μm,

respectively.

Soil-organisms: in order to establish potential relationships between particle size and species of fungi, small amounts (0.03 ± 0.002 g) of soil collected from each mesh size were plated in triplicate on malt-extract-agar to isolate the fungi (Warcup 1960). Fungi were also isolated in triplicates according to Warcup (1960) on malt-extract-agar from the total sample of windblown and nonblown sand. The numbers of the various species of nematodes in 100 ml of windblown and nonblown sand were assessed after collecting them in duplo by elutriation (Oostenbrink 1960).

Pot-experiment: the effects of the pathogens in windblown and nonblown soil on the productivity of *A. arenaria* were tested in a pot-experiment. The biomass production of seedlings in windblown soil was compared with that in nonblown, unsterilized and sterilized soil.

Seeds collected from a vigorous stand at Voorne were germinated on glass beads at an 8/16 hour dark/light regime of 10/30°C. Six pots of 1.5 l were either filled with 1500 g of unsterilized windblown, unsterilized nonblown or sterilized nonblown soil (10% soil moisture (w/w)). Each pot was planted with six seedlings of two weeks old (3 to 5 cm tall). The sand surface was covered by tinfoil to prevent desiccation of the sand. The pots were placed at 23°C (± 2°C) in the greenhouse. A day length of 14 hours was achieved by additional illumination (250 μmol m^{-2} h^{-1}).

Once a week, the soil moisture content was set at 10% (w/w) with demineralized water. To avoid nutrient deficiency during plant growth, increasing amounts of nutrients were added: weeks 1 to 4: 15 ml, weeks 5 to 6: 25 ml and weeks 7 to 10: 50 ml full strength Hoagland nutrient solution.

After two weeks, the plants were randomly thinned to a density of 4 plants per pot. The plants were harvested after a growing period of 6 weeks (experiment with soil sample V1) or 10 weeks (experiment with soil sample V2), respectively. The bioassay of soil sample V1 was carried out in early autumn, whereas soil sample V2 was tested in late autumn.

After harvesting, biomass of shoots and roots were determined after drying at 70°C for 48 hours. Nematodes in soil were determined after decantation (Oostenbrink 1960).

Effects of scouring

In Februari 1993, 75 kg of root-zone soil were collected. To study the effects of scouring on the numbers of nematodes and fungi, soil was stirred using a propeller mixer for 15 minutes at a velocity of 0, 300, 600, 900 or 1500 rpm, respectively. During stirring, soil temperatures increased from 10-15°C to about 20°C.

A pot-experiment, similar to that in experiment 2, was done with the scoured soil. In each treatment, 5 replicate pots were planted with seedlings and harvested after 8 weeks. A control with unscoured sterilized soil was also included. The growth of *A. arenaria* seedlings was compared with that in unscoured unsterilized and sterilized soil. To assess the initial number of nematodes, 3 replicate pots were decanted (Oostenbrink 1960) after 4 days. Fungi were isolated by soil-dilution before the pot-experiment. For soil-dilutions, 10 g of dry soil was suspended in 90 ml of sterilized water. One ml of the dilutions 10^{-2}, 10^{-3} and 10^{-4} was spread in triplicate on malt-extract-agar. At harvest, nematodes were isolated from whole pots by decantation (Oostenbrink 1960).

Identification of soil organisms

Nematodes were identified to generic level according to Bongers (1988). Nomenclature according to Domsch et al. (1980) was used throughout this study for all fungi except *Fusarium*. Species of the latter were identified according to Nelson et al. (1983).

Data analyses

The data were analyzed statistically by one-way analysis of variance after testing for homogeneity of variances by means of Cochran's Q test. If required the biomass data and the numbers of fungi were ln-transformed and numbers of nematodes were square-root-transformed to achieve homogeneity. The treatment means were compared using Tukey's test ($P < 0.05$).

With both soil samples in experiment 2, Wilcoxon's rank sum test was used to analyse the effects of aeolian transport (windblown versus nonblown soil) on the dominant species of fungi. The Pearson rank correlation coefficient and its significance were calculated of correlations between the number of colony forming units of the most dominant fungal species and particle size. Significant differences were set at the 5% level.

RESULTS

Drifting sand in blowouts

In the blowout at Goeree, sand was only trapped during one hour of the six hours period of measurement, after which no further movement occurred. In the blowout at Julianadorp, however, drifting sand was trapped during the whole 6 hours of measurement. Nevertheless, the amount of sand trapped during the short term at Goeree was more than 10 times higher than during the 6 hours at Julianadorp (Fig. 6.2). At the centre of the blowout and on the depositional slope, sand drifted just above the soil surface, whereas at the top of the depositional lobe, the amount of trapped sand was more evenly distributed over the different heights (Fig. 6.2).

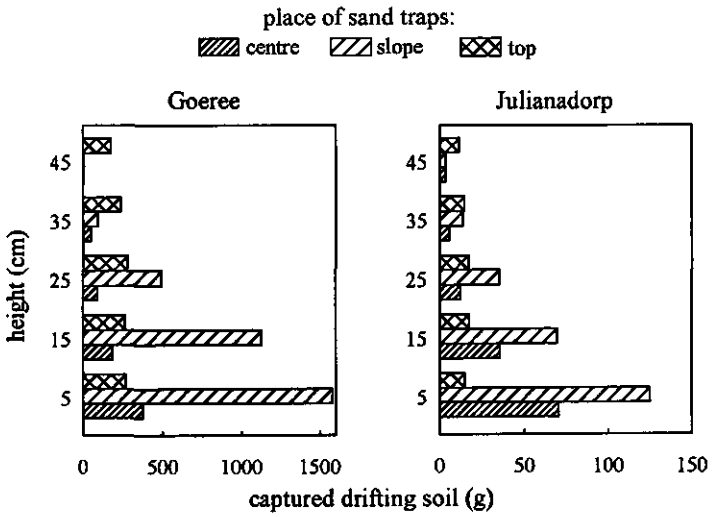


Figure 6.2. Drifting sand captured (g) during 6 hours at different heights (cm) above the soil surface in blowouts at Goeree (January 18th 1994) and Julianadorp (January 20th 1994). For place of sand traps see figure 6.1.

The living nematodes caught in the cotton bags were mainly non-plant-feeding nematodes (Table 6.1). The dorylaimids and the very few plant-parasitic nematodes that were found in the trapped soil were dead. Also in the samples taken near each sand trap, hardly any plant-parasitic nematodes were found.
At Goeree, the number of fungal propagules (counted as colony forming units) in soil trapped in the bags was in line with that in soil sampled from the surface (Table 6.1). However, at Julianadorp the numbers of fungal propagules in the surface soil were generally 1.5 to 4 times higher than in soil trapped in the bags (Table 6.1). No relationship between the height of the bags and the number of fungal propagules in the trapped soil could be detected (Table 6.1). Direct microscopic observations of the wind-transported sand caught on Petridishes showed that the majority of fungi and bacteria were attached to the soil particles (data not shown).

Table 6.1. Nematodes (numbers per 10 g dry soil) and fungi (number of colony forming units per g dry soil) in sand collected in the sand traps at different heights above the soil surface and from the soil sample taken near the sand trap (height=-10-0). Blowouts were sampled in January 1994 at Goeree and at Julianadorp. The number of plant-parasitic nematodes are presented in brackets. * lumped with 45-50

height above soil surface	nematodes in sand of traps at			fungi in sand of traps at		
(cm)	bottom	slope	top	bottom	slope	top
Goeree						
-10-0	3	1	0	828	934	874
5-10	2	0	5	1805	713	874
15-20	1	0	0	954	1046	1517
25-30	2 (1)	7	4	1483	1471	1379
35-40	3 (1)*	7*	4	1195	839	1264
45-50			7	1000	1230	2218
Julianadorp						
-10-0	4 (1)	1	2	2264	2103	2345
0-5	5 (1)	5 (1)	5 (1)	1425	563	1149
15-20	2 (1)	7	3 (1)	1471	828	943
25-30	2	2 (1)	4 (1)	989	908	1414
35-40	12 (2)*	2*	3*	1632	770	1000
45-50				945	517	1713

Sand movement by wind in a wind-tunnel

In spite of initial differences in the soil moisture content of the soil samples, the soil moisture content had decreased to a level of about 1 % in windblown soil (data not shown). The relatively moist soil of soil sample V1 could only be blown at wind-speeds higher than 18 m s^{-1}, whereas aeolian transport of "dry" soil (soil sample V2) started at a velocity of 6 m s^{-1}.

Although the data did not allow for statistic testing, windblown sand appeared to contain proportionally less fine soil particles than nonblown sand (Fig. 6.3). This difference appeared to be enhanced at increasing wind speed.

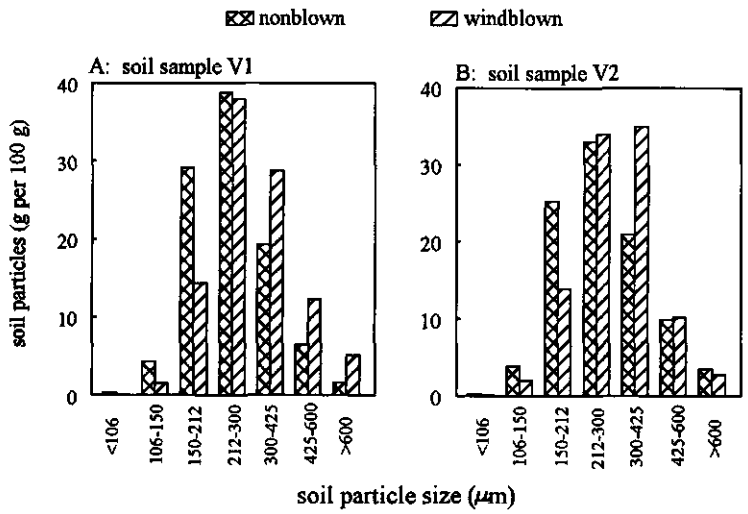


Figure 6.3. Proportional particle size distribution of not-transported and wind-transported sand that was blown in a windtunnel at wind speeds of 22 m s^{-1} (A; soil sample V1) and 12 m s^{-1} (B; soil sample V2).

In both pot-experiments, dry weights of plants grown in unsterilized soil were significantly lower than those grown in sterilized soil (Fig. 6.4). After soil had been transported at a wind speed of 22 m s^{-1}, the biomass production of *A. arenaria* in unsterilized soil was significantly higher than that in unsterilized, nonblown soil (Fig 6.4A). Also the growth of the seedlings in sterilized,

windblown soil was significantly lower than that in sterilized, nonblown soil. However, when soil was blown at a wind speed of 12 m s^{-1} (soil sample V2), productivity of plants in unsterilized windblown soil was not significantly improved with regard to the biomass in unsterilized nonblown soil (Fig. 6.4B).

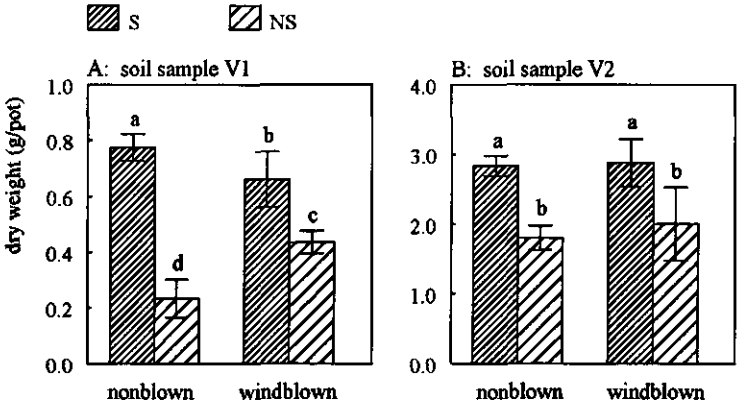


Figure 6.4. Biomass production of *Ammophila arenaria* seedlings planted in windblown or nonblown soil. Soil was blown in a wind-tunnel at a wind speed of 22 m s^{-1} (A; soil sample V1) and 12 m s^{-1} (B; soil sample V2), respectively. The growth of the seedlings in sterilized soil (S) was compared to that of non-sterile soil (NS). The biomass was assessed after a growing period of 6 weeks for soil sample V1 or 10 weeks for soil sample V2. Bars bearing different letters are significantly different at $P = 0.05$.

The total number of fungal colonies in windblown soil did not differ from that in nonblown soil at both tested wind-velocities (Table 6.2). However, when soil was blown at 22 m s-1, the total number of fungal propagules excluding *Penicillium* spp. was reduced to about a third of that in nonblown soil. At low wind speeds, numbers were halved (data not shown). These differences were, however, not significant (Table 6.2). When soil was blown at 22 m s^{-1} (soil sample V1), the numbers of *Acremonium* spp., *Fusarium culmorum* and *Mortierella* spp. were significantly lower than in nonblown soil. Only the number of *Phoma* spp. was higher in the windblown soil than in nonblown soil (Table 6.2). The numbers of *Fusarium culmorum*, the other *Fusarium* species, *Penicillium* spp. and *Phoma* spp.

as well as the total number of fungal colonies with or without the number of *Penicillium* spp. colonies were negatively correlated with particle size (Table 6.2). This indicates a decrease in CFU's as the soil particles sizes increase. In soil blown at 12 m s^{-1} (soil sample V2), only the number of *Fusarium culmorum* colonies was significantly reduced by aeolian transport. All tested fungi, including the total number of fungal colonies, were not significantly correlated with particle size in soil sample V2 (Table 6.2).

Table 6.2. Correlation between fungi (number of colony forming units per g dry soil) and treatment (windblown and nonblown) particle size. Wilcoxon's rank sum test was used for comparing the two treatments (P_w). For correlation (=corr.) between particle size distribution and fungi the Pearson rank correlation coefficient and its significance are presented. Two soil samples (V1 and V2) were taken in August and October 1993 and were blown at a wind velocity of 22 (soil sample V1) or 12 m s^{-1} (soil sample V2). Only dominant fungal species were tested.

	soil sample V1			soil sample V2		
	treatment	particle size		treatment	particle size	
	P_w	corr.	P	P_w	corr.	P.
Acremonium spp.	<0.001	-0.26	0.114	0.063	-0.02	0.450
Cladosporium spp.	0.333	0.05	0.410	0.055	-0.05	0.363
Fusarium culmorum	<0.001	-0.42	0.021	0.038	-0.09	0.236
other *Fusarium* spp.	0.259	-0.55	0.003	0.506	0.18	0.085
Mortierella sp.	0.014	-0.31	0.443	0.106	-0.19	0.076
Mucor hiemalis	0.445	0.04	0.427	0.270	0.01	0.484
Penicillium spp.	0.112	-0.45	0.014	0.127	-0.20	0.060
Phoma spp.	0.028	-0.48	0.009	0.999	-0.09	0.248
Trichoderma harzianum	0.768	0.28	0.095	0.951	0.04	0.377
Total colonies	0.729	-0.50	0.006	0.107	-0.21	0.056
Total without *Penicillium*	0.083	-0.38	0.033	0.082	-0.01	0.470

Before the pot-experiment, wind-driven sand movement tended to reduce the numbers of plant-parasitic nematodes, dorylaimids and non-plant-feeders (Table 6.3). No nematodes could be detected in soil blown at 22 m s^{-1} (soil sample V1) (Table 6.3). After conducting the bioassay, all types of nematodes were isolated, even in sand blown at 22 m s^{-1}. Nevertheless, after conducting the bioassay, numbers of plant-parasites and dorylaimids were lower in windblown soil than in

nonblown soil (Table 6.3). In contrast to soil sample V1, the non-plant-feeders in windblown soil of soil sample V2 was larger than in nonblown soil.

Table 6.3. Nematodes (No per 100 ml soil) in nonblown and windblown soil directly after being transported by wind (N=2) and at harvest (N=6) of the pot-experiment. Soil, collected in August (V1) and October (V2) 1993 was blown in a wind-tunnel at wind speeds of 22 and 12 m s^{-1} respectively. The numbers of nematodes determined directly after the wind-tunnel experiments are averages of duplicate samples of 200 ml. At harvest, nematodes were assessed from 6 replicate pots. Significant differences between the numbers of nematodes of nonblown and windblown sand are marked with * (Tukey: $P < 0.05$).

	soil sample V1		soil sample V2	
windblown	nonblown	windblown	nonblown	
directly after wind transport				
Plant-parasites	3.5	0	2.5	0.3
Dorylaimids	16.5	0	13.5	0.3
Non-plant-feeders	45	0	28	1.5
after pot-experiment				
Plant-parasites	3	1 *	2.2	1 *
Dorylaimids	34	0.3 *	31.9	0.9 *
Non-plant-feeders	143	130 *	245	348

Effects of scouring

After a growing period of 8 weeks in soil stirred at a speed of minimally 900 rpm, shoot dry weights were significantly higher than the control (Fig. 6.5A). The shoot dry weight of plants grown in sterilized soil was significantly higher than that of plants in any of the other treatments. The root biomass developed at a lower rate than the shoot biomass, but also here root biomass increased significantly when the soil was stirred at an increasing intensity (Fig. 6.5B).

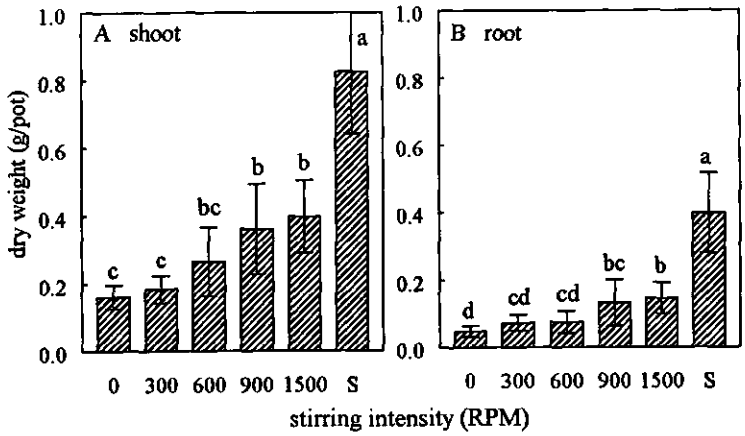


Figure 6.5. Biomass production (g dry weight per pot) of shoots (A) and roots (B) of *Ammophila arenaria* seedlings after a growing period of 8 weeks in soil stirred at a speed of 0, 300, 600, 900 or 1500 rpm. The growth of the seedlings planted in the treated soils was compared to that of not-treated sterilized soil (S). Bars bearing different letters indicate significant differences at $P = 0.05$.

Increasing stirring intensities resulted in decreasing numbers of fungal propagules (Fig. 6.6). The numbers were significantly lower than in unscoured soil when soil had been stirred at a speed of at least 900 rpm.

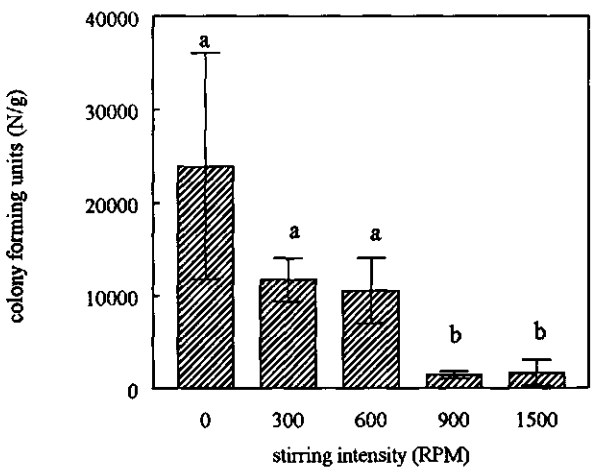


Figure 6.6. Numbers of fungi (colony forming units per g dry soil) in soil stirred at speeds of 0, 300, 600, 900 or 1500 rpm, respectively. The numbers were assessed directly after stirring the soil. Bars bearing different letters indicate significant differences at $P = 0.05$.

Numbers of nematodes had significantly decreased when soil had been stirred (Table 6.4). Most plant-parasitic nematodes decreased rapidly as the stirring intensity increased. However, the numbers of juveniles of *Heterodera* sp. and *Meloidogyne maritima* were not affected by stirring the soil. Stirring apparently enhanced the hatching of juveniles from cysts and egg-masses, therefore, higher numbers of juveniles were found when soil was stirred at 600 rpm (Table 6.4). At harvest, roots in this soil showed higher numbers of cysts and root-knots than roots in soil stirred at 300 or 900 rpm (data not shown). These cysts and root-knots were not yet fully grown, so that at harvest no new juveniles could be isolated (Table 6.4). This resulted in a significantly lower number of plant-parasitic nematodes at harvest than at the start of the experiment (Table 6.4; ANOVA not shown). The non-plant-feeding nematodes had increased significantly during the pot-experiment (ANOVA not shown) whereas the numbers of dorylaimids remained stable.

DISCUSSION

The present study showed that aeolian transport of sand from the root zone of *A. arenaria* reduced the numbers of pathogenic soil organisms. Plants benefit from sand deposition especially when the sand does not contain pathogens and parasites (chapter 4). Rejuvenation of stands of *A. arenaria* along the edges of blowouts where windblown sand accumulates may, therefore, be explained by the reduced inoculum pressure of plant-pathogenic organisms in the deposited soil.

In the blowouts studied at Goeree and Julianadorp, sand was mostly moved just a few cm above the surface. It also occurred in the wind-tunnel. This type of movement implies heavy bouncing and saltation of soil particles (Arens 1994). The severity of scouring of soil particles in blowouts, simulated in experiment 3, is unknown.

Scouring of soil particles probably crushed and destroyed the free-living nematodes between the sharply edged sand particles. This process was especially devastating in the wind-tunnel experiments when wind-velocities were high. A decrease in numbers of nematodes could only be proven when soil was blown at high wind-speeds. However, after 6 weeks of plant growth in soil blown at high wind-velocity, plant-parasitic nematodes were again found. This demonstrates that there were still some plant-parasites present at the start of the experiment and, although at the time of planting no nematodes were found, they were not completely

Table 6.4. Nematodes 5 days after mechanical stirring of the soil and after a pot-experiment with this soil during 8 weeks. The numbers are averages of 3 replicates. Soil was stirred at speeds of 0, 300, 600, 900 or 1500 rpm, respectively for 15 minutes. jv= juveniles. Significant differences in the total number of plant-parasites, Dorylaimidae or non-plant-feeding nematodes are marked with different letters at $P = 0.05$.

	directly after stirring (5 days)				
	0	300	600	900	1500
Pratylenchus sp.	1.8	0.5	0.7	0.6	0.6
Rotylenchus goodeyi	22.1	0.7	0.4	3.1	0.2
Filenchus sp.	0.5	0.2	0.5	0.2	0
Telotylenchus ventralis	1.2	0.6	0.5	0.4	0.1
Hemicriconemoides sp.	0.2	0	0	0	0
Heterodera sp. (jv)	14.8	13.7	18.4	14.4	13.3
Meloidogyne maritima (jv)	19.1	29.1	40.2	21.4	7.5
Heteroderidae (males)	1	1	0	0.5	0.2
Aphelenhus sp.	1.2	2.5	0.1	0.1	1.6
Plant-parasites	60.8 a	45.9 ab	60.7 a	40.6 ab	22.1 b
Dorylaimidae	4.4 a	4.7 a	0.6 b	1 b	0.7 b
Non-plant-feeders	32.5 a	28.4 a	19.3 b	12.1 b	11.4 b
	at harvest of pot-experiment (8 weeks)				
	0	300	600	900	1500
Pratylenchus sp.	0.1	0	0	0	0
Rotylenchus goodeyi	19.1	0.1	0.1	1.1	0.1
Helicotylenchus sp.	0.5	0.1	0	0.2	0.1
Filenchus sp.	0	0.1	0.1	0.2	0
Telotylenchus ventralis	0.1	0	0.1	0.4	0
Hemicriconemoides sp.	0.5	0	0	0	0
Heterodera sp. (jv)	0	0.6	0	0	0
Heteroderidae (males)	0.2	0.2	0.7	0.6	2.3
Aphelenhus sp.	0	0	0.5	0	0
Plant-parasites	20.6 a	1.2 b	1.1 b	2.6 b	2.6 b
Dorylaimidae	1.7 ab	3.2 ab	4.1 a	1.9 ab	1.5 b
Non-plant-feeders	53.3 a	88.9 a	65.6 a	117.9 a	113.6 a

eradicated by aeolian transport. It may have been that nematode numbers were below the detection threshold of the elutriation method or that nematodes were in an anhydrobiotic state. The latter seems less plausible because anhydrobiosis only occurs after slow desiccation of the soil (Simons 1973). This was not the case for soil sample V1.

After conducting the bioassay, especially in windblown soil of soil sample V2, numbers of non-plant-feeding nematodes had increased. The numbers of bacteria had increased as a result of root growth, which subsequently led to an increase in the numbers of nematodes that feed on them (Yeates 1987). The higher amount of biomass in the pot-experiment of soil sample V2 due to a longer growth period may have led to even larger numbers of non-plant-feeders.

Stirring of soil killed most plant-parasites and dorylaimids. However, mechanical damage of cysts of *Heterodera* spp. and egg-masses of *Meloidogyne maritima* increased juvenile hatching. Stirring may have facilitated hatching by breaking the tough cyst-wall or by disintegrating the gelatinous mass. In absence of other plant parasites, which may inhibit successful infections of roots (Eisenback and Griffin 1987, Eisenback 1993), more Heteroderidae juveniles were able to infect the roots in stirred soil than in unscoured soil. This, however, did not result in further growth inhibition of the test plants.

Unlike nematodes, fungi and bacteria were transported mainly attached to the soil particles. Fungi are known to form small aggregates of sand particles (Forster and Nicolson 1981). Wind-driven sand movement was expected to disintegrate these aggregates and to rub off and to destruct the fungal hyphae. Most aggregates were broken after sand had been blown in the blowouts (P.C.E.M. de Rooij-van der Goes, personal observation), although the results of the wind-tunnel experiment showed no decrease in the total number of fungal propagules when transported by wind. If, however, the most dominantly present fungi, i.e. *Penicillium* spp., were excluded from the analysis, fungal numbers tended to decrease when soil was blown. Only numbers of *Fusarium culmorum*, *Acremonium* spp. and *Mortierella* spp. were negatively affected when soil was blown at 22 m s^{-1}. As *F. culmorum* is associated with the smaller particles, this fungus may also have been blown from the soil as small particles in suspension. The numbers of the propagules of the other two groups of fungi, however, were not correlated with particle size. Their reduction after wind-borne sand transport implied that they were most likely destroyed by scouring of sand grains. This mechanism, tested in experiment 3, proved to reduce the numbers of fungal propagules. As a consequence, plant growth in the scoured soil is enhanced due to a reduction in numbers of plant-

parasitic nematodes and fungal propagules.

Apart from scouring, soil organisms may also disappear from the deposited sand when they are transported in suspension over long distances. Vertical air movement above the vegetation (Arens 1994) resulted in a more even distribution of the amount of soil caught in the sand trap. By the upward air movement above the vegetation, sedimentation of these small soil particles will only take place when the wind speed drops to zero (Zingg 1952, Arens 1994). The small soil particles will, therefore, be transported over long distance.

The proportion of small-sized soil particles tended to decrease when soil was blown in the wind-tunnel. This meant that most large particles were caught in the cotton bags and smaller particles drifted away. Also sand deposited at the accumulation site of blowouts and on the seaward slope of coastal foredunes had a larger proportion of large sized sand particles than at the deflation site (P.D. Jungerius, unpublished results). The disappearance of small soil particles from the sand deposited along the edge of blowouts implies that nematodes and fungal propagules that are not attached to soil particles may also easily be sifted from the soil. Transport of fungi by wind over large distances is well known (Gregory 1961, Zadoks and Schein 1979), and also nematodes, when in anhydrobiotic state or in cysts or egg-masses, can easily be transported by wind (Orr and Newton 1971).

In accordance with Zingg (1952), our wind-tunnel experiments showed that the proportion of large soil particles increased when sand was blown at increasing wind speeds. The subsequent pot-experiments showed that soil blown at a wind speed of 22 m s^{-1} was less heavily infested than when soil was blown at 12 m s^{-1}. Both tested wind-velocities in the wind-tunnel commonly occur in the Dutch coastal foredunes (Arens 1994). In blowouts intensive scouring of soil particles will kill soil organisms in windblown sand. Further reduction of the plant-pathogenic inoculum is caused by separation of the fine particles from the relatively course sand grains.

The reduced biomass of plants grown in windblown sterilized soil compared to that in nonblown sterilized soil may have been a side-effect of the larger soil particles. Movement by wind changes the texture of the soil. However, the growth reduction of plants (sterilized vs. unsterilized) in nonblown soil was still larger than in windblown soil.

Furthermore, a prolonged absence of vegetation may contribute to the rejuvenation as numbers of soil organisms will slowly decline in absence of their hosts. This phenomenon of reduction of the numbers is the essence of crop rotations in

agriculture. The absence of plant-parasitic nematodes in the soil samples from the soil surface in the blowouts, especially of the one at Goeree, may have been due to the absence of host roots.

In conclusion, the effects of scouring and elimination of small particles, which increases at high wind velocities, will all contribute to the reduction in the numbers of soil organisms in sand originating from blowouts. Consequently, at the accretion site sand relatively free of soil organisms is deposited upon the vegetation. The vigorous response of declining *A. arenaria* at the depositional lobe of blowouts (Van Dieren 1934, Willis 1989) can, therefore, be explained by the elimination of soil-borne pathogens from the deposited sand. Besides the physiological effects of burial by sand (Baye 1990, Yuan et al. 1993), also deposition of clean or moderately infested sand will contribute to the vigour response of *A. arenaria* (chapter 4). As windblown soil becomes less infested by soil organisms, serious considerations should be given to the re-activation of blowouts to rejuvenate the declining *A. arenaria* in stabilized foredunes. These rejuvenated stands will provide an adequate degree of stability to coastal foredunes and maintain the value as a dynamic natural defence against the sea.

CHAPTER 7
GENERAL DISCUSSION

Disease complex

A survey was carried out at nine locations along the Dutch coast. From the root zone of vigorous and early declining *A. arenaria* at these locations, a wide range of potential fungal pathogens and plant-parasitic nematodes have been isolated (chapter 2). These organisms were tested experimentally for their role in the degeneration process. The inoculation experiments described in chapter 3 gave no conclusive answer as to the involvement of fungi. Although *Fusarium culmorum* was the most common fungus isolated from young plants that died during pot-experiments (unpublished results), single inoculation of this fungus, or any other fungus, did not result in growth reduction of the plants. However, the combination of all fungi that were commonly found in the root zone of *A. arenaria* reduced growth to about 80 percent of that in sterilized soil (chapter 3). The absence of growth-reducing effects when inoculated separately and the presence of such effects when inoculated in mixtures suggested synergistic effects between the commonly found fungi. Inoculation with 2.5 percent sand-oatmeal-cultures of the same group of fungi doubled the number of root segments infected with *F. culmorum*, but did not result in significantly more growth reduction as compared to inoculation with fungal spores (unpublished results).

The involvement of nematodes, especially of *Telotylenchus ventralis*, the only species that thus far has been successfully cultured on *A. arenaria* in the laboratory, was evident when added in high numbers. Inoculations with the endoparasitic species *Heterodera* sp. and *Meloidogyne maritima* were less conclusive (chapter 3). These sedentary endoparasitic species were frequently found on roots of *A. arenaria* in natural soil (chapter 2). Also in the experiments in which plants were buried artificially with various sand types (chapters 4 and 5) and after scouring of the soil (chapter 6), both species became established, multiplied and were, at least in part, the cause of reduced plant growth.

The success of infection by the endoparasitic nematode species may depend on a minimum root size (Rawsthorne and Hague 1985). This hypothesis was tested on *A. arenaria* plants that were grown for 4 weeks in sterilized soil. Inoculation of these plants with juveniles of *Heterodera* sp. led to infections of roots but only males were produced (chapter 3). The development of only males indicates that growing conditions were not optimal (Yeates 1987) and is in contrast to successful

infections of roots in non-sterile soil that were grown under identical abiotic conditions. Besides the differences between sterilized and unsterilized sand, the applied inoculation method may as well have been responsible for the lack of establishment by the endoparasitic nematodes. In culture trials, the *Heterodera* sp. was inoculated either as juveniles, as young full-grown cysts or as old, but viable, cysts. *Meloidogyne maritima* was added either as juveniles or in eggmasses. Unfortunately, all these inoculation-trials did not result in production of juveniles. Growth and multiplication of the nematodes appeared to be suppressed by some factor coinciding with sterilized soil. Therefore, the growth conditions in sterilized soil and the method of inoculation need to be studied in more detail before the role of sedentary endoparasitic nematodes in the decline of *A. arenaria* can be experimentally studied. Because under more natural conditions both nematodes are able to infect plants, their role in the degeneration may be misinterpreted from ineffective inoculations in pot trials.

Visual observations showed that the numbers of cysts and root-knots on roots were higher when soil was stirred than on roots in non-sterile soil (chapter 6). Stirring may have facilitated hatching by breaking the tough cyst-wall or by disintegrating the gelatinous mass. In the absence of other plant-parasites which may inhibit successful infections of roots (Eisenback and Griffin 1987, Eisenback 1993), more Heteroderidae were able to infect the roots in stirred than in unscoured soil. The potential interactions between infections of Heteroderidae and other plant-parasites have, in the case of *A. arenaria*, not been studied.

From the results described in chapter 3, it became clear that the growth reduction of *A. arenaria* was not caused by infection by one single organism alone, but required an assemblage of organisms. This assemblage, particularly those groups containing plant-parasitic nematodes, also includes plant-pathogenic fungi. Combinations of nematodes and fungi are common in many plant-disease-complexes and are, especially for endoparasitic nematodes such as *Heterodera* and *Meloidogyne* in combination with *Fusarium* species, well documented for agricultural crops (e.g. Powell et al. 1971, Mai and Abawi 1987). Besides pathogens and parasites, also saprophytic fungi contributed to some growth reduction in pot trials when inoculated with plant-pathogens and parasitic nematodes. Because several combinations of pathogens and parasitic nematodes may be harmful to *A. arenaria*, a single cause of the disease could not be established.

Impact of soil-borne diseases on plant vigour in relation to sand accretion

The response of *Ammophila* species to burial has, until now, been explained by physiological mechanisms within the plant tissue. These mechanisms, ruled by plant hormones like cytokinins and ethylene, are involved in the response of *A. breviligulata* to sand accretion (Baye 1990, Yuan et al. 1993, Seliskar 1994). Another explanation for the vigorous response of *A. arenaria* to burial is that upward growth enables the plants to escape the harmful effects of soil organisms (Van der Putten et al. 1988). This escape hypothesis has been tested in the present thesis (chapters 4 and 5).

A. arenaria plants responded to sand accretion by upward growth as a result of internode elongation and root formation. This process was independent of the presence of harmful soil organisms in the deposited layer (chapter 4). This suggests that, similar to *A. breviligulata*, physiological mechanisms are involved in the upward growth. However, in the absence of soil organisms in the deposited layer of sand, *A. arenaria* plants were able to produce more above and below ground biomass than when soil organisms were present (chapter 4). This implies that plants benefit from avoiding infections of roots by soil organisms, thus confirming the escape-hypothesis.

After sand burial, fungi immediately grew through the soil and along the buried shoots (chapter 5). It is likely that these fungi utilized and degraded the buried, non-functional leaves. Plant-parasitic nematodes migrated upwards only after new roots have been formed. If sand is deposited in spring, plant-parasitic nematodes may migrate directly after roots have been formed. If, however, sand is deposited during autumn or early winter, plants may escape from rapid infections by nematodes for a longer period because of the plants' ability to elongate and form new roots in late winter. Due to low soil temperatures, most nematodes are dormant at this time of the year (Wallace 1966). Thus, plants that form roots in the deposited layer when the nematodes are inactive, will benefit the most from upward growth (chapter 5). The amount of sand deposited on the vegetation also appeared to be important for the chances of plants to escape infections (chapter 5).

During summer, buried plants continue to form new roots (Baye 1990, Eldred and Maun 1982, chapter 5 of this thesis). Obviously, the production of new roots enables the plants to absorb water and nutrients that are needed for growth. The prolonged formation of new roots provides the soil organisms with fresh substrate, so their numbers will increase. It has been found that stabilized soils contained higher numbers of fungi than mobile soils with continuous sand deposition (Venkateswarlu and Rao 1981, Marchant 1970). The endoparasitic nematodes

Heterodera and *Meloidogyne* usually have only one life-cycle per year (Yeates 1987), but they benefit from a higher supply of nutrients by producing more juveniles per adult. After two years without sand burial, a reduced vigour was evident (Baye 1990), suggesting that pathogen and parasite population sizes had increased to damaging levels.
In the inner dunes, both *A. arenaria* and *A. breviligulata* regain their vigour when sand accretion starts again. These rejuvenated stands can, among others, be found along the edges of blowouts (Van Dieren 1943, Willis 1989). Unlike beach sand, sand from the dunes is usually infested by pathogenic soil organisms. The vigour response of plants to burial with unsterilized root-zone sand was less than that of plants buried with sterilized soil or sand from the beach (chapter 4). It was, therefore, hypothesized that aeolian transport of infested soil from the deflation site of a blowout towards the deposition zone reduced the numbers of soil organisms (chapter 6). Experiments in a wind-tunnel demonstrated that in windblown soil the numbers of plant-parasitic nematodes were reduced. Fungi were less affected by sand movement by wind probably because they are attached to the sand particles. Nevertheless, windblown sand contained lower numbers of fungal propagules due to sifting and heavy scouring of the sand particles. These will be the most likely mechanisms involved in reducing fungal and nematode densities during sand movement by wind. Consequently, relatively pathogen- and parasite-free sand is deposited at the accumulating edges of blowouts which may explain the response of vigorous growth by *A. arenaria* (chapter 6).

Because the experiments in chapters 4 and 5 were done with unsterilized soil, also beneficial organisms may have been involved. Arbuscular mycorrhizal (AM) fungi are assumed to play an important role in the nutrient supply of plants, especially when growing in relatively nutrient poor soils (Pugh 1977, Koske and Halvorson 1981, Forster and Nicolson 1981, Nicolson and Johnston 1979, Ernst et al. 1984). Nonetheless, the experiments described in the present thesis suggest that the harmful effects of soil organisms were larger than the beneficial effects.

Implications for plant ecology
The question why *A. arenaria* is such a susceptible plant to infections remains unanswered. For *A. arenaria*, seedling establishment may depend on the ability of individual plants to survive sand burial (Huiskes 1977). This selection for burial-tolerant individuals may result in a trade-off at the cost of susceptibility to root damage caused by soil organisms. Such trade-offs have also been found in

another pioneer plant, reed (*Phragmites australis*), where individuals with properties benefiting tolerance for flooding were sensitive for adverse effects caused by accumulation of organic material (Clevering 1995). Seedlings of *A. arenaria* can establish in the outer foredune area, where sand accretion may be relatively high or in wet dune slacks where sand accretion is minimal (Huiskes 1977). However, the genetics of *Ammophila* spp. in relation to seedling establishment and plant morphology is, as yet, unknown (Huiskes 1977, 1979, Gray 1985, Laing 1967) and should be studied preferably in relation to disease susceptibility.

Succession in the vegetation regarding soil-borne pathogens and parasites
The experiments described in the present thesis clearly demonstrate that the vigour of *A. arenaria* in coastal sand dunes is closely related to infections by harmful soil organisms and is influenced by the timing and amount of sand accumulation. Besides *A. arenaria*, also the degeneration of *Hippophaë rhamnoides* (Sea buckthorn), a species dominating the foredune vegetation after *A. arenaria* has disappeared, has shown to be related to infections of roots by soil-borne pathogens (Oremus 1982, Zoon et al. 1993, Zoon 1995). When succeeding plant species of the coastal foredunes were grown in non-sterile root zone soil of each other, plants grown in their own soil or in that of species succeeding them demonstrated poor growth as compared to those in soil of their predecessors (Van der Putten, Van Dijk & Peters 1993). Thus, a considerable specificity of soil-borne diseases must be present in the different successional stages affecting the rate and direction of succession in coastal foredunes (Van der Putten, Van Dijk & Peters 1993). This conclusion has two implications: first, succeeding plant species have to be tolerant to the soil-borne diseases of their predecessors and, secondly, the community of soil organisms changes when one plant species is replaced by another. A succession of species of fungi has been shown to be correlated with a changing habitat (Brown 1958, Wohlrab et al. 1963, Rose 1988). Also a succession in nematode species was found to occur in vegetation succession in coastal foredunes (Bussau 1990a, 1990b, Zoon et al. 1993). However, the effects of nematodes and fungi in relation to the vigour of different plant species remains to be studied experimentally by inoculation experiments. Besides the potentially harmful effects of soil organisms, also competition between and within plant species, and the soil development will have their influence on plant succession. The net result of these abiotic and biotic factors on natural plant populations is that the distribution of plant species becomes non-continuous (Augspurger 1988). This will lead to an

apparently chaotic pattern of the structure in the plant community resulting in patches of monocultures in coastal foredunes. This process is at least partially governed by infections by soil organisms (Dobson and Crawley 1994).

Management practices in coastal foredunes with respect to soil-borne diseases
At sites with little or no sand accumulation, e.g. at stable or regressive coasts, plants degenerate and bare patches within the vegetation may develop. In the absence of succeeding plant species, these exposed sites are vulnerable to wind-erosion and therefore form a threat to the function of dunes as a natural sea-wall. However, bare patches in coastal foredunes are frequently covered by algae and mosses (Pluis and De Winder 1994). As trampling causes breaking of these crusts, wind erosion can easily occur at such sites. Replanting *A. arenaria* often fails because plants do not establish (Van der Putten 1990). In the case of *A. breviligulata*, plants could not be introduced in such a bare area for up to five years (Seliskar 1995). Therefore, planting successional plant species would be an effective control measure to prevent wind-erosion. The success of establishment of these species depends largely on the water availability of the soil, the season of planting and exclusion of rabbit grazing (Van der Putten and Peters 1995).
In spite of the potential danger of an ineffective sand-fixing vegetation, wind erosion also proved to enhance the vigour of *A. arenaria* as soil-borne pathogens and parasites are killed during sand movement by wind. Allowing development of blowouts will be useful to the vegetation, especially at those sites where they do not damage the function of the sea-wall or cause intolerable sand drift. Blowouts can become naturally fixed by horizontal rhizomes of *A. arenaria*, colonizing the blowout from the vegetated surroundings, or by colonization by a succeeding plant species, *Carex arenaria* (Sand sedge) (personal observation). However, when blowouts become too large, they may jeopardize the defence against the sea. Until now, these blowouts were reshaped and replanted. By reshaping, root-zone sand is deposited in the centre of the blowout resulting in poor establishment of the replanted culms. Based on the results of the experiments in this thesis, it should be considered not to reshape blowouts but to replant *A. arenaria* in the centre where soil-borne pathogens are nearly absent. As the side-walls become less exposed to wind erosion, the blowout will stabilize. By further colonization through horizontal rhizomes, the whole blowout becomes vegetated. Allowing blowouts to develop will, therefore, be an effective way to maintain a vigorous stand of *A. arenaria* and acting as an effective sand-fixing vegetation.
Another way to enhance the sand-catching capacity of the vegetation at regressive

coasts is to stimulate deposition of sea sand on the beach and to allow this deposited sand to be blown into the vegetation. This relatively expensive method can be used at those sites with a degrading coast consisting of only a small dune ridge and is already put into practice. In conclusion, by understanding growth of plants in relation to infections by soil-organisms, management practices can be exploited fruitfully.

References

Anonymous. 1990. *Kustverdediging na 1990.* Tweede Kamer, vergaderjaar 1989-1990, 21136, nrs. 5-6.

Alexander, H.M. 1990. Dynamics of plant-pathogen interactions in natural plant communities. In: *Pests, pathogens and plant communities.* J.J. Burdon, and S.R. Leather (eds). Blackwell Scientific Publications, Oxford. pp. 31-45.

Arens, S.M. 1994. *Aeolian processes in Dutch foredunes.* PhD. Thesis. University of Amsterdam. Amsterdam.

Augspurger, C.K. 1988. Impact of plant pathogens on natural plant populations. In: *Plant population ecology.* A.J. Davy, M.J. Hutchings and A.R. Watkinson (eds). Blackwell scientific publications, Oxford. pp. 431-433.

Bagnold, R.A. 1954. *The physics of blown sand and desert dunes.* 2nd edition. Methuen, London.

Baye, P.R. 1990. *Comparative growth responses and population ecology of European and American beachgrasses (*Ammophila *spp.) in relation to sand accretion and salinity.* PhD. thesis. University of Western Ontario, London.

Beckman, C.H. 1987. *The nature of wilt diseases of plants.* American Phytopathological Society Press, St. Paul, Minnesota.

Bever, J.D. 1994. Feedback between plants and their soil communities in an old field community. *Ecology* **75**: 1965-1977.

Bollen, G.J. 1974. Fungal recolonization of heat-treated glasshouse soils. *Agro-ecosystems* **1**: 139-155.

Bongers, T. 1988. *De nematoden van Nederland.* Stichting uitgeverij Koninklijke Nederlandse Natuurhistorische vereniging. Utrecht.

Boorman, L.A. 1978. Sand-dunes. In: *The coastline.* R.S.K. Barnes (ed). John Wiley and Sons, Chichester. pp. 161-197.

Brown, J.C. 1958. Soil fungi of some British sand dunes in relation to soil type and succession. *Journal of Ecology* **46**: 641-664.

Brown, V.K. 1990. Insect herbivory and its effect on plant succession. In: *Pests, pathogens and plant communities.* J.J. Burdon and S.R. Leather (eds). Blackwell Scientific Publications, Oxford. pp. 275-288.

Brown, A.C. and McLachlan, A. 1990. *Ecology of sandy shores.* Elsevier. Amsterdam.

Bussau, C. 1990 a. Freilebende Nematoden aus Küstendünen und angrenzenden Biotopen der deutschen und dänischen Küsten. I. Gesamtüberblick und Chromadorida (Nematoda). *Zoologische Anzeiger* **225**: 161-188.

Bussau, C. 1990 b. Freilebende Nematoden aus Küstendünen und angrenzenden Biotopen der deutschen und dänische Küsten. IV. Rhabditida und Tylenchida. *Zoologischer Anzeiger* **226**: 114-148.

Carmi, A. and Heuer, B. 1981. The role of roots in control of bean shoot growth. *Annals of Botany* **48**: 519-527.

Carter, R.W.G., Hesp, P.A. and Nordstrom, K.F. 1990. Erosional landforms in coastal dunes. In: *Coastal dunes. Forms and Process*. K.F. Nordstrom, N. Psutty and R.W.G. Carter (eds) John Wiley and Sons Ltd, Chichester. pp. 217-249.

Coleno, A., Rapilly, F., Pionnot, J.C. and Auge, G. 1965. Incidence des variations de la microflore d'un sol désinfecté sur la genèse de certains maladies des plantes. *Annal Epiphyties* **16**: 267-278.

Clevering, O.A. 1995. Ecological significance of genetic variability of *Phragmites australis* in relation to nutrient status and occurrence of dieback. In: *Reednews. The newsletter of the EC-Eureed project*. W.H. van der Putten (ed). NIOO, Heteren. pp.1-10.

Dennis, R.W.G. 1983. Fungi of *Ammophila arenaria* in Europe. *Revista de Biologia* **12**: 15-47.

Den Toom, T. 1988. Influence of temperature and soil moisture on the relation between *Tylenchorhynchus dubius* and *Lolium perenne*. *Netherlands Journal of Plant Pathology* **94**: 33-44.

Disraeli, D.J. 1984. The effect of sand deposits on the growth and morphology of *Ammophila breviligulata*. *Journal of Ecology* **72**: 145-154.

Dobson, A. and Crawley, M. 1994. Pathogens and the structure of plant communities. *TREE* **9**: 393-398.

Domsch, K.H., Gams, W. and Anderson, T-H. 1980. *Compendium of soil fungi. Vol. 1 & 2.* Academic Press, London.

Draga, M. 1983. Eolian activity as a consequence of beach nourishment-observations at Westerland (Sylt), German North Sea coast. *Zeitschrift für Geomorphologie* **45**: 303-319.

Eisenback, J.D. 1993. Interactions between nematodes in cohabitance. In: *Nematode interactions*. M.W. Khan (ed). Chapman and Hall, London. pp. 134-174

Eisenback, J.D. and Griffin, G.D. 1987. Interactions with other nematodes. In: *Vistas on nematology: a commemoration of the 25th anniversary of the Society of Nematologists*. J.A. Veech and D.W. Dickson (eds). Society of nematologists, De Leon Springs, Florida. pp. 313-320.

Eldred, R.A. and Maun, M.A. 1982. A multivariate approach to the problem of decline in vigour of *Ammophila*. *Canadian Journal of Botany* **60**: 1371-1380.

Ernst, W.H.O., Van Duin, W.E. and Oolbekking, G.T. 1984. Vesicular-arbuscular mycorrhiza in dune vegetation. *Acta Botanica Neerlandica* **33**: 151-160.

Forster, S.M. 1979. Microbial aggregation of sand in an embryo dune system. *Soil Biology and Biochemistry* **11**: 537-543.

Forster, S.M. and Nicolson, T.H. 1981. Microbial aggregation of sand in a maritime dune succession. *Soil Biology and Biochemistry* **13**: 205-208.

Gams, W. and Van Laar, W. 1982. The use of solacol (validamycin) as a growth retardant in the isolation of fungi. *Netherlands Journal of Plant Pathology* **88**: 39-45.

Gemmel, A.R., Greig-Smith, P. and Gimmingham, C.H. 1953. A note on the behaviour of *Ammophila arenaria* (L.) Link in relation to sand dune formation. *Transactions and Proceedings of the Botanical Society of Edinburgh* **36**: 132-136.

Goodey, T. 1937. Two methods for staining nematodes in plant tissue. *Journal of Helminthology* **15**: 137-144.

Gray, A.J. 1985. Adaptation in perennial coastal plants: with particular reference to heritable variation in *Puccinella maritima* and *Ammophila arenaria*. *Vegetatio* **61**: 179-188.

Gregory, P.H. 1961. *Microbiology of the atmosphere*. Leonard Hill, London.

Harper, J.L. 1990. Pests, pathogens and plant communities: an introduction. In: *Pests, pathogens and plant communities*. J.J. Burdon and S.R. Leather (eds). Blackwell Scientific Publications, Oxford. pp. 3-14.

Heald, C.M. 1987. Classical nematode management practices. In: *Vistas on nematology. A commemmoration of the 25th anniversary of the society of nematologists*. J.A. Veech and D.W. Dickson (eds). Society of nematologists. DeLeon Springs, Florida. pp. 100-104.

Hill, M.O. 1979. *TWINSPAN - a FORTRAN program for arranging multivariate data in an ordered two-way table by classification of individuals and attributes*. Cornell University, Ithaca, New York.

Hooper, D.L. 1986. Extraction of free-living stages from soil. In: *Laboratory methods for work with plant and soil nematodes. Reference book 402*. J.F. Southey (ed). Ministry of Agriculture, Fisheries and Food, Her Majesty's Stationary Office, London. pp. 5-30.

Hope-Simpson, J.F. and Jefferies, R.L. 1966. Observations relating to vigour and debility in marram grass (*Ammophila arenaria* (L.) Link). *Journal of Ecology* **54**: 271-275.

Huiskes, A.H.L. 1977. The natural establishment of *Ammophila arenaria* from seed. *Oikos* **29**: 133-136.

Huiskes, A.H.L. 1979. Biological flora of the British isles: *Ammophila arenaria* (L.) Link (*Psamma arenaria* (L.) Roem. et Shult.: *Calamagrostis arenaria* (L.) Roth). *Journal of Ecology* **67**: 363-382.

Jepson, S.B. 1987. *Identification of root-knot nematodes (*Meloidogyne *species).* C.A.B. International, Wallingford, Oxon.

Jungerius, P.D. and Van der Meulen, F. 1989. The development of dune blowouts, as measured with erosion pins and sequential air photos. *Catena* **16**: 369-376.

Jongman, R.H.G., Ter Braak, C.J.F. and Van Tongeren, O.F.R. 1987. *Data analysis in community and landscape ecology.* Pudoc, Wageningen.

Koske, R.E. and Halvorson, W.L. 1981. Ecological studies of vesicular-arbuscular mycorrhizae in a barrier sand dune. *Canadian Journal of Botany* **59**: 1413-1422.

Knutson, P.L. 1978. Planting guidelines for dune creation and stabilization. *Coastal Zone* **1978**: 14-16.

Labruyère, R.E. 1979. Resowing problems of old pastures. In: *Soil-borne plant pathogens.* B. Schipper and W. Gams (eds). Academic Press, London. pp. 313-326.

Laing, C.C. 1967. The ecology of *Ammophila breviligulata.* II. Genetic change as a factor in population decline on stable dunes. *American Midland Naturalist* **77**: 495-500.

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.

Mai, W.F. and Abawi, G.S. 1987. Interactions among root-knot nematodes and *Fusarium* wilt fungi on host plants. *Annual Review of Phytopathology* **25**: 317-338.

Marchant, R. 1970. The root surface of *Ammophila arenaria* as a substrate for micro-organisms. *Transactions of the British Mycological Society* **54**: 479-506.

Marshall, J.K. 1965. *Corynephorus canescens* (L.) P. Beauv. as a model for the *Ammophila* problem. *Journal of Ecology* **53**: 447-465.

Maun, M.A. and Baye, P. 1988. The ecology of *Ammophila breviligulata* Fern. on coastal dune systems. *Critical Review in Aquatic Science* **1**: 661-681.

Maun, M.A. and Lapierre, J. 1984. The effects of burial by sand on *Ammophila breviligulata. Journal of Ecology* **72**: 827-839.

Moreau, M. and Moreau, F. 1941. Première contribution à l'étude de la microflore des dunes. *Revue de Mycologie* **6**: 49-94.

Murray, D.I.L. and Gadd, G.M. 1981. Preliminary studies on *Microdochium bolleyi* with special reference to colonization of barley. *Transactions of the British Mycological Society* **76**: 397-403.

Nelson, P.E., Toussoun, T.A. and Marasas, W.F.O. 1983. Fusarium *species. An illustrated manual for identification.* Pennsylvania State University Press. University Park, London.

Newman, E.I. 1978. Root microorganisms: their significance in the ecosystem. *Biological Reviews* **53**: 511-554.

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. *Transactions of the British Mycological Society* **72**: 261-268.

Nombela, G., Navas, A. and Bello, A. 1993. Spatial and temporal variation of the nematode fauna in representative soils of the central region of the Iberian Peninsula. *Nematologica* **39**: 81-91.

Old, K.M and Nicolson, T.H. 1975. Electron microscopical studies of the microflora of roots of sand dune grasses. *New Phytologist* **74**: 51-58.

Oostenbrink, M. 1960. Estimating nematode populations by some selected methods. In: *Nematology*. J.N. Sasser and W.R. Jenkins (eds). Chapel hill, University of North Carolina Press, p. 85-102.

Oremus, P.A.I. 1982. *Growth and nodulation of* Hippophaë rhamnoides *L. in the coastal sanddunes of the Netherlands*. PhD. Thesis, University of Utrecht, Utrecht.

Orr, C.C. and Newton, O.H. 1971. Distribution of nematodes by wind. *Plant Disease Reporter* **55**: 61-63.

Palmer, M.W. 1993. Putting things in even better order: the advantages of canonical correspondence analysis. *Ecology* **74**: 2215-2230.

Papendick, R.I. and Cook, R.J. 1974. Plant water stress and development of *Fusarium* foot rot in wheat subjected to different cultural practices. *Phytopathology* **64**: 358-363.

Rawsthorne, D. and Hague, N.G.M. 1985. The relationship between size of barley roots and nematode invasion by the cereal cyst nematode *Heterodera avenae*. *Nematologica* **31**: 79-87.

Rose, S.L. 1988. Above and belowground community development in a marine sand dune ecosystem. *Plant and Soil* **109**: 215-226.

Pluis, J.L.A. and De Winder, B. 1990. Natural stabilization. In: *Dunes of the European coasts*. T.W.M. Bakker, P.D. Jungerius and J.A. Klijn (eds). Catena supplement 18: 195-208

Powell, N.T., Melendez, P.L. and Batten, C.K. 1971. Disease complexes involving *Meloidogyne incognita* and certain soil-borne fungi. *Phytopathology* **61**: 1332-1337.

Prot, J.C. 1980. Migration of plant-parasitic nematodes towards plant roots. *Revue Nematologie* **3**: 305-318.

Pugh, G.J.F. 1980. Strategies in fungal ecology. *Transactions of the British Mycological Society* **75**: 1-20.

Pye, K. 1990. Physical and human influences on coastal dune development between the Ribble and Mersey estuaris, northwest England. In: *Coastal dunes. Forms and Process*. K.F. Nordstrom, N. Psutty and R.W.G. Carter (eds). John Wiley and Sons Ltd, Chichester. pp. 339-359.

Rozema, J., Laan, P., Ernst, W.H.O. and Appelo, C.A.J. 1985. On the lime transition and decalcification in the coastal dunes of the province of North Holland and the island of Schiermonnikoog. *Acta Botanica Neerlandica* **34**: 393-411.

Seliskar, D.M. 1994. The effect of accelerated sand accretion on growth, carbohydrate reserves, and ethylene production in *Ammophila breviligulata* (Poaceae). *American Journal of Botany* **81**: 536-541.

Seliskar, D.M. 1995. Coastal dune restoration: a strategy for alleviating dieout of *Ammophila breviligulata*. *Restoration ecology* **3**: 54-60.

Seliskar, D.M. and Huettel, R.N., 1993. Nematode involvement in dieout of *Ammophila breviligulata* (Poaceae) on the mid-atlantic coastal dunes of the United States. *Journal of Coastal Research* **9**: 97-103.

Sewell, G.W.F. 1981. Soil-borne fungal pathogens in natural vegetation and weeds of cultivation. In: *Pests, pathogens and vegetation. The role of weeds and wild plants in the ecology of crop prests and diseases*. J.M. Tresh (ed). Association of applied biologists. Pitman Press, Bath. pp. 175-190.

Simons, W.R. 1973. *Nematode survival in relation to soil moisture.* Mededelingen Landbouwhogeschool Wageningen 73-3.

's Jacob, J.J. and Van Bezooijen, J. 1984. *Manual for practical work in nematology*. Practicumhandleiding vakgroep nematologie, Landbouw Universiteit Wageningen.

Steen, E. and Larsson, K. 1986. Carbohydrates in roots and rhizomes of perennial grasses. *New Phytologist* **104**: 339-346.

Ter Braak, C.J.F. 1988. *CANOCO - a FORTRAN program for canonical community ordination by [partial] [detrended] [canonical] correspondence analysis, principal components analysis and redundancy analysis (version 2.1)*. Groep Landbouwwiskunde Wageningen.

Trivedi, P.C. and Barjker, K.R. 1986. Management of nematodes by cultural practices. *Nematropica* **16**: 213-236.

Van der Goes, P.C.E.M. and Van der Putten, W.H. 1992. Are nematodes involved in the degeneration of *Ammophila arenaria* (marram grass)? *Mededelingen van de Faculteit van de Landbouwwetenschappen Universiteit van Gent*, **57/3a**: 847-856.

Van der Putten, W.H. 1990. Establishment and management of *Ammophila arenaria* (marram grass) on artificial coastal foredunes in the Netherlands. *Proceedings Canadian Symposium on coastal sand dunes* **1990**: 367-387.

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 Biology and Biochemistry* **22**: 845-852.

Van der Putten, W.H. and Peters, B.A.M. 1995. Possibilities for management of coastal foredunes with deteriorated stands of *Ammophila arenaria* (Marram grass). *Journal of Coastal Conservation* (in press).

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*. *Canadian Journal of Botany* **68**: 1560-1568.

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 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 der Werf-Klein Breteler, J.T. and Van Dijk, C. 1989. Colonization of the root zone of *Ammophila arenaria* by harmful soil organisms. *Plant and Soil* **120**: 213-223.

Van Dieren, J.W. 1934. *Organogene Dünenbildung. Eine geomorphologische Analyse der Dünenlandschaft der West-Friesischen Insel Terschelling mit pflanzensoziologischen Methoden*. Martinus Nijhoff, Den Haag.

Venkateswarlu, B. and Rao, A.V. 1981. Distribution of microorganisms in stabilised and unstabilised sand dunes of the Indian desert. *Journal of Arid Environments* **4**: 203-207.

Wallace, H.R. 1966. Factors influencing the infectivity of plant-parasitic nematodes. *Proceedings of the British Royal Society of London. Series B.* **164**: 592-614.

Wallén, B. 1980. Changes in structure and function of *Ammophila* during a primary succession. *Oikos* **34**: 227-238.

Warcup, J.H. 1960. Methods for the isolation and estimation of activity of fungi. In: *The ecology of soil fungi*. D. Parkinson and J.S. Waid (eds). Liverpool University Press, Liverpool. pp. 3-21.

Webley, D.M., Eastwood, D.J. and Gimmingham, C.H. 1952. Development of a soil microflora in relation to plant succession on sand-dunes, including a 'rhizosphere' flora associated with colonizing species. *Journal of Ecology* **40**: 168-178.

Willis, A.J. 1965. The influence of mineral nutrients on the growth of *Ammophila arenaria*. *Journal of Ecology* **53**: 735-745.

Willis, A.J. 1989. Coastal sand dunes as biological system. *Proceedings of the Royal Society of Edinburgh* **96B**: 17-36.

Windham, M.T., Elad, Y. and Baker, R. 1986. A mechanism for increased plant growth induced by *Trichoderma* spp.. *Phytopathology* **76**: 518-521.

Wohlrab, G., Tuveson, R.W. and Olmsted, C.E. 1963. Fungal populations from early stages of succession in indiana dune sands. *Ecology* **44**: 734-740.

Wyss, U. 1973. Feeding of *Tylenchorhynchus dubius*. *Nematologica* **19**: 125-136.

Yeates, G.W. 1968. An analysis of annual variation of the nematode fauna in dune sand, at Himatangi Beach, New Zealand. *Pedobiologia* **8**: 173-207.

Yeates, G.W. 1987. How plants affect nematodes. *Advances in Ecological Research* **17**: 61-113.

Yeates, G.W., Bongers, T., De Goede, R.G.M., Freckman, D.W. and Georgieva, S.S. 1993. Feeding habits in soil nematode families and genera - an outline for soil ecologists. *Journal of nematology* **25**: 315-331.

Yuan, T., Maun, M.A. and Hopkins, W.G. 1993. Effects of sand accretion on photosynthesis, leaf-water potential and morphology of two dune grasses. *Functional Ecology* **7**: 676-682.

Zadoks, J.C. and Schein, R.D. 1979. *Epidemiology and plant disease management*. Oxford University Press, New York.

Zingg, A.W. 1952. Wind-tunnel studies of the movement of sedimentary material. *Proceedings of the 5th Hydraulic Conference Iowa University* **34**: 111-135.

Zoon, F.C. 1995. *Biotic and abiotic soil factors in the succession of Sea buckthorn,* Hippophaë rhamnoides *L. in coastal sand dunes*. PhD. Thesis. Agricultural University Wageningen, Wageningen.

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. Fundamental and Applied *Nematology* **16**: 247-258.

SUMMARY

Ammophila arenaria is the most dominant sand-fixing plant species in the Dutch coastal foredunes. The plants have a natural ability to emerge from being buried and are therefore used to stabilize the coastal foredunes. On seaward slopes, *A. arenaria* retains its vigour when it is regularly buried but the species starts to degenerate when sand accumulation diminishes. These degenerated patches are vulnerable to erosion by wind; thus threatening the functioning of coastal foredunes as natural sea-walls.
Several factors have been reported as the cause of degeneration of *A. arenaria* at stabilized dunes: nutrients are limiting, plants become outcompeted, the functioning of roots is reduced or plants degenerate due to physiological ageing. Previous experiments with biocides demonstrated the involvement of nematodes and fungi in the declined growth of *A. arenaria*. Burial by fresh windblown sand may enable the plants to overcome these harmful soil organisms. The nature of the disease (caused by nematodes and fungi) and its relationship with sand deposition is studied in the present thesis.

A survey was carried out at nine locations in the Dutch coastal foredunes to identify the species of soil-borne fungi and nematodes associated with *A. arenaria*. A wide range of nematodes and fungi was isolated. Several assemblages of coexisting nematode and fungal species were identified using canonical correspondence analysis (CCA) and two-way indicator species analysis (TWINSPAN) (chapter 2).
Adding single species of fungi did not reduce the growth of the seedlings, but the combination of all fungal species that were commonly found in the Dutch coastal foredunes significantly reduced growth to about 80% of that in sterilized soil. This indicates synergistic effects among the plant-pathogenic fungi. The addition in a density of 80 times higher than present in natural soil of the nematode *Telotylenchus ventralis* reduced plant growth to about the same level as that in natural soil. Adding relatively large numbers of *T. ventralis* in combination with the commonly occurring fungi also reduced plant growth in an additive way. The involvement of other plant-parasitic nematode species, such as *Heterodera* and *Meloidogyne maritima*, could not be established in the inoculation experiments (chapter 3). Nevertheless, they could successfully infect and multiply on roots of *A. arenaria* plants that were buried with sand (chapters 4 and 5) or that were planted in scoured soil (chapter 6). It seems likely, that several different

combinations of soil organisms are harmful to *A. arenaria*, and thus, that the decline is not caused by one single well defined pathosystem (chapter 3).

A. arenaria plants were buried with sand to test the hypothesis that upward growth following sand accretion enables plants to escape from infections by soil organisms (chapters 4 and 5). If plants in a degenerated field stand or potted plants grown in sterilized soil were covered with sand, the stems elongated and new shoots and roots were formed in the deposited sand layer (chapter 4). This response to burial, based on physiological mechanisms within the plant tissue, enabled the plants to emerge from the deposited sand and escape temporarily from plant-pathogenic fungi and nematodes (chapters 4 and 5).
After burial with sand, fungi immediately colonized the added soil where they probably grew on the buried non-functional leaves that surround the stems. Plant-parasitic nematodes migrated only after roots had been formed which were infected soon after being formed (chapter 5). If sand is deposited in spring, nematodes migrate directly after new roots have been formed. However, if sand is deposited in autumn, *A. arenaria* may escape from rapid infection by plant-parasitic nematodes due to the plants' ability to grow and form new roots during winter while the nematodes are largely inactive. During the summer period, plants continue to form roots (chapter 5). As a result of this prolonged formation of new roots, numbers of pathogens and parasites will increase. Without a renewed burial of the plants by sand, increasing populations of pathogens and parasites will eventually lead to their degeneration.
Degenerated stands of *A. arenaria* rejuvenate when sand is deposited on the vegetation. This phenomenon is observed along the accumulating edges of blowouts. This deposited sand is not fresh beach sand but originates from foredunes (chapter 6). Unlike sand from the beach, sand from the coastal foredunes is usually infested by soil-borne pathogens and parasites. When *A. arenaria* plants were buried with sand from its own root zone, they responded less vigorous than when plants were buried with pathogen-free sand (chapter 4). Therefore, it is hypothesized that during the proces of sand movement by wind the pathogens and parasites will, in some way, disappear. Field measurements in blowouts and experiments in a wind-tunnel demonstrated that numbers of plant-parasitic nematodes and, to a lesser extent, numbers of fungal propagules were reduced during aeolian transport. The nematodes and fungi may have been killed by scouring of sand particles and are sifted from the sand fraction. However, fungi were also transported attached to the soil particles (chapter 6).

Mechanical damaging of nematodes and fungi was simulated by thorough stirring in infested soil. This treatment affected the numbers of nematodes and fungi negatively and led to an enhanced growth of the test plants planted in stirred soil compared to that in unscoured soil. Also, sand deposited on the accumulating edges of blowouts was coarser than the original sand, implying a disappearance of the smaller and lighter soil particles, including nematodes and fungi. The small particles were transported over long distance. Consequently, sand relatively free of soil organisms is deposited on the vegetation. Thus the increased vigour of *A. arenaria* at the accumulating edges of blowouts will be due to burial by soil that has lost pathogenic propagules during sand movement by wind (chapter 6).

It is concluded that the growth of *A. arenaria* is affected by infections of several groups of soil organisms, especially those groups including plant-parasitic nematodes. Furthermore, it could be demonstrated that burial with unsterilized root zone sand is less beneficial for plant growth than burial with sterilized or beach sand. This implies that plants are able to escape infections by soil organisms through upward growth following sand accumulation. Fungi colonized the freshly deposited layer of sand faster than plant-parasitic nematodes. The season of sand accretion and the amount of sand accumulation are important factors for the chances of plants to escape infections.

Additionally, it could be shown that in windblown soil numbers of fungal propagules and nematodes were reduced. Management practices aimed at increased burial with windblown sand can increase the vigour of *A. arenaria* stand. This increases the sand-catching capacity of the vegetation. Besides that these management practices will result in a natural appearance of coastal foredunes, it will also sustain the value of the Dutch coastal foredunes as a natural defence against the sea.

SAMENVATTING

Helm (*Ammophila arenaria*) is de belangrijkste zandvastleggende plantesoort in de Nederlandse duinen. In de zeereep neemt vanaf het strand landinwaarts de vitaliteit van helm af. Deze afname in vitaliteit is gerelateerd aan de afnemende aanvoer van vers zand. Na overstuiving groeit de plant omhoog en worden uit de ondergrondse knoppen nieuwe spruiten en wortels gevormd. Deze eigenschap om met het zand mee omhoog te komen wordt benut voor de vastlegging van zand in de duinen. Als echter geen zandaanwas heeft plaatsgevonden, kunnen nieuwe wortels slechts in de oude (al eerder begroeide) zandlaag worden gevormd. Hierdoor neemt de effectiviteit van de stuifwerende begroeiing af en het gevaar van ongewenste zandverstuivingen toe.
De verminderde vitaliteit van *A. arenaria* werd voorheen toegeschreven aan verschillende faktoren waaronder beperking van voedingsstoffen, concurrentie met andere planten, een verminderd functioneren van wortels of fysiologische veroudering waardoor planten niet goed uitgroeien. Experimenten met biociden hebben echter aangetoond dat na selectieve uitschakeling van nematoden of schimmels in de grond de groei van helm verbetert. Indien de planten worden overstoven, zouden ze aan deze schadelijke bodemorganismen kunnen ontsnappen. De aard van de bodemziekte (veroorzaakt door nematoden en schimmels) en hun relatie met zandoverstuiving wordt in dit proefschrift beschreven.

Om de aard van de schadelijke bodemorganismen vast te stellen zijn langs de Nederlandse kust negen locaties bemonsterd (hoofdstuk 2). Uit deze inventarisatie bleek, dat een groot aantal nematoden en schimmels de potentie heeft helmplanten aan te tasten. Om een overzicht te krijgen van de organismen die samen kunnen voorkomen is gebruik gemaakt van canonische correspondentie analyse (CCA) en twee-weg indicatorsoorten-analyse (TWINSPAN). Hiermee zijn verschillende combinaties van potentieel schadelijke bodemorganismen vastgesteld.
Na de inventarisatie zijn inoculatieproeven uitgevoerd (hoofdstuk 3). In deze experimenten werden de te onderzoeken organismen individueel of groepsgewijs aan gezonde helmzaailingen toegediend, waarna de groeiremming werd gemeten. Toevoeging van afzonderlijke schimmelsoorten resulteerde niet in een groeiafname van helm, maar als alle algemeen voorkomende schimmelsoorten gelijktijdig werden toegevoegd, werd de groei van de zaailingen met 20% geremd. Dit suggereert een synergistische, ofwel elkaar versterkende werking tussen schimmels onderling. De toevoeging van 80 maal zoveel individuen van de semi-endoparasi-

taire nematode *Telotylenchus ventralis* als in natuurlijke grond voorkomt reduceerde de groei van de zaailingen net zo sterk als natuurlijke grond. Wanneer een redelijk groot aantal individuen van deze nematodesoort in combinatie met de algemeen voorkomende schimmels werd toegevoegd, werd de groei van de planten ook geremd. De rol van andere plant-parasitaire nematoden als *Heterodera* sp. (cyste-alen) of *Meloidogyne maritima* (wortelknobbel-alen) kon in de inoculatie-experimenten niet worden vastgesteld. Desalniettemin konden ze wel wortels infecteren en zich vermenigvuldigen op wortels van planten die waren overstoven (hoofdstukken 4 en 5) en op wortels in zand dat gemengd was (hoofdstuk 6). De resultaten van de inoculaties zijn niet glashelder. Zeer waarschijnlijk zijn er een aantal combinaties van bodemorganismen schadelijk voor helm en is er dus niet één ziektecomplex aan te wijzen.

Helmplanten zijn overstoven met verschillende zandtypen (besmet en niet besmet met pathogenen) om te toetsen of planten kunnen ontsnappen aan hun belagers door in de aangestoven laag zand te groeien (hoofdstukken 4 en 5). Wanneer gedegenereerde planten in het veld of planten gekweekt in gesteriliseerd zand in potten met wel of niet besmet zand werden overstoven, strekte de stengel zich en werden nieuwe spruiten en wortels gevormd in de aangebrachte laag. Deze reactie is gebaseerd op fysiologische veranderingen in het planteweefsel. Dit helpt de plant om tijdelijk te ontsnappen aan de bodemorganismen (hoofdstukken 4 en 5). Na zandaanwas groeiden schimmels direct in de aangestoven laag waar zij waarschijnlijk de ondergestoven, niet-functionele bladeren koloniseerden (hoofdstuk 5). Plant-parasitaire nematoden migreerden pas naar boven als er wortels in de nieuw overstoven laag gevormd waren, waar ze deze direct infecteerden (hoofdstuk 5). Wanneer pas in het voorjaar zand rond de helmbegroeiing accumuleert, kunnen nematoden direct na wortelvorming omhoog kruipen. Maar als aanstuiving al in het najaar plaatsvindt, krijgt helm door z'n vroege groei (de eerste strekking en wortelgroei beginnen vaak al in de winter) een grotere voorsprong op de nematoden. Gedurende de zomer blijven planten nieuwe wortels vormen. Hierdoor blijft steeds nieuw voedsel voorradig voor de bodemorganismen en zullen deze dus in aantal toenemen. Zonder overstuiving van planten, zullen de toenemende aantallen bodemorganismen meer schade veroorzaken aan de planten en uiteindelijk zal dit tot degeneratie van de helm leiden.
De vitaliteit van helm wordt verbeterd als helmplanten het door de wind aangevoerd zand invangen. Dit fenomeen wordt waargenomen langs de randen van stuifkuilen waar helm weer spontaan opbloeit wanneer het wordt overstoven

(hoofdstuk 6). Zand uit de zeereep bevat, in tegenstelling tot strandzand, gewoonlijk schadelijke bodemorganismen. Wanneer helmplanten worden overstoven met zand waarin nog pathogenen aanwezig zijn, reageert helm minder positief dan wanneer de planten worden overstoven met steriel zand (hoofdstuk 4). Door verstuiving van besmet zand werden wel de aantallen nematoden en, in mindere mate, de aantallen schimmels gereduceerd. Dit is getoetst zowel in stuifkuilen in de buitenduinen als experimenteel in een windtunnel (hoofdstuk 6). De reductie van de aantallen nematoden werd zeer waarschijnlijk veroorzaakt doordat nematoden werden kapot gewreven tussen de zandkorrels en/of doordat ze uit het zand werden geblazen. Schimmels bleken hoofdzakelijk aan zandkorrels vast te zitten en werden dus met het zand meegevoerd. Desondanks werd ook de schimmeldichtheid tijdens het zandtransport gereduceerd. Deze mechanische beschadiging werd ook in een meng-experiment nagebootst. Een grondige menging van zand had een negatief effect op de aantallen bodemorganismen, waardoor helmplanten in gemengd zand beter groeiden dan in niet-gemengd zand. Het zand dat op de accumulatiezijde van stuifkuilen door helm werd ingevangen was grover van samenstelling dan het oorspronkelijke zand. Dit suggereert dat de lichte delen, waaronder ook nematoden en schimmels, niet werden ingevangen en over een grotere afstand met de wind werden meegevoerd. Hierdoor bevatte het geaccumuleerde verstoven zand minder schimmels en nematoden dan niet-verstoven zand, hetgeen een verbetering van de groei van helm mede verklaard.
Geconcludeerd wordt dat de groei van helm wordt belemmerd door aantastingen door verschillende groepen bodemorganismen, speciaal die groepen die plantparasitaire nematoden bevatten. Verder kon worden aangetoond dat overstuiven met niet-steriel zand de groei van helm minder werd gestimuleerd dan overstuiven met strandzand of gesteriliseerd zand. Dit impliceert dat na overstuiven, helmplanten kunnen ontsnappen aan schadelijke bodemorganismen. Schimmels bleken de aangebrachte laag sneller te koloniseren dan nematoden. Het seizoen waarin overstuiving plaatsvindt en de hoeveelheid overstuiving zijn belangrijke factoren in de ontsnapping van helm aan bodemorganismen.
Daarnaast bleek dat door secundaire verstuiving van zand uit de zeereep het aantal nematoden en de schimmeldichtheid werden verminderd. Beheersmaatregelen die gericht zijn op stimulatie van de dynamiek in de duinen zal de vitaliteit van helm verbeteren. Hierdoor zal de zand invangende capaciteit van de helmvegetatie verbeterd worden. Buiten dat deze beheersmaatregelen zullen uitmonden in een natuurlijke zeereep, zal het ook geen negatieve gevolgen hebben voor de veiligheid van de Nederlandse duinen.

NAWOORD

Voor de totstandkoming van dit werk zijn 6 contracten versleten. De schimmelgegevens, gedocumenteerd in dit proefschrift, zijn verzameld door 177 grondverdunningen uit te voeren, 442 maal de Warcup-techniek, 7690 stukjes wortel en 1760 stengelstukjes te wassen in gesteriliseerd gedemineraliseerd water en uit te leggen. Hierbij is gebruik gemaakt van minimaal 50 liter mout-extract-agar, een even zo grote hoeveelheid aardappel-glucose-agar en op z'n minst 11.000 Petrischalen. De nematodengegevens zijn verzameld door 545 maal gebruik te maken van de Oostenbrink-trechters, minimaal 625 maal is de decanteertechniek gebruikt en zijn 230 wortelmonsters geanalyseerd met Baerman-trechters. Dit alles had nooit kunnen plaatsvinden zonder de financiële steun van Rijkswaterstaat en de kustbeherende waterschappen: Hoogheemraadschap Noordhollands Noorderkwartier, Hoogheemraadschap van Delfland, Hoogheemraadschap van Rijnland, Waterschap De Brielse Dijkring, Waterschap Goeree-Overflakkee, Waterschap Schouwen-Duiveland, Waterschap Noord-en Zuid-Beveland, Waterschap Walcheren en Waterschap Het vrije van Sluis ten behoeve van de Technische adviescommissie Waterkeringen (TAWC*natduin). Hiervoor mijn hartelijke dank.

Maar, onderzoek is niet iets dat je alleen doet of zelfs alleen kunt doen. Ofschoon er één naam op de kaft van dit proefschrift staat zijn er in de loop der jaren veel mensen, zowel direkt als indirekt, bij betrokken geweest. Vanaf deze plaats wil ik ze bedanken.
Allereerst Wim van der Putten, hij was de initiator van het onderzoek en zonder zijn begeleiding was dit nooit rond gekomen. Prof. dr. L. Brussaard, Prof. dr. ir. J.W. Woldendorp, mijn beide promotoren, en Kees van Dijk jullie commentaren hebben dit huzarenstukje stukken leesbaarder gemaakt.
Mijn dank gaat speciaal uit naar de begeleidingscommissie van mijn project (TAWC*natduin): ing. J. van Beek (Rijnland), ir. J.J. van der Kolff (Delfland), mw. drs. M. Löffler (RWS-DWW), dr. F. van der Meulen (FGBL, UvA), ir. J.W.B. Nienhuis (Brielse Dijkring), ir. J.J. Pilon (RWS-DGW), mw. drs. G. Veenbaas (RWS-DWW), ir. H.J. Verhagen (IHE), ing. H. Voogt en dhr. van der Zwan (Rijnland). Ook Wim en Kees maakten deel uit van deze commissie.
Marieke Eleveld, Daphne van der Wal en natuurlijk Pim Jungerius, dankzij jullie inspanningen ben ik iets wegwijs geworden in de fysische geografie. Verder wil ik Frank Verschoor, Carlijn Zweekhorst, Roos Duyvesteijn (stagaires van het

MLO te Delft), Sonja Honders, Mardi Lensink (stagaires van de HLO, toen Wageningen), Marcel van der Heijden (stagair van de LUW), Remko Voogt en bovenal Bas Peters bedanken voor hun doorzettingsvermogen om veel van die (bijna ontelbare) aantallen schimmels en nematoden te isoleren. Claudia, bedankt voor het verlenen van onderdak.
Mam, je wilde het niet, maar je staat er toch: bedankt voor het maken van de zandvangzakjes. Frank en Willemijn, het is af.

Petra.

CURRICULUM VITAE

Petronella Cornelia Elizabeth Maria de Rooij-van der Goes werd op 30 juni 1964 geboren te Delft. Na het behalen van HAVO en VWO begon zij in 1983 met de studie planteziektenkunde aan de toenmalige Landbouwhogeschool te Wageningen. In de doctoraalfase deed zij onderzoek bij de vakgroep Fytopathologie naar de microbiële herkolonisatie van gestoomde kasgrond. Voor dezelfde vakgroep heeft zij in Engeland aan de fysiologie van de schimmel *Coniothyrium minitans*, een biologisch bestrijdings agens, gewerkt. Voor stage en onderzoek voor de vakgroep Nematologie heeft zij in Costa Rica de mogelijkheden voor biologische bestrijding van nematoden in bananenplantages onderzocht alsmede een service-laboratorium voor identificatie van planteziekten opgezet. Het ingenieursdiploma behaalde zij in 1989.

In december 1989 kwam zij in dienst bij het toenmalige Instituut voor Oecologisch Onderzoek (IOO), eerst in Oostvoorne, later in Heteren. In opdracht van Rijkswaterstaat en de kustbeherende waterschappen voerde zij een onderzoek uit naar de oorzaak van helmmoeheid. In 1991 volgde aansluitend een onderzoek naar de mogelijkheden voor de verbetering van de vitaliteit van gedegenereerde helmbegroeiing door gecontroleerde verstuiving in de zeereep. De resultaten van beide onderzoeken zijn weergegeven in dit proefschrift.

Van augustus 1994 tot augustus 1995 werkte zij als postdoctoraal onderzoeker bij het Nederlands Instituut voor Oecologisch Onderzoek, Centrum voor Terrestrische Oecologie (NIOO-CTO) te Heteren aan de rol van schimmels in de successie van planten in de Nederlandse duinen.