ESTABLISHMENT, GROWTH AND DEGENERATION OF AMMOPHILA ARENARIA IN COASTAL SAND DUNES

W.H. van der Putten

Promotor   : dr. ir. L 't Mannetje
                 hoogleraar in de graslandkunde

Co-promotor   : dr. C. van Dijk
                 wetenschappelijk hoofdmedewerker
                 Instituut voor Oecologisch Onderzoek

Monument te Bloemendaal (J.W. Havermans 1935): *Den onbekende Hollander gewijd die het eerst het stuivende duinzand door helmbeplanting bedwong* (foto W. Verholt).

VOORWOORD

Deze studie naar "vestiging, groei en degeneratie van *Ammophila arenaria* (helm)" is uitgevoerd op verzoek van het waterschap De Brielse Dijkring en gefinancierd door Rijkswaterstaat. In een plezierige samenwerking met de technische dienst en het bestuur van het waterschap De Brielse Dijkring en met de diensten Weg- en Waterbouwkunde, Getijdewateren, Meetkundige Dienst en de Directie Zuid-Holland van Rijkswaterstaat zijn zowel praktische toepassingen als nieuwe fundamentele inzichten tot stand gekomen.
Na rapportage van de onderzoeksresultaten aan de opdrachtgever was er weinig tijd over voor het schrijven van dit proefschrift. De directeur van het IOO, dr. ir. J.W. Woldendorp, stond mij toe de schrijfwerkzaamheden tijdens mijn nieuwe functie af te ronden.

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De heren S.L. Dob, R. Dekker, A. van Marion en de kantonniers van het

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

Immediate cause of the study

In 1953, after the south-western part of the Netherlands had been partially flooded the Dutch Government enacted the 'Delta Act' which prescribed minimum dimensions for coastal foredunes and sea dykes. As a consequence, sea arms were closed by barrier dams and both dykes and foredunes were reconstructed (the Delta-Plan). The foredune on the island of Voorne did not conform to minimum dimensions. The planning stage of measurements and studies, however, took so much time ahead of the actual reconstruction works that in the meanwhile weak parts of the foredunes had to be fortified in order to provide the minimum security (Voogt 1988). *Ammophila arenaria* (L.) Link, as is common practice, was used to stabilize the reconstructed foredunes during these preliminary works. However, the estabilishment was subject to failures as plants frequently died. Usually, *A. arenaria* had to be replanted 3 to 5 times before an effective sand-stabilizing vegetation could be obtained (Voogt 1988). Meanwhile, measures had to be taken to protect the wet dune slacks, behind the foredunes, from drifting sand. These measures were very expensive, but without any protection a unique nature reserve would have lost much of its value. In order to avoid both high expenses and damage to the inner dunes during large-scale reconstruction of the foredunes, an investigation on establishment, growth, and development of *A. arenaria* was undertaken. Results of the study are presented in the present thesis.

The ecology of *Ammophila arenaria*

Ammophila arenaria occurs in coastal foredunes in north-western Europe, the Mediterranean, Australia and along the west coast of North America (Knutson 1978, Huiskes 1979). The biology of *A. arenaria* has been described by Huiskes (1979), and taxonomic aspects concerning the genus *Ammophila* have been presented in a recent review on *Ammophila breviligulata* (American beachgrass; Maun and Baye 1989). In the present introduction relevant aspects for the establishment, growth and

degeneration of *A. arenaria* are reviewed.

A. arenaria has special properties which enable it to survive the severe conditions that occur in coastal foredunes, such as salt spray, drought, sand erosion, and burial by windblown sand (Huiskes 1979). This species tolerates burial by windblown sand of 0.8 to 1 m (Ranwell 1958, Huiskes 1979), which also holds for the North American *A. breviligulata.* It was supposed that rhizomes of *A. arenaria* change geotropy when they are buried (Gemmell et al. 1953). Experimental burying of *A. breviligulata* resulted in elongation of its internodes and at more than 60 cm sand accretion, to the production of new nodes with dormant buds (Maun and Lapierre 1984). At sites where for several years considerable amounts of sand have been deposited, underground stems can become 4 to 6 m (Adriani and Terwindt 1974). However, since several fluxes of tillers are produced yearly (Huiskes and Harper 1979) these long stems apparently consist of a number of succeeding tillers. However, the demography of these underground stems of *A. arenaria* has not yet been studied in detail.

A. arenaria mainly forms vertical underground stems (Fig. 1), but it produces few rhizomes. According to some papers rhizomes can be numerous (Greig-Smith et al. 1947, Gemmell et al. 1953) which may, however, be dependent on local environmental conditions (A.H.L. Huiskes, personal communication). In contrast, *A. breviligulata* produces long rhizomes which are able to rapidly colonize the beach, whereas in areas with moderate sand accretion 'diving rhizomes' are formed (Maun 1985). This formation of rhizomes results in a rather open stand and is more similar to *Calammophila baltica* (Fluegge ex Schrader) Brand (purple, or Baltic marram grass; Rihan and Gray 1985) than to *A. arenaria.*

In the present thesis, vertical underground stems of *A. arenaria* will be called rhizomes, as 'real' rhizomes are virtually absent in this species and the vertical stems fit the definition of rhizomes as suggested by Wareing (1964) and Berg (1972).

Ammophila usually reproduces vegetatively by pieces of rhizomes, which are deposited on the beach after water action has eroded the foredune (Gemmell et al. 1953, Maun 1984). This species can also establish from seeds (Huiskes 1977), which only occurs occasionally (Gemmell et al. 1953).

The colonization of bare dune sand by *Ammophila* leaves questions unanswered concerning its nutrition. In this context, the importance of micro-organisms beneficial to colonizing plants has been suggested (Webley et al. 1952, Nicolson 1960, Harley 1970, Ernst et al. 1984). The occurrence of the free-living N_2 fixing bacteria *Azotobacter* sp. and *Bacillus* sp. along the roots was supposed to be related to the nitrogen nutrition of *A. arenaria* (Hassouna and Wareing 1964, Abdel Wahab 1975, Ahmad and Neckelman 1975, Abdel Wahab and Wareing 1980). However, it has also

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spike

soilsurface

culms

$\dfrac{\triangle}{\triangledown}$

rhizomes

5cm
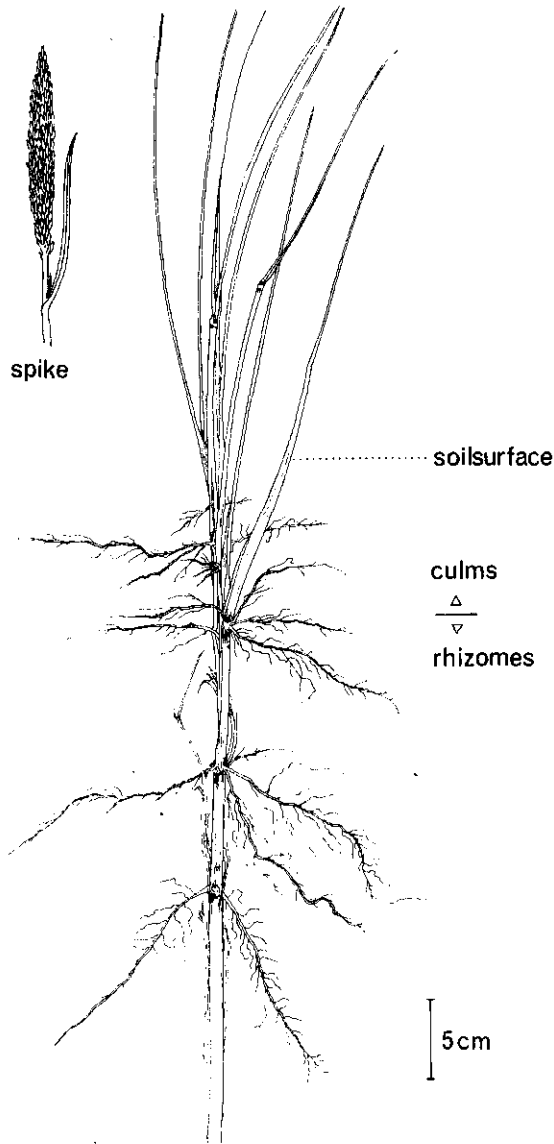
Fig. 1. *Ammophila arenaria* with underground vertical stems that are formed by upward growth of the plant through windblown sand.

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been demonstrated that the supply of carbon to these organisms was limiting the fixation of atmospheric nitrogen (Abdel Wahab and Wareing 1980). Field measurements showed that normally 0.1 kg N/ha.year and maximally 10 kg N/ha.year was fixed asymbiotically in the rhizosphere of *A. arenaria* (Akkermans 1971). Hence, the contribution of free-living N_2 fixing micro-organisms to the nitrogen nutrition of *A. arenaria* will be insignificant. Also, the role of vesicular-arbuscular mycorrhizae (VAM) has been examined, and it was suggested that VAM contributes to the supply of water and phosphorus to the plants (Nicolson 1960, Forster and Nicolson 1981a, b, Ernst et al. 1984).

Many studies on *A. arenaria* have been into aspects of its response to sand burial (Van Dieren 1934, Greig-Smith et al. 1947, Greig-Smith 1961, Lux 1964, Marshall 1965, Hope-Simpson and Jefferies 1966, Huiskes 1979, Huiskes and Harper 1979). Plant growth is vigorous when it is buried regularly by windblown sand from the beach, whereas it degenerates when the sand deposition ceases (Hope-Simpson and Jefferies 1966). A similar ecology is known of *A. breviligulata* (Laing 1967, Eldred and Maun 1982, Disraeli 1984, Maun and Lapierre 1984, Maun and Baye 1989). Several hypotheses have been formulated in order to clarify the relationship between sand burial and vigour of *Ammophila* (Table 1). However, these hypotheses cannot explain replant failures of culms which are planted on reconstructed foredunes in sand originating from the rhizosphere of *A. arenaria*. The plants showed poor growth and a high percentage mortality, even when fertilizers were applied and competing plant species were absent. Similar observations have been made in natural foredunes (Willis 1963, 1965, Hope-Simpson and Jefferies 1966). Hence, some other unknown factor should be involved, which may also be involved in natural degeneration of *A. arenaria* at stable sites.

Many plant species occurring naturally in coastal sand dunes have establishing, optimal, and declining phases, e.g. *Carex arenaria* (sand sedge; Tietema 1981), *Calammophila baltica* (Olsson 1974, Wallén 1980) and *Hippophaë rhamnoides* (sea buckthorn; Oremus 1982). In the latter, degeneration has been related to occurrence of the nematodes *Tylenchorhynchus microphasmis* and *Longidorus dunensis* (Oremus and Otten 1981, Maas, Oremus and Otten 1983). However, other soil organisms such as soil fungi were also supposed to be involved in the degeneration of *H. rhamnoides* (Oremus and Otten 1981, Zoon 1986). Possibly, harmful soil organisms are a more important factor in regulation of vegetation succession of coastal sand dunes than has thus far been assumed.

In the present investigation some attention has been given to *Calammophila baltica*, as this coastal foredune species is also (both planned and unplanned) used for erosion control. Much less information on *C. baltica*, a sterile hybrid of *A. arenaria* x *Calamagrostis epigejos*

Table 1. Review of theories for explaining the relationship between burial by windblown sand and vigour of _Ammophila arenaria_ and _A. breviligulata_ (an extension of Table 1 in Marshall 1965 and Table 1 in Eldred and Maun 1982).

Explanation	Authority
Causes of decline in absence of sand accretion	
(a) Dead and decaying organic matter accumulates	Buchenau 1889; Waterman 1919; Farrow 1919[+]; Wallen 1980
(b) Interspecific competition increases	Benecke 1930; Tansley 1949[+]; Salisbury 1952; Willis et al. 1959a, 1959b; Marshall 1965; Watkinson et al. 1979; Huiskes and Harper 1979
(c) Ageing of plants	Westgate 1904[+]; Wallén 1980
(d) Soil acidity increases by leaching of calcium carbonate	Van Dieren 1934; Salisbury 1952
(e) Toxic substances accumulate	Carey and Olivier 1918[*]; Tansley 1949[+]
(f) Aeration of roots and rhizomes decreases	Carey and Olivier 1918[*]; Tansley 1949[+]; Salisbury 1952; Szafler 1966[+]
(g) Roots lose cortex	Halwagy 1953[*]; Marshall 1965
(h) No new roots are formed	Marhall 1965
Causes of vigour due to sand accretion	
(a) Promotion of adventitious root growth	Willis 1965; Marshall 1965
(b) Increase in moisture and lowering of soil temperature around the nodes	Olson 1958
(c) Supply of nutrients from deposited sand	Van Dieren 1934; Westhoff 1947; Willis 1965; Lux 1969
(d) Association of N$_2$-fixing bacteria and mycorrhizal fungi with roots	Webley et al. 1952; Nicolson 1960; Hassouna and Wareing 1964; Abdel Wahab 1975; Ralph 1978[+]; Pugh 1979[+]; Abdel Wahab and Wareing 1980; Ernst et al. 1984
(e) Genotypes in mobile dunes differ from stable dunes	Laing 1967; Gray 1985

[*] cited in Marshall 1965

[+] cited in Eldred and Maun 1982

(Westergaard 1943) is available than on *Ammophila*. In the Baltic area three subspecies occur (ssp. *intermedia*, ssp. *epigejoida*, and ssp. *subarenaria*; Westergaard 1943). According to the description by Westergaard (1943) ssp. *intermedia* dominates in Dutch foredunes.

A. arenaria is one of the most important species being established artificially in order to control sand erosion in foredunes, as its natural occurrence and behaviour results in the formation and the fixation of sand dunes (Brown and Hafenrichter 1948a,b,c, Augustine et al. 1964, Adriani and Terwindt 1974, Hobbs et al. 1983). A very old method of stabilizing sand by *A. arenaria* is to plant bundles of culms. *A. arenaria* was planted according to this method as early as 1423 on Voorne (Pilon 1988). Records in the United Kingdom indicate that the planting of dune grasses has been carried out since the fourteenth or fifteenth century (Hobbs et al. 1983).

In the Netherlands, local differences exist in the method of collecting and planting of *A. arenaria*. Sometimes, culms are pulled, but usually a hand shovel is used to cut them before collecting. Culms, however, are always obtained from natural stands in opposite to growing them in nurseries, as is the case in the USA (e.g. Brown and Hafenrichter 1948a,b,c, Augustine et al. 1964, Knutson 1978). On Voorne planting holes are made using a small spade, whereas on the northern islands a hand shovel ('graaf') is used. The numbers of culms that are planted per hole is variable (6-15) and at some locations dry reed (*Phragmites australis*) is planted for temporary sand stabilization. Nevertheless, the planting of *Ammophila* is well established and the general principle is the same everywhere. Only very few studies have been undertaken on the development of new planting methods (Hobbs et al. 1983). However, the artificial establishment of *A. arenaria* from seed has been examined to some extent (Adriani and Terwindt 1974, Tsuriell 1974, Mitchell 1974, Barr and McKenzie 1976).

A number of studies deal with fertilizing stands of *Ammophila* (Brown and Hafenrichter 1948c, Augustine et al. 1964, Adriani and Terwindt 1974, Schwendimann 1977). Plant growth is greatly stimulated by fertilizing, as dune sand is poor in nutrients (Willis and Yemm 1961, Lux 1964, 1965). Conventional fertilizers are subject to leaching due to the low absorption capacity of dune sand (Willis and Yemm 1961), but little attention has been paid to the application of slow-release fertilizers (Brown and Hafenrichter 1948c).

The planting of *Ammophila* is expensive. In the Netherlands, 5 million Dutch guilders are spend anually on regular sand stabilization by *A. arenaria* (WLD 1987), on about 200 ha of foredune. Irregularly, *A. arenaria* has to be established on a large scale on new or reconstructed dunes. Between 1985 and 1989, in the province of Zuid-Holland 270 ha of raised and new dunes were planted with *A. arenaria*. The improvement of

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establishment, growth, and degeneration of *A. arenaria* is therefore of economic importance.

Purpose of the present study

The study reported in this thesis was carried out to develop methods for the establishment of *A. arenaria* on reconstructed coastal foredunes, to avoid shortage of plant materials, and to examine the origin of replant failures. Therefore, the traditional method of planting was improved and new planting methods were developed in order to use the available amount of plant materials efficiently. Additionally, application of slow-release fertilizer was studied to stimulate growth of new plantings. In order to clarify causes of replant failures the relation between vigorous growth of *A. arenaria* and burial by windblown sand was studied, as well as the degeneration of the plants in stable dunes.

Outline of the thesis

In chapter 2 experiments on germination of seeds and on temporary sand stabilizers are discussed. A description is given of establishment of *A. arenaria* from culms (traditional method), seeds and rhizomes in small-scale field trials. The effects of the sowing rate of seeds, planting density of rhizomes, and of slow-release fertilizer on shoot production are discussed.
In chapter 3 the traditional, as well as new planting methods have been compared in large-scale field trials in order to examine their applicability in practice. Biomass production and percentage cover of the experimental plantings was evaluated by airborne remote sensing (false colour photography).
In chapter 4 growth of seedlings of *A. arenaria* in sand from the root zone of a natural stand of *A. arenaria* is compared with growth in fresh (sea) sand. The origin of growth reduction in the dune sand is examined by sterilizing both sand types by gamma irradiation. An outdoor experiment is carried out in order to verify the results of the greenhouse experiments under semi-natural conditions. A hypothesis is formulated in order to relate the degeneration of *A. arenaria* to the occurrence of harmful soil organisms in its rhizosphere and to relate the vigour of *A. arenaria* to the deposition of windblown sand.
In chapter 5 the effect of application of biocides (bactericides, fungicides and nematicides) on the growth of seedlings of *A. arenaria* in dune sand is described. These biocides have been applied in order to characterize the nature of the soil organisms, which are harmful to *A. arenaria*.
In chapter 6 three extreme stages in foredune vegetation succession (beach,

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the mobile, and the stable foredunes) have been examined for the presence of harmful soil organisms. The susceptibility of *C. baltica* to the harmful organisms is compared outdoors with that of *A. arenaria*.

In chapter 7 the distribution of harmful organisms in soil profiles of foredunes is described. The impact of the soil organisms on the morphology of the root system is quantified.

In chapter 8, the results of this study are discussed with respect to the ecology of *A. arenaria*, especially the relationship between vigour and burial by windblown sand.

ESTABLISHMENT OF *AMMOPHILA ARENARIA* (MARRAM GRASS) FROM CULMS, SEEDS, AND RHIZOMES

W.H. van der Putten

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CHAPTER 2

ESTABLISHMENT OF AMMOPHILA ARENARIA (MARRAM GRASS) FROM CULMS, SEEDS, AND RHIZOMES

SUMMARY

(1) A study was made of three different methods of establishing *Ammophila arenaria* (Marram grass): planting bundles of culms (the traditional method), sowing of seeds, and disk-harrowing of rhizomes. In addition, the effects of applying slow-release fertilizer were examined.

(2) Before the methods of establishment were compared, a preliminary field experiment was carried out to examine the relative merits of different methods of stabilizing the sand, which is required when sowing seeds or disk-harrowing rhizomes. Comparisons were made of the use of straw, Carboxy Methyl Cellulose, compost, reed, and a protective cover comprising a mixture of crop species. Of all methods only straw was effective.

(3) A higher sowing rate (rates applied were: 200, 400 and 600 seeds m^{-2}) and a higher density of rhizomes (densities applied were: 20, 40, and 60 pieces m^{-2} of 15 cm length, each with at least 2 buds) resulted in a higher number of seedlings and primary shoots. However, after one growing season production of biomass and tillers was only increased by fertilization.

(4) Although the recovery in above-ground plant parts of N and P was fairly low, slow-release NPK fertilizer (80-20-20 kg ha^{-1}) increased biomass significantly, when compared with unfertilized plants. Rhizomes fertilized with 160-40-40 kg NPK ha^{-1} produced significantly more dry matter than with 80-20-20 kg NPK ha^{-1}. This was not the case, however, for sown or planted *A. arenaria*.

(5) Rhizomes produced more tillers and biomass than bundles of culms and seedlings when fertilizer was applied. Without application of fertilizer culms produced highest biomass, whereas seedling growth was very poor.

(6) To clarify germination under field conditions, experiments were carried out in the laboratory. A high rate and percentage of germination was obtained at fluctuating temperatures and by supplying light. At fluctuating low temperatures stratification increased germination of seeds. At fluctuating high temperatures seeds germinated well and stratification gave no improvement.

INTRODUCTION

Ammophila arenaria (L.) Link (Marram grass) is a species that naturally dominates the foredunes of north-western Europe, the Mediterranean, Australia, and the west coast of the USA (Knutson 1978, Huiskes 1979). Because of its natural ability to colonize and stabilize sand dunes, *A. arenaria* is often used for erosion control. Traditionally, bundles of *A. arenaria* with 3-6 culms (usually collected from vigorous stands in foredunes) are planted in 15-20 cm deep holes in a grid, 30-60 cm apart. New plants then emerge from basal stem buds. The tradition of planting *Ammophila* is very old, and is applied in many parts of the world (Brown & Hafenrichter 1948a, b, c, Augustine et al. 1964, Lux 1969, Atkinson 1971, Adriani & Terwindt 1974, Knutson 1978, Hobbs et al. 1983). As early as 1423, *A. arenaria* was planted on the island of Voorne (the Netherlands) to protect the dunes from wind erosion (Pilon 1988).

At present, in the Netherlands large parts of the foredunes have to be raised and, subsequently, planted with *A. arenaria* to stablize the sand-surface. These works are being carried out under the so-called Delta Act, which was enacted after the flooding of the south-western part of the Netherlands in 1953. The traditional planting method is very expensive, as it is carried out manually. The method could be improved by harvesting and planting mechanically (Atkinson 1971, Knutson 1978) and by planting the stems horizontally (Hobbs et al. 1983). In large-scale dune reconstructions, however, shortage of plant material often occurs.

Since seeds and rhizomes can also be used for plant propagation, more planting material could be collected from natural stands of *Ammophila* than is obtained when only the traditional planting method is applied. Sowing seeds of *A. arenaria* has been applied with reasonable success (Adriani & Terwindt 1974, Tsuriell 1974, Mitchell 1974, Barr & McKenzie 1976). On very exposed sites, however, failures often occur owing to wind erosion (Mitchell 1974). Temporary sand stabilization after sowing should be carried out, e.g. by spraying emulsions of bitumen (Tsuriell 1974) or disk-harrowing of straw (Mitchell 1974, Adriani & Terwindt 1974).

Underground vertical stems (that can be called rhizomes according to Wareing (1964) and Berg (1972)) have never been used for artificial establishment of *A. arenaria*. A recently developed method concerns the disk-harrowing of rhizomes of *A. arenaria* (Van der Putten & Van Gulik 1987). It simulates the natural colonization of the beach by rhizome pieces with axillary buds, which can regenerate by forming primary shoots after fragmentation by waves, dispersal, and sand burial (Gemmell et al. 1953, Maun 1984). The natural regeneration from rhizomes of *A. arenaria* has not been studied, but similar studies have been undertaken on *Ammophila*

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breviligulata (Maun 1984) and *Elymus farctus* (Harris & Davy 1986). Although the regeneration percentage of buds is highest on single-bud fragments most primary shoots that actually emerge originate from fragments with 2-5 buds (Maun 1984, Harris & Davy 1986).

In the present paper, the traditional planting method (1) is compared with the application of seeds (2) and rhizomes (3) of *A. arenaria*. Because the latter two methods (2 and 3) require an effective stabilization of the sand surface (Van der Putten & Van Gulik 1987), a preliminary field trial (experiment 1) has been carried out first, in which several ways of temporary sand stabilization were compared. The method for temporary sand stabilization that turned out to be best has been applied in a field trial in order to compare the traditional planting method with sowing of seeds and disk-harrowing of rhizomes at different rates of plant material and slow-release fertilizer (experiment 2). During the course of experiment 1, dormancy of seeds caused a considerable delay of seedling emergence. In experiment 2, however, dormancy appeared to be much less severe than in experiment 1. In order to clarify these differences in seed behaviour, a germination experiment has been carried out in the laboratory (experiment 3).

MATERIALS AND METHODS

Experimental area

The field experiments were carried out on the coastal foredune ridge of the island of Voorne, the Netherlands (51.5 N, 4.05 E). Because of the Delta-works this foredune was raised with dredged sea sand. Plant material and seeds were collected from the foredune before reconstruction with sea sand was finished.

Field experiment 1. *Effect of short-term sand fixation and protective cover by nurse crops on establishment of A. arenaria seedlings.*

In May 1984, protective cover by nurse crops and short-term sand fixation were studied in a preliminary field experiment by measuring emergence and establishment of seedlings of *A. arenaria*. Plots of 3 x 6 m^2 were arranged in a Latin square design with three replicates. Seeds of *A. arenaria*, collected the previous year and dry stored at 12 °C, were sown at a rate of 580 seeds m^{-2} (20 kg ha^{-1}; maximum germination percentage 95%). One half of each of the plots was sown with a mixture of five nurse crops, that were supposed to emerge quickly and protect the seedlings of

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A. arenaria. The mixture (241 kg/ha) contained *Secale cereale* (70 kg/ha), *Hordeum vulgare* (70 kg/ha), *Triticum aestivum* (70 kg/ka), *Lupinus perennis* (30 kg/ha), and *Trifolium repens* (1 kg/ha). The seeds were buried by harrowing the sand.

Five methods for short-term sand fixation were examined: (1) disk-harrowed straw (0.5 kg m^{-2}), (2) bundles of dry reed (*Phragmitis australis*; planted in a grid at 50 x 75 cm^2 apart), (3) compost ('edelcompost' type, 4 kg m^{-2}), and (4) Carboxy Methyl Cellulose (CMC, 'average viscosity' type, 4 g m^{-2}), and (5) none. Both compost and CMC were suspended in water and sprayed on the sand surface.

Method of evaluation. In August and November 1984, and in August and September 1985, the numbers of emerged seedlings were counted in all plots (per plot a random 0.5 x 1.0 m^2 area was examined).

Field experiment 2. *Comparison of three methods of establishing A. arenaria and effects of slow-release NPK fertilizer.*

Experimental design.
In March 1987, *A. arenaria* was established at experimental field plots of 5x5 m^2 (excluding a 1-m margin all around) from: (1) culms (traditional method), (2) seeds, and (3) rhizomes. The traditional method was carried out at one density; seeds were applied at 3 rates: 200 (low), 400 (medium), and 600 (high) seeds m^{-2} (7.5, 15, and 22.5 kg ha^{-1}, respectively); and rhizomes at 3 densities: 20 (low), 40 (medium), and 60 (high) 15-cm pieces m^{-2}. Before planting of culms, sowing of seeds, or disk-harrowing of rhizomes, a broadcast placement of slow-release fertilizer was applied at three levels: 0, 80-20-20, and 160-40-40 kg NPK ha^{-1}.

All treatments (combinations of planting method, density, and fertilizer level) were carried out in four replicates.

Methods of establishing A. arenaria.
(1) *Planting of culms* (the traditional method). Culms of *A. arenaria* were cut 10 cm below soil surface from a vigorous stand. Bundles of 13 culms each were planted in 15-cm deep holes in a grid 50 x 75 cm^2 apart. 95 Per cent of the tillers had at least 2 apparently viable buds.

(2) *Sowing of seeds.* Seeds of *A. arenaria* (from the same batch as used later on in the germination experiment) were mixed with sand to improve their distribution, and sown. The sand surface was disk-harrowed superficially to bury the seeds at a depth of 3-5 cm.

(3) *Disk-harrowing of rhizomes.* Rhizomes were collected from the top 2-m soil layer in a vigorous stand of *A. arenaria*. In order to obtain pieces with 2-5 viable buds (Maun 1984), the rhizomes generally had to be cut into pieces with a length of approximately 15 cm. This was accomplished by disk-harrowing the sand surface after the rhizomes had been spread,

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which resulted in a chopping of the rhizomes and burying them at a depth of 10-15 cm.

Fertilization and sand fixation.

The applied 24-6-6 NPK fertilizer ('Osmocote', active for 12-14 months at a soil temperature of 21°C) consisted of 14% N as urea, 10% N as ammonium nitrate and ammonium phosphate, 6% P_2O_5 as ammonium phosphate and calcium phosphate, and 6% K_2O as potassium sulphate. Hereafter, P and K will be used to indicate P_2O_5 and K_2O.

The fertilizer was broadcast before establishing *A. arenaria* and disk-harrowed to a depth of 10-15 cm. After disk-harrowing the fertilizer and the sowing and disk-harrowing of seeds and rhizomes, the sand surface of all plots was fixed by means of superficially (5 cm) disk-harrowed straw (0.5 kg m^{-2}). Finally, the culms were planted. In some additional plots, fertilizer was applied directly in the planting holes instead of by broadcasting; in this case two levels of NPK were supplied: 80-20-20 and 120-30-30 kg NPK/ha.

Method of evaluation.

The field experiment was evaluated twice. In July 1987, numbers of emerged seedlings, primary shoots (i.e. emerged buds of the rhizomes), and tillers (from culms and rhizomes) were counted. Plant parameters were determined according to a stratified random sampling method. The sampled area, or numbers of plants counted, depended on the variability within the treatment. In every plot with seedlings 5x1 m^2 has been examined; in every plot with rhizomes 8x0.18 m^2; and 25 bundles were examined in every plot with planted culms. Numbers of tillers per bundle were estimated for each level of NPK by means of linear regression between the circumference and the numbers of tillers per bundle.

In October 1987, a strip of 3x1 m^2 was cut at ground level in each plot with seedlings or rhizomes and the total amount of fresh material was weighed. A subsample was weighed fresh and the numbers of tillers were counted before drying for 48 hrs at 70 °C in order to determine the percentage dry matter. Numbers of tillers of the bundles of culms were assessed as indicated above and dry weight was obtained by linear regression.

Analysis of plant material.

Plant samples were dried at 70 °C and ground finely (1-mm mesh). After digestion of a subsample in a concentrated sulphuric acid/salicylic acid mixture with hydrogen peroxide, N and P were determined colorimetrically (indophenol-blue method and molybdenum-blue method, respectively), and K by atomic absorption.

Recovery of N, P, and K

Recovery of N, P, and K via above-ground plant parts was determined in order to assess effectiveness of the applied slow-release fertilizer.

Recovery of N, P, and K was calculated according to:
$$R = 100\% \times (U-Z) \times (Y-X)^{-1}$$
R = recovery (%); U = uptake of N, P, or K $(g.m^{-2})$ in a fertilized plot;
Z = uptake of N, P, or K $(g.m^{-2})$ in an unfertilized plot;
Y = the amount of N, P, or K in the applied slow-release fertilizer $(g.m^{-2})$;
X = the amount of N, P, or K in the slow-release fertilizer $(g.m^{-2})$, still present after one growing season. Fertilizer granules were collected in April 1988 and digested and analysed similarly to the plant material.

Data analysis. The results were statistically analysed by analysis of variance (ANOVA) after Cochran's test for homogeneity of variances (Sokal & Rohlf 1981). If necessary, data were logarithmic (ln) or square root (sqrt) transformed to obtain homogeneity of variances.

Laboratory experiment. *(experiment 3) Seed germination*

Collecting of seeds. In July 1986, spikes of *A. arenaria* were collected, air-dried and threshed. After dry storage for 5 months at 12 °C, the seeds (1000-kernel weight of 3.56 g) were placed in Petri-dishes on filter paper (40 seeds per dish).

Experimental design
A 10x3x2 factorial experiment was carried out with three replicates per treatment. Factors were: (1) *Seed pre-treatment.* The Petri-dishes with seeds were stored at 4 °C, either (i) moistened with demineralized water ('stratification') or (ii) kept dry ('dry cold'), for 0, 2, 3, 5, or 7 weeks. (2) *Germination temperature.* Following the period of storage, the pre-treated seeds were exposed to three night/day (16/8 hours) germination regimes: (i) 20/20 °C, (ii) 10/20 °C, and (iii) 20/30 °C. (3) *Illumination.* Half the Petri-dishes were wrapped in light-proof bags. The remaining Petri-dishes were illuminated during the 8-hour period of high temperatures.
Apart from the germination experiment, six extra Petri-dishes were each filled with 40 seeds, from which palea and lemna had been removed. In three dishes (replicates) an incision was made in the seed coat without damaging the embryo. Seeds were germinated in light/dark at 8/16 hours 20/10 °C.

Watering and counting
The dry seeds were moistened immediately before the germination experiment started and, if necessary, demineralized water was supplied every other day. To determine the percentage of germination, seeds were counted and removed when the plumula became visible. Moistening and counting of all seeds was carried out in green light (see Blom 1978), to protect the seeds of the 'dark' treatment from illumination (Wesson & Wareing 1969).

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Table 1. Numbers of seedlings of A. arenaria (No.m^{-2}) in plots with and without short-term sand fixation and protective crops. Because the numbers of seedlings in the treatments reed, CMC, and compost were all low, these were averaged. Data presented are the means of three countings; Ranges are indicated in parentheses.

Sand fixation	Nurse crops[a]	1-August-1984	14-November-1984	1-August-1985	24-September-1985
None	+	14 (4-32)	6 (0-26)	0	0
Reed/CMC/Compost	-	24 (8-64)	6 (0-28)	0	0
Reed/CMC/Compost	+	34 (6-78)	16 (0-36)	0	0
Straw	-	58 (50-66)	16 (12-22)	114 (86-142)[b]	112 (78-148)[b]
Straw	+	32 (18-62)	8 (0-14)	158 (66-290)[c]	142 (34-326)[c]

a A mixture of cereals (Secale cereale, Hordeum vulgare, Triticum aestivum), Lupinus perennis, and Trifolium repens. Application of nurse crops is indicated with +.

b Slow-release NPK fertilizer (Osmocote, 40-10-10 kg NPK/ha) applied in May 1985.

c Slow-release NPK fertilizer (Osmocote, 120-30-30 kg NPK/ha) applied in May 1985.

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RESULTS

Field experiment 1. *Effect of short-term sand fixation and protective cover by nurse crops on establishment of A. arenaria seedlings.*

The numbers of seedlings (means, minima and maxima) in Table 1, in which the results with reed, CMC and compost are combined, show that only very few seedlings had established in 1984. A new germination flush occurred in 1985, which was, however, restricted to the plots stabilized by straw. In the plots with reed, CMC, and compost, the seedlings had disappeared completely by 1985. The highest emergence of nurse crops occurred in plots with sand fixation and 15-cm stubbles remained until the end of 1985. Their presence, however, did not affect the number of *Ammophila* seedlings (Table 1).

Table 2. Effect of fertilization (and sowing rate/rhizome density) on three methods of artificially establishing A. arenaria. In the table, average numbers of emerged seedlings, primary shoots, and tillers (No.m^{-2}) in July 1987 are presented as calculated over the main factors NPK and density. Per column, data followed by the same letter are not significantly different (P > 0.05). F-values and degrees of freedom (df) of emerged seedlings, and primary shoots and tillers from rhizomes were calculated by two-way ANOVA with the factors NPK (fertilizer) and sowing rate (or rhizome density). F-values and degrees of freedom of tillers from bundles of culms were calculated by one-way ANOVA with the factor NPK.

NPK(kg.ha^{-1})		seeds — emerged seedlings (No.m^{-2})		rhizomes — primary shoots (No.m^{-2})		tillers (No.m^{-2})		bundles of culms[2] — tillers (No.m^{-2}) B	P
0-0-0		50	a	21	a	31	a	14 a	
80-20-20		57	ab	21	a	70	b	38 ab	27 ab
120-30-30									45 c
160-40-40		67	b	25	a	77	b	49 c	
Rate or Density[1]									
low		36	a	12	a	33	a		
medium		62	b	22	b	57	b		
high		69	b	33	c	87	c		
Factor	df	F		F		F		df	
NPK	2	5.14 *		1.20 NS		38.9 ***		4	5.89 **
Density	2	23.9 ***		31.4 ***		41.4 ***			
NPKxDensity	4	2.63 NS		0.80 NS		1.01 NS			
MS (Error)		183		42.8		0.81		138	

1 sowing rate of seeds: low = 200 seeds m^{-2}; medium = 400 seeds m^{-2}; high = 600 seeds m^{-2}
   density of rhizomes: low = 20 pieces m^{-2}; medium = 40 m^{-2}; high = 60 m^{-2}
2 only one density (50 x 75 cm); B = broadcast and P = plant-hole placement of fertilizer
NS not significant; * P < 0.05; ** P < 0.01; *** P < 0.001

Field experiment 2. *Comparison of three methods of establishing A. arenaria and effects of slow-release NPK fertilizer.*

Emergence of seedlings and primary shoots and production of tillers in July 1987 were analysed statistically for the effects of sowing rate of seeds (or density of rhizomes) and NPK level. Sowing rate of seeds and planting density of rhizomes had a significantly positive effect on the emergence of seedlings and primary shoots (P<0.001; Table 2).

The intermediate sowing rate resulted in significantly more seedlings than the low rate, but there was no further increase from the high rate (Table 2). The numbers of seedlings increased by fertilization (P<0.05). In July 1987, the rhizomes produced significantly more tillers when rhizome density and NPK-level were increased (P<0.001; Table 2). Fertilization also increased tiller production of the bundles. There were no significant differences between broadcast and plant-hole application of NPK.

In October 1987, after one growing season, the above-ground dry matter production, the numbers of tillers, and the weight per tiller were no longer affected by sowing rate or density of rhizomes (Table 3). Only the application of NPK led to significant increases in these plant parameters (P<0.001).

Table 3.   Effect of fertilization and density on two methods of artificially establishing A. arenaria. In the table, F-values of dry matter production, numbers of tillers, and weight per tiller of seedlings and rhizomes in October 1987 were calculated by two-way ANOVA with the factors NPK (fertilizer) and sowing rate (or rhizome density). Numbers of tillers of seedlings were sqrt transformed and tiller weight was ln transformed.

		seedlings			rhizomes		
		dry matter production	tillers (No)	tiller weight	dry matter production	tillers (No)	tiller weight
Factor	df	F	F	F	F	F	F
NPK	2	21.0 ***	20.1 ***	27.8 ***	35.1 ***	17.1 ***	9.25 ***
Density	2	3.16 NS	1.94 NS	2.87 NS	1.88 NS	0.55 NS	2.65 NS
NPKxDensity	4	2.73 NS	0.80 NS	2.55 NS	0.31 NS	0.57 NS	2.92 NS
Mean square of error		750	8.62	0.078	2587	14.3	0.021

NS not significant; *** P < 0.001

Without application of NPK, bundles of culms produced the most dry matter (Fig.1A) owing to the relatively high dry weight per tiller (Fig.1C). Mean tiller weight, however, may be over-estimated because of remnants of the originally planted bundles. Since no significant differences were observed between plant-hole application and broadcast placement of NPK, data of the former are not shown.

The application of 80-20-20 kg NPK ha^{-1} increased the dry matter production and numbers of tillers in all cases (Fig. 1A, B), whereas weight
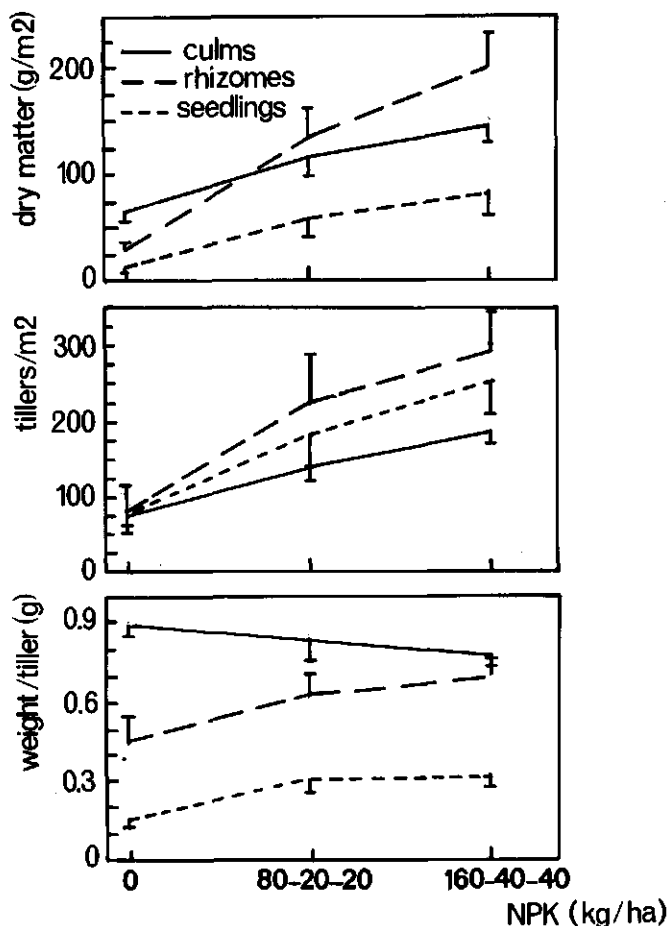
Fig. 1. Plant parameters after one growing season (October 1987) of bundles of culms, seedlings, and rhizomes at three levels of NPK: 0, 80-20-20, and 160-40-40 kg NPK/ha. $\perp$ = Standard Error.
A: Above-ground dry matter production (g.m^{-2}).
B: Number of tillers (No.m^{-2}).
C: Weight per tiller (g).

per tiller only increased for seedlings and rhizomes (Fig. 1C). Doubling of the amount of fertilizer from 80-20-20 to 160-40-40 kg NPK ha^{-1} caused a significant increase of dry matter production in the case of rhizomes ($P<0.05$), but did not significantly affect the numbers of tillers and weight per tiller. Responses of numbers of tillers and tiller weight upon NPK fertilization were similar for seedlings and rhizomes. In the planted

Table 4. Recovery (%) of nitrogen, phosphorus, and potassium in above-ground plant parts of _A. arenaria_ from seedlings, rhizomes, and bundles of culms (October 1987) after fertilizing with NPK (Osmocote, slow-release fertilizer).

Fertilization (kg NPK/ha)	Recovery of nitrogen				Recovery of phosphorus				Recovery of potassium			
	seed-lings	rhizomes	bundles[a] B	P	seed-lings	rhizomes	bundles B	P	seed-lings	rhizomes	bundles B	P
80-20-20	9	15	7	5	6	13	4	3	79	160	72	46
120-30-30				8				3				57
160-40-40	7	11	10		4	9	5		59	122	79	

a B = broadcast placement of fertilizer
   P = plant-hole placement of fertilizer

bundles, significant differences in dry matter production and numbers of tillers occurred only between the unfertilized stands and those supplied with 160-40-40 kg NPK ha^{-1}. Weight per tiller was not affected significantly by fertilizing. The rhizomes tended to produce the greatest number of tillers, whereas tiller production of the bundles of culms was lowest (Fig.1B). Seedlings produced the smallest tillers (Fig.1C).

In the fertilizer residue no urea-N was left. Of the total amount of fertilizer applied, 9.4% of N, 40.5% of P and 52.4% of K was still present in the fertilizer granules after one growing season (data not listed). The maximum recovery of N and P in the plants did not exceed 15% (above-ground plant parts only; Table 4). Recovery of K, however, was very high and exceeded 100% in the case of rhizomes. Generally, the recovery of N, P, and K in above-ground plant parts of the rhizomes was 1.5 - 2 times higher than in the seedlings and in the bundles of culms (Table 4). Doubling the amount of fertilizer from 80-20-20 to 160-40-40 kg ha^{-1} reduced recovery of N and P in the seedlings and rhizomes. In culms, however, recovery of N and P increased with higher rate of fertilization.

Laboratory experiment 3. *Seed germination*

(1) *Effect of pre-treatment of seed.* Effect of pre-treatment depended on the germination temperature. A stratification period of 5 and 7 weeks increased the percentage of germination only when the germination temperature fluctuated daily between 10 and 20 °C (Fig. 2A, C). Dry cold storage had no effect on the germination (Fig. 2B, D). After incision of the seed coat and a germination period of 22 days at 10/20 °C there was a 94% germination as compared to 14% in untreated seeds (data not shown in Fig. 2).

(2) *Effect of temperature.* All pre-treatments resulted in very low germination percentages at a constant temperature of 20 °C (Fig. 2A$_1$, B$_1$, C$_1$, D$_1$). In light and at daily fluctuating germination temperatures of 20/30 °C there was an 80% germination in 10-15 days, irrespective of pre-treatment of the seed (Fig. 2A$_3$, B$_3$). At a low daily fluctuating temperature of 10/20 °C, percentage of germination depended on the effectiveness of pre-treatment of the seed (see under 1).

(3) *Effect of light.* Germination in darkness was generally considerably lower than in light (Fig. 2C, D). Only with seeds that were stratified for 5 and 7 weeks, and that were subsequently exposed to fluctuating temperatures of 10 and 20 °C, a relatively high germination percentage was obtained in darkness (Fig. 2C$_2$).
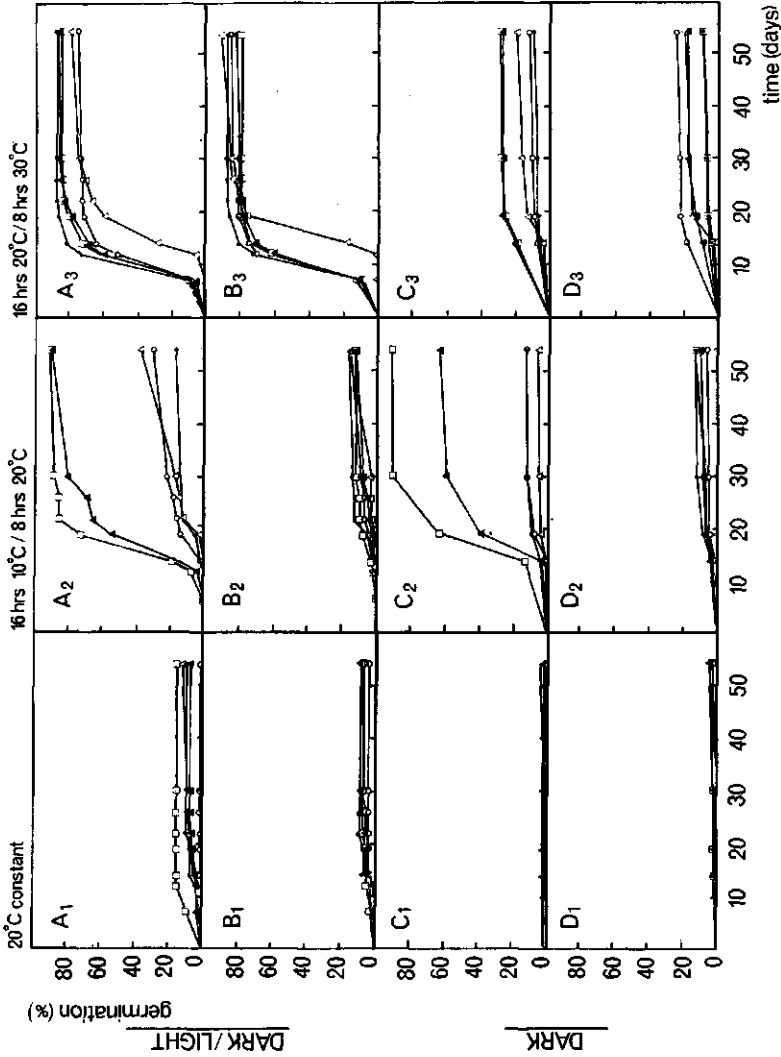
Fig. 2. Germination (%) of seeds of *A. arenaria* with time (days) at different temperature regimes, illumination, and pre-treatment • =0, Δ =2, o =3, ▲ =5 and □ =7 weeks of stratification (A and C) or dry cold (B and D).

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DISCUSSION

In this paper three methods of artificially establishing *A. arenaria* are compared i.e. planting of culms, sowing of seeds, and disk-harrowing of rhizomes, and the effect of temporary sand stabilizers was studied. The evaluation of temporary sand stabilization, however, interacted with a poor germination rate due to seed dormancy. Only straw remained long enough to enable the seeds to germinate. In plots stabilized with CMC, compost or reed, the sand surface had been eroded during winter. Protective cover by nurse crops had no effect on numbers of seedlings. In another, large-scale, experiment seed dormancy did not occur. In that experiment compost was found to work out more favourable (Van der Putten and Kloosterman Chapter 3).

As compared to the investigated methods of establishing *A. arenaria*, vegetatively propagated *A. arenaria* produced more robust tillers and more above-ground biomass than seedlings. Disk-harrowing of rhizomes, if supplied with fertilizer, resulted in the highest biomass production (Fig. 1A) and numbers of tillers (Fig. 1B). Sixty rhizome pieces m^{-2} tended to produce more biomass than 40 or 20 m^{-2}, but the effect of rhizome density had disappeared after one growing season (Table 3). A similar effect has been observed with different densities of planted culms (Brown & Hafenrichter 1948b,c, Augustine et al. 1964). As planting is usually carried out in early winter, a planting density of at least 40 rhizomes m^{-2} is to be preferred in order to compensate for e.g. frost damage.

As establishment of seedlings was significantly enhanced by fertilizing (Fig. 1A, Table 2), low seedling survival observed by other authors (Huiskes 1977, Maun & Baye 1989) may also have been due to low nutrient concentrations in the soil.

Plantings of *Ammophila* can benefit from conventional fertilizers if supplied during the growing season (Brown & Hafenrichter 1948c, Augustine et al. 1964, Lux 1965, Adriani & Terwindt 1974). However, the application of conventional fertilizer at planting has not been successful due to leaching before the plants even have formed roots (Van der Putten & Van Gulik 1987). Application of slow-release fertilizer resulted in a significantly increased biomass production, although recovery of N and P in above-ground biomass did not exceed 15%, and were usually considerably lower (Table 4). High percentages of recovery of K were found, exceeding 100% in the case of rhizomes. Apparently, absorbed K partly had been derived from the soil. As the sand contained about 15 g K m^{-2} in the upper 20 cm, there was probably no direct need for K fertilization. Immobilization of nutrients due to straw decomposition can be virtually excluded as the fertilizer was applied at greater depth.

Therefore, other factors such as leaching may be responsible for the low percentages of recovery, especially of N. Biomass production of bundles of culms with either broadcast or plant-hole dressed slow-release fertilizer did not differ significantly. Apparently, the root system exploits the total upper part of the soil volume rather efficiently. Therefore, in order to increase the efficiency of fertilization of dune grasses, the release of nutrients from slow-release fertilizer could still be improved.

It was supposed that the low germination of seeds during the first season of field experiment 1 could have been due to the late start of the experiment (May), which prevented effects of some natural pre-treatment. To test this hypothesis germination was studied in the laboratory. At low, fluctuating temperatures (10/20 °C) germination of seeds of *A. arenaria* was improved by 5-7 weeks of stratification (Fig. 2A_2 and C_2), which is about twice as long as was recorded for *Ammophila breviligulata* (Maun & Baye 1989). Incision of the seed coat also improved the rate and percentage of germination of *A. arenaria*, which is in accordance with observations for *A. breviligulata* (Laing 1958 and Seneca 1969). These results suggest that the seed-coat of *Ammophila* has to be pre-treated in order to enable imbibition (Spurny 1973). Germination in darkness of stratified seeds was found to be temperature dependent. However, this may have been an artefact caused by the use of green light during watering and counting (Baskin & Baskin 1979, W.H. van der Putten, unpublished results).

In practice, sowing of *A. arenaria* in winter or in early spring will result in a natural stratification which was apparently the reason for germination in field experiment 2. Although a lower light availability reduces germination of buried seeds (Huiskes 1979) this has to be accepted, because superficially sown seeds are subject to wind erosion or dessication (Huiskes 1977).

From the data presented in this paper it can be concluded that good results are obtained by the disk-harrowing of rhizomes in combination with sand fixation by straw and the application of slow-release fertilizer. However, other aspects are also involved, such as costs and long-term vegetation development in the artificial plantings. Therefore, the three methods of establishing *A. arenaria*, i.e. planting of culms, sowing of seeds and disk-harrowing of rhizomes, are being applied experimentally on a large scale in the Netherlands at present. The techniques and results will be reported in a subsequent paper.

LARGE-SCALE ESTABLISHMENT OF *AMMOPHILA ARENARIA* AND QUANTITATIVE ASSESSMENT BY REMOTE SENSING.

W.H. van der Putten and E.H. Kloosterman

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CHAPTER 3

LARGE-SCALE ESTABLISHMENT OF AMMOPHILA ARENARIA AND QUANTITATIVE ASSESSMENT BY REMOTE SENSING

SUMMARY

In order to examine the applicability of new methods for the establishment of *Ammophila arenaria*, the use of culms (traditional method) was compared with the use of seeds, rhizomes, and culms in combination with rhizomes. A large-scale (120 ha) experiment was undertaken and approximately 40 per cent of the total area (45 ha) was evaluated by airborne remote sensing (false colour photography). At the end of the first growing season, planted culms had produced lower amounts of biomass and percentage cover than seeds, rhizomes, and culms in combination with rhizomes. At the end of the second growing season, however, biomass production was highest in the planted culms. Nevertheless, all methods were successful for sand stabilization and less than 5 per cent of the total area had to be replanted. The need for replanting was highest in the fields that were sown with seeds. Biomass and soil cover were more affected by temporary stabilization of the sand surface, origin of the plant material and other factors, than by the method of planting. The botanical composition of the stands from seeds was less diverse than of those from culms and rhizomes.

INTRODUCTION

Ammophila arenaria (L.) Link (marram grass) occurs dominantly on foredunes in the Netherlands, as well as in other parts of north-western Europe, the Mediterranean, Australia and the west coast of the USA (Knutson 1978, Huiskes 1979). Because of the natural potentials of this grass to promote formation and stabilization of dunes it is widely used to control erosion of sand dunes.

In 1953, after large-scale flooding of the south-western part of the Netherlands, the Dutch Government enacted the so-called Delta Act which prescribed minimum heights and widths for barrier dunes and dykes. Initially, the weak parts of the foredunes were fortified. Only recently have large parts of the foredunes (120 ha on the island of Voorne) been raised and strengthened to meet the Delta-Act standards and the *A. arenaria* vegetation had to be re-established to stabilize the sand surface.

In planning these large-scale works it became apparent that there would be insufficient material to plant the whole newly raised foredune area with *A. arenaria* in the traditional way, i.e. by planting bundles of culms (Brown and Hafenrichter 1948a,b,c, Adriani and Terwindt 1974, Knutson 1978). Moreover, the traditional planting method is usually carried out manually and thus very expensive. Therefore, alternative planting methods were developed and slow-release fertilizers were applied to promote plant growth (Voogt and Van der Putten 1988). The investigation concentrated on the application of seeds and rhizomes (in this case: underground vertical stems) of *A. arenaria*. Seeds have only occasionally been used to stabilize sand dunes (Adriani and Terwindt 1974, Tsuriell 1974, Mitchell 1974, Barr and McKenzie 1976). However, as far as we know rhizomes have never previously been used for artificial establishment although these are very important in natural establishment of *Ammophila* (Webley et al. 1952, Maun 1984).

In the laboratory and in small-scale experimental fields, the use of seeds and rhizomes for the establishment of *A. arenaria* was found to be full of promise (Van der Putten chapter 2). However, as the value of a method also depends on its applicability and costs it was decided to apply the traditional as well as the newly developed methods in practice on a large scale and to evaluate the planting results after the first and second growing seasons.

For an adequate comparison of the planting methods, quantitative data were required on biomass production and percentage cover. Traditional field sampling on a large scale scale would have been very time-consuming and would have caused inadmissible damage to the young plantings. Therefore, a remote sensing technique (false color aerial photography) was

used for data assessment. The applied remote sensing technique is based on the relations between plant biomass and - to a certain extent - percentage cover and colour densities on a false colour diapositive (Meulstee et al. 1986, Kloosterman and Van Stokkom 1988).

This paper presents the evaluation of planting methods by remote sensing. Field data on biomass production and percentage cover were used to calibrate airborne false colour photographs. Therefore, a comparison could be made of large-scale plantings of *A. arenaria* from culms (traditional method), seeds, rhizomes, and a combination of culms with rhizomes.

MATERIALS AND METHODS

Description of the location.

The experimental planting methods were carried out at the coastal foredune ridge of the island of Voorne, the Netherlands (51.5 N 4.05 E). Between 1985 and 1988, 9.4 km of this foredune ridge had been raised and strengthened by sand of sea-floor origin. The raised foredune had a minimal height of 8.30 m above 'normal Amsterdam level' (Normaal Amsterdams Peil) and a minimal top width of 20 m. The total area to be planted was 120 ha. The large-scale plantings of *A. arenaria* reported on in this paper covered a total area of 45 ha: 32 ha in the north-west section, and 13 ha in the south-west section (Fig. 1). The remaining area, 70 ha with *A. arenaria* and 5 ha with *Hippophaë rhamnoides* (sea buckthorn), was not included in the present evaluation.

Experimental design.

Plantings were carried out in fields varying in size from 2 to 10 ha. Methods usually were not duplicated in the same year.

(1) *North-west section.* In the winter of 1984-1985, dredged sea sand was deposited on the north-west beach. In the following winter, the foredune was raised with this sand and planted with *A. arenaria*. The present evaluation refers to fields on top of the raised foredune. Since failures in plant growth occurred on the outer seaward slope (talus) due to a relatively high degree of soil compaction, fields from the talus were excluded from the quantitative evaluation (Van der Putten and Van Gulik 1988).

(2) *South-west section.* In the winter of 1985-1986, dredged sea sand had been deposited on the south-west beach and the foredune was raised and planted in the following winter. The same methods were applied as used in the north-west section, except that in the sowing method the spraying of

compost was replaced by the disk-harrowing of straw.

Fertilization.

Before *A. arenaria* was established, slow-release fertilizer (Osmocote, 12 to 14 months active at 21 °C) had been broadcast mechanically at a rate of 325 kg.ha^{-1}. It contained 24% of N (10% N as ammonium nitrate and ammonium phosphate, and 14% N as urea), 6% P_2O_5 (ammonium phosphate and calcium phosphate; 5.4% H_2O-soluble), and 6% K_2O (potassium sulphate). In a previous field experiment with the same type of fertilizer, about 90% of the N, 60% of the P, and 50% of the K was released during the first growing season (Van der Putten Chapter 2).

field numbers	planting methods
1	seeds + compost
6	seeds + straw
2,7	culms + reed
3,5,8	culms + rhizomes + reed
4,9	rhizomes + reed
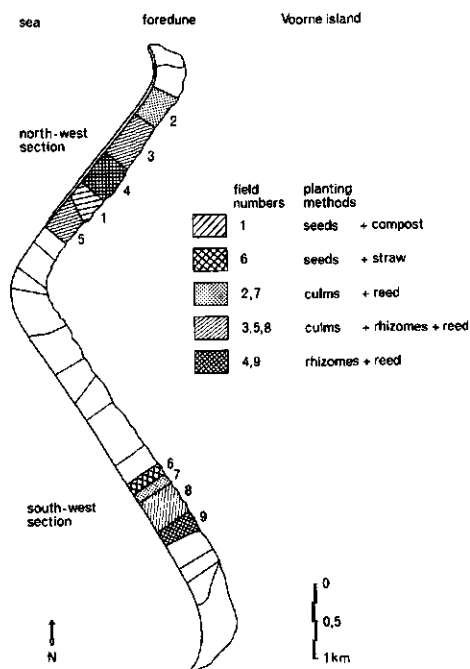
Fig. 1. The experimental area on the foredune at Voorne, the Netherlands where the traditional and new methods of establishing *Ammophila arenaria* have been carried out. For specification see Table 1.

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Table 1. Field numbers and areas, densities of plant material, sowing rates, methods and amount of material for sand stabilization used in the establishment of _Ammophila arenaria_ from culms (traditional method), seeds, rhizomes, and a combination of culms and rhizomes. Field numbers correspond with those in Fig. 1.

Field number	Field area (ha)	Plant material	Plant density	Sand fixation	Amount of material
north-west section					
1	2.80	seeds	15 kg/ha	compost	4.10^4 kg/ha
2	9.70	culms (tradional)	0.5×0.75 m^2	reed	0.5×0.75 m^2
3	7.11	culms + rhizomes	0.75×1 m^2 + 4.10^5/ha	reed	0.5×0.75 m^2
4	6.87	rhizomes	6.10^5/ha	reed	0.5×0.75 m^2
5	5.90	culms + rhizomes	0.75×1 m^2 + 4.10^5/ha	reed	0.5×0.75 m^2
south-west section					
6	1.72	seeds	20 kg/ha	straw	5.10^3 kg/ha
7	1.38	culms (tradional)	0.35×0.7 m^2	reed	0.5×0.7 m^2
8	7.34	culms + rhizomes	0.7×0.7 m^2 + 4.10^5/ha	reed	0.5×0.7 m^2
9	2.45	rhizomes	6.10^5/ha	reed	0.5×0.7 m^2

Planting methods.

Ammophila arenaria is almost exclusively planted in winter (October until April), as this gives the best survival. During the winters of 1985-1986 (north-west section) and 1986-1987 (south-west section), *A. arenaria* was established according to four different methods: i.e. (1) planting of culms, (2) sowing of seeds, (3) disk-harrowing of rhizomes, and (4) a combination of planting of culms and disk-harrowing of rhizomes.

(1) *Planting of culms (traditional method).*
Culms of *A. arenaria* were cut at 10 cm below soil surface in an optimal stand (for definition of optimal stand see: Greig-Smith 1961) using a hand shovel. Bundles of 6 culms were planted at a depth of 15 cm and bundles of dry reed (*Phragmites australis*), with a circumference of 6 cm, were planted in between to stabilize the sand surface. See Table 1 for further details.

(2) *Sowing of seeds.*
Seeds were harvested in July, air-dried and threshed. Percentage of germination (determined at 8/16 hours of 30/20 °C in a light/dark regime) was 95% and 1000-kernel weight was in the range of 2.5 to 3.4 g. The hairs at the basal part of the seeds were removed to enable sowing by a drill and sowing depth was 3 to 5 cm. Two methods of sand stabilization were applied: disk-harrowing wheat straw and spraying compost (type 'edelcompost' of 'Vuil Afvoer Maatschappij', Wijster) by hydro-seeding method. The latter method has been developed by an enterprise (Groenvoorziening b.v., Diepenveen). See Table 1 for further details.

(3) *Disk-harrowing of rhizomes.*
In order to collect the rhizomes of *A. arenaria*, above-ground plant parts of a vigorous stand were removed and the upper 2 to 4 m of the soil profile was dug by a crane and spread on the beach. This sand was levelled out by a mechanical shovel to a layer of 20 to 30 cm and rhizomes were sifted by an adapted bulb harvesting machine. The rhizomes were used on the day of collection and if not, they were stored underground or kept in cold storage (3 to 4 °C) to protect the buds from high temperatures or frost damage (Van der Putten and Van Gulik 1988).
To establish *A. arenaria*, the rhizomes were spread over the sand surface, and disk-harrowed twice to a depth of 10 to 15 cm. This ensured that the rhizomes were cut into 15-cm pieces – each with 2 to 5 viable buds – and buried (Van der Putten and Van Gulik 1987). In order to stabilize the sand surface, wheat straw was disk-harrowed or dry reed was planted. The rhizome densities used are based on previous experiments (Van der Putten chapter 2) and these, as well as further details, are presented in Table 1.

(4) *Planting culms in combination with disk-harrowing rhizomes.*
This procedure consisted of a combination of two previously mentioned

methods: the planting of culms and the disk-harrowing of rhizomes (Table 1). First rhizomes were disk-harrowed, secondly culms were planted, and finally the sand surface was stabilized by dry reed.

Visual evaluation.

One year after establishment, plantings were inspected by the local dune manager to assess the need for replanting. There are no strict regulations for deciding whether to replant or not. Generally, bare areas in excess of 10 m^2 were replanted. Sand erosion was assessed qualitatively by observing the accumulation of shells on the soil surface after sand grains had been blown away, and sand deposition by observing its accumulation around plants or temporary sand stabilizers (straw and reed).

Evaluation by means of Remote Sensing.

In September 1986 and 1987, at the end of the growing season of *A. arenaria* the results of the large-scale plantings were examined.
(1) *Parameters to evaluate.*
The results of the planting methods were expressed in biomass production, percentage cover, and heterogeneity of the distribution of the biomass within each stand. The relative contributions to the stands by *Ammophila arenaria, Calammophila baltica*, and other, spontaneously established, plant species were assessed on the basis of their proportion in the biomass as collected from the sampling plots in the fields.
(2) *Lay out of sampling plots within fields.*
In each field, plots (3x3 m^2) were marked with a disk (diameter of 33 cm) that could be recognized on the image. In 1986, all fields in the north-west section were stratified according to relief, i.e. (1) tops, (2) slacks, (3) seaward slopes, (4) landward slopes, and (5) sideward slopes. Four plots per relief type were chosen randomly. In 1987, each field was stratified according to (1) patches with homogeneous biomass and (2) relief. Random plots were chosen within strata: In the north-west section, 20 plots (fields 1, 2, and 4) or 10 plots (fields 3 and 5) per planting method were examined. In the south-west section, 12 plots per planting method were examined; 10 at the same relief types as in the north-west section and 2 plots at the talus along the beach.
(3) *The applied Remote Sensing technique.*
In 1986 and 1987, aerial photographs were taken from the study area on false colour diapositive film, which is sensitive for green, red (R) and near infra-red (NIR) (see Table 2 for technical data). Since only R and NIR were of importance, a filter was used to expel the green radiation.
The applied remote sensing technique (adapted from Meulstee et al. 1986)

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is based on the relations between above-ground biomass and the intensity of reflected sun radiation (Rousse et al. 1973), as well as between the intensity of reflectance and density (darkness) of the photographic emulsion (Lillesand et al. 1979, Meulstee et al. 1986). This results in a relationship between biomass and colour density ratio (DENRAT):

$$\text{Biomass} = f\,(\text{DENRAT}) = \frac{10^{(Dg-Dr)}-1}{10^{(Dg-Dr)}+1} \qquad \text{(Meulstee et al. 1986)}$$

Dg = colour density in the green layer of the film (R-sensitive)
Dr = colour density in the red layer of the film (NIR-sensitive)
First the model was established by combining the measurements on the diapositive with the data of the corresponding field plots on biomass. Secondly, the biomass of a given field was calculated based on measurement of colour densities on the image (Kloosterman and Van Stokkom 1988). It was also possible to estimate quantitatively the percentage cover per field by relating the assessed percentages cover to the corresponding colour densities on the image (Kloosterman and Van Stokkom 1988).

Table 2. Technical specifications of the airborne remote sensing method and of the densitometer applied in the analysis of the false colour film.

Dates of photography	: 4-10-1986 and 8-10-1987
Scale	: 1 : 2.000
Platform	: in 1986: 600 m; in 1987: 300 m
Camera	: Zeiss forward motion control camera
Focal length	: in 1986: 30 cm; in 1987: 15 cm
Shutter speed	: 1/125 sec.
Aperture	: 5.6
Filmtype	: Kodak infra-red aerographic film 2424
Negative size	: 23 x 23 cm.
Filter	: in 1986: Zeiss H filter (cut-off level 635 nm), no anti vignetting filter applied. in 1987: Wratten 29 (cut-off level 635 nm), anti vignetting filter
Overlap	: 80 %
Densitometer	: McBeth TD 504
Opening sensor	: 2 mm (i.e. diameter in field of 4 m)

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(4) *Field sampling and treatment of the plant material.*

Percentage cover. In each field plot, percentages cover of individual species were estimated according to a scale of: 1%, 2.5%, 5%, 5-10%, 10-15%, etc. Total percentage cover could be determined by adding up all individual percentages, as there was very little overlap.

Biomass. After photographing plants were cut at ground level, separated into *A. arenaria*, *C. baltica*, and other plant species, and total fresh weights (F) were determined. Subsamples were taken randomly, weighed fresh (f), dried during 48 hours at 70 °C, and weighed again (d). Before drying the subsamples, numbers of tillers and length of 20 tillers of *A. arenaria* were determined. Total above-ground dry weights per plot (D) of individual species and of the total stand were calculated as D = F x d/f kg/9 m^2.

(5) *The relationship between colour density ratio, biomass, and soil cover.*

Within the range of observations, regression equations were not significantly influenced by either method of planting or relief (Kloosterman and Van Stokkom 1988). However, the relationships between colour density ratio and biomass, as well as between colour density ratio and percentage cover were found to be affected by the age of the *A. arenaria* stand (Fig.2). Therefore, relations between biomass productions, percentages cover and colour density ratios were calculated separately for one- and two-year-old plantings (Table 3).

Table 3.   Parameters of the regression equations that were used for calculation of the biomass (g/m^2) and percentage cover of the soil (%) of new stands of <u>Ammophila arenaria</u> in the north-west and south-west sections from the colour densities measured on the false colour film. In the regression equation Y = b X + a, X = colour density and Y = biomass or soil cover. All R^2 are significant at the 0.01 level. Results obtained from Kloosterman and Van Stokkom (1988).

		Biomass			Percentage cover		
Area	Year	b	a	R^2	b	a	R^2
north west	1986	749	-207	0.75	57.5	-11.1	0.50
north west	1987	1769	-525	0.69	87.5	-20.0	0.67
south west	1987	729	-201	0.82	63.7	-16.2	0.83
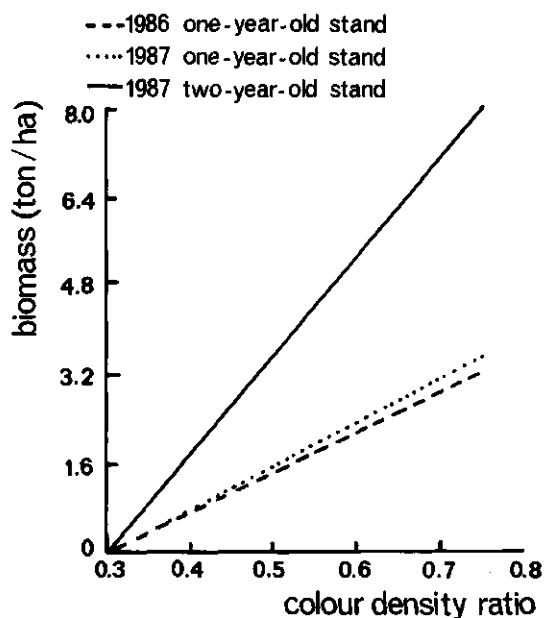
Fig. 2. The relationship between colour density on the aerial photographs and the biomass production (ton/ha) of one-year-old and two-years-old stands of established *Ammophila arenaria*.

Statistical analysis of the data.

Colour density ratios of relection panels (situated in the field when pictures were taken) were used for power analysis in order to calculate the number of measurements required. The standard deviation of the measurements of the vegetation had to be smaller than the error specification of the densitometer obtained from replicate measurements on the panels. Power analyses were undertaken for every planting method.
Since both biomass and soil cover per field had been derived from colour density ratios on the image, differences among the latter are supposed to be representative for differences among biomass productions, as well as percentages cover. Therefore, colour densities of different fields were compared and as variances were not homogeneous according to Cochrans Q test, Kruskal-Wallis non-parametric analysis of variance was applied (Sokal and Rohlf 1981). Biomass and percentage cover (Figs. 3 and 4) were obtained from colour density ratios by linear regression (Table 3) with 80 per cent confidence level.

RESULTS

Visual field evaluation

The most effective stabilization of the sand surface was obtained with disk-harrowed straw, which allowed hardly any sand deflation. Both seaward slopes with compost and talus with reed plantings were to some extent susceptible to wind erosion. However, in reed only superficial sand erosion occurred, whereas the erosion of compost resulted in the formation of blow-outs.
Deposition of windblown sand occurred only along the beach on the lowest 1 to 5 m of the talus. During the two years of investigation more than 1 m of sand had accreted around well established plants.

Replanting

No more than 5 per cent of the total area had to be replanted and most failures occurred in stands established from seeds. Locally, the establishment and growth of seedlings of *A. arenaria* was very poor due to (1) abundant growth of the cereal rye, which had been sown at the talus in the north-west section as a nurse crop providing the seedlings of *A. arenaria* with a protecting cover and (2) blow-outs in the field stabilized by compost.
At one site, where rhizomes had been disk-harrowed at an air temperature of -9 °C, no plants emerged probably because the buds froze. Emergence of plants from rhizomes was reduced locally on the talus stabilized with reed, probably because of superficial sand erosion.

Quantitative evaluation

In 1986, the false colour pictures of the north-west section revealed two discrete parts within fields with culms (2), culms and rhizomes (3), and rhizomes (4). It appeared that part of the plant material in these fields originated from stable foredunes, instead of being collected (as usual) from mobile foredunes. Plant material originating from stable foredunes produced significantly less biomass and percentage cover than plant material from mobile foredunes (P<0.05; Fig. 3A). These differences remained in the second growing season (Fig. 3B). Plant materials from the stable dune had been used because there was a shortage of plants at the start of the work. Since using plants from stable dunes has to be regarded as an artefact, only those parts of the stands which had been established

from plant materials originating from mobile foredunes were used for the quantitative evaluation.
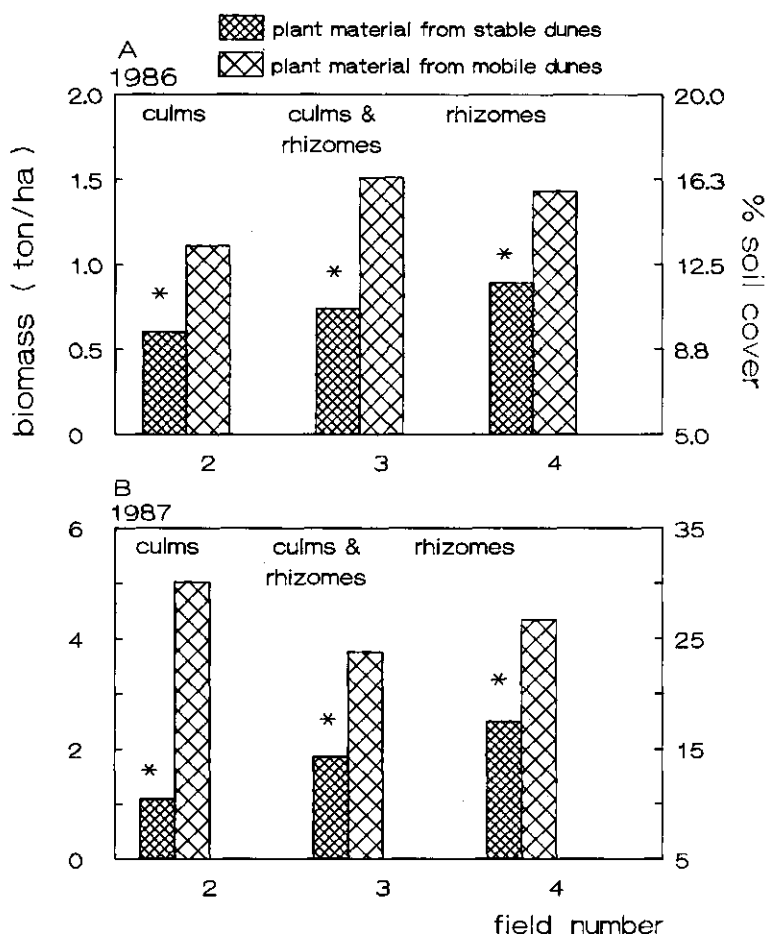
Fig. 3. The effect of plant materials of *Ammophila arenaria* originating from stable and mobile dunes on the production of biomass and percentage cover after one year (A: 1986) and after two years (B: 1987). 2 = traditionally planted culms, 3 = culms in combination with rhizomes and 4 = rhizomes. Per two bars differences marked with * are significant (P<0.05).

In 1986, biomass production and percentage cover of the traditional method were significantly lower than those of rhizomes, or a combination of culms and rhizomes (Fig. 4A). Biomass production and percentage cover of seeds + compost were not significantly different from the traditional

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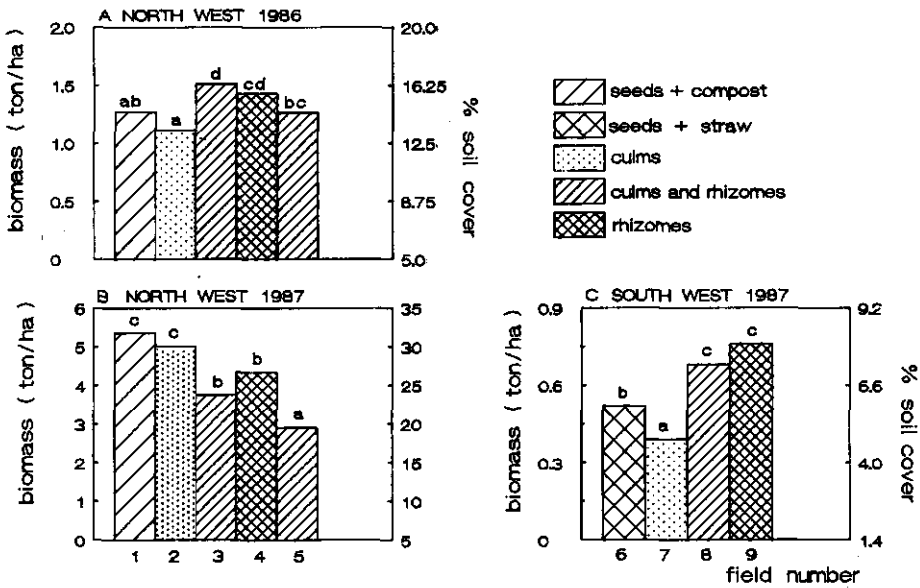
Fig. 4. Biomass production and percentage cover of established stands of *Ammophila arenaria*. 1 = seeds with compost, 2, 7 = culms (traditional method), 3, 5, 8 = culms in combination with rhizomes, 4, 9 = rhizomes, 6 = seeds with straw (see also Table 1).

     A. North-west area in 1986 (one-year-old stands)

     B. North-west area in 1987 (two-year-old stands)

     C. South-west area in 1987 (one-year-old stand).

Significant differences between the bars per plot are indicated by different characters.

method. Differences between the two fields with a combination of culms and rhizomes (3 and 5) were significant (Fig.4A). Density of tillers was highest in the stand established from seeds (Table 4). However, the weight and length of these tillers were considerably less than of those originating from culms or rhizomes, which were also fewer in number (Table 4). In 1987, in the north-west section two-year-old stands established from culms (traditional method) and from seeds + compost produced more biomass and higher percentages cover than stands from rhizomes or a combination of culms and rhizomes (P<0.05; Fig.4B). As was the case in 1986, significant differences occurred between the two stands that were established from culms in combination with rhizomes. In 1987 only planted culms (traditional method) produced more tillers than in 1986. Tillers in 1987 weighed 2 to 5 times as much as in 1986 and were slightly longer (Table 4).

Table 4. Densities, dry weights, and lengths of tillers in one- and two-years-old stands of _Ammophila arenaria_ (1986 and 1987, respectively) established from culms, seeds, and rhizomes, and culms with rhizomes in the north-west section.

Field number	Planting method	1986			1987		
		Density $(n.m^{-2})$	Dry weight $(mg.tiller^{-1})$	Length $(cm.tiller^{-1})$	Density $(n.m^{-2})$	Dry weight $(mg.tiller^{-1})$	Length $(cm.tiller^{-1})$
1	seeds + compost	458	227	37.4	451	1188	62.0
2	culms + reed (traditional)	148	750	58.4	317	1580	70.5
3	culms + rhizomes + reed	198	763	56.8	209	1795	62.4
4	rhizomes + reed	345	414	45.7	285	1521	58.9
5	culms + rhizomes + reed	228	553	54.1	131	2207	71.2

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In 1987, biomass production and percentage cover in the south-west section were about half as high as those of the one-year-old stand in the north-west section (Figs.4C and 4A, respectively). Again, production of biomass and percentage cover were lowest in planted culms (traditional method), and differed significantly from seeds + straw, a combination of culms and rhizomes, and rhizomes alone (P<0.05; Fig.4C). Biomass production and percentage cover of a combination of culms and rhizomes were not significantly different from rhizomes alone, and both methods produced more biomass and soil cover than seeds + straw (P<0.05). Numbers of tillers were highest in the stand from seeds and lowest in the traditionally planted stand (Table 5). However, differences were less pronounced than in the one-year-old stand in the north-west section.

Table 5.   Densities, dry weights, and lengths of tillers in the one-year-old stand of Ammophila arenaria (1987) established from culms, seeds, rhizomes, and culms with rhizomes in the south-west section.

Field number	Planting method	Density $(n.m^{-2})$	Dry weight $(mg.tiller^{-1})$	Length $(cm.tiller^{-1})$
6	seeds + straw	155	335	46.2
7	culms + reed (tradional)	88	441	53.5
8	culms + rhizomes + reed	140	484	46.9
9	rhizomes + reed	123	617	52.1

Vegetation pattern and composition.

The frequency distribution of the colour densities that have been obtained from the false colour images shows that the stand originating from the traditional method was the most homogeneous (Fig. 5). In the north-west section the stand from seeds + compost was the most heterogeneous, which is reflected by the shape of the frequency distribution as well as by the large number of measurements required for obtaining a standard deviation within the error specification of the densitometer. However, in the south-west section the stand from seeds + straw apparently was not more heterogeneous than the other stands. Within the fields, biomass production and percentage cover were lower on the tops than on the other relief types (data not shown).
The botanical composition of the new stands was not surveyed systematically. However, data of the plots in the experimental fields allowed some assessment of it. *C. baltica* did not occur in the sown stands,
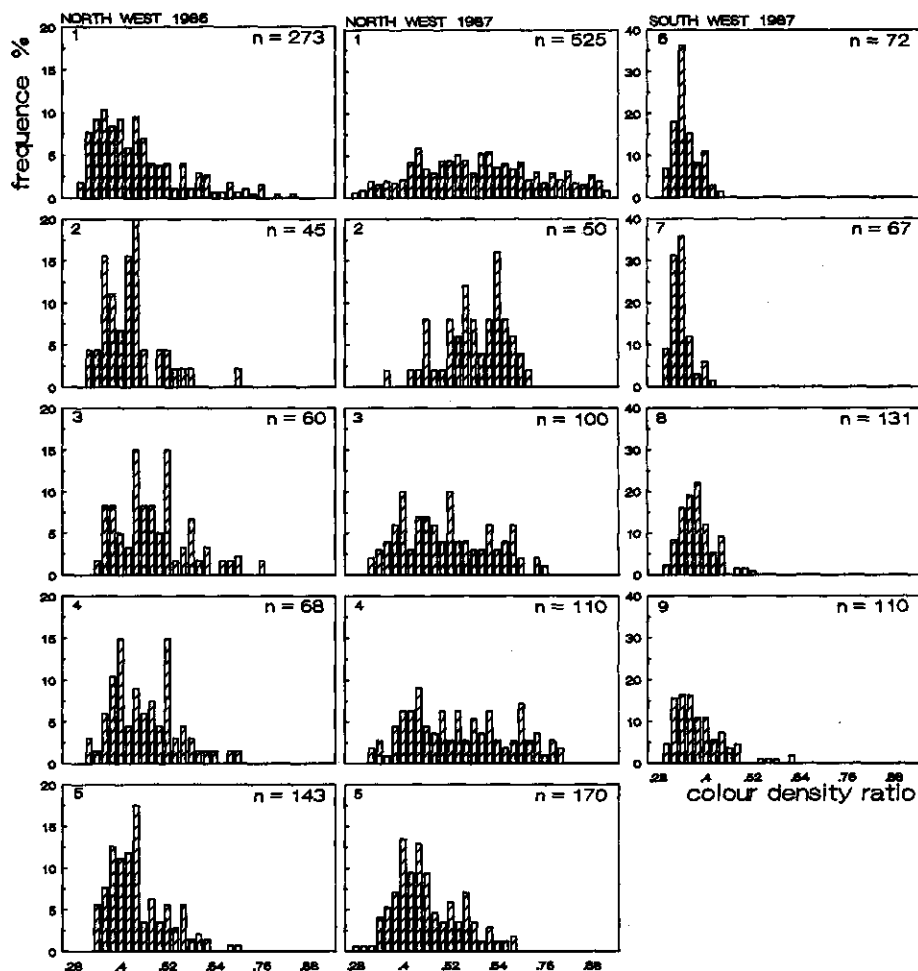
Fig. 5. Frequency distributions of colour density ratios obtained from the images of all experimental plantings at the north-west section (1986 and 1987) and south-west section (1987). The numbers of measurements required for obtaining a standard deviation within the error specification of the densitometer (n) is given in each separate plot.

1 : seed + compost
2, 7 : culms + reed
3, 5, 8: culms with rhizomes + reed
4, 9 : rhizomes + reed
6 : seed + straw

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whereas it was relatively more present in stands from rhizomes than in stands planted from culms (data not presented). Stands from seeds had, besides *Ammophila arenaria* and *Calammophila baltica* the least other species. The species occurring mostly were: *Cakile maritima**, *Cirsium arvense*, *Corispermum leptopterum**, *Erigeron canadensis*, *Festuca rubra*, *Senecio sylvaticus*, *Solanum triflorum*, *Sonchus arvensis*, and *Sonchus oleraceus* (* presence largely restricted to the first growing season).

DISCUSSION

In addition to the traditional method (the planting of culms), *A. arenaria* can be established on a large scale from seeds or rhizomes. Differences between planting methods were affected by the age of the stands. Nevertheless, since 5 per cent of the total area had to be replanted, all planting methods demonstrated their practical value. Between 1985 and 1988, costs of planting of culms + reed (traditional method), seeds + straw, rhizomes + straw, and culms with rhizomes + reed, amounted to 16500, 4500, 10500, and 18000 Dutch guilders per ha, respectively (Van der Putten and Van Gulik 1988). Hence, the costs of using seeds or rhizomes were much lower than those of the traditional method. However, the use of the various methods should be dependent on more than price alone.
The stand obtained from seeds + compost was heterogeneous, as compared to the other stands, which was due to a combination of complete absence of plants in local blow-outs and relatively high dry matter yields at unexposed sites. The latter is probably caused by extra release of nutrients from the compost. Temporary sand stabilization by straw did not allow blow-outs being formed and heterogeneity was comparable to stands established from culms and rhizomes. Nevertheless, since the slightest erosion or deposition of sand causes mortality of the seeds (Huiskes 1977) the sowing method involves the highest risks. Disk-harrowed rhizomes were much less susceptible to sand erosion. One reason for this may be the deeper planting of rhizomes. On the other hand stems and buds contain higher amounts of reserve materials than seeds, resulting in more robust tillers that enable plants to emerge from greater depths. The plants established from vegetative material are therefore less sensitive for sand deposition. Similar observations have been made for other rhizomatous grasses from coastal sand dunes, viz. *Ammophila breviligulata* (Maun 1985) and *Elymus farctus* (Harris and Davy 1986), as well as for weed grasses, e.g. *Agropyron* (now *Elymus*) *repens* (Hakanson 1968a, b).
The production of biomass and soil cover of the plantings were more affected by quality of the plant material than by method of planting.

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Culms and rhizomes collected from stable foredunes produced less than half the amount of dry matter and soil cover compared with plant material which had been obtained from mobile foredunes. Differences between *A. arenaria* collected from mobile and stable dunes have been related to genetic differentiation (Gray 1985). It is more likely, however, that they are due to harmful soil organisms in the rhizosphere of *A. arenaria* (Van der Putten and Troelstra chapter 6). In order to obtain a high biomass production and percentage cover, plant material has therefore to be collected from mobile dunes.

Biomass productions of one-year-old stands were very different in the two years. Stands planted in 1987 produced half as much biomass as stands planted in 1986. Notwithstanding these annual differences in production, the differences between the planting methods remained unchanged. Brown and Hafenrichter (1948a) showed for one winterperiod that if air temperatures after planting were high ($\geq$ 12 °C), survival of the planted culms was less than when temperatures were low and there was ample rainfall. However, as the present large-scale planting had been carried out during two whole winter periods and at two different sections of the foredune, differences in production between the one-year-old stands of 1986 and 1987 must be attributed to other factors.

The development of the botanical composition of newly established stands of *A. arenaria* takes at least 5 to 10 years (Hewett 1970, Hansen and Vestergaard 1986). Data from field plots already showed some trends, e.g. *Cakile maritima* and *Corispermum leptopterum* were abundant during the first growing season, whereas they had disappeared in the second year. *C. baltica* was completely absent in the sown stands which is due to sterility of this hybrid species. The development of the vegetation is being surveyed and the results will be presented in a subsequent paper (D. Van der Laan, in prep.).

The applied remote sensing technique gave very satisfactory results. However, in 1986 a lower correlation was obtained for heterogeneous stands as compared with more homogeneous stands (Kloosterman and Van Stokkom 1988). Since the densitometer measures a circle (diameter of 4 m) in the field plot (3 x 3 m^2), the error in a stand with heterogeneous biomass was expected to be larger than when biomass was distributed homogeneously. In 1987, therefore, the area was stratified to patches with homogeneous biomass, which resulted in a more reliable relationship between biomass (or percentage cover) and colour densities (Kloosterman and Van Stokkom 1988).

Other methods that are used in the assessment of tiller densities and botanical composition of grassland are completely carried out in the field (e.g. Mannetje and Haydock 1963, Haydock and Shaw 1975). Most grass species, however, have high tiller densities and overlapping leaves. As

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biomass production is not correlated with colour density at high leaf area ratios the used remote sensing method will be less applicable in high-producing pasture. However, high leaf area ratios will be reached rarely in sand dunes and therefore, the method of Kloosterman and Van Stokkom (1988) could be used for monitoring the condition of the vegetation at coastal foredunes, as is already done anually for the coast-line position.

BIOTIC SOIL FACTORS AFFECTING THE GROWTH AND DEVELOPMENT OF *AMMOPHILA ARENARIA*

W.H. van der Putten, C. van Dijk and S.R. Troelstra

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CHAPTER 4

BIOTIC SOIL FACTORS AFFECTING THE GROWTH AND DEVELOPMENT OF AMMOPHILA ARENARIA

SUMMARY

To study the origin of replant disease of *Ammophila arenaria* (L.) Link the growth and development in sand originating from the rhizosphere of a natural *Ammophila* vegetation was compared with the growth in sand from the sea-floor. In a greenhouse experiment, the growth of *Ammophila* seedlings in rhizosphere sand, when compared with that in sea sand, was significantly reduced. As sterilization by means of gamma-irradiation increased the biomass production of *Ammophila* seedlings signific ntly, it was concluded that the rhizosphere sand contained biotic factors that were harmful to *Ammophila*. In rhizosphere sand the roots of *Ammophila* were brown and poorly developed, and the specific uptake rates of N, P and K were reduced. The shoot weight proportion of the total plant dry matter was hardly influenced. In an outdoor experiment with *Ammophila* seedlings and cuttings, using both sands, the mortality was high and the plants were feeble in rhizosphere sand whereas plants in sea sand grew vigorously. It seems plausible that the plants in rhizosphere sand were dessicated because the root system was shallow and badly developed. In the greenhouse experiments, *Ammophila* cuttings were less sensitive to the inhibiting factors in the rhizosphere than seedlings. This was confirmed in the outdoor experiment. *Calammophila baltica* (Fluegge ex Schrader) Brand, however, was hardly affected by the harmful biotic factors in the greenhouse. These results are discussed with reference to the ecology of *Ammophila*. It is assumed that the catching of fresh wind-blown sand provides *Ammophila* with a way to escape from harmful biotic soil factors, and it was concluded that degeneration of *Ammophila* is caused mainly by self-intolerance due to these biotic soil factors.

INTRODUCTION

Ammophila arenaria (L.) Link (marram grass) is a grass species that stimulates dune building. It dominates the outer dune ridges along the North West European sea-coast, around the Mediterranean, and along Australian and North-American coasts (Huiskes 1979, Barr & McKenzie 1976 and Brown & Hafenrichter 1948b). *Calammophila baltica* (Fluegge ex Schrader) Brand, a hybrid between *A. arenaria* and *Calamagrostis epigejos* (L.) Roth (Westergaard 1943), occurs in the North West European coastal dunes, be it less frequently than *A. arenaria* (Rihan & Gray 1985).
Both *Ammophila* and *Calammophila* are intensitively planted to prevent the sand dunes from erosion. They also establish naturally from rhizome fragments and seed (Adriani & Terwindt 1974, Huiskes 1977, Maun 1984). *A. arenaria* can withstand sand accretion up to one metre a year, and in this respect resembles its North-American cogener *A. breviligulata* Fern. (Laing 1958, Maun & Lapierre 1984). Both *Ammophila* species not only withstand burial by sand, but they need it in order to grow vigorously (Willis 1963, Marshall 1965, Huiskes 1979, 1980, Eldred & Maun 1982, Disraeli 1984). On fixed dunes, where hardly any sand accretion occurs, *Ammophila* degenerates and disappears from the vegetation.
In order to explain the relationship between the vigour of *Ammophila* and the catching of drifting sand, many experiments have been carried out in the past. The results have been listed by Marshall (1965), Laing (1967) and Eldred & Maun (1982). The beneficial effects of fresh, windblown sand on the growth and development of *Ammophila* have been related to (1) ageing, (2) competition, and (3) nutrition.
(1) *Ageing.* On sites which do not get much fresh sand, *Ammophila* plants degenerate, as the uptake function of the root system declines and no new roots are formed (Willis 1965, Marshall 1965, Wallén 1980).
(2) *Competition.* Because of its ability to survive excessive burial by sand, the weakly competitive *Ammophila* was thought to avoid interspecific competition by colonizing sites where there were no other species (Huiskes 1979). As sand accretion decreases, mobile dunes become fixed and *Ammophila* is expelled from the vegetation by invading plant species (Huiskes & Harper 1979, Huiskes 1979, 1980).
(3) *Nutrition.* Drifting sand contains nutrients such as phosphorus and potassium, and has a high pH. It was assumed to be a kind of natural fertilizer (Lux 1969). The growth and development of *Ammophila* may benefit from the accumulation of windblown sand as the plant can produce new roots which exploit new sand depositions. Although this sand contains few nutrients, exploitation of a large volume of it enables the plant to collect the necessary nutrients (Willis 1963, 1965, Marshall 1965). More

recently, the possible role of soil micro-organisms in the nutrition was emphasized. The ecology of *Ammophila* has been related to the activity of Azotobacter (Ahmad & Neckelman 1978, Abdel Wahab & Wareing 1980), and the occurrence of Vesicular-Arbuscular Mycorrhizae (Nicolson 1960, Nicolson & Johnston 1979, Ernst et al. 1984).

The experiments described in this paper deal with the growth and development of *A. arenaria* and *C. baltica* in relation to biotic factors in the rhizosphere. Sand from *Ammophila* dunes was compared with sand originating from the sea-floor. In the Netherlands the latter is used to strengthen foredune ridges (Van der Putten & Van Gulik 1987). Originally, it was supposed that sand from the rhizosphere of *Ammophila* contains micro-organisms that benefit plant nutrition. To examine this *Ammophila* was grown in various mixtures of sea and rhizosphere sand. The growth of *Ammophila* on sea sand turned out to be superior. An experiment with gamma-irradiated rhizosphere and sea sand was carried out to determine the origin of the inferior growth in rhizosphere sand. The results of an experiment under semi-natural conditions underlined the ecological importance of the first and second experiment. Finally, a new hypothesis is put forward that connects the presence of pathogenic, or harmful, soil micro-organisms in *Ammophila* dunes with the ecological behaviour of *Ammophila arenaria*.

MATERIALS

Two types of sand were used in the experiments:
- rhizosphere sand i.e. sand originating from the root zone (10-30 cm below soil surface) of a foredune, covered by a vigorous *Ammophila* vegetation (Rockanje, the Netherlands, 51.52N, 4.05E).
- sea sand i.e. sand from the sea-floor (20-40 m below sea level) off the island of Voorne, the Netherlands (51.55N, 4.05E).

The seeds, as well as the cuttings, of *A. arenaria* and *C. baltica* were collected at the same location as the rhizosphere sand.

METHODS

Three experiments were carried out: (1) a greenhouse experiment with various sand mixtures, and with different NPK-levels; (2) a greenhouse experiment with both sand types, with and without gamma-irradiation treatment; (3) an outdoor experiment with both sand types under natural

rainfall conditions.

Experiment 1

To examine the effect of the substrate on *A. arenaria*, seedlings were planted in five soil mixtures (100% rhizosphere and 100% sea sand, and sea sand mixtures with 1%, 15%, and 50% rhizosphere sand; percentages were based upon air-dry weight), that were supplied weekly with 100 ml 1/2 Hoagland non-NPK elements and 0, (O) 1/8, (L) or 1/2 (H) Hoagland NPK (Hewitt 1966), resp.
Rhizosphere and sea sand were sieved using a 5-mm sieve. The *Ammophila* roots, separated from the rhizosphere sand, were chopped and homogenised with the sand. During sieving and mixing the sand and roots were not allowed to dry out. Demineralized water was added to all soil mixtures to give a final moisture content of 18%. Pots of 1.5 l were filled with sand (1500 g dry weight/pot) and planted with four *Ammophila* seedlings that were 2 weeks old. The pots were placed in a greenhouse (October 1984 to January 1985), maintained at $21^{o}C$ ($\pm$ 3) and a day length of 16 hours by additional illumination with Philips HLRG - 400W (4.8 W m^{-2}).
The pots were watered every second day with demineralized water. Four times, every three weeks, plants of randomly selected pots (three replicates per treatment) were harvested. After drying for 24 hours at $70^{o}C$, the weights of roots and shoots were determined. Numbers of tillers and leaves, and the length of the longest tiller were measured. Shoot weight ratios (SWRs) were calculated as dry shoot/total weight.

Experiment 2

To determine whether the growth inhibition of seedlings on rhizosphere sand in experiment (1) was of biotic origin or not, rhizosphere and sea sand were sterilized by means of gamma-irradiation (2.5 Mrad; see Oremus 1982). Four *Ammophila* seedlings (four replicates per treatment), three *Ammophila* cuttings, or two *Calammophila* cuttings (five replicates per treatment) were planted in 1.5 litre pots, filled with sterilized or non-sterilized sand (1500 g dry weight/pot). The cuttings were prepared from 4-cm segments of vertical underground stems, each bearing one viable bud (Pavlik 1983). The segments were placed in flats, covered with a 2-cm layer of fine sand, regularly supplied with demineralized water. Pots were planted with either seedlings or cuttings, randomly selected to achieve comparable plant material per treatment. These pots were placed in a heated greenhouse (see experiment (1)) from February - April 1985. Each pot was watered every second day with demineralized water and supplied

weekly with 100 ml 1/2 Hoagland solution.

Ammophila seedlings were harvested four times at intervals of three weeks; each time four pots per treatment were selected randomly and harvested. *Ammophila* and *Calammophila* cuttings were harvested three times at intervals of four weeks; each time five pots per treatment were selected randomly and harvested. The same parameters were determined as described for experiment (1), and the plant material was analyzed for N, P, and K. The specific root uptake rates (SURs) of N, P and K were calculated according to:

$$I = (M_2 - M_1) (\ln R_2 - \ln R_1) / (R_2 - R_1) (t_2 - t_1)$$

M represents the quantity of N (μmol), P or K (μeq); R is the root weight (g), and t is the time (day). t_1 and t_2 represent two successive harvest dates (Williams 1948). The specific root uptake rate between planting and the first harvest was calculated by supposing $M_0 = 0$. Because amount of plant material per pot was small, combined samples per treatment were analyzed.

Experiment 3

To determine the ecological relevance of the results of the greenhouse experiments a simple outdoor experiment was carried out. Rhizosphere and sea sand were planted with *Ammophila* seedlings and cuttings, supplied with slow-release NPK-fertilizer (8 g N, 2 g P, and 2 g K per m^2; Osmocote). There were no replicates. A frame-work of twelve compartments (four of 1 x 1 x 0.7 m^3 and eight of 1 x 0.25 x 0.7 m^3) was placed outdoors. Two large and four small compartments were filled with rhizosphere sand. The other compartments were filled with sea sand. One large and two small compartments of each sand-type were planted with *Ammophila* seedlings that were two weeks old at a density of 300 m^{-2} and the others with 100 cuttings m^{-2}. The cuttings were obtained in the same way as in experiment (2). After three weeks the seedlings were thinned to a density of 100 m^{-2}, and the few cuttings that had died were replaced.

The sand-surface was kept moist daily with tap water for the first three weeks, thereafter rainfall was the only source of water. At twelve and eighteen weeks after planting date one small compartment per treatment was harvested. The large compartments were harvested after 24 weeks. The plant material was dried and weighed as before.

Analysis of soil and plant material

After drying (35°C) and sieving (2 mm), bulk soil samples were mechanically subdivided. Part of the samples was ground in a mortar mill,

Table 1. Chemical properties of rhizosphere and sea sand.

Sand type	pH H$_2$O	pH KCl	organic matter (%)	CaCo$_3$ (%)	electrical conductivity (µS.cm^{-1})	chloride a	tot-P a	tot-N a	Mg b	K b	Na b
rhizosphere sand	8.9	8.8	0.20	5.2	60	1.6	13.0	8.1	0.60	0.04	0.04
sea sand	8.8	8.7	0.27	8.5	131	2.9	16.8	6.2	0.69	0.10	0.17

a (mg/100 g)
b (meq/100 g)

Table 2. Distribution of grain size (in %) of rhizosphere and sea sand.

	Grain size (µm)													
	<2	2–16	16–53	53–75	75–106	106–150	150–212	212–300	300–425	425–600	600–850	850–1400	>1400	D50[a]
rhizosphere sand	1.00	0.69	0.47	0.33	2.54	35.7	48.7	10.1	0.41	0.06	0.01	0	0	150–212
sea sand	1.09	0.41	0.62	1.64	17.1	36.6	25.2	9.46	4.29	2.32	0.89	0.25	0.12	106–150

a Grain size at which 50% of the sand grains are equal or smaller

and, depending on the type of determination, analyses were performed on either ground or unground samples.

The pH of the soil was measured potentiometrically in 1:2.5 (W/V) suspensions of H_2O or 1 M KCl. Carbonates were measured gas-volumetrically by treating samples with 4 M HCl. Organic matter was determined as loss-on-ignition, i.e. weight loss after ignition at $430^{\circ}C$ for 24h. Total N and total P were measured colorimetrically in single soil digests (Novozamsky et al. 1984). Exchangeable cations were determined by atomic absorption spectophotometry after shaking soils with neutral ammonium acetate. Chloride and electrical conductivity analyses were carried out on 1:5 water extracts. The granular composition (soil texture) of the samples was determined by dry sieving (fractions $> 53 \mu$), and a pipette method (fractions $< 53 \mu$).

Plant samples dried at $70^{\circ}C$ were used for analysis. In a sulphuric acid digest, N (total) and P were determined colorimetrically (indophenol-blue method and molybdenum-blue method, respectively), and K by atomic absorption.

Data analysis

The data were analyzed statistically by means of analysis of variance (ANOVA) after testing homogeneity of variances by means of Cochran or F_{max}. Treatment means were compared using the mean significant range (MSR; Sokal & Rohlf 1981)

RESULTS

Chemical properties and grain size distribution

The chemical properties of rhizosphere and sea sand were roughly similar (Table 1). As the sea sand had recently been dug from the sea floor electrical conductivity, chloride, potassium and sodium values of the sea sand were higher. The grain size of rhizosphere sand was concentrated mainly in the 106 - 212 μ range (Table 2), those of the dredged sea sand was somewhat larger, ranging from 75 - 212 μ, and the D50 was lower.

Ammophila development on rhizosphere and sea sand (experiment 1)

The total dry matter production on either 100% rhizosphere sand or 100% sea sand is shown in Fig. 1. The F-values of the total dry matter (biomass) production of both the factors and interaction are listed in Table 3. The

Table 3. Total dry weight (mg.pot^{-1}) of seedlings on five sand mixtures and three nutrient levels: $\underline{0}$ 0 NPK; $\underline{L}$ 1/8 Hoagland NPK and $\underline{H}$ 1/2 Hoagland NPK (all 100 ml.pot^{-1}.week^{-1}). Per block dry weights followed by the same letter are not significantly different (P $<$ 0.05). Significance of F-values, and degrees of freedom (df), and mean squares of error from 3-factor ANOVA of biomass and Shoot Weight Ratio (SWR) with the factors: sand mixture, nutrients (NPK) and harvest (experiment 1). Before analysis the biomass data were transformed with ln(x) and the SWR data with arcsin (square root (x)).

harvest	rhizo-sphere (%)	sea sand (%)	nutrient (NPK) level		
			0	L	H
1	0	100	36 a	57 a	64 a
	1	99	49 a	53 a	63 a
	15	85	30 a	40 a	55 a
	50	50	27 a	–	39 a
	100	0	23 a	32 a	37 a
2	0	100	66 ab	241 c	292 b
	1	99	68 b	179 bc	270 b
	15	85	39 ab	86 ab	213 ab
	50	50	38 ab	–	213 ab
	100	0	31 a	60 a	118 a
3	0	100	158 c	532 c	842 b
	1	99	99 bc	390 bc	717 b
	15	85	62 ab	215 ab	463 ab
	50	50	59 ab	–	319 a
	100	0	42 a	114 a	260 a
4	0	100	105 ab	838 c	1914 c
	1	99	161 b	588 bc	1617 bc
	15	85	53 a	384 ab	1226 abc
	50	50	63 a	–	887 ab
	100	0	68 a	205 a	731 a

Factor	Biomass	SWR	df
sand	98.8 ***	4.6 ***	4
NPK	812 ***	252 ***	2
harvest	730 ***	30.0 ***	3
sand x NPK	16.6 ***	4.6 ***	8
sand x harvest	2.9 **	3.1 ***	12
NPK x harvest	62.4 ***	3.9 **	6
sand x NPK x harvest	1.2 ns	1.7 *	24
mean squares of error	0.0532	0.0024	120

62 ns not significant   * P $<$ 0.05   ** P $<$ 0.01   *** P $<$ 0.001

effect of sand type on biomass production is highly significant (P < 0.001). The data of total dry matter production on rhizosphere and sea sand and the various sand mixtures (Table 3) show that if there was a significant difference between treatment means, plants in sea sand always ranked higher than in rhizosphere sand. The amount of biomass produced decreased as more rhizosphere sand was added to the sea sand. Interactions between sand and NPK, and sand and harvest date were significant (Table 3). The NPK treatments resulted in a significantly higher dry matter production in sea sand compared with rhizosphere sand eight, eleven, and fourteen weeks after planting (Fig. 1 and Table 3).
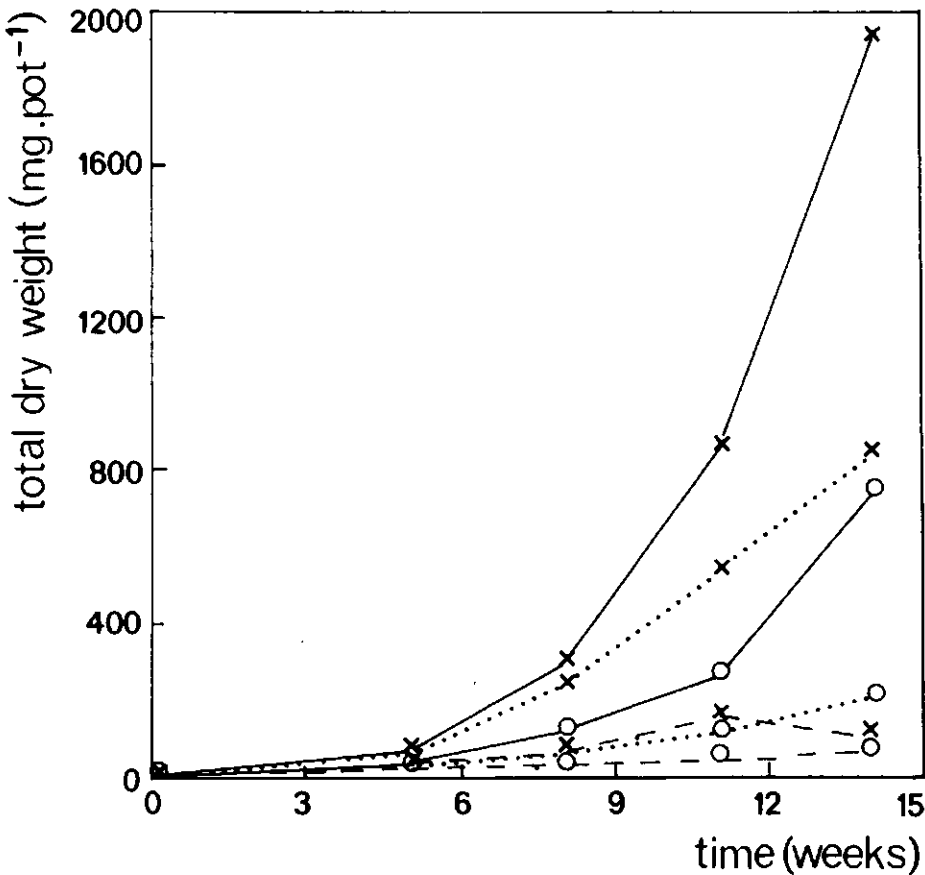

Fig. 1. Total dry weight (mg.pot^{-1}) of *Ammophila* seedlings on rhizosphere (O) and sea (X) sand, supplied with 0 (O - - -) 1/8 (L . . .) or 1/2 (H ---) Hoagland NPK.

The effect of irradiation of rhizosphere and sea sand on seedlings and cuttings (Experiment 2)

(1) *Ammophila seedlings*. F-values of factors and interactions are listed in Table 4. Both sand and irradiation affected the biomass production significantly ($P < 0.001$), but the interaction ($P < 0.001$) showed that the effect of irradiation did not occur on both sand types. The biomass production of seedlings on rhizosphere sand was significantly lower than that on irradiated rhizosphere or irradiated sea sand ($P < 0.05$; Fig. 2A). No significant differences occurred between sea sand, irradiated rhizosphere and irradiated sea sand. As the RGR (data not presented) of seedlings on rhizosphere sand first tended to be lower, then equal and later higher than the other treatments the growth inhibited plants seemed to recover.

The tiller length, as well as the numbers of leaves and tillers of the seedlings on rhizosphere sand were affected significantly ($P < 0.05$) by irradiation.

Table 4. Significance of F-values, and degrees of freedom (df), and mean squares of error from 3-factor ANOVA of biomass of <u>Ammophila</u> seedlings, <u>Ammophila</u> cuttings, and <u>Calammophila</u> cuttings with the factors: sand type, irraditation and harvest. Before analysis the data were transformed with ln(x).

Factor	Ammophila seedlings		df	Ammophila cuttings		df	Calammophila cuttings		df
sand	30.5	***	1	3.53	ns	1	4.01	*	1
irradiation	165	***	1	74.2	***	1	8.17	*	1
harvest	1431	***	3	736	***	2	538	***	2
sand x irradiation	27.4	***	1	6.79	**	1	13.2	*	1
sand x harvest	1.39	ns	3	0.04	ns	2	0.78	ns	2
irradiation x harvest	1.38	ns	3	1.42	ns	2	0.67	ns	2
sand x irradiation x harvest	1.11	ns	3	0.26	ns	2	0.60	ns	2
mean squares of error	0.0325		48	0.0358		45	0.0484		47

ns not significant   * $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

The appearance of the root system of plants in rhizosphere sand differed strongly from that in the other treatments. The roots were brown in rhizosphere sand, short branched, and badly developed. However, no evidence of macroscopic disease spots could be found. In order to find an explanation of the differences in biomass production between plants on rhizosphere sand and plants from the other treatments, the SURs of N, P and K were calculated for two intervals (Table 5). During the first two weeks, uptake rates of plants in rhizosphere sand were two to three times lower than those in irradiated rhizospere sand. Between the third and fourth harvest, however, the SURs of plants in rhizosphere sand were two to three times higher than those of the plants in irradiated rhizosphere

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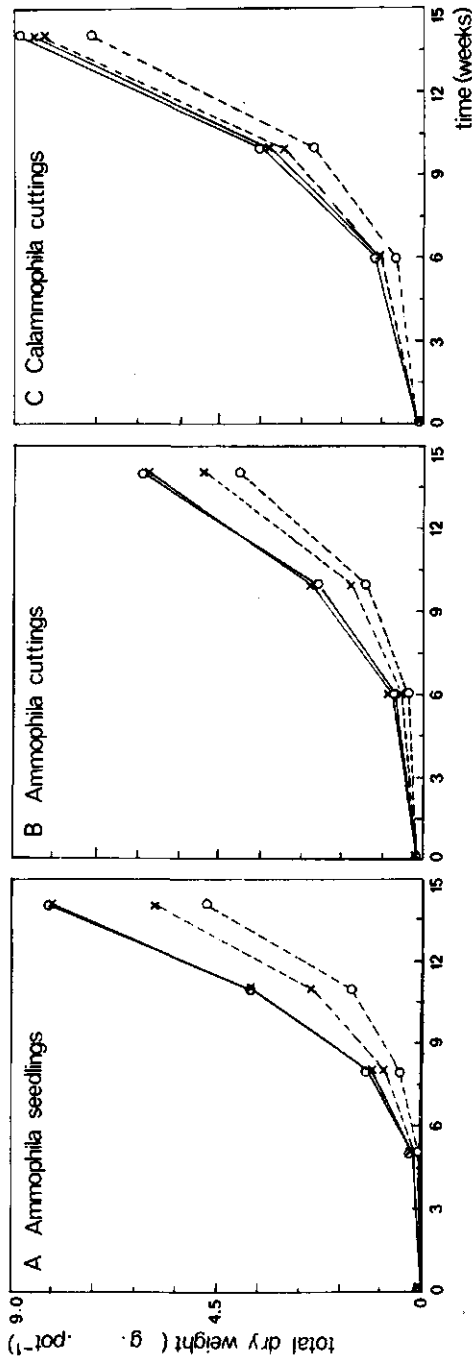
Fig. 2. A–C. Total dry weight (mg.pot^{-1}) of *Ammophila* seedlings A, *Ammophila* cuttings B, and *Calammophila* cuttings C on irradiated (———) and not-irradiated (- - -) rhizosphere (O) and sea (X) sand.

sand. Between these harvests, the uptake rates of plants in rhizosphere sand diminished less than in the other treatments. Between planting and the first harvest the SURs of plants in both sea sand and irradiated sea sand resembled each other, however, between the third and fourth harvest date the uptake rates of plants in sea sand tended to be intermediate between rhizosphere sand and both irradiated sand types.

Table 5. Specific root uptake rates of N (μmol.g^{-1}.day^{-1}), P and K (μeq.g^{-1}.day^{-1}) based on dry weight of <u>Ammophila</u> seedlings between t=0 (planting date) and t=1 (first harvest), and between t=3 (third harvest) and t=4 (fourth harvest) in four sand types. t_0-t_1 = 5 weeks and t_3-t_4 = 3 weeks.

time interval sand type	N		P		K	
	t_0-t_1	t_3-t_4	t_0-t_1	t_3-t_4	t_0-t_1	t_3-t_4
rhizosphere sand						
not irradiated	401	151	19	11	141	104
irradiated	870	65	53	3	355	49
sea sand						
not irradiated	796	93	40	5	269	72
irradiated	821	49	41	3	297	44

(2) *Ammophila cuttings*. F-values of biomass production are listed in Table 4. Irradiation affected the biomass production significantly (P < 0.001). The irradiation effect occurred only in rhizosphere sand (Fig. 2B), and interaction with sand type was significant (P < 0.01). Either seedlings or cuttings in rhizosphere sand always had lower dry matter yields than those of the other treatments, although the differences for the cuttings did not reach statistic significance (P < 0.05; Fig. 2A, B and Table 4). The recovery in RGR's (data not presented), which was apparent for seedlings, was much less evident for cuttings. There was less difference between the average number of tillers and leaves, and between the tiller lengths of the cuttings than amongst the seedlings. Shoot weight ratios were not significantly different and the uptake rates for cuttings were less pronounced than for seedlings.

(3) *Calammophila cuttings*. The F-values (Table 4) show that the biomass production was affected significantly (P < 0.05) by both sand types and irradiation treatment. However, the significance of the F-value of the different sand types was not confirmed when the treatment means were compared. As for the cuttings and seedlings of *Ammophila* the growth of

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Calammophila was only affected significantly (P < 0.05) by irradiating rhizosphere sand (Fig. 2C). SWR's were not significantly different (P < 0.05) and RGR's, and uptake rates only showed less pronounced trends than *Ammophila* cuttings. Irradiation did not cause any significant effect on any of the aboveground plant parameters (P < 0.05).
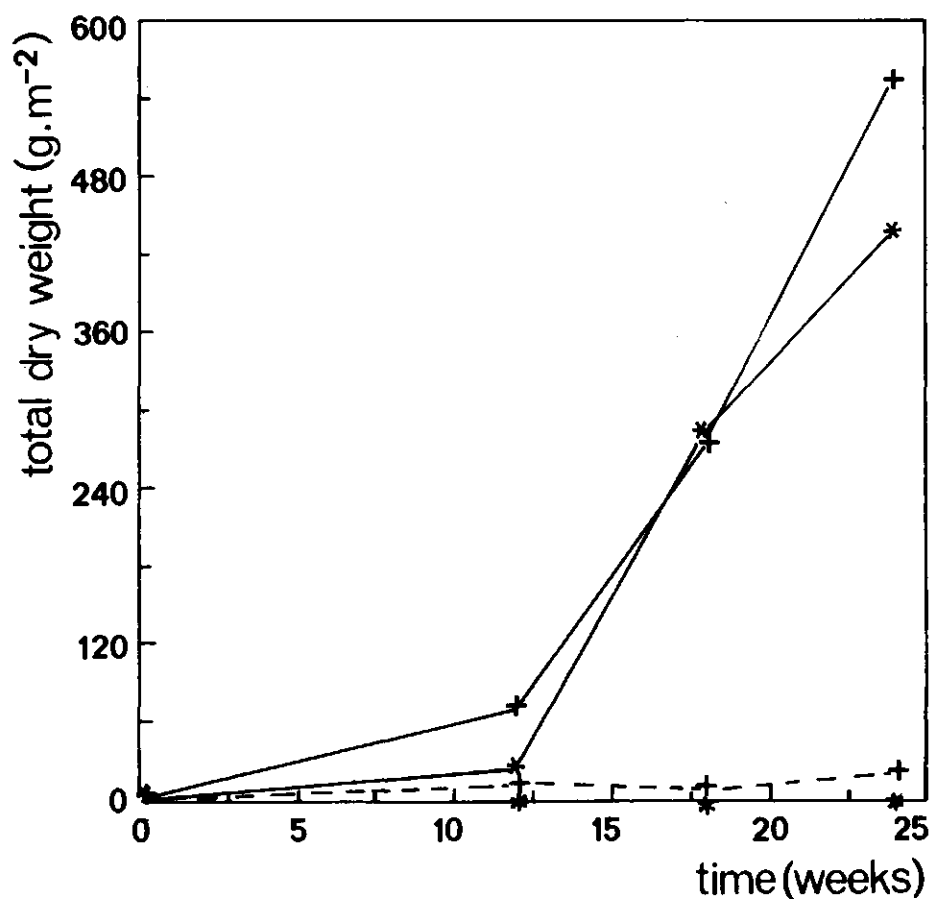

Fig. 3. Total dry weight (g.m^{-2}) of *Ammophila* seedlings (*) and cuttings (+) on rhizosphere (- - -) and sea (——) sand.

Experiment 3

The biomass development of cuttings and seedlings on sea sand was similar and appeared to be normal, whereas the development on rhizosphere sand was very poor (Fig. 3). Plant mortality on sea sand was zero. On

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rhizosphere sand 50% of the cuttings and 92% of the seedlings had died after a growth period of 24 weeks. The highest mortality occurred during a dry period six to nine weeks after transplanting. The roots of the plants on rhizosphere sand were shallow, brownish and short. The roots of the plants on sea sand, on the other hand were deep, long, white and wiry. After 24 weeks the SWR of seedlings (0.82) and cuttings (0.91) on rhizosphere sand tended to be higher than on sea sand (0.67 for both seedlings and cuttings). The seedlings on sea sand produced 4 to 16 tillers (average of 8.1). The average plant length of seedlings was 42 cm on sea sand and 11 cm on rhizosphere sand. The cuttings on sea sand produced 3 to 21 tillers (average of 8.6). The average plant length of cuttings on sea sand was 52 cm compared with 24 cm on rhizosphere sand. Both seedlings and cuttings on rhizosphere sand failed to produce more than one tiller.

DISCUSSION

The results of experiment 1 indicate the presence of a growth inhibiting factor in the rhizosphere of an *Ammophila* vegetation. Plant growth increased on both rhizosphere and sea sand after nutrients (NPK) were added, however plants in sea sand always produced more biomass than those in rhizosphere sand. Without the addition of NPK *Ammophila* did not show any increased growth on rhizosphere sand as compared with sea sand. The suggested beneficial effect of rhizosphere micro-organisms on the nutrition of *Ammophila* (Hassouna & Wareing 1964, Abdel Wahab 1975, Abdel Wahab & Wareing 1980, Nicolson 1960, Ernst et al. 1984), if present at all, was overshadowed by a strong growth reducing factor. Growth reduction could not be related to the mineral composition of both sand types. There was a significant growth reduction when 15% to 50% rhizosphere sand was mixed with sea sand. Hoestra (1968) obtained similar results with apple seedlings on mixtures of untreated and steam-sterilized apple soils.
Gamma-irradiation of the rhizosphere sand (experiment 2) showed that the inhibiting factor was of a biotic nature. Growth differences due to nutrient flushes after sterilization (Powlson & Jenkinson 1976, De Nooij et al. 1986) were avoided by adding nutrients. As growth of *Ammophila* on sea sand (either sterilized or not) and on sterilized rhizosphere sand did not differ significantly it seems obvious that the growth inhibition was not due to the accumulation of toxic chemical compounds.
When compared to non-inhibited plants, the relative growth rate of the growth-inhibited plants tended to be lower at the start and higher at the end of the experiment. It must, therefore, be concluded that the inhibition

in the experimental situation was most severe in the early stages of growth of the plants.

Cuttings of *Calammophila baltica* and (to a lesser extent) *Ammophila* were less affected by the biotic factor than seedlings of *Ammophila*. An explanation for this could be that the cuttings initially use metabolic reserves present in the stems. Furthermore, the fact that *Calammophila* is a hybrid of *Ammophila* and *Calamagrostis epigejos* (Westergaard 1943) also may contribute to its reduced sensitivity to harmful factors.

The different response of *Ammophila* seedlings and cuttings in rhizosphere sand in the greenhouse experiment 2 was confirmed by the results of the outdoor experiment (3). *Ammophila* cuttings developed poorly when planted in rhizosphere sand supplied with slow-release fertilizer, whereas nearly all *Ammophila* seedlings died. As both cuttings and seedlings showed luxurious growth in sea sand, it is very plausible to relate the differences between the two sand types to the occurrence of biotic factors in the rhizosphere sand. In the outdoor experiment the luxurious growth of *Ammophila* in sea sand was expressed by biomass production as well as by the number of tillers (and therefore by the number of leaves) per plant, and by the tiller length. As in the greenhouse experiment (2) irradiation affected the non-biomass plant parameters significantly a causal relation of these parameters with biotic factors would seem plausible.

In experiment 3 the large differences between the growth of plants on sea sand and on rhizosphere sand cannot primarily be connected with nutritional differences as the chemical and physical properties of both sand types hardly differed. Moreover slow-release fertilizer had been added to the top layer. Unlike in the greenhouse experiments, water was not added regularly. A more satisfactioning explanation would, therefore, be that in this experiment, the plants in rhizosphere sand grew poorly due to dessication because of the shallow and badly developed root system.

In its natural environment, *Ammophila* degenerates on fixed dunes where sand deposition has stopped (Olson 1958). Here, interspecific competition for nutrients and water with species, such as *Festuca rubra* spp. *arenaria*, may occur (Huiskes 1980 and Marshall 1965). But this does not explain degeneration of *Ammophila* at locations where competitors are absent (Hope-Simpson & Jefferies 1966, Eldred & Maun 1982, and Disraeli 1984). The positive effect of sand deposition on growth of *Ammophila* was explained by stimulation of growth of new roots in the fresh sand supplies (Marshall 1965). Experiments by Willis (1965) showed that accumulation of sand stimulated growth of *Ammophila* on fixed dunes, which could not be explained by nutritional effects. The occurrence of harmful biotic factors in the rhizosphere of *Ammophila*, presented in this paper, allows us to formulate an adapted version of Marshalls hypothesis. During its development, *Ammophila* needs a continual supply of fresh sand to

stimulate the growth of new roots not because of physical ageing, but to escape from harmful biotic soil factors. Marshalls (1965) argument about the physical ageing of *Ammophila* can be countered by the fact that a grass plant cannot age, only the tillers and leaves age and die, but are replaced. *Ammophila* roots, growing in stable sand dunes, are infected by harmful micro-organisms that reduce the root absorption function, this in turn reduces the formation of new tillers. By catching wind-blown sand, however, *Ammophila* continually receives fresh substrate, which prevents the build up of the pathogens. As soon as sand deposition stops, the root system is in some way impaired by the harmful biotic factor and the *Ammophila* vegetation starts degenerating because of self-intolerance. Self-intolerance, which is due to accumulation of pathogens in the rhizosphere (Scholte & Kupers 1977, 1978, Salt 1979, Schippers et al. 1985) also causes yield depressions in narrow rotation schemes of crops grown in monoculture. In natural ecosystems self-intolerance is almost unknown. It is known to occur in *Hippophaë rhamnoides* (Sea Buckthorn) a species which, like *Ammophila*, shows vigorous as well as degenerating phases in coastal sand dunes (Oremus 1982). Plant-parasitic nematodes (*Longidorus* sp. and *Tylenchorhynchus* sp.) could be related to a decline in vigour of *Hippophaë* (Oremus & Otten 1981, Maas et al. 1983). As plant-parasitic nematodes may also occur in the rhizosphere of *Ammophila* (Kisiel 1970, K. Kuiper pers. comm.) roots and rhizosphere sand of experiment 3 were analyzed. There were few plant-parasitic nematodes and it was, therefore, supposed that in this experiment nematodes were not the chief cause of self-intolerance in *Ammophila* (P.W.Th. Maas & H. Brinkman, unpublished).

The development of the root system of *Ammophila* on rhizosphere sand was restricted and the roots were brown. As no distinct macroscopic disease symtoms could be found, minor pathogens (Salt 1979) or harmful rhizosphere micro-organisms (Schippers et al. 1985) may cause the self-intolerance in *Ammophila*. The lowest specific uptake rates of N, P and K coincided with the severest growth reduction. It may be questioned if this low uptake rate is a cause or a result of the growth inhibition due possibly to the activity of toxin producing soil micro-organisms (Schippers et al. 1985).

To be able to manage coastal fore-dune ridges, it is obvious that more research needs to be done to uncover ecological relationships of *Ammophila* and its soil pathogens. In order to do this the organism(s) need to be identified and the mechanisms of parasitism, by which the degeneration occurs in the economically important *Ammophila*, need to be clarified.

CHARACTERIZATION OF SOIL ORGANISMS INVOLVED IN THE
DEGENERATION OF *AMMOPHILA ARENARIA*

W.H. Van der Putten, P.W.Th. Maas, W.J.M. Van Gulik and H. Brinkman

CHAPTER 5

CHARACTERIZATION OF SOIL ORGANISMS INVOLVED IN THE DEGENERATION OF AMMOPHILA ARENARIA

SUMMARY

In stable stages of coastal foredunes harmful soil organisms are supposed to be involved in the degeneration of *Ammophila arenaria* (marram grass). In an attempt to characterize the harmful organisms, biocides were applied to sand from the root zone of *A. arenaria* in which seedlings of *A. arenaria* were grown. In this way, sand from a stable and a mobile foredune (degenerated and vigorous *A. arenaria,* respectively) were examined.

Harmful soil organisms occurred in sand from stable, as well as from mobile foredunes. As bactericides (streptomycin and penicillin) had no effect on growth of seedlings of *A. arenaria* the involvement of harmful bacteria was excluded. The fungicide propamocarb only showed a very weak growth stimulating effect, whereas the fungicide benomyl increased growth significantly. However, as benomyl also prevented root infection by the nematodes *Heterodera* (*avenae* group) and *Meloidogyne maritima,* it could not be established to which extent soil fungi are actually involved in the degeneration of *A. arenaria.*

The highest increase in yield was obtained with the nematicide oxamyl, which prevented the roots from infection by endo-parasitic nematodes (*Heterodera avenae* group, *Meloidogyne maritima,* and *Pratylenchus* sp.). Oxamyl also reduced the numbers of ecto-parasitic nematodes. However, not all growth reduction could be prevented by applying oxamyl. Therefore, it is concluded that plant parasitic nematodes play an important role in the degeneration of *A. arenaria,* but that interactions with other groups of soil organisms, such as soil fungi, cannot be excluded.

INTRODUCTION

Ammophila arenaria (L.) Link (marram grass) occurs dominantly in coastal foredunes of north-western Europe, the Mediterranean, Australia, and along the west coast of the USA (Knutson 1978, Huiskes 1979). It is vigorous when regular burial by windblown sand occurs and degenerating at sites where sand deposition has stopped (Willis 1963, 1965, Marshall 1965, Hope-Simpson and Jefferies 1966, Huiskes 1979 and 1980). A similar response to sand burial is also shown by the North American species *Ammophila breviligulata* (Laing 1967, Eldred and Maun 1982, Disraeli 1984).

Several theories (listed by: Marshall 1965, Laing 1967, Eldred and Maun 1982) have been presented to explain the relationship between burial by windblown sand and vigour of *Ammophila*. In summary, it has been suggested that windblown sand (1) provides *Ammophila* with nutrients, (2) keeps away competing species, or (3) enables *Ammophila* to avoid physiological ageing by developing new adventitious roots. However, it has recently been demonstrated that the root zone of *A. arenaria* contains soil organisms which may strongly reduce plant growth, and it has been suggested that these organisms are involved in the degeneration of *A. arenaria* (Van der Putten et al. 1988).

Whereas some research has been done on the role of above-ground pathogens in vegetation succession (Weste 1981, Burdon 1987, Jarosz and Burdon 1988), only a few studies on the relationship between soil-borne pathogens and succession are known. In coastal sand dunes, e.g. the degeneration of *Hippophaë rhamnoides* (sea buckthorn) has been related to the occurrence of plant parasitic nematodes in the root zone (Oremus and Otten 1981, Maas, Oremus and Otten 1983). These harmful organisms apparently occur in specific stages of dune succession (Oremus and Otten 1981). The occurrence of soil micro-organisms in certain succession stages of coastal sand dunes has been related to physical and chemical soil factors (Brown 1958), and to plant growth (Webley et al. 1952). Similarly, the occurrence of harmful soil organisms in the root zone of *A. arenaria* (Van der Putten et al. 1988) may be related to certain succession stages of foredunes. Therefore, the present study on characterization of soil organisms harmful to *A. arenaria* was undertaken in two different stages of foredune succession i.e. a mobile foredune (10 to 30 cm of sand accretion per year and vigorous *A. arenaria*), and a stable foredune (no sand accretion and degenerated *A. arenaria*).

Characterization of the harmful soil organisms can either be performed by selective elimination of groups of organisms from rhizosphere soil, or by isolating specific organisms and, subsequently, inoculating them into

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sterilized, planted soil (Oostenbrink 1969, Bouhot 1979, 1982). In the present paper the effect of selective elimination - or inactivation - of soil bacteria, soil fungi, and nematodes from sand of mobile and stable foredunes on biomass production of planted *A. arenaria* is discussed.

MATERIALS AND METHODS

Study sites

Sand samples were collected from mobile stable foredunes at Rockanje, the Netherlands (51.52 N, 4.05 E). The mobile foredune was covered by tussocks with vigorous *A. arenaria* and was subject to burial by windblown sand (10 to 30 cm year^{-1}), originating from the beach. The stable foredune was not subject to sand deposition, and covered by an open and degenerated stand of *A. arenaria*. In this stage of succession the *A. arenaria* vegetation had been invaded locally by other species, mainly *Festuca rubra* ssp. *arenaria*.

Soil sampling and treatments

In February 1986 and in February 1987 (experiment 1 and 2, respectively), sand from mobile and stable foredunes was collected. Soil samples were taken randomly from the 0 to 20 cm layer containing roots of *A. arenaria*. The sand was sieved (5 mm mesh) and homogenized. Sifted roots were chopped and mixed through the soil. One half of each mixture was sterilized by gamma irradiation (2.5 Mrad).
Phytotoxicity of the biocides had been examined previously by testing different concentrations on seedlings of *A. arenaria* grown in quartz sand at pH 6. The highest concentrations not causing significant growth reduction were applied (concentrations of active material in mg.kg^{-1} dry soil):
Experiment 1: bactericides (a mixture of streptomycin sulphate, 50 mg.kg^{-1} and penicillin G Sodium 25 mg.kg^{-1}) and fungicides (a mixture of benomyl, 20 mg.kg^{-1} as Benlate and propamocarb, 20 mg.kg^{-1} as Previcur N, 722 g l^{-1} propamocarb-hydrochloride).
Experiment 2: fungicide (propamocarb, see experiment 1) fungicide (benomyl, see experiment 1) and nematicide (oxamyl, 100 mg.kg^{-1} as Vydate 10G).
Bactericides and fungicides were applied in suspension, and oxamyl in granular form.

Experimental design

In two pot experiments seedlings of *A. arenaria* were grown in the mixtures of soil and biocides.
Experiment 1: Twelve treatments were carried out in a 2x2x3 factorial design with factors: 'site' (i.e. succession stage), 'sterilization' and 'biocide' (none, bactericide mixture and fungicide mixture). Each treatment was carried out in twelve-fold.
Experiment 2: Sixteen treatments were carried out in a 2x2x4 factorial design with the factors: 'site' (i.e. succession stage), 'sterilization', and 'biocide' (none, propamocarb, benomyl, and oxamyl). Each treatment was carried out in twelve-fold.

Growing of plants

Experiments 1 and 2: Seeds of *A. arenaria* were collected at the foredune near Rockanje, stratified during 5 weeks at 4 °C, and germinated on glass beads at an 8/16 hour light/dark regime of 20/10 °C. Pots of 1.5 l were filled with 2170 g of sand (18% soil moisture), through which the biocides had been mixed. Each pot was planted with four *A. arenaria* seedlings of two weeks old and covered by tinfoil to protect the sand from desiccation. The pots were placed in a greenhouse at a temperature of 23 °C ($\pm$ 2 °C). Day length of 14 hours was obtained by additional illumination with Philips HLRG-400W (4.8 $W.m^{-2}$).
Soil moisture was maintained by supplying the pots every other day with demineralized water by weight. Nutrient solution (Hoagland; Hewitt 1966) was added weekly. To avoid depletion because of increased plant growth, amounts of supplied nutrients were raised regularly (weeks 1 to 5: 25 ml half strength; weeks 5 to 7: 25 ml full strength (1H); weeks 7 to 9: 50 ml 1 H; and after 9 weeks: 75 ml 1 H).

Plant harvests

Experiment 1: Four pots (replicates) were selected randomly at 6, 10, and 14 weeks after planting. Weights of roots and shoots were determined after drying for 48 hours at 70°C.
Experiment 2: Six pots (replicates) were selected randomly at 4 and 10 weeks after planting. The pots were examined as in experiment 1 and numbers of tillers and leaves, and tiller length were determined.

Analyses on nematodes (experiment 2 only)

On both harvest dates, two random pots were used for identification and

Table 1. ANOVA results (F-values) of shoot and root dry weight of <u>Ammophila arenaria</u> for the factors site (i.e. dune stage), sterilization and biocides at three harvest dates (growth period of 6, 10, and 14 weeks, respectively; experiment 1). MSE is mean squares of error.

Source of variation	df	harvest 1		harvest 2		harvest 3	
		Shoot	Root[1]	Shoot	Root	Shoot	Root
site	1	18.2 ***	57.2 ***	68.4 ****	8.1 **	6.6 *	15.1 ***
sterilization	1	652 ***	714 ***	309 ***	208 ***	44.6 ***	121 ***
biocide	2	13.5 ***	22.6 ***	60.3 ***	3.8 *	17.7 ***	15.5 ***
site x sterilization	1	0.4 ns	17.2 ***	0.2 ns	0.5 ns	8.1 **	3.0 ns
site x biocide	2	13.6 ***	9.8 ***	7.8 **	1.9 ns	1.9 ns	0.8 ns
sterilization x biocide	2	70.0 ***	37.0 ***	54.2 ***	29.4 ***	3.2 ns	2.8 ns
site x sterilization x biocide	2	9.6 ***	36.0 ***	7.3 **	0.3 ns	4.1 *	3.7 *
MSE	36	0.0021	0.0376	0.0555	0.0505	1.005	0.4837

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$ ns not significant

1) ln transformation

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counting of nematodes. The soil was examined according to Oostenbrink (1960) and large nematode species were collected according to an Oostenbrink method modified by Maas and Brinkman (1980). Nematodes from root tissue were collected by means of a maceration and floatation method (Coolen and d'Herde 1972).

Data analyses

The data were analysed statistically by means of analysis of variance (ANOVA), after testing for homogeneity of variances by means of Cochran's Q. Treatment means were compared by Tukey's test (Sokal and Rohlf 1981).

RESULTS

Experiment 1

In sand from the stable, as well as from the mobile dunes the dry matter production of shoots and roots was increased significantly by soil sterilization ($P<0.05$, Fig.1). In unsterilized sand, the production was significantly lower in the mobile stage than in the stable stage, whereas no difference between the two stages occurred when the sand was sterilized ($P<0.05$; these differences are not indicated in the figure). At the end of the growing period, the effect of soil sterilization diminished, which is shown by the decreasing F-values in the ANOVA (Table 1). Consequently, the effects of the biocides became less apparent and, therefore, only the results of harvests 1 and 2 have been presented (Fig. 1). At the first harvest the effect of the biocidal treatment depended strongly on the soil sterilization and also on the site ($P<0.001$; Table 1). The importance of the interactions, however, became less significant at the end of the growing period (Table 1).
Application of bactericides to sand from the mobile and stable dunes did not affect shoot and root production significantly ($P<0.05$, Fig.1). On the other hand, in sand from the mobile stage the fungicides increased growth of shoots and roots significantly ($P<0.05$, Fig.1). However, in sand from the stable stage, the fungicides only increased shoot growth significantly at the second harvest (Fig.1).
In unsterilized sand from the mobile stage with fungicides, shoot production was not significantly different from the sterilized sand, but in the former root production was significantly lower ($P<0.05$; Fig.1). However, since this root production was not lower ($P>0.05$) than in

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Fig.1. Mean shoot and root dry matter (g.pot^{-1}) of *Ammophila arenaria* grown in unsterilized and sterilized sand from the mobile and the stable foredune at two harvest dates 6 and 10 weeks after planting (experiment 1). The sand had been treated with bactericides (streptomycin + penicillin) or funcicides (benomyl + propamocarb). Per individual plot, significant differences (P<0.05) are marked with different letters.

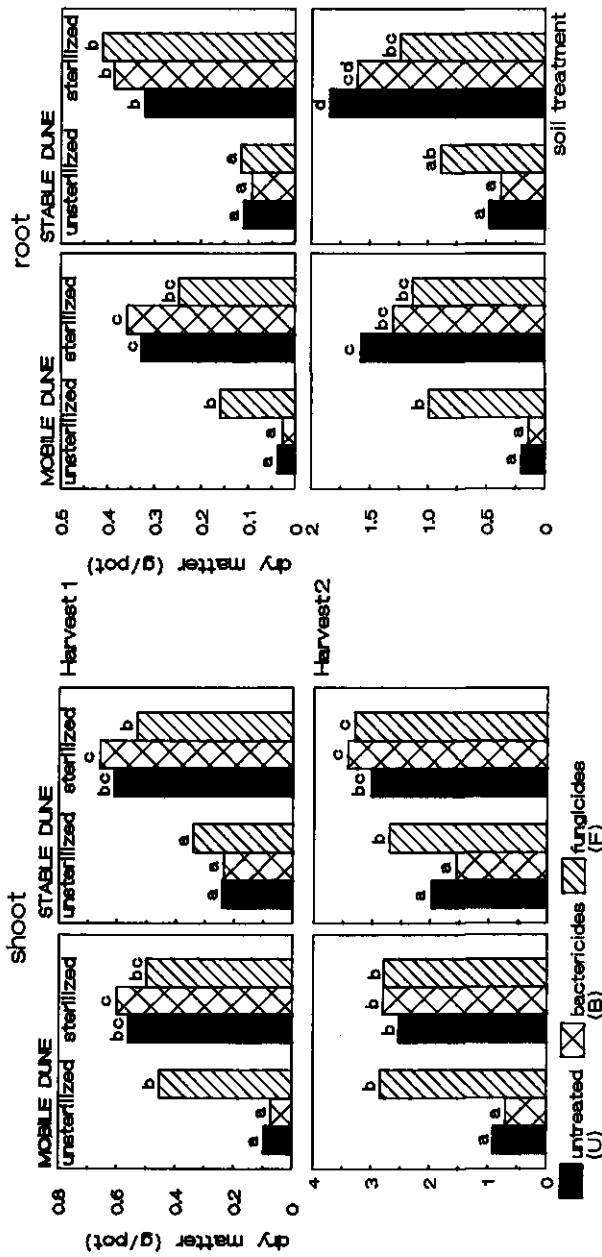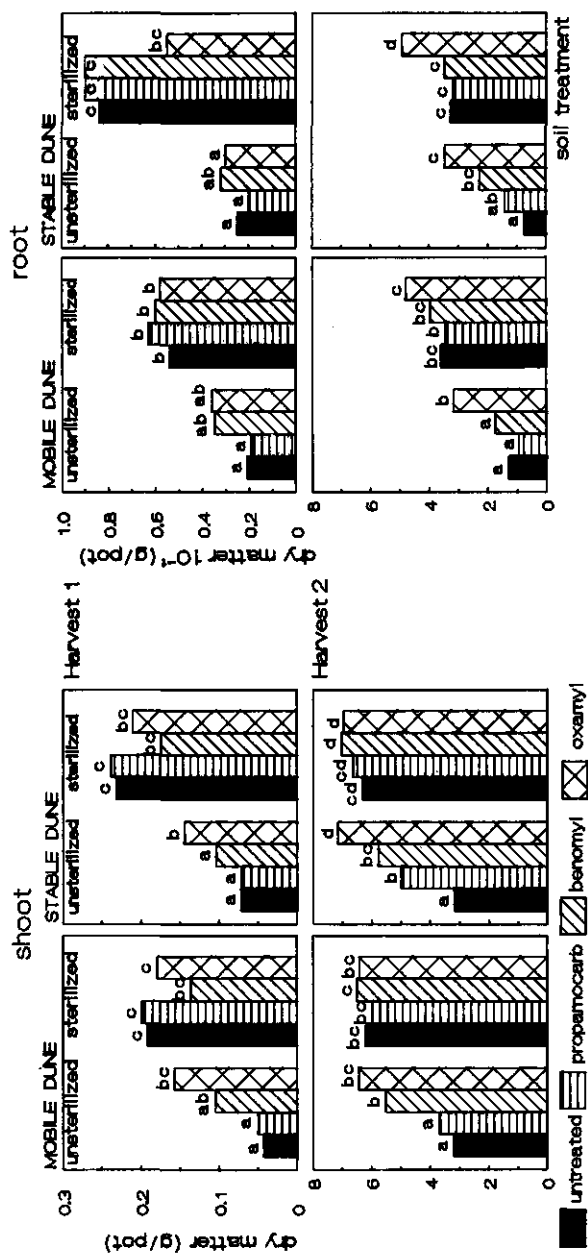Fig.2. Mean shoot and root dry matter (g.pot⁻¹) of *Ammophila arenaria* in unsterilized and sterilized sand from the mobile and the stable foredune at two harvest dates 4 and 10 weeks after planting (experiment 2). The sand had been treated with the fungicides propamocarb and benomyl, or the nematicide oxamyl. Per individual plot significant differences (P<0.05) are marked with different letters.

sterilized sand with fungicides apparently the fungicides caused some slight reduction in root growth (Fig. 1).

Experiment 2

Plant growth. As was shown in experiment 1, soil sterilization increased production of shoots as well as roots significantly in sand from the stable and the mobile dunes (P<0.05; Fig.2). The factor soil sterilization was highly significant for the production of shoots and roots (P<0.001; Table 2). However, in this experiment no significant growth differences occurred between the two sites either unsterilized or sterilized (P<0.05, this is not indicated in Fig.2).
At the first harvest, treatment with oxamyl significantly increased shoot biomass (P<0.05; Fig.2; Table 2). In the sand from the mobile dune the same production was reached with oxamyl as in the sterilized sand (Fig.2). The effect of oxamyl on the number of tillers and on the shoot length differed per sand type, whereas in both sand types the addition of oxamyl, as well as benomyl resulted in a higher number of leaves (P<0.05; Table 3).

Table 2. ANOVA results (F-values) of shoot and root dry weight of Ammophila arenaria for factors site (i.e. dune stage), sterilization and biocides at two harvest dates (growth period of 4 and 10 weeks, respectively; experiment 2). MSE is mean squares of error.

Source of variation	df	harvest 1		harvest 2	
		shoot	root[1]	shoot	root
site	1	5.57 *	1.11 ns	29.1 ***	0.02 ns
sterilization	1	250.6 ***	174.6 ***	245.2 ***	227.6 ***
biocide	3	10.89 ***	1.17 ns	87.35 ***	45.04 ***
site x sterilization	1	9.68 **	6.70 *	0.16 ns	2.68 ns
site x biocide	3	1.20 ns	2.09 ns	3.75 *	1.23 ns
sterilization x biocide	3	22.01 ***	5.58 **	47.34 ***	2.37 ns
site x sterilization x biocide	3	1.00 ns	1.01 ns	1.25 ns	1.14 ns
MSE	68[2]	1108	0.1305	0.2232	0.4018

* P < 0.05   ** P < 0.01   *** P < 0.001   ns not significant
1) ln transformation
2) df = 64 at harvest 2

The factor biocides again affected the production of shoots and roots at the second harvest (P<0.001; Table 2). In both sand types a higher dry matter yield of shoots and roots was obtained by the application of oxamyl (P<0.05; Fig.2). Oxamyl increased the numbers of tillers and leaves, whereas increase of shoot length was significant in sand from the stable dune only (P<0.05; Table 3). In the sterilized sand, however, root growth

Table 3. Numbers of tillers and leaves, and shoot length of Ammophila arenaria grown in sterilized and unsterilized sand from the mobile and the stable dune at two harvest dates (growth period of 4 and 10 weeks, respectively; experiment 2). The sand has been treated with the fungicides propamocarb, benomyl, or the nematicide oxamyl. Per sand origin, data followed by the same character are not significantly different (P<0.05).

biocide	sterilization	harvest 1			harvest 2		
		tillers ($n.pot^{-1}$)	leaves ($n.pot^{-1}$)	shoot length (cm)	tillers ($n.pot^{-1}$)	leaves ($n.pot^{-1}$)	shoot length (cm)
sand mobile dune							
none	–	4.0 a	10.3 a	12.2 a	26.0 a	88.3 a	57.0 a
	+	7.8 bc	22.0 d	22.0 c	30.3 abc	115 abc	64.7 a
propamocarb	–	4.0 a	12.3 ab	13.6 ab	27.8 ab	95.8 ab	60.1 a
	+	8.2 c	22.2 d	22.4 c	30.3 abc	120 bc	59.6 a
benomyl	–	5.0 ab	15.8 bc	15.2 ab	33.5 abc	114 abc	63.1 a
	+	7.0 abc	20.2 cd	19.2 bc	35.7 bc	130 c	67.1 a
oxamyl	–	6.0 abc	18.0 cd	19.8 bc	36.3 bc	132 c	58.0 a
	+	8.3 c	21.5 d	19.6 bc	38.3 c	139 c	64.2 a
sand stable dune							
none	–	4.3 a	12.3 a	16.4 a	21.8 a	77.8 a	44.7 a
	+	8.5 b	23.3 d	23.3 b	25.3 ab	101 ab	69.3 bc
propamocarb	–	2.7 a	13.5 ab	17.0 a	34.0 cd	117 bc	55.8 b
	+	10.0 b	25.7 d	23.3 b	31.0 bc	123 bc	66.4 bc
benomyl	–	3.8 a	17.5 bc	18.8 ab	40.3 d	143 c	60.2 bc
	+	9.3 b	24.7 d	20.4 ab	38.2 cd	142 c	68.2 c
oxamyl	–	8.3 b	22.0 cd	17.1 a	35.5 cd	132 c	65.0 bc
	+	9.7 b	25.8 d	20.2 ab	39.8 d	145 c	60.6 bc

was also increased by oxamyl. Benomyl increased shoot production significantly in sand from the stable dune resulting in a higher number of tillers and leaves as well as greater shoot length. The shoot yield in unsterilized sand with benomyl, however, was lower than in sterilized sand with benomyl (P<0.05; Fig.2, Table 3). Soil treatment with benomyl increased root yield and supply of propamocarb resulted in a higher shoot yield, as well as more tillers and leaves, and increased shoot length in sand from the stable dune only (P<0.05; Fig.2, Table 3).

Nematodes. In both sand types the endo-parasitic nematodes *Heterodera* (*avenae* group) and *Meloidogyne maritima* occurred, whereas *Pratylenchus* sp. was restricted to the stable dune (Table 4). Roots grown in soil treated by oxamyl did not contain plant parasitic nematodes. Benomyl protected roots from infection with *Heterodera* sp. and *Meloidogyne maritima*, but it gave no protection from infection with *Pratylenchus* sp. (Table 4).

In sand from the stable dune the numbers of *Pratylenchus* sp. per pot had increased between first and second harvest if not treated with oxamyl. In the pots with benomyl, the numbers of *Pratylenchus* sp. were about ten times higher than in the pots with propamocarb or without biocides (Table 4). Infection levels of *Heterodera* sp. and *Meloidogyne maritima* in the roots were very low.

DISCUSSION

Harmful soil organisms occurred in sand from the stable, as well as from the mobile foredunes. However, notwithstanding their presence in mobile foredunes, *A. arenaria* grows vigorously at this stage of succession. To explain this vigour it has been suggested that the colonization of windblown sand allows *A. arenaria* to escape continually from its soil-borne pathogens (Van der Putten et al. 1988). This 'escape' theory is supported by observations that the degeneration of *A. arenaria* starts very soon after sand deposition has stopped (Marshall 1965, Hope-Simpson and Jefferies 1966). More evidence for this escape theory will be given in subsequent papers.

The effect of biocides on plant growth may be due to elimination of their target organisms (specificity; e.g. Edgington et al. 1980), by elimination of non-target organisms (non-specificity; Rodriguez-Kabana and Curl 1980, Parker et al. 1985, Weischer and Müller 1985), or to artefacts, e.g. enhanced mineralization or the occurrence of growth-promoting (or inhibiting) compounds in the biocides (Powlson 1975). Therefore, the effects of application of biocides on plant growth in unsterilized sand has been compared with those in sterilized sand with and without biocides, and

Table 4. Nematodes in sand from the mobile and the stable dune in 100 ml of soil before the experiment was started (T$_0$), and in 100 ml of soil and in 1 g of fresh roots of <u>Ammophila arenaria</u> at two harvest dates (growth period of 4 and 10 weeks, T$_1$ and T$_2$, respectively; experiment 2). n = no biocides, p = propamocarb, b = benomyl, o = oxamyl.

	Sand from mobile dune									Sand from stable dune								
	T$_0$	T$_1$				T$_2$				T$_0$	T$_1$				T$_2$			
	-	n	p	b	o	n	p	b	o	-	n	p	b	o	n	p	b	o
Soil																		
Paratylenchus sp.	0	0	0	0	0	0	0	0	0	2.5	0.3	0	0.3	1.3	0	0.3	2.8	0
Paratylenchus	0	0	0	0	0	0.3	0	2.8	0	0	0	0	0	0	1.8	0	0	0
Telotylenchus ventralis	1	1.3	0.3	0	0		1	0.5	0	4	1	0.8	0	0	2.8	13.5	0.3	0
Rotylenchus goodeyi	4	6.4	4.5	2.5	0	1.8	1.8	1	0.5	6	6.8	3.5	4.8	0.5	3.3	4.5	1	0.8
Criconematidae	0.5	0.9	0.3	0.5	0	1.5	1.5	0	0	0.5	0	0.3	0	0	0	0	0	0
Heterodera (avenae gr.)[1]	11.5	0.9	0.3	15.5	7.5	0	0	0.3	0	1	1.5	0.8	3	0.5	0	0	0.8	0
Heterodera (avenae gr.)[2]	25	113	23	59	38	0	45	38	85	0	188	0	18	13.5	0	25	120	10
Meloidogyne maritima	4.5	0.3	0.3	2	2	0	0	0	0	24.5	0	0	4.5	0	0	0	0	0
Saprobiotic	188	97	150	13.5	25	127	286	149	136	157	149	178	10.8	15	226	236	139	257
Roots																		
Pratylenchus sp.	-	0	0	0	0	0	0	0.2	0	-	**57**	**80**	**86**	0	**7**	**5.1**	**47**	**0.5**
Heterodera (avenae group)	-	27	56	0	0	0.4	0.5	0.2	0.2	-	**87**	**133**	0	0	0	0.2	0.2	0
Meloidogyne maritima	-	4.5	20	0	0	0.6	2.8	0	0	-	**28**	**50**	0	0	0	0.1	0	0
Saprobiotic	-	121	139	0	11.5	7	2.8	12.3	6.8	-	**80**	41	0	2.3	6.9	5.8	19.1	2.1

1) free larvae   2) larvae in cysts

the fungicides have been checked for nematotoxicity.

Soil treatment with bactericides did increase plant growth neither in sand from the stable nor in sand from the mobile dune. Anderson and Domsch (1975) found high percentages of elimination of soil bacteria with 250 mg/kg of streptomycin. The complete absence of growth stimulation by bactericides in the present experiments, although the applied concentrations were lower than 250 mg/kg, at least gives no support to the possible involvement of bacteria in the degeneration of *A. arenaria*. Moreover, the results of the experiments with the biocides suggested that other groups of soil organisms were responsible for the poor growth of *A. arenaria*, since an increase in growth was observed in the plants treated by the fungicides and oxamyl.

The growth stimulating effect of the fungicide mixture was mainly due to benomyl, which has a broad spectrum and is active on fungi belonging to *Ascomycetes*, *Deuteromycetes*, and *Basidiomycetes* (Bollen and Fuchs 1970). However, the involvement of soil fungi in growth reduction of *A. arenaria* cannot be established unequivocally by the experiments described in this paper, because benomyl also eliminated the nematodes *Heterodera* (*avenae* group) and *Meloidogyne maritima*. Nematotoxicity of benomyl has been demonstrated for *Heterodera avenae* (Weischer and Müller 1985, and Rodriguez-Kabana and Curl 1980), as well as for some *Meloidogyne* species (Rodriguez-Kabana and Curl 1980). On the other hand, propamocarb generally had no effect on plant performance. Therefore, since propamocarb is specific for *Phycomycetes* (Sijpestijn et al. 1974), these fungi will be of little, or no, importance for the degeneration of *A. arenaria*.

In both sand types, the nematicide oxamyl increased shoot production significantly, whereas root yield was increased only at the second harvest. However, since in sterilized sand from the stable dune the application of oxamyl also caused a significant increase in growth, the effect of oxamyl on root yield may have been partly due to a direct stimulation, such as has also been observed by Tu (1980) and Mathur et al. (1980). Hence, in the unsterilized sand oxamyl increased shoot yield more than root yield.

In general, oxamyl has little effect on non-target organisms (Bunt 1975, Bollen 1979, Tu 1980, Parker et al. 1985), although some effects on non-target organisms have been reported (Mathur et al. 1980). However, such effects mostly occur in specific situations, e.g. *in vitro* conditions poor in nutrients (Hofman and Bollen 1987), or in non-target organisms that are dependent on nematodes, e.g. for transport from the soil to the roots (Bunt 1975). Therefore, at least part of the growth reduction of *A. arenaria* can be related to the activity of nematodes. Oxamyl effectively protected the roots from being colonized by the endo-parasitic nematodes *Pratylenchus* sp., *Heterodera* (*avenae* group) and *Meloidogyne maritima*. It even

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eliminated part of the ecto-parasitic nematode population in the soil. As the yield-increasing effect of benomyl was also correlated with a complete absence of the endo-parasitic nematodes *Heterodera* (*avenae* group) and *Meloidogyne maritima*, apparently these species are strongly involved in growth reduction of *A.arenaria*. The *Heterodera* species resembles *H. hordecalis* (Anderssen 1974), but lengths of juveniles and of stylets were not indentical (H. Brinkman, unpublished results). *M. maritima* has been described as parasitising roots of *A. arenaria* (Jepson 1987). Causal relationships between growth reduction of plants and numbers of *Pratylenchus* sp. occurring in the roots cannot be unequivocally be established, since in sand from the stable dune supplied with benomyl numbers of *Pratylenchus* sp. increased between the first and second harvest, whereas growth of roots and shoots was also increased.

In the present experiment, growth reduction of the roots could not be completely nullified by elimination of the endo-parasitic nematodes with oxamyl or benomyl. Apparently, some additional biotic factor was involved in the growth reduction of *A.arenaria*. Preliminary inoculation experiments with nematodes and fungi collected from the roots of *A. arenaria* have indicated that the presence of both nematodes and soil fungi may enhance growth reduction as compared to when soil fungi are present only (W.H. Van der Putten and W.J.M. Van Gulik, unpublished results). Hence, degeneration of *A. arenaria* may be related to a complex of plant parasitic nematodes and soil fungi.

HARMFUL SOIL ORGANISMS IN COASTAL FOREDUNES CAUSING DEGENERATION OF *AMMOPHILA ARENARIA* AND *CALAMMOPHILA BALTICA*

W.H. Van der Putten and S.R. Troelstra

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CHAPTER 6

HARMFUL SOIL ORGANISMS IN COASTAL FOREDUNES CAUSING DEGENERATION OF AMMOPHILA ARENARIA AND CALAMMOPHILA BALTICA

SUMMARY

The presence of harmful soil organisms in the root zone of *Ammophila arenaria* (marram grass) was examined by biotesting. For this investigation three locations along the sandy shoreline of the Netherlands were chosen: Voorne, Texel, and Schouwen. At all three locations harmful organisms had been detected in sand from stable dunes, as well as in sand from mobile dunes (degenerated and vigorous *A. arenaria*, respectively). In beach sand, however, no harmful organisms occurred. Since *A. arenaria* shows vigorous growth only when it is buried regularly by windblown sand from the beach, it is concluded that this sand deposition enables the plants to escape from harmful soil organisms.
Ammophila arenaria and *Calammophila baltica* (purple or hybrid marram grass) from the Voorne location were grown outdoors in containers filled with sand from the beach, the mobile, and the stable dunes, and sterilized sand from the stable dunes. Biomass production of both species was highest in sterilized sand from the stable dune, followed by (in descending order) beach sand, sand from the mobile dune, and unsterilized sand from the stable dune. As compared with *A. arenaria*, however, growth of *C. baltica* was reduced less and without mortality of cuttings. The degree of growth reduction by the harmful soil organisms could not be related to the numbers of plant parasitic nematodes.

INTRODUCTION

At coastal sand dunes the vegetation is subject to a constant change in species composition. This process of succession of species has been studied intensively. Environmental factors, such as wind, salt spray, nutrient enrichment, and decalcification, were found to be initiating the succession of coastal dune vegetation (Cowles 1899, Van Dieren 1934, Oosting and Billings 1942, Salisbury 1952, Olson 1958, Wallén 1980).
Ammophila arenaria L (Link) (marram grass) is a perennial grass species occurring naturally on coastal foredune ridges in Europe, the Mediterranean, Australia, and along the west coast of North America (Knutson 1978, Huiskes 1979). *Calammophila baltica* (purple or hybrid marram grass, Rihan and Gray 1985) is a hybrid between *A. arenaria* and *Calamagrostis epigejos*. It occurs in foredunes of north-western Europe, but less dominant than *A. arenaria* (Westergaard 1943, Kubien 1970). In the field *C. baltica* can be distinguished from *A. arenaria* because the former produces less dense tussocks, has more flattened, supple and greener leaves, and infertile flowers (Olsson 1974, Heukels and Van der Meijden 1984). *Ammophila arenaria* and *C. baltica* are planted to control erosion of coastal foredunes.
Ammophila arenaria is well known because of its ability to survive sand burial of 80 to 100 cm per year (Huiskes 1979). It not only withstands sand burial, but it even requires the accretion of fresh, windblown sand for the maintenance of vigour, because the plants decline soon after the sand accumulation ceases (Marshall 1965, Hope-Simpson and Jefferies 1966, Huiskes 1979). In its response to burial by sand, *A. arenaria* resembles *Ammophila breviligulata* (American beach grass), a native grass species of the east and west coast and of the Great Lakes of North America (Laing 1958, 1967, Eldred and Maun 1982, Disraeli 1984, Maun and Lapierre 1984, Maun and Baye 1989). *Calammophila baltica* shows an ecological response that is similar to *Ammophila* (Olsson 1974, Wallén 1980), although the relation between its vigour and sand deposition has not been studied experimentally.
Many efforts have been made to explain the relationship between accumulation of fresh, windblown sand and vigour of *Ammophila*. Possible explanations, regarding drifting sand as a source of nutrients, at the same time removing competing plant species, or enabling *Ammophila* to avoid physiological ageing, have been summarized by Marshall (1965), Laing (1967), and Eldred and Maun (1982). Recently, it has been found that the root zone of *A. arenaria* contained harmful soil organisms, which impaired its growth (Van der Putten et al. 1988). It was concluded that the decline of *A. arenaria* is mainly due to the development of harmful organisms in

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its root zone (Van der Putten et al. 1988). Consequently, the accumulation of fresh, windblown sand (usually originating from the beach) may allow *A. arenaria* to escape from these harmful soil organisms. Degeneration at sites where sand deposition has ceased could be explained by the absence of an escape route (Van der Putten et al. 1988).

In the above-mentioned hypothesis two assumptions have been made:

(1) If harmful soil organisms are involved in the decline of *Ammophila* they are expected to be ubiquitous in randomly chosen locations with degenerating plants.

(2) If fresh sand enables *Ammophila* to escape from harmful soil organisms in its root zone, these organisms are assumed to be absent in the windblown sand originating from the beach.

These two assumptions will be examined more closely in the present paper. At different locations (Voorne, Texel, and Schouwen), sand from three sites (beach, mobile dune, and stable dune) was examined on the presence of harmful soil organisms. In addition, the response of plant populations from the Voorne, Texel and Schouwen locations to harmful soil organisms from remote locations was studied. Moreover, the susceptibility of *A. arenaria* to the harmful soil organisms was compared outdoors with that of *C. baltica*. In the greenhouse, *C. baltica* had been less sensitive to these organisms than *A. arenaria* (Van der Putten et al. 1988). However, since growth of the latter was stronger reduced outdoors than in the greenhouse (Van der Putten et al. 1988), it was assumed that growth of *C. baltica* is also reduced outdoors.

The results of the experiments are discussed with respect to the interaction between harmful soil organisms and natural environmental factors in coastal foredunes, and its impact on succession in foredune vegetation.

MATERIALS AND METHODS

Experiment 1

Study sites

Sampling sites were chosen at three island locations in the Netherlands: Voorne (432-62; co-ordinates refer to the Dutch State Survey Grid, SSG), Texel (561-109), and Schouwen (416-38). Voorne and Schouwen are situated in that part of the Dutch coastal dune area which has a high calcium carbonate content, whereas the calcium carbonate content of the dunes of Texel is low. At each location *A. arenaria* is planted frequently in order to protect the foredunes from sand erosion. The local wardens have for a long time almost exclusively planted locally gathered *A. arenaria*

plant material.

At these locations, three extreme stages in the foredune vegetation succession could be distinguished: the beach above the average high-water level (about 20 m in front of the first plant growth), the mobile dune (vigorous *A. arenaria* and *C. baltica*, no other dominant plant species), and the stable dune (degenerating *A. arenaria* and *C. baltica* with *Festuca rubra* ssp *arenaria* as the main other species.). At Voorne, the beach had been artificially supplied with sea sand three years before sampling, to protect the foredune against erosion by the sea. At the sampling location Texel, sand erosion and sand accretion on the beach were roughly in balance, whereas foredune erosion occurred occasionally at Schouwen. In all areas under study, the mobile dune was subject to moderate rates of sand accretion (10-30 cm year^{-1}). The stable dune rarely received any windblown sand.

Analysis of soil characteristics

After drying (35 °C) and sieving (2-mm mesh), bulk soil samples were mechanically subdivided. Part of the samples was ground in a mortar mill, and, depending on the type of determination, analyses were performed on either ground or unground samples.

The pH of the soil was measured potentiometrically in 1:2.5 (w/v) suspensions of H_2O or 1 *M* KCl. Carbonates were measured gas-volumetrically by treating samples with 4 *M* HCl. Organic matter was determined as loss-on-ignition at 430 °C for 24 h. Total N and P were measured colorimetrically in single soil digests (Novozamsky et al. 1984). Exchangeable cations were determined by atomic absorption spectrophotometry after shaking soils with neutral ammonium acetate. Chloride and electrical conductivity analyses were carried out on 1:5 water extracts. The granular composition (soil texture) of the samples was determined by dry-sieving.

Collecting of the seeds

In July 1987, spikes of *A. arenaria* were collected from a 100x20 m^2 area in the mobile dunes of Voorne, Texel, and Schouwen, which were also the sites where the sand was to be sampled. The spikes were dried and threshed. The 1000-kernel weights of the seeds were determined in 5-fold (100 seeds at each time).

Sampling, sand treatment, and experimental design

In September 1987, samples were taken at each location from the beach, as well as from the mobile and from the stable dunes. Sampling was carried out along four parallel transects (replicates), perpendicular to the shoreline and 25 m apart. At each sampling site, sand was collected from the upper part of the soil profile (0-20 cm) within a 1-m^2 plot. Roots of *A. arenaria* were present in this layer at the mobile and stable sites. The sand samples were sieved (5-mm mesh), coarse fractions such as shells were removed

and half of each sand sample was sterilized by means of gamma irradiation (2.5 Mrad, see Oremus 1982).

Treatments were carried out in a 3x3x2 factorial design with factors: location (Voorne, Texel, and Schouwen), sand type (beach, mobile, and stable dune), and sterilization (sterilized and unsterilized). As indicated above, each treatment was replicated four times.

Growing and harvesting of plants

Pots of 1.5 l were filled with 1950 g of sand containing 15% moisture, based on dry sand weight. Seeds were germinated at 8/16 hour 30/20 °C in light/dark. Uniform, two-week-old seedlings (plumula length 3-5 cm) were selected and planted in the pots (four per pot). Voorne seedlings were planted in sand from Voorne, Texel seedlings in sand from Texel, and Schouwen seedlings in sand from Schouwen. The pots were placed in a greenhouse at 23 ± 2 °C (September - October 1987), and illumination (Philips HLRG-400 W, 4.8 $W.m^{-2}$) was supplied to keep the photoperiod at 12-14 $hours.day^{-1}$. Every other day the pots received demineralized water to maintain the soil moisture content at 15%. Hoagland nutrient solution (Hewitt 1966) was supplied weekly to each pot at the following rates (1H = full strength solution): 25 ml 1/2 H (weeks 1+2), 25 ml 1 H (weeks 3+4), and 50 ml 1 H (weeks 5+6). The plants were harvested after 44 days. Dry weights of shoots and roots were determined after drying for 48 hours at 70 °C. Shoot/weight ratio (SWR) was calculated as shoot dry weight/ (shoot + root dry weight).

Experiment 2

Simultaneously with experiment 1, seedlings of the Voorne population were planted in pots with sterilized and unsterilized sand from the mobile and stable dunes of the locations Texel and Schouwen. In the same way, seedlings of the Texel and Schouwen populations were planted in sand from remote origin. The sampling of the sand, collecting of the seeds, and the growing and harvesting of the plants were performed as in experiment 1.

Treatments were carried out in a 3x3x2x2 factorial design with factors: location (Voorne, Texel, and Schouwen), population (Voorne, Texel, and Schouwen), sand type (mobile and stable dune), and sterilization (sterilized and unsterilized). Each treatment was replicated four times.

Experiment 3

Sampling, sand treatment, and experimental design

In May 1986, sand was collected at location Voorne from the beach, and the mobile and stable dunes (same sites as in experiments 1 and 2). At each

site, sand was collected from the 0-20 cm depth and within an area of 5x5 m^2. A subsample was taken for identification and counting of nematodes (see below). Containers of 50 l were filled with 70 kg of sand (moisture content 4%, based on dry weight). Additional containers were filled with sand from the stable dune that had been sterilized by means of gamma irradiation (2.5 Mrad). Containers were planted either with *A. arenaria* or with *C. baltica*. Treatments were carried out in a 4x2 factorial design with factors: sand type (beach, mobile, stable unsterilized, stable sterilized), and plant species (*A. arenaria* and *C. baltica*). Each treatment was replicated four times.

Collecting, growing, and harvesting of plants

Cuttings of *A. arenaria* and *C. baltica* were obtained from locally collected plants. Underground stem parts of 4 cm length, each with one bud, were grown in large flats (Van der Putten et al. 1988). Five two-week-old cuttings were planted per container. One of these cuttings was planted in a gauze case (height 30 cm, diameter 2 cm, and mesh size 100 μ), situated in the centre of the pot in order to collect nematodes from roots.

The containers were supplied with 3.25 g slow-release NPK fertilizer (Osmocote, 24-6-6, active for 9 to 12 months at 21 °C) to avoid interaction with possible nutrient flushes due to soil sterilization.

The plants were grown outdoors from June 4 until November 3, 1986. The containers were supplied daily with tap water until two weeks after planting, thereafter the plants were completely dependent on natural rainfall. On July 10, the plants with gauze cases were removed and the remaining pits filled with sand from the corresponding place of origin. The sand and roots in the gauze cases were examined on the presence and identity of nematodes. In November, the remaining four plants were harvested. Numbers of shoots and rhizomes were counted. Roots and shoots were dried for 48 hours at 70 °C and weighed. A random subsample of the fresh root material was used to determine the root length (Comair root length scanner, Australia). Roots and rhizosphere soil of one plant per container were collected in a cylinder (diameter 8 cm, height 20 cm) and examined for nematodes.

Sampling and identification of nematodes

Nematodes were identified in the sand before planting (May), 5 weeks (July 10) and 22 weeks (November 3) after planting the cuttings (see previous paragraph).

The active nematodes were separated from the soil samples (300 ml of sand, specific volume of 0.7 ml.g^{-1}) according to the method of Oostenbrink (1960). Nematodes present in and on the roots of one plant (July) or 5 g of the harvested root system (November) were identified and counted. The roots were macerated and centrifuged. Nematodes were removed by a floatation technique (Coolen and d'Herde 1972).

Plant analysis
Plant samples dried at 70 °C were used for analysis. In a sulphuric acid/salicylic acid digest, N and P were determined colorimetrically (indophenol-blue method and molybdenum-blue method, respectively), and K by atomic absorption. Ethanol-soluble carbohydrates were extracted from dried material with 80% ethanol. The ethanol-insoluble carbohydrates (starch) were hydrolysed by boiling in 3% HCl. Carbohydrates were determined using a modified Anthrone reagent (Fales 1951) and a calibration curve for glucose.

Data analysis

Data were analysed by means of analysis of variance (ANOVA) and, if necessary, they were transformed to obtain homogeneity of variances. Treatment means were compared by means of Tukey's test (Sokal and Rohlf 1981).
In experiment 2, the effect of sand sterilization was tested by pairwise

Table 1. Grain size distribution (%) of sand (0–20 cm depth) originating from the beach, the mobile, and the stable dunes, at the locations Voorne, Texel, and Schouwen.

Location	Sampling site	Grain size (μ)					
		< 75	75–106	106–150	150–212	212–300	300 $<$
Voorne	beach	2.1	20	44	21	8.4	4.5
	mobile dune	1.2	13	32	35	15	4.4
	stable dune	0.3	3.0	21	51	20	5.4
Texel	beach	0.1	0.1	3.3	51	43	2.9
	mobile dune	0	0.1	4.6	61	32	2.0
	stable dune	0.1	0.1	7.3	67	24	1.3
Schouwen	beach	0	0.2	3.0	38	53	5.7
	mobile dune	0	0.3	9.3	57	31	1.8
	stable dune	0.2	0.3	8.0	54	36	2.1

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Table 2. Chemical properties of sand (0-20 cm depth) from the beach, the mobile and the stable dunes, at locations Voorne, Texel, and Schouwen.

Location	Sampling site	pH(KCl)	CaCO$_3$ (%)	organic matter(%)	tot-N (mg/100 g)	tot-P	K (	Na meq/100 g	Mg)	Cl (mg/100g)	electro conductivity (dS.m^{-1})
Voorne	beach	8.8	7.4	0.27	7.8	8.0	0.08	0.47	0.69	4.4	0.09
	mobile dune	8.9	5.7	0.21	4.2	7.2	0.06	0.09	0.49	1.6	0.03
	stable dune	8.6	4.6	0.28	6.7	7.7	0.06	0.08	0.30	1.4	0.06
Texel	beach	8.8	0.3	0.07	3.6	3.7	0.04	0.32	0.16	3.0	0.04
	mobile dune	8.7	0.6	0.08	3.6	5.9	0.03	0.08	0.13	1.3	0.05
	stable dune	8.4	0.6	0.20	6.6	4.3	0.03	0.05	0.14	1.3	0.04
Schouwen	beach	9.3	2.7	0.15	2.7	7.2	0.06	0.60	0.38	14.8	0.13
	mobile dune	8.9	2.5	0.18	4.9	7.3	0.05	0.11	0.22	2.1	0.06
	stable dune	8.7	3.1	0.36	7.9	10.5	0.03	0.07	0.24	1.4	0.04

t-tests. Contrasts were calculated in the main factors 'location' and 'population', premised that interactions with other factors were absent, by splitting them into components with single degrees of freedom in the ANOVA (Sokal and Rohlf 1981).

RESULTS

Soil characteristics of the sampling locations

The soil texture of the locations Texel and Schouwen was very similar (Table 1). Compared to these locations the Voorne sand contained a higher percentage of fine to very fine sand (75–150 μ). Beach sand from Texel and Schouwen was slightly coarser than sand from the corresponding mobile and stable sites. At Voorne, however, beach sand was finer than sand from the other sites (Table 1).

The pH of all sand samples was high ($\geq$ 8.4; Table 2). Organic matter, potassium, total N, and total P were low in all sand samples, but generally lowest in the sand from Texel (Table 2). $CaCO_3$ content was highest in the Voorne sand and lowest in the Texel sand (Table 2). Per location, no large differences were observed in nutrient levels among the sampling sites; in general total N was highest in sand from the stable site, whereas salinity level was highest in sand from the beach.

Experiment 1

At all three locations a significant effect of sterilization on the dry matter yield of shoots and roots was observed (Table 3). The effect of sand type

Table 3. Degrees of freedom and F-values according to two-factor ANOVA (sand type and sterilization) of shoot and root dry matter of A. arenaria that was grown in sand from the locations Voorne, Texel, and Schouwen. MSE: mean squares of error.

Source of variation	df	Voorne		Texel		Schouwen	
		shoot	root	shoot	root	shoot	root
Sand type	2	5.32 *	16.2 ***	1.23 ns	0.63 ns	0.47 ns	1.50 ns
Sterilization	1	41.9 ***	39.2 ***	15.3 **	7.96 *	23.1 ***	15.7 ***
Sand x Sterilization	2	14.9 ***	4.01 *	4.67 *	0.13 ns	5.54 *	0.89 ns
MSE	18	31.4	22.5	55.3	69.5	70.9	39.1

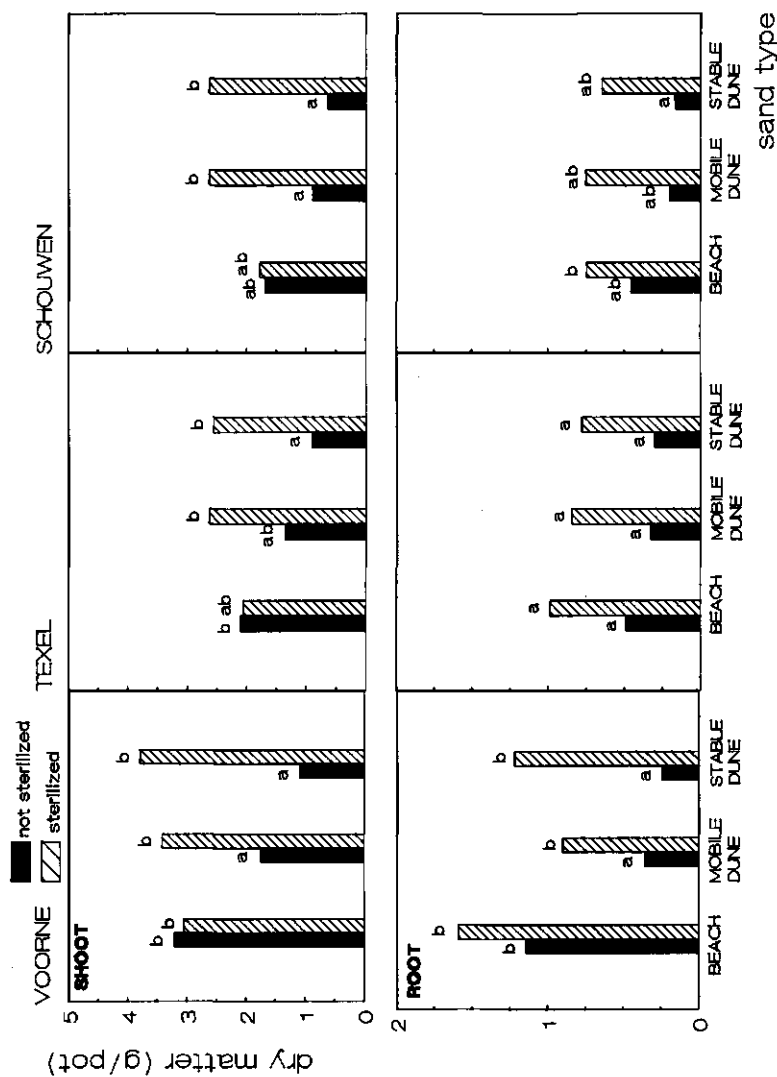* P < 0.05   ** P < 0.01   *** P < 0.001   ns not significant

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Fig.1. Dry matter production (g/pot) of shoots and roots of seedlings of *Ammophila arenaria* in sterilized and unsterilized sand samples from the beach, the mobile, and the stable dunes. The samples originated from the locations Voorne, Texel, and Schouwen. In each plot significant differences are indicated by different letters (P<0.05).

on the dry yield of roots and shoots, however, was significant at the Voorne location only. With regard to the production of shoots, the effect of soil sterilization was not the same for all sand types due to interaction between sand and soil sterilization (Voorne: $P<0.001$; Texel and Schouwen: $P<0.05$). Sterilization caused an increase in shoot production only in the sand from the mobile and the stable dune, and not in the beach sand (Fig.1).

At location Voorne, root yield had been increased significantly in sand from the mobile and stable dunes by sterilization, but not in beach sand ($P<0.05$; Fig.3). The same trend was observed for locations Texel and Schouwen. However, notwithstanding the significant sterilization effect in the ANOVA (Table 3), the effect of sterilization was not significant for the individual sand types ($P<0.05$; Fig. 1).

The shoot/weight ratio (SWR) was significantly affected by the factors sand type and sterilization at the location Voorne only (data and results of ANOVA not shown). The SWR was lower in beach sand than in sand from the mobile and the stable dunes, and lower in sterilized than in unsterilized sand ($P<0.05$).

Experiment 2

In the experiment with seedlings of *A. arenaria* grown in sand of local and foreign origin, soil sterilization increased shoot and root growth significantly in all cases ($P<0.05$; Fig. 2; results of pairwise t-tests are not indicated). The shoot yield was affected by all main factors (location: $P<0.05$; population: $P<0.001$; sand type: $P<0.01$; and soil sterilization: $P<0.001$; Table 4). Calculation of contrasts showed that the significant location effect in the ANOVA was caused by a higher shoot production in sand from Texel, as compared to that in sand from Voorne and Schouwen ($P<0.05$; Table 4). Root weight and SWR generally showed the same trend in the ANOVA as shoot dry matter. However, the location effect was not significant and root production showed no significant effect of sand type (Table 4).

The effect of soil sterilization on shoot yield was not the same for the mobile and the stable dune, due to interaction between sand type and sterilization ($P<0.01$; Table 4). Nevertheless, soil sterilization increased shoot and root yields of all populations significantly ($P<0.05$), irrespective of the origin of the sand (Fig. 2). The absence of a significant interaction between the factor population and the other factors emphasizes the generally similar reaction of the three populations of *A. arenaria* with respect to different sand origins and soil sterilization (Table 4). There was a significant difference, however, in dry matter production among the populations ($P<0.001$; Table 4). Calculation of contrasts in the ANOVA
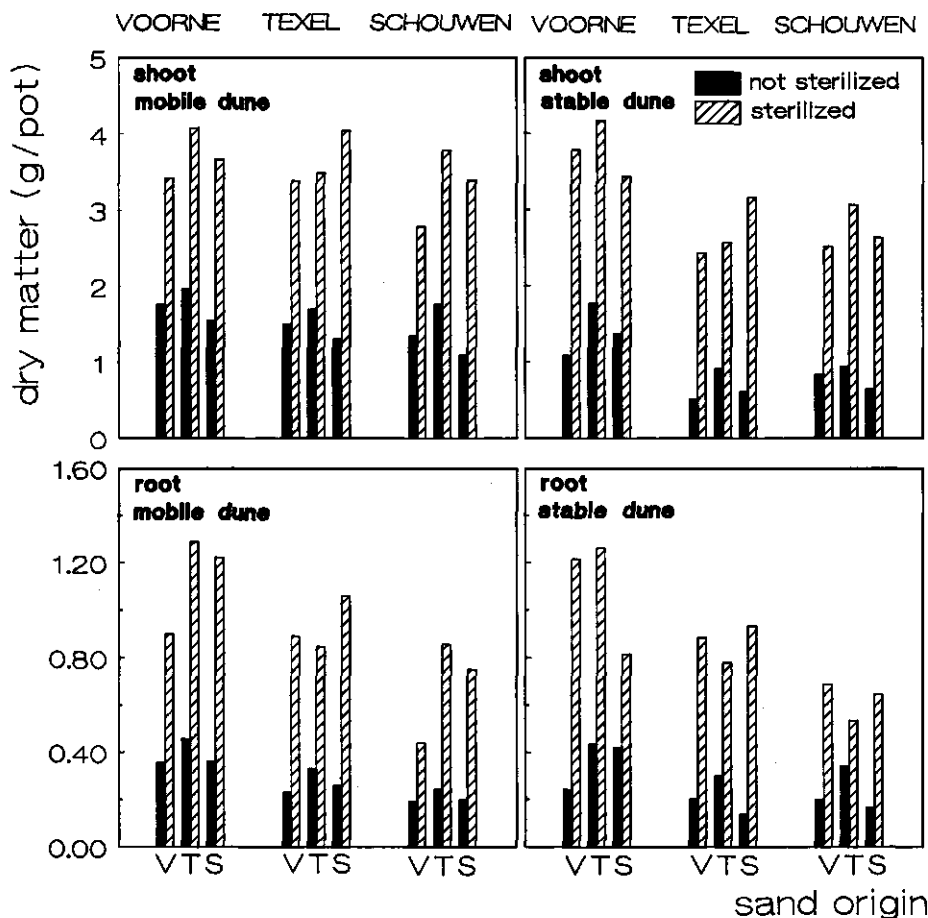
Fig. 2. Dry matter production (g/pot) of seedlings of three *Ammophila arenaria* populations (Voorne, Texel, and Schouwen), grown in sterilized and unsterilized sand collected from the mobile and stable dunes of the original and reciprocal locations. In all pairs of bars, the dry matter yield in the sterilized sand is significantly higher (P<0.05) than in the unsterilized sand.

demonstrated that the population of Voorne produced more shoot and root dry matter than those of Texel and Schouwen (P<0.001; Table 4), whereas the latter two populations showed no significant differences. These differences in dry matter production correlated in part with the 1000-kernel weights (g) of the seeds: Voorne 3.78[a] > Schouwen 3.36[ab] > Texel 3.28[b] (significant differences are indicated by different letters; results of

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Table 4. Degrees of freedom and F-values according to ANOVA with four factors (location, population, sand type, and soil sterilization) of shoot and root dry matter, and SWR of A. arenaria. Contrasts were calculated for the main factors location and population: V = Voorne, T = Texel and S = Schouwen. MSE: mean squares of error.

Source of variation	Contrast	df	Shoot		Root		SWR	
Location (L)		2	4.68	*	1.48	ns	0.30	ns
	T> [V,S]	1	4.66	*				
	V=S	1	0.02	ns				
Population (P)		2	27.1	***	15.0	***	3.73	*
	V> [T,S]	1	27.1	***	13.3	***	0.40	ns
	T>S	1	0.01	ns	1.76	ns	3.34	*
Sand type (T)		1	7.50	**	0.81	ns	4.87	*
Sterilization (S)		1	341	***	166	***	2.30	ns
L x P		4	0.92	ns	0.38	ns	0.62	ns
L x T		2	0.10	ns	1.04	ns	1.62	ns
L x S		2	1.56	ns	0.55	ns	0.71	ns
P x T		2	0.39	ns	0.21	ns	0.26	ns
P x S		2	0.09	ns	1.40	ns	0.70	ns
T x S		1	10.41	**	0.01	ns	9.33	**
L x P x T		4	0.17	ns	0.19	ns	0.18	ns
L x P x S		4	0.52	ns	0.57	ns	0.08	ns
L x T x S		2	0.50	ns	1.17	ns	0.39	ns
P x T x S		2	0.25	ns	0.10	ns	0.20	ns
L x P x T x S		4	0.27	ns	0.89	ns	1.73	ns
MSE		105	48.6		34.8		0.006	

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$ ns not significant

the one-way ANOVA not shown). The SWR of population Voorne was not significantly different from the other two populations, whereas the SWR of population Texel was significantly higher than that of population Schouwen ($P < 0.05$; Table 4).

Experiment 3

Both factors 'plant species' and 'sand type' were highly significant for total, shoot, root, and rhizome dry matter production of *A. arenaria* and *C. baltica* in the outdoor experiment ($P < 0.001$; Table 5). *C. baltica* produced more dry matter than *A. arenaria* ($P < 0.001$), and dry yield of both plant species was significantly higher in beach sand than in sand from the mobile dune, whereas production was lowest in unsterilized sand from the stable dune ($P < 0.05$; Fig. 3). Following sterilization of sand from the stable dune, however, total dry yield of both plant species was significantly higher than in the unsterilized sand types ($P < 0.05$; Fig. 3).

Table 5. Degrees of freedom and F-values according to two-factor ANOVA of total, shoot, root, and rhizome dry matter of A. arenaria and C. baltica that were grown in four sand types (beach, mobile, stable unsterilized and stable sterilized). MSE: mean squares of error.

Source of variation	df	Total	Shoot	Root	Rhizome
Plant species	1	154 ***	74.9 ***	132 ***	53.1 ***
Sand type	3	148 ***	114 ***	114 ***	12.2 ***
Plant x Sand	3	1.37 ns	7.93 ***	0.06 ns	1.23 ns
MSE	24	0.431	0.237	0.283	0.271

ns not significant   *** P < 0.001

Although biomass production of *C. baltica* was also reduced in the unsterilized sand, this species did not show any mortality, whereas 25 to 44 per cent of the cuttings of *A. arenaria* died after transplanting in unsterilized sand (Table 6). Mortality of *A. arenaria* was highest in unsterilized sand from the stable dune and shoot length of both plant species was lowest in this sand type (Table 6). Reduction of shoot length in unsterilized, as compared to sterilized sand, was most severe in *A. arenaria*. In beach sand and in sterilized sand from the stable dune, *A. arenaria* produced more green tillers than *C. baltica*. In the other sand types the production of tillers was reduced in both species, but more severe in *A.arenaria* than in *C. baltica* (Table 6). *C. baltica* produced most rhizomes in the sterilized sand, whereas in *A. arenaria* rhizomes were rare (Table 6). Root length corresponded with root weight, being highest in sterilized sand from the stable dune and lower (in descending order) in beach, mobile dune and stable dune (data not presented).
In agreement with differences in total biomass, uptake of N, P, and K by *C. baltica* was higher than by *A. arenaria* (Fig. 3; Table 6). Plants in sterilized sand took up 1.5 to 2 times as much N, P, and K as those in beach sand.
Sugar and starch concentrations in shoots were quite similar for both species (Table 6). Lowest sugar concentrations, however, were found in *A. arenaria* growing in unsterilized sand from the stable dune. Sugar and starch concentrations in other plant parts revealed a similar pattern (data not shown).
Numbers of nematodes in the soil in May (before the experiment was started) have been listed in Table 7, and numbers in soil and in roots during the course of the experiment in Table 8. No data from beach sand

Table 6. Mortality, shoot length, numbers of tillers and rhizomes, uptake of N, P, and K per container and concentrations of sugars and starch in the shoots of A. arenaria and C. baltica grown in sand from the beach, the mobile, and the stable dune, and in sterilized sand from the stable dune. Standard deviations are given in parentheses.

| Plant species	Sand type	Mortality (%)	Length (cm)	Tillers (N.plant⁻¹)	Rhizomes (N.plant⁻¹)	N	P mg.pot⁻¹	K	Sugars (%)	Starch (%)
A. arenaria	beach	25 (20)	72.5 (3.9)	9.8 (2.5)	0.6 (0.7)	305	35	387	7.5 (1.0)	14.6 (0.1)
	mobile dune	25 (20)	59.8 (16.6)	3.1 (0.5)	0.4 (0.1)	72 (56)	7.4 (6)	78 (54)	7.5 (1.0)	14.2 (0.2)
	stable dune	44 (24)	24.8 (7.5)	1.1 (0.2)	0.1 (0.2)	-	-	-	6.3 (1.0)	14.3 (0.8)
	stable dune, sterilized	6 (13)	69.5 (7.9)	12.1 (1.8)	0.5 (0.1)	436 (237)	69 (15)	656 (133)	9.2 (1.0)	14.4 (0.4)
C. baltica	beach	0	82.0 (4.2)	4.7 (0.5)	2.3 (0.6)	390 (36)	53 (8)	555 (45)	7.9 (0.9)	13.6 (1.1)
	mobile dune	0	71.5 (5.8)	3.9 (0.6)	1.5 (0.7)	251 (71)	28 (10)	317 (118)	8.9 (0.4)	13.5 (0.7)
	stable dune	0	69.8 (8.4)	3.5 (0.8)	1.2 (0.4)	224 (66)	26 (9)	207 (78)	8.5 (0.7)	12.7 (0.7)
	stable dune, sterilized	0	94.0 (1.8)	8.0 (1.0)	4.2 (0.3)	669 (89)	103 (11)	941 (68)	8.9 (0.4)	14.5 (2.1)

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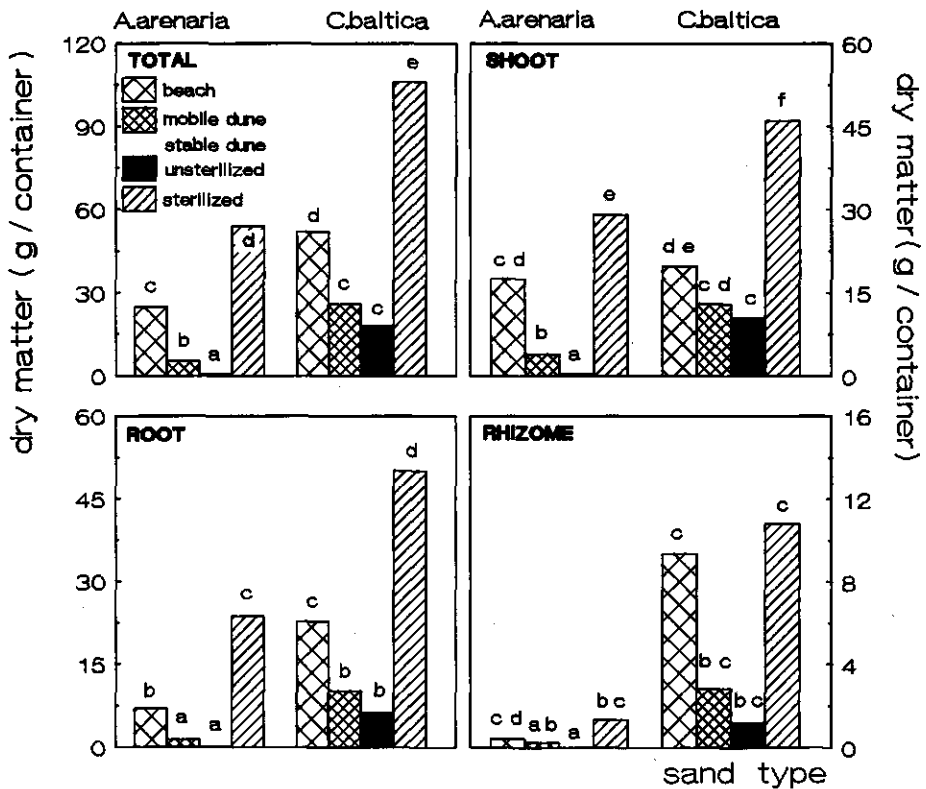
Fig. 3. Dry matter production (g/container) of cuttings of *Ammophila arenaria* and *Calammophila baltica* in sand from the beach, the mobile, and the stable dune, and in sterilized sand from the stable dune. Sand samples and plants originated from the location Voorne. Significant differences are marked with different letters (P<0.05).

and sterilized sand from the stable dunes are presented, since only saprobiotic nematodes were present in soil and roots. In July and in November, the numbers of nematodes were highest in containers with sand from the mobile site, being mainly *Pratylenchus* sp. and *Tylenchorhynchus* sp. (Table 8). Compared to the numbers in the soil in May, these nematode species had increased 10- to 20-fold, whereas saprobiotic nematodes had increased 5- to 10-fold. *Rotylenchus goodeyi, Paratrichodorus nanus* and *Criconematidae*, if present in the soil samples, were restricted to the sand from the stable site, and their numbers had not (or hardly) increased between May and July (Tables 7 and 8). *Pratylenchus* sp. was the most abundant species in the roots, but numbers declined strongly between July and November (Table 8). The numbers of *Meloidogyne maritima* and

Table 7. Numbers of nematodes in the soil (per 100 ml; specific volume of 0.75 ml.g^{-1}) from the mobile and the stable dunes, in May at location Voorne. Some species presented in Table 8 were not found in May.

	mobile	stable
Pratylenchus sp.	2.7	2.7
Tylenchorhynchinae	5.0	0
Rotylenchus goodeyi	0	10
Heterodera sp.[1] (larvae in cysts)	0	25
Heterodera sp.[1] (larvae in soil)	0	5.0
Saprobiotic	282	392

[1] avenae group

Heterodera (*avenae* group) were very low in both soil and roots.
The numbers of nematodes in replicate samples varied highly. In July, e.g. numbers of *Pratylenchus* sp. in roots from containers with sand from the mobile site varied between 0 and 7050 and between 230 and 29100 per g of fresh root for *A. arenaria* and *C. baltica*, respectively. In July and November, the numbers of plant parasitic nematodes were higher in soil and roots from containers with sand from the mobile dune than with sand from the stable dune (Table 8). Growth reduction of *A. arenaria* and *C. baltica*, however, was severest in the latter sand type (Fig. 3). Therefore, no positive correlation could be detected between numbers of nematodes and severity of growth reduction.

DISCUSSION

In soils from all three foredune areas, soil sterilization improved growth of test seedlings in sand samples collected from root zones of vigorous and degenerated *A. arenaria* (mobile and stable dunes, respectively). Since in the greenhouse ample nutrients had been supplied, the effect could not be related to extra release of nutrients due to soil sterilization. Therefore, reduction in plant growth in the unsterilized sand had to be attributed to harmful soil organisms, which were eliminated by the sterilization

Table 8. Numbers of nematodes in the soil (per 100 ml soil; specific volume of 0.7 ml.g⁻¹) and in the roots (per g fresh weight) of A. arenaria and C. baltica. Data are presented as average and lowest-highest number (in parentheses) found in replicates (n = 4). Soil and roots were collected from the containers in July and November (experiment 3).

	July				November			
	A. arenaria		C. baltica		A. arenaria		C. baltica	
	mobile	stable	mobile	stable	mobile	stable	mobile	stable
Soil								
Pratylenchus sp.	3(1.7-4)	0.3(0-0.3)	25(6.3-47)	0.3(0-0.3)	1.3(0-5)	0	2.7(0-10)	0
Tylenchorhynchinae	96(47-158)	1.3(0-2)	62(35-130)	2.7(0-4)	37(0-70)	0	58(10-130)	2(0-5)
Rotylenchus goodeyi	0	2.7(0-6)	0	1.3(0-1.3)	0	1.3(0-5)	0	0
Paratrichodorus nanus	0	0	0	1(0.7-1.7)	0	0	0	0
Criconematidae	0	6.3(0.3-19.7)	0	1(0-2)	0	0	0	0
Paratylenchus sp.	0	0.3(0-0.3)	1(0-4.3)	0	0	0	0	0
Hemicycliophora sp.	0.7(0.7-1.3)	0	2.3(0-7.7)	0	0	0	0	0
Meloidogyne maritima	0.3(0.3-1.7)	0	0	0.3(0-0.3)	2.7(0-10)	0	0	0
Heterodera (avenae gr.)	0	0.3(0-0.3)	0	0.3(0-0.3)	0	0	0	0
Saprobiotic	569(196-838)	299(249-360)	530(431-757)	299(217-400)	1350(440-3765)	389(230-500)	1083(758-1720)	497(303-688)
Roots								
Pratylenchus	2360(0-7050)	400(0-1090)	13500(230-29100)	10(0-10)	60(0-130)	0	480(40-940)	0
Meloidogyne maritima	0	0	0	0	10(0-10)	10(0-100)	0	0
Heterodera (avenae gr.)	10(0-20)	0	0	40(10-70)	10(0-30)	0	0	0
Saprobiotic	230(0-350)	150(10-300)	1060(480-1650)	280(70-830)	200(80-230)	280(0-400)	830(300-1670)	690(420-1060)

procedure. The locations Voorne, Texel, and Schouwen are representative for the two parts of the Dutch dune district, that are poor and rich in calcium carbonate, respectively. Since harmful soil organisms were present in the root zone of *A. arenaria* at all three locations, it can be concluded that their occurrence is quite common in coastal foredunes. These organisms were detected in sand from both the mobile and the stable dunes, but they did not occur in sand from the beach (Fig.1). Thus, the colonization of windblown beach sand will enable *A. arenaria* to keep its young roots free from harmful organisms. Although these experiments give circumstantial rather than causal evidence, they support the theory relating vigorous growth by colonizing windblown sand to the avoidance of harmful soil organisms (Van der Putten et al. 1988). A subsequent paper (Van der Putten et al. Chapter 7) will discuss the rate of colonization of fresh sand by soil organisms after the sand has been explored by plant roots.

The harmful soil organisms were not specific to certain populations of *A. arenaria*, as all three examined populations were reduced by harmful soil organisms of both local and remote origin. The three populations produced different amounts of dry matter. The highest dry yield (population Voorne) appeared to be correlated with the highest initial seed weight. Seed weight may be a population characteristic, but it may also be the result of better growing conditions, e.g. the amount of sand accretion (Laing 1958, Huiskes 1979, Maun 1985, Eldred and Maun 1982). Since *A. arenaria* can establish from seed (Huiskes 1977), micro-evolution resulting in differentiation between populations needs to be considered seriously (Silander Jr. 1985). Until now, only Gray (1985) has demonstrated differences between populations of *A. arenaria*. His results, however, may have been due to artefacts (Van der Putten et al. Chapter 7). A thorough study of populations of *A. arenaria*, therefore, might contribute to knowledge of possible differentiation among populations.

On the average, the three populations of *A. arenaria* produced more biomass in sand from Texel, than in sand from Voorne and Schouwen. The main difference between these locations was the relatively low calcium carbonate content in the sand from Texel (Table 2). This observation is at variance with the suggestion that *A. arenaria* needs calcium carbonate to maintain vigour (Van Dieren 1934, Lux 1969), which has been based on field observations, however, and any experimental evidence for this is lacking.

In the outdoor experiment, growth of both *A. arenaria* and *C. baltica* was reduced in sand from the mobile dune and in unsterilized sand from the stable dune, as compared to sterilized sand. Both species also produced more biomass in sterilized sand from the stable dune than in beach sand. Uptake of N and P did not exceed the amounts supplied by the fertilizer

(780 mg.pot^{-1} N and 195 mg.pot^{-1} P, respectively). Recovery of the applied fertilizer in the total biomass of *C. baltica* was nearly 100 per cent in sterilized sand, taking into account total releases of N and P in the fertilizer of 90% and 60%, respectively (Van der Putten Chapter 2). Soil sterilization may have caused a nutrient flush, which was during the course of the experiment not entirely compensated for by fertilization, since an uptake by the total plant of 100% of the supplied nutrients may be quite high. Therefore, growth differences between sterilized sand from stable dune and beach sand may be explained by a flush of nutrients in the stable dune sand after soil sterilization. Uptake of potassium exceeded the applied amount up to 5 times. This was in accordance with the results of a field trial (Van der Putten Chapter 2), demonstrating that potassium in dune sand is not a limiting factor for growth of *A. arenaria*.

It has been shown that the elimination of nematodes from dune sand results in an increased growth of planted seedlings of *A. arenaria* (Van der Putten et al. Chapter 5). In the present experiments, however, the variation in nematode counts among replicates was large (Table 8) and the numbers of nematodes could not always be related to the reduction in growth. The strongest growth reduction was observed in the sand from the stable dune which contained only low numbers of nematodes. Low numbers of nematodes may become pathogenic if they interact synergistically with other soil organisms (e.g. Agrios 1978, Rowe et al. 1985, Caperton et al. 1986). Synergism between nematodes and soil fungi has also been shown for *A. arenaria* (W.H. Van der Putten and W.J.M. Van Gulik, unpublished results).

Succession in coastal sand dunes has been related to changes in the environment, i.e. changing soil fertility (Olson 1958, Lux 1969), loss of calcium carbonate (Van Dieren 1934, Lux 1969, Baldwin and Maun 1983), accumulation of organic matter (Salisbury 1952, Olson 1958, Willis et al. 1959, Baldwin and Maun 1983), reduced salt spray (Oosting and Billings 1942), and competition (Huiskes 1979). However, most of these changes take more time than the degeneration of *A. arenaria* (Olson 1958, Desmukh 1979, Wallén 1980), or did not occur in the experiments described in this paper, e.g. salt spray and interspecific competition. By comparing the growth reduction of well-nursed test plants in the greenhouse with that outdoors it can be concluded that the latter is far higher. Even *C. baltica* showed a significant growth reduction in unsterilized sand outdoors, which had not become manifest in the greenhouse (Van der Putten et al. 1988). When plants fail to produce long roots outdoors, stress of drought and high temperatures occurring in upper sand layers (Baldwin and Maun 1983) may become more serious. Therefore, in addition to the synergistic interaction between different groups of soil organisms, environmental stress factors may affect the functioning of the roots of *A. arenaria* negatively, thus

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enforcing the process of degeneration at stable sites. *C. baltica* appears to be less sensitive to these stress factors because it did not show any mortality. With continued growth of *C. baltica* at the same site, however, harmful soil organisms may develop and attack this species just as severely as *A. arenaria*. Systematic comparisons of *A. arenaria* and *C. baltica* in the field have not yet been carried out. Field observations, however, suggest that *C. baltica*, although being relatively more vigorous at stable sites than *A. arenaria*, also declines when the dune becomes stabilized (Olsson 1974, Wallén 1980).

In the Netherlands, *Hippophaë rhamnoides* (Sea buckthorn) is the successive species dominating the foredune vegetation when *A. arenaria* and *C. baltica* have disappeared. Degeneration of *H. rhamnoides* can also be related to harmful soil organisms, e.g. *Tylenchorhynchus microphasmis* and *Longidorus kuiperi* (Oremus and Otten 1981, Maas, Oremus and Otten 1983, Zoon 1986, Brinkman et al. 1987). Therefore, it has to be concluded that vegetation succession at coastal foredunes can be regarded as a process being influenced by abiotic factors, as well as by harmful biotic factors.

COLONIZATION OF THE ROOT ZONE OF *AMMOPHILA ARENARIA* BY HARMFUL SOIL ORGANISMS

W.H. Van der Putten, J.T. Van der Werf-Klein Breteler and C. Van Dijk

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CHAPTER 7

COLONIZATION OF THE ROOT ZONE OF AMMOPHILA ARENARIA BY HARMFUL SOIL ORGANISMS

SUMMARY

The role of harmful soil organisms in the degeneration of *Ammophila arenaria* at coastal foredunes was examined by the growing of seedlings of *A. arenaria* in soil samples collected from the root zone. Three sites, each representing a successive stage in foredune succession were examined: (1) a highly mobile dune (sand accretion of 80 cm year^{-1}) with vigorous *A. arenaria*, colonizing only the upper 30 cm of the annually deposited layer of sand, (2) a mobile dune with vigorous *A. arenaria* and a 1-metre soil profile completely colonized by roots (sand accretion of 22 cm year^{-1}) and (3) a stable dune with degenerated *A. arenaria* and young roots mainly present in the upper 0-10 cm.
In the upper 1-metre of the highly mobile site, the presence of harmful soil organisms was confined to the root layers. At the mobile site for all depth layers a significant growth reduction of *A. arenaria* was observed due to the activity of harmful soil organisms, however, at the stable site growth had only been reduced in some of the depth layers. Newly formed roots of *A. arenaria* had been colonized by harmful soil organisms within one year. It was assumed that the colonization of fresh windblown sand in mobile dunes enables *A. arenaria* to maintain vigour, as the plants continuously form new roots in sand that does not contain harmful organisms.
Harmful soil organisms reduced root length and root hair formation severely and they enhanced branching of the roots if they were present in sand prior to root growth. It is suggested that harmful soil organisms initiate degeneration of *A. arenaria* in stable dunes by attack of the root system, which makes the plants suffer from stress caused by drought, high soil temperatures, and shortage of nutrients.

INTRODUCTION

Ammophila arenaria (L.) Link (marram grass) is a dominant grass species in the coastal foredunes of north-western Europe, the Mediterranean, Australia, and the west coast of N. America (Knutson 1978, Huiskes 1979). The vigour of *A. arenaria* is related to the rate of sand accretion. Plants grow vigorously on sites where fresh sand is deposited by wind. When sand drift diminishes, however, plants become feeble and they degenerate (Marshall 1965, Willis 1965, Hope-Simpson and Jefferies 1966, Huiskes 1979, 1980). Degeneration starts soon after the deposition of windblown sand comes to an end (Hope-Simpson and Jefferies 1966).

In North America a similar relationship between sand accumulation and plant vigour has also been demonstrated for *Ammophila breviligulata* Fern. (American beachgrass) (Laing 1967, Eldred and Maun 1982, Disraeli 1984, Maun and Baye 1989). During the last 100 years several theories have been presented to explain this ecological aspect of *Ammophila* (summarized by: Marshall 1965, Laing 1967, and Eldred and Maun 1982). The accumulation of windblown sand is thought to provide *Ammophila* with nutrients (Van Dieren 1934, Lux 1969), to enable the plants to renew their root system and thus to overcome physiological ageing (Marshall 1965), and to allow *Ammophila* to escape from competition, as no other plant species withstand strong sand accretion (Huiskes and Harper 1979, Huiskes 1980). In recent papers it has been shown that the root zone of *A. arenaria* usually contains harmful soil organisms (Van der Putten et al. 1988), which are supposed to be a combination of nematodes and soil fungi (Van der Putten et al. Chapter 5). It has been suggested that windblown sand provides *A. arenaria* with the opportunity to escape the harmful organisms (Van der Putten et al. 1988). Following the deposition of windblown sand and the formation of roots, however, the new roots will again be colonized by harmful soil organisms, since these organisms can be detected in the root zones of both stable and mobile foredunes (Van der Putten and Troelstra Chapter 6).

In this paper the colonization of the soil profile of a foredune by harmful organisms is presented for dune stages with different rates of sand accretion. To this end the presence of harmful soil organisms in different depths at sites with various amounts of sand deposition was examined by comparing the growth of seedlings of *A. arenaria* in unsterilized and sterilized soil samples. In two experiments the general hypothesis has been tested that the natural colonization of fresh sand by roots of *A. arenaria* is followed by a rapid colonization of organisms which are harmful to the plant. The distribution of harmful soil organisms in both stable and mobile dunes has been examined without regarding the distribution of the roots on forehand (experiment 1). In a highly mobile dune the stratified distribution

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of the roots in horizontal layers gave rise to a selective sampling in and between these layers. Both rooted and rootless soil layers have been examined on the occurrence of harmful organisms (experiment 2). Subsequently the root zone of a one-year-old plantation of culms of *A. arenaria* in dredged sea sand has been examined (experiment 3), since this sand usually does not contain harmful organisms (Van der Putten et al. 1988). Consequences of the observed presence of harmful organisms in soil profiles of foredunes and their colonization on new roots of *A. arenaria* will be discussed with respect to degeneration of *A. arenaria* and vegetation succession in stabilized coastal foredunes.

MATERIALS AND METHODS

Study area

The study sites were located at the coastal foredunes on the island of Voorne, the Netherlands (51.5 N, 4.1 E). These foredunes are about 100 m wide and 9.4 km long and a large dune area with wet slacks extends behind it. The shoreline of Voorne is subject to wave-erosion and part of the beach had been raised artificially by dredged sea sand. The foredunes only expand naturally in a small area of 1 km parallel to the shoreline. This dune development is due to the colonization of the beach by *Elymus farctus*, *Leymus arenarius*, and - in the higher parts - *A. arenaria.*

Table 1. Percentage of vegetation cover at the mobile and stable foredune succession stages of A. arenaria and other plant species (mainly Sonchus arvensis, Euphorbia paralias, Sedum acre, and Festuca rubra ssp. arenaria). Deposition of windblown sand (cm) at both sites during the period October 1986 - October 1987.

Succession stage	Vegetation cover (%)		Sand deposition (cm)	
	Ammophila arenaria	Other species	October–May	May–October
mobile dune	25–30	0	21.2±5.6	2.2±5.4
stable dune	20–25	35–40	1.6±1.6	0.5±2.0

Sampling, sand treatment and experimental design

Experiment 1
In the expanding part of the foredune between km land-marks 15 and 16,
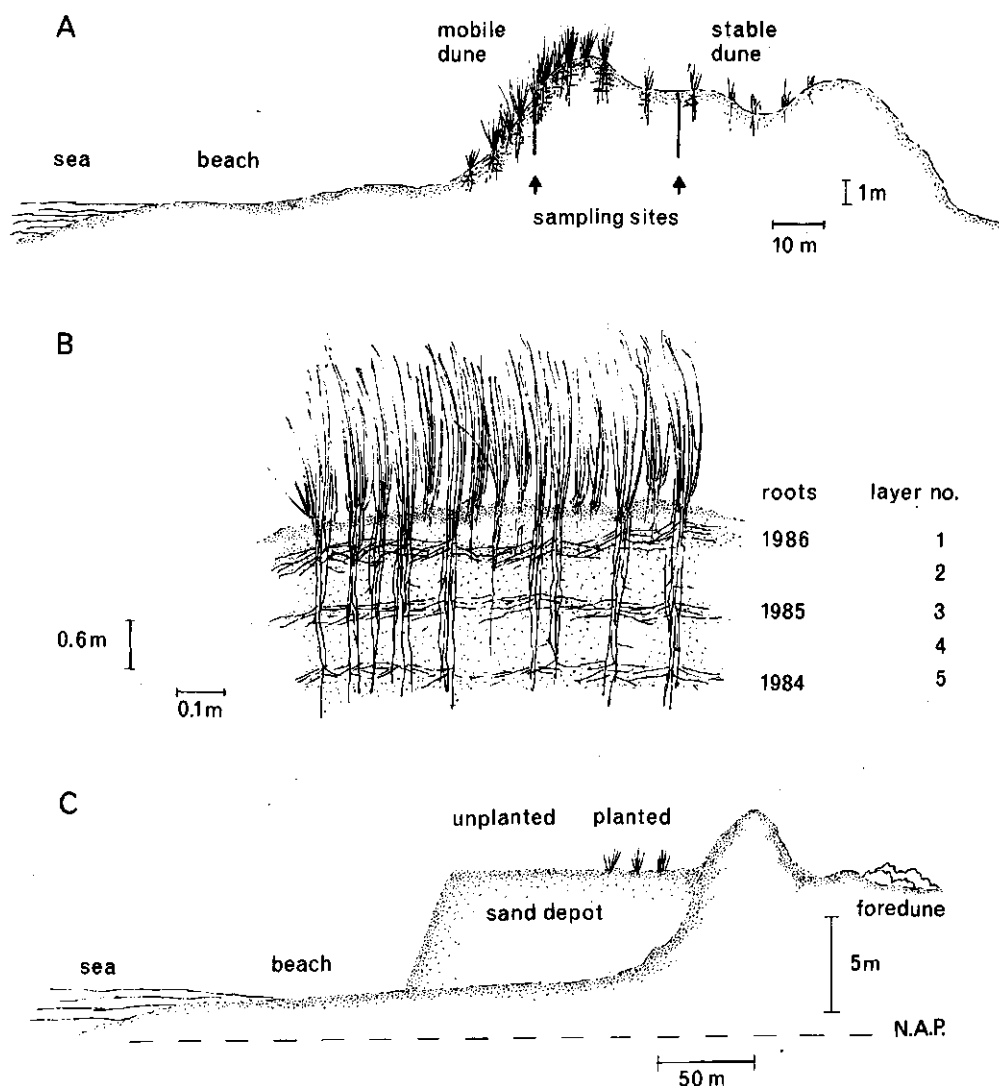
Fig.1.   Schematic cross-section of the sampling sites.
A: Dune sere with different stages of foredune succession. Successive
        depths up to 1 m were sampled at the mobile and stable dune stages
        (vigorous and degenerated *Ammophila arenaria*, respectively).
B: Soil profile of a highly mobile dune stage with successive root
        layers of *Ammophila arenaria*.
C: Sea sand depot on the beach, to be used to strengthen the foredune.
        A small part had been planted experimentally with culms of
        Ammophila arenaria.

two sites were examined: a mobile dune stage with vigorous *A. arenaria*, and a stable dune stage with degenerated *A. arenaria* (dune stage nomenclature according to Krajnyk and Maun 1981; Fig.1A). In September 1986, percentages of vegetation cover were determined and sand accretion was measured from October 1986 to October 1987 in order to characterize both sites (table 1). At the mobile site, *A. arenaria* occurred in a pattern of dense tussocks, and roots were distributed all over the uppermost 1 m part of the soil profile. At the stable site there was a degenerated and open stand (see also Greig-Smith 1961) and white, fresh roots were present mainly in the upper 0.1-m layer.
On September 1986, soil samples were collected from the root zone of *A. arenaria* in 6 successive layers of 10 cm and a 7th layer at a depth of 60 - 100 cm. The sand was sieved (5 mm) and homogenized. One half of each sample was sterilized by gamma irradiation (2.5 Mrad; Oremus 1982). In this way, twenty-eight treatments were carried out in a 2x7x2 factorial design with the factors: site (or: succession stage), layer, and sterilization, respectively. Each treatment was replicated six times.
Experiment 2
In 1983, at km land-mark 12 the beach (the bare part of the shoreline between low and high water) had been raised artificially with dredged sea sand and it became frequently subject to wind erosion during the successive winters (November to April). The windblown sand had been deposited upon the vegetation which was dominated by *A. arenaria*. The plants followed sand deposition by vigorous upward growth. In this way, alternating layers of roots (30 cm year^{-1}) and uncolonized sand (50 cm year^{-1}) were formed. In December 1986, at two sites along the high-tide line (sites I and II), soil samples were collected from three successive root layers of *A. arenaria*, and from two non-root layers in between (Fig.1B). The soil samples were stored at a temperature of 5 to 10°C. In April 1987, the samples were sieved (5 mm mesh) and homogenized. Half of each sample was sterilized by gamma irradiation (2.5 Mrad). In this way, twenty treatments were carried out in a 2x5x2 factorial design with the factors: site, depth, and sterilization, respectively. Each treatment was replicated six times.
Experiment 3
In October 1984, between km land-marks 6 and 9, sea sand for dune reconstruction (3.10^6 m^3) had been deposited in a depot on the beach. In February 1985, a plot of 10x20 m^2 was planted with *A. arenaria* bundles of 6-10 culms in a grid of 0.5x0.75 m^2 (Fig. 1C). The culms that were used for planting were cut at 10 cm depth in a semi-mobile dune with a vigorous *A. arenaria* vegetation.
In October 1985, soil samples were collected from the root zone (0-20 cm below the soil surface) of the planting, and from a similar layer in the

unplanted part of the depot. Per site, samples were collected randomly from an area of 5x10 m². Half of each sample was sterilized by gamma irradiation (2.5 Mrad). In this way four treatments were carried out in a 2x2 factorial design with the factors: site and sterilization, respectively. Each treatment was replicated four times.

Growing of test plants in the soil samples

Experiments 1 and 2
Seedlings of *A. arenaria* were obtained by germination of seeds on glass beads in a 8/16 hour light/dark regime at 30/20°C. Pots of 450 ml containing 700 g of sand (soil moisture 18% of the dry weight) were planted with four *A. arenaria* seedlings that were two weeks old, and placed in a greenhouse at a temperature of 23 °C ($\pm$ 2 °C). The sand surface was covered with tinfoil to prevent the soil from drying out. Day length of 16 hours was obtained by additional illumination with Philips HLRG-400W (4.8 $W.m^{-2}$). The moisture content of the sand was maintained by supplying the pots with demineralized water every other day. Every week, the pots were amply provided with Hoagland nutrient solution (Hewitt 1966) to overcome differences in soil nutrients, due to sterilization. Each fortnight, the amount of added nutrients was raised because of increased plant growth (25 ml half strength, and 25 ml, 50 ml, and 75 ml full strength Hoagland, respectively.

Experiment 3
Plants were grown as in the other experiments, except that pots of 1.5 l (2170 g of sand of 18% soil moisture) were used.

Harvesting and treatment of the plant material

Experiment 1
Eight weeks after planting, the seedlings were harvested and root length was measured (Comair root length scanner, Australia). After drying at 70 °C, for 48 hours, the shoots and roots were weighed. In order to quantify the presence of root hairs, a random part of the root system was excluded from drying. In this sample, length of roots without root hairs was estimated as a percentage of total root length, as has been suggested by Biermann and Linderman (1981). Similarly, the percentage of root length with a disintegrated cortex was determined. The same determinations were carried out on roots that had been collected from the field. The degree of branching (i.e. the numbers of branching points per metre of root length) of the roots was determined by photographing a root sample of a known length and by counting the numbers of root tops on projected slides.

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Experiments 2 and 3
Seven weeks after planting, the seedlings were harvested and dry weights of shoots and roots were determined after drying at 70°C for 48 hours.

Soil analysis

(Experiment 1 only)
After drying (35 °C) and sieving (2 mm), bulk soil samples were mechanically subdivided. Part of each sample was ground in a mortar mill and, depending on the type of determination, analyses were performed on either ground or unground samples.
The pH of the soil was measured potentiometrically in 1:2.5 (W/V) suspensions of 1 *M* KCl. Carbonates were measured gas-volumetrically by treating samples with 4 *M* HCl. Organic matter was determined as loss-on-ignition at 430 °C for 24 h. Total N and P were measured colorimetrically in single-soil digests (Novozamsky et al. 1984). Exchangeable cations were determined by atomic absorption spectrophotometry after shaking soils with neutral ammonium acetate. Chloride and electrical conductivity analyses were carried out on 1:5 water extracts. The granular composition (texture) of the samples was determined by dry-sieving (fractions > 53 μ) and a pipette method (fractions < 53 μ).
The soil of all depth layers was analysed immediately after collecting the samples for the occurrence of nematodes according to Oostenbrink (1960), and for the large nematode species an Oostenbrink method modified by Maas and Brinkman (1980) was used.

Table 2. Analysis of variance for shoot and root dry matter, root length, and degree of branching of <u>Ammophila arenaria</u> (experiment 1).

Source of variation	df	F-values			
		Shoot weight	Root weight	Root length	Degree of branching
Site	1	0.36 ns	8.24 **	4.51 *	10.1 **
Sterilization	1	617 ***	161 ***	141 ***	28.7 ***
Depth	6	4.35 ***	1.55 ns	1.28 ns	1.96 ns
Site x sterilization	1	83.6 ***	39.7 ***	28.7 ***	5.08 *
Site x depth	6	2.71 *	0.52 ns	0.74 ns	1.62 ns
Sterilization x depth	6	2.90 *	0.87 ns	1.65 ns	0.10 ns
Site x sterilization x depth	6	5.96 ***	1.72 ns	2.58 *	0.24 ns
MSE	140	0.025	0.066	0.023[1]	0.026

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$ ns not significant

[1] df (MSE) = 56

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Data analyses

The data were analysed statistically by means of analysis of variance (ANOVA), after testing homogeneity of variances by means of Cochran's Q test. Treatment means were compared by Tukey's test. In experiment 1, (sand x sterilization) and depth were correlated by Spearmans rank correlation (Sokal and Rohlf 1981).

RESULTS

Experiment 1

The shoot production of the seedlings of *A. arenaria* in sand from the mobile, as well as from the stable site was increased by soil sterilization (Fig. 2). The factors sterilization and depth were significant for the shoot yield (P<0.001; Table 2). Sterilization increased shoot yield more in sand from the mobile dune stage than in sand from the stable dune stage (Fig. 2). This is demonstrated by the strong site x sterilization interaction in the ANOVA (P<0.001; Table 2). However, the shoot yield also depended on the sampling depth, as three-way interaction was significant (P<0.001). In unsterilized sand from the mobile dune, shoot yield was correlated negatively with sampling depth (R = -0.89; P<0.01). In sterilized from the mobile and the stable dunes and in unsterilized sand from the stable dune, however, this correlation was not significant (R = 0, -0.3, and -0.29, respectively).
Factors site and sterilization were significant for root yield (P<0.01 and P<0.001, respectively) and there was strong interaction between them (P<0.001; Table 2). This interaction was due to a significant increase of root yield in sterilized sand from the mobile dune, whereas sterilization did not affect root yield in sand from the stable dune (P<0.05; Fig. 2).
The degree of branching of the roots grown in the greenhouse depended on the factors site and sterilization (P<0.01 and P<0.001), and interaction existed between them (P<0.05; Table 2). The degree of branching of the roots was significantly higher in unsterilized sand from the mobile site than in the other treatments (P<0.05; data not shown).
Roots of the plants grown in sterilized sand (from both stable and mobile sites) in the greenhouse were almost completely covered with hairs, as were the roots in unsterilized sand from the stable site (Table 3). In unsterilized sand from the mobile site, however, only 2 to 43 per cent of the total root length possessed hairs. On the other hand in the field-collected roots from the mobile stage more fresh, white roots could be observed than in the
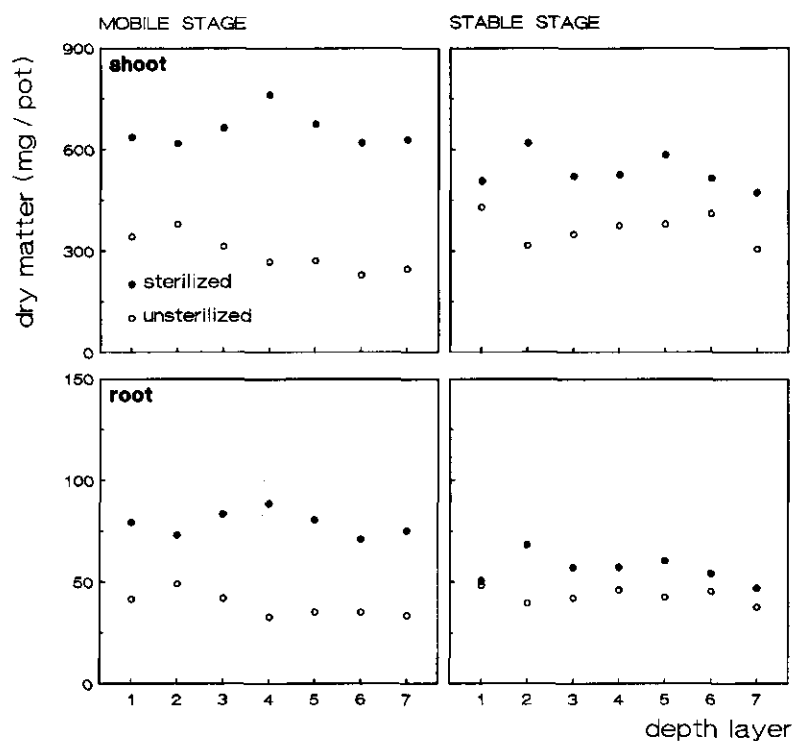
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Fig.2. Shoot and root dry weights of *Ammophila arenaria* in sand samples from the mobile and stable dune with (from left to right) increasing sampling depths (experiment 1). Layers 1 to 6 are 0-10 to 50-60 cm deep, and layer 7 is 60-100 cm deep.

stable stage. On roots collected from the field, hairs were virtually absent below a depth of 40 cm (mobile stage) or below 10 cm (stable stage). Root hairs were present in the uppermost layers, however, the percentage of the total root length with hairs was considerably higher at the mobile than at the stable site.

In the greenhouse cortex disintegration hardly occurred and in the field it was low in the upper layers, but increasing with sampling depth (Table 3). At the stable site, a disintegration of more than 70 per cent occurred already in the soil below 10 cm depth, whereas at the mobile site this percentage was not reached before a depth of 30 to 40 cm.

Comparison of the chemical soil properties of both succession stages shows that some calcium carbonate had been leached from the stable stage, whereas in the top soil layer of the stable site the accumulation of organic matter had started (Table 4). At this site the amount of total-N decreased

Table 3. Percentage of root length covered with root hairs, and percentage of length with a degenerated cortex of roots of *Ammophila arenaria* from the greenhouse and from the field (experiment 1).

		mobile site			stable site		
		Field material	Pot experiment		Field material	Pot experiment	
Layer	Depth (cm)		Unsterilized sand	Sterilized sand		Unsterilized sand	Sterilized sand
Root hairs (%)							
1	0–10	82.5	37.5	99.5	17.5	92.5	97.5
2	10–20	82.5	2.5	99.5	2.5	92.5	97.5
3	20–30	72.5	22.5	99.5	2.5	57.5	87.5
4	30–40	17.5	2.5	97.5	0	67.5	97.5
5	40–50	2.5	17.5	99.5	0	52.5	87.5
6	50–60	2.5	42.5	97.5	0	67.5	92.5
7	60–100	2.5	12.5	97.5	0	32.5	47.5
Cortex desintegration (%)							
1	0–10	7.5	7.5	0	22.5	2.5	0
2	10–20	17.5	12.5	0	72.5	2.5	0
3	20–30	12.5	2.5	0	82.5	2.5	7.5
4	30–40	72.5	2.5	0	100	2.5	0
5	40–50	92.5	2.5	0	100	12.5	2.5
6	50–60	87.5	2.5	0.5	100	7.5	2.5
7	60–100	87.5	2.5	0.5	100	12.5	2.5

Table 4. Some chemical soil properties of mobile and stable sites.

Succession stage	Layer	Depth (cm)	pH(KCl)	CaCO$_3$ (%)	Organic matter(%)	Tot-N 1)	Tot-P 1)	K 2)	Na 2)	Mg 2)	Cl$^-$ 1)	Electro conductivity (μS.cm^{-1})
Mobile dune	1	0-10	9.1	3.2	0.15	3.5	10.1	0.07	0.22	0.31	4.1	78
	2	10-20	9.1	3.5	0.19	4.3	11.3	0.07	0.23	0.30	4.8	83
	3	20-30	9.1	3.5	0.15	4.6	10.9	0.08	0.21	0.31	4.8	80
	4	30-40	9.1	3.3	0.23	5.5	10.9	0.08	0.19	0.35	2.1	64
	5	40-50	9.2	3.6	0.09	4.5	11.0	0.05	0.21	0.34	2.1	66
	6	50-60	9.1	3.5	0.19	4.2	10.8	0.05	0.21	0.31	1.7	60
	7	60-100	9.2	3.1	0.19	5.2	10.7	0.05	0.19	0.29	1.3	55
Stable dune	1	0-10	8.9	2.8	0.36	9.8	10.2	0.05	0.20	0.23	1.1	43
	2	10-20	9.0	2.7	0.22	4.7	8.8	0.04	0.18	0.18	1.0	40
	3	20-30	9.0	2.7	0.18	3.8	8.5	0.03	0.19	0.17	1.2	40
	4	30-40	9.0	2.7	0.17	3.2	8.3	0.03	0.19	0.18	1.0	41
	5	40-50	9.1	2.8	0.17	3.7	9.8	0.03	0.20	0.21	1.0	43
	6	50-60	9.1	2.7	0.09	1.7	9.7	0.03	0.18	0.22	1.0	41
	7	60-100	9.0	2.9	0.16	1.9	8.5	0.03	0.21	0.23	1.1	46

1) mg / 100 g   2) me / 100 g

with depth and the amount of magnesium was somewhat lower than at the mobile site. At the mobile site the amounts of nutrients did not show trends which could be related to depth, with the exception of chloride which decreased with depth, apparently as a result of leaching.

The grain size distribution of the different layers per site was quite similar, so that only averages are presented. Sand from the mobile site was relatively fine, which is expressed by the low D_{50} (150 to 212 μ; i.e. the median of the grain size distribution), as compared to the sand from the mobile site (212 to 300 μ).

Numbers of saprobiotic nematodes in both succession stages diminished with increasing depth (Table 5). Highest numbers were present in the upper layers of sand from the mobile site. Mainly saprobiotic nematodes were present in sand from the stable site, whereas in the mobile site below 30 cm relatively high numbers of *Meloidogyne maritima* occurred. Numbers of the other plant parasitic nematodes were low.

Table 5. Nematode counts in successive soil layers from mobile and stable sites (numbers/100 ml).
Tyl = Tylenchorhynchus sp., Rot = Rotylenchus sp., Het = Heterodera avenae group, Mel = Meloidogyne maritima, Sap = saprobiotic nematodes, Pra = Pratylenchus sp., Cri = Criconemata sp.

Layer	Depth (cm)	Mobile site					Stable site			
		Tyl	Rot	Het	Mel	Sap	Pra	Mel	Cri	Sap
1	0–10	3	0	0	0	238	0	1	1	95
2	10–20	3	0	0	0	160	0	0	1	23
3	20–30	3	0	0	1	143	0	1	1	13
4	30–40	4	0	0	53	168	0	0	1	5
5	40–50	0	1	1	23	50	0	0	1	3
6	50–60	1	1	0	38	60	0	1	0	3
7	60–100	0	0	2	23	25	1	0	1	3

Experiment 2

In sand from the highly mobile dune growth of seedlings of *A. arenaria* was affected mainly by soil sterilization, followed by sampling layer (Table 6). The site effect was not significant. There was, however, interaction between all three above-called factors (shoots: P<0.001 and roots: P<0.01) and data of shoot and root dry matter production, therefore, are presented separately for both sites, I and II.
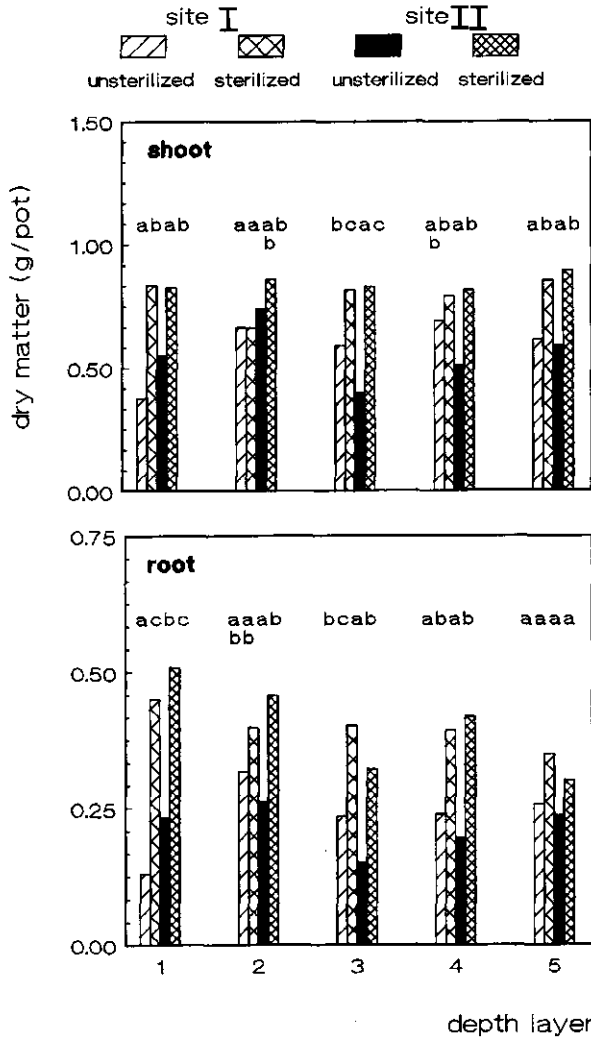
Fig.3. Shoot and root dry matter of *Ammophila arenaria* grown in sand collected from five successive depths in a highly mobile dune: 1, 3, 5 are root layers and 2, 4 are rootless layers (experiment 2). Significant differences (P<0.05) per group of 4 bars are indicated by different symbols.

Table 6. Analysis of variance for shoot and root dry matter of *Ammophila arenaria* (experiment 2).

Source of variation	df	F-values dry weights	
		Shoot	Root
Site	1	0.13 ns	1.08 ns
Layer	4	6.41 ***	6.37 ***
Sterilization	1	192 ***	242 ***
Site x layer	4	9.03 ***	8.42 ***
Site x sterilization	1	5.62 *	0.97 ns
Layer x sterilization	4	12.3 ***	11.77 ***
Site x layer x sterilization	4	7.06 ***	4.21 **
MSE	99	0.022	0.042

* P < 0.05   ** P < 0.01   *** P < 0.001   ns not significant

The shoot production in sand from the uppermost rooted layers (1 and 3) increased significantly after soil sterilization, whereas in the upper rootless layer (2) soil sterilization had no significant effect (P<0.05; Fig. 3). In the lower layers of site I increase of shoot production was also confined to the sterilized rooted layer, however, not in site II. Soil sterilization also increased root growth significantly in the soil samples collected from the upper root layers (1 and 3; Fig. 3). In the upper rootless layer (2), a sterilization effect was absent only in site I, and in the lower layers (4 and 5) the growth increasing effect of soil sterilization was not confined to the rooted layer at all. Hence, in the upper three layers (1-3) the growth-increasing effect of soil sterilization was mainly found in the sand collected from the root layers, whereas this relationship was less evident in the lowest layers (4 and 5).

Experiment 3

Sea sand that (1) had been planted with culms of *A. arenaria* and (2) had not been planted were both examined. In sand from the part of the depot where the culms had grown, shoot and root dry matter yields of the seedlings of *A. arenaria* were significantly lower (P<0.05) than when the sand was sterilized prior to the growing of the seedlings (Fig.4). However, no sterilization effect was observed in sand collected from the unplanted part of the sand depot. Therefore, growth reduction occurred only in the
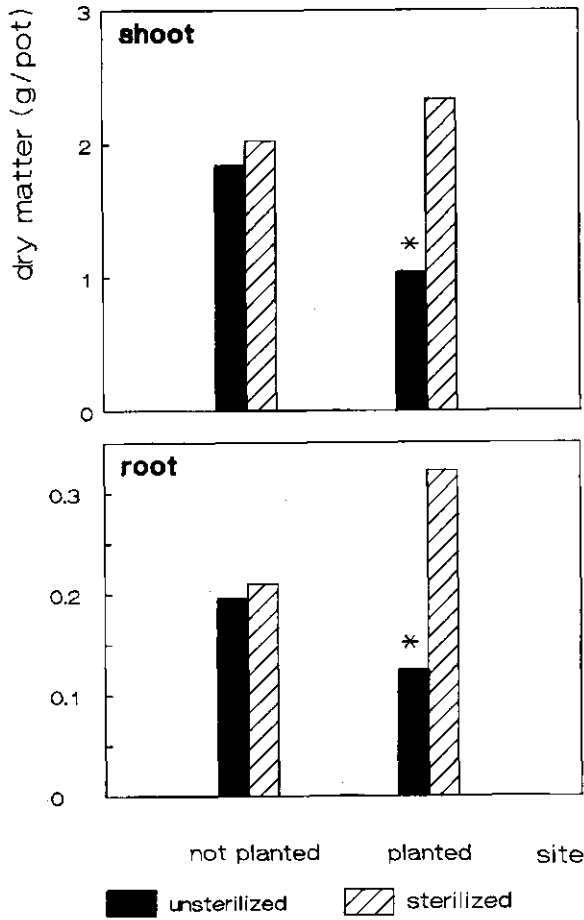
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Fig.4. Shoot and root dry matter of *Ammophila arenaria* in sterilized and unsterilized sand samples from an area planted with culms of *Ammophila arenaria* and an unplanted area on fresh sea sand (experiment 3). Significant differences (P<0.05) between sterilized and unsterilized sand have been indicated (*).

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Table 7. Analysis of variance for shoot and root dry
matter of <u>Ammophila arenaria</u> (experiment 3).

		F-values of dry weights	
Source of variation	df	Shoot	Root
Site	1	1.22 ns	0.01 ns
Sterilization	1	8.51 *	11.0 **
Site x sterilization	1	5.35 *	7.47 *
MSE	12	0.095	0.099

* P ⟨ 0.05   ** P ⟨ 0.01   ns not significant

sand that had been planted with culms prior to the examination. This site-dependent sterilization effect was confirmed by the ANOVA results ($P<0.05$; Table 7).

DISCUSSION

Research on the relationship between soil micro-organisms and succession of the vegetation in coastal foredunes has been focussed on the description of species in the different stages (Brown 1958, Kisiel 1970, Dennis 1983), or on their beneficial effect to colonizing plants (Webley et al. 1952, Nicolson 1960, Hassouna and Wareing 1964, Harley 1970, Abdel Wahab 1975, Ahmad and Neckelman 1978, Abdel Wahab and Wareing 1980, Ernst et al. 1984). Only recently, the possible impact of harmful soil organisms in dune vegetation succession was demonstrated for *Hippophaë rhamnoides* (Oremus and Otten 1981, Oremus 1982), as well as for *A. arenaria* (Van der Putten et al. 1988). In mobile dunes *A. arenaria* is supposed to maintain vigour by continuously colonizing windblown sand from the beach that does not contain harmful soil organisms (Van der Putten and Troelstra Chapter 6). Since harmful soil organisms occurred in both mobile and stable foredune stages (vigorous and degenerated *A. arenaria*, respectively) it is obvious that constant colonizing of windblown sand is essential for maintenance of vigour.

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The strongest growth reduction, increasing with depth, occurred in sand collected from the mobile stage, and not in that from the stable (degenerative) stage. Apparently, in the mobile dune vigorous growth enables *A. arenaria* to maintain a high sink activity resulting in strong root growth and storage of assimilates in underground plant parts. Soil organisms (both harmless and harmful), in turn, are not restricted by food resources and are stimulated to multiply. However, they will be relatively harmless to *A. arenaria* in the mobile dune, because the plants can continuously produce new roots in recently deposited windblown sand. The new roots in fresh sand are long and have abundant root hairs (Table 3). When the dune system becomes stabilized new roots can no longer be formed in fresh soil, since even the one-year-old root zone has been colonized by harmful soil organisms (experiment 2). Therefore, after sand deposition stops roots will be formed in soil containing the harmful soil organisms already. These organisms attack the roots, which results in deformation of the root system. According to the respons of seedlings in the greenhouse in sand from the mobile dune the total length of newly formed roots will be reduced, the roots will be heavily branched, and possess few root hairs. Such roots are restricted to the surface layers of the sand (Halwagy 1953 cited by Marshall 1965, Marshall 1965, Van der Putten et al. 1988), whereas formation of new roots in deeper soil layers does not occur, as is evident from examination of the condition of the cortex of the roots collected from the field (Table 3). Unfavourable environmental factors, such as drought and extreme soil temperatures (Baldwin and Maun 1983), will refrain the plants at stable sites from functioning optimally. Because of the reduced sink activity of the plants, the population of harmful soil organisms will be reduced in size and possibly the species composition will change (e.g. Brown 1958, Kisiel 1970). The low degree of growth reduction of plants in the greenhouse in sand from the stable dune can be related to a reduced inoculation potential of the harmful soil organisms at this succession stage. However, notwithstanding reduction and changes in composition of harmful soil organisms their presence in combination with unfavourable environmental factors is apparently sufficient to prevent *A. arenaria* from becoming vigorous again. This can be due to synergism between abiotic and biotic stress (Van der Putten et al. 1988, Van der Putten and Troelstra Chapter 6).

It is not yet clear which soil organisms reduce the vigour of *A. arenaria*. Disease symptoms mentioned above may be caused by a variety of organisms: bacteria, fungi, and nematodes (Bowen and Roviera 1961, Hornby and Fitt 1981, Oremus and Otten 1981, Oremus 1982). Elimination of plant parasitic nematodes in dune sand greatly increased the growth of test plants (Van der Putten et al. Chapter 5). However, in the present paper no functional relationships between nematodes and growth reduction could

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be detected. Numbers of *Meloidogyne maritima*, if present, were high enough to harm the plants in some soil samples from the mobile dune stage (P.W.Th. Maas, pers. comm.). On the other hand, in samples where parasitic nematodes were almost completely absent, soil sterilization was still beneficial to plant growth. A preliminary growth experiment using sterilized sand inoculated with soil fungi and nematodes, both obtained from roots of *A. arenaria* in the foredune, suggested that a combination of both groups was more pathogenic than each group separately (W.H. Van der Putten and W.J.M. Van Gulik, unpublished results). These results, however, need confirmation and more detailed study.

In the latter two experiments of the present paper it was shown that colonization of fresh sand by harmful soil organisms depended on the development of a root zone of *A. arenaria*. Whereas in experiment 1 the soil profile had been sampled into layers without regarding the distribution of the roots on forehand, in the latter experiments both rooted and rootless sand had been examined. In a highly mobile foredune successive horizontal root layers had been separated by layers of sand without roots of approximately 50 cm thickness. In the uppermost metre of the soil profile harmful organisms were restricted to the root layers. In the rootless zone in-between there was relatively little activity by harmful organisms.

In the half-year-old root zone of planted culms of *A. arenaria* harmful soil organisms were also found in dredged sea sand that did not contain them before planting the culms (Fig.4). The soil organisms apparently were introduced together with the culms. Conclusions concerning genetic differentiation of *A. arenaria* within a dune sere based on the growth of transplanted culms originating from different dune stages (Gray 1985) should, therefore, be viewed with caution. The differences observed by Gray (1985) will, at least partially, be related to the transplanting of different plant-micro-organism complexes.

The soil samples examined in the present paper were all collected during the winter period, when both plants and soil organisms will be in a steady state. Since the harmful soil organisms demonstrated the ability to colonize newly formed roots of *A. arenaria* during one growing season, it will be interesting to examine this plant - micro-organism relationship more in detail.

CHAPTER 8

GENERAL DISCUSSION

Each chapter of the present thesis has been supplied with a discussion of the results. This chapter focuses on the hypotheses reviewed in chapter 1 which concern the relationship between burial by windblown sand and vigour of *Ammophila arenaria*. A number of these hypotheses were at variance with the results of the study presented in this thesis.

Explanations of the relationship between burial by windblown sand and the maintenance of vigour of *Ammophila* can be summarized into 4 groups:
(1) *Windblown sand as a fertilizer.*
The deposition of windblown sand in stands of *Ammophila* has been considered as a supply of nutrients to the plants (Van Dieren 1934, Lux 1964, 1969). However, there was a lower growth increase when degenerated *A. arenaria* was fertilized than when it was buried with windblown sand, which contained less nutrients than the fertilizer (Willis 1963, 1965). This could have been due to leaching of the applied conventional fertilizer that was used by Willis (1965). However, addition of slow-release fertilizer to sand taken from degenerated *A. arenaria* stands also failed to promote growth of planted cuttings (Chapter 4). On the contrary, vigorous growth was obtained by fertilizing plants that were grown in sea sand (Chapter 4).
Another suggestion for the degeneration of *A. arenaria* has been the leaching of calcium carbonate from the sand (Van Dieren 1934, Lux 1964, 1969). A positive correlation between degeneration of *Ammophila* and decalcification of the sand could, however, not be established (Eldred and Maun 1982, Chapters 6 and 7).
The suggested role of free living N_2-fixing bacteria in the nutrition of *A. arenaria* (Hassouna and Wareing 1964, Ahmad and Neckelman 1978) was doubtful as it is impossible for the roots to supply the micro-organisms with sufficient carbon (Akkermans 1971, Abdel Wahab and Wareing 1980). Since the impact of non-symbiotic N_2-fixation requires a high level of photosynthetic capacity, high soil temperatures, and an ample supply of

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water, this process is more likely to be substantially in certain tropical environments (Dommergues et al. 1973) than in dry coastal sand dunes.

In the experiments described in chapter 4 plant roots were rarely infected by vesicular-arbuscular mycorrhizae (VAM). Inoculation of *A. arenaria* with *Glomus fasciculatum* did not result in root infection by this VAM species (R. Baas and W.H. Van der Putten, unpublished results) irrespective of the P-level in the sand. Therefore the suggested contribution of VAM to P-nutrition of *A. arenaria* (Nicolson 1960, Nicolson and Johnston 1979, Ernst et al. 1984) could not be demonstrated experimentally. Moreover, in a greenhouse experiment seedlings of *A. arenaria* produced less biomass in rhizosphere sand than in fresh sea sand irrespective of the amount of NPK supplied with the nutrient solution (Chapter 4). If both sand types were mixed, plant growth was reduced in mixtures containing more than 15 per cent rhizosphere sand. Therefore, an effect of beneficial micro-organisms could not be established. On the other hand, by sterilizing the sand it was shown that micro-organisms in dune sand may be harmful to *A. arenaria* (Chapters 4, 5, 6, and 7).

(2) *The role of windblown sand in eliminating competing species.*

It has been suggested that *A. arenaria* has lost competitive ability because of specialization for surviving sand burial (Huiskes 1979, Huiskes and Harper 1979). Hence, it was concluded that invading species, e.g. *Festuca rubra* and *Hippophaë rhamnoides*, expel *A. arenaria* from the vegetation by interspecific competition (Halwagy 1953 (cited by Marshall 1965) Huiskes 1979, 1980, Huiskes and Harper 1979). The degeneration of *Ammophila*, however, cannot be due to interspecific competition alone, since degeneration also takes place at sites where no other species are present (Hope-Simpson and Jefferies 1966, Eldred and Maun 1982, Disraeli 1984). In chapters 4 and 6, in outdoor experiments growth of *A. arenaria* in dune sand was found to be poor, although slow-release nutrients had been supplied and inter-specific competition did not occur. Therefore, interspecific competition, which positively will increase as soon as sand dunes become stabilized (Huiskes 1979) is probably not a major factor in the degeneration of *A. arenaria*.

(3) *Windblown sand as a means of preventing the plants from physiological ageing.*

A. arenaria was supposed to decline in stabilized dunes because of physiological ageing, as nodes can only form a few roots and the formation of new nodes is reduced as soon as the dune becomes stabilized (Gemmell et al. 1953, Marshall 1965). Old roots of *A. arenaria* lose their cortex (Purer 1941, Halwagy 1953 (cited by Marshall 1965), Marchant 1970, Gemmell et al. 1953, Marshall 1965) which results in reduced functioning (Marshall 1965). However, this theory does not explain why survival of vigorous culms or cuttings was less successful in sand from the root zone of *A.*

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arenaria (Chapters 4 and 6) than in sea sand or sterilized dune sand (Van der Putten and Van Gulik 1985, chapters 4 and 6). Therefore, it is more likely that some other factor is responsible for the reduced tiller formation (chapter 4).

(4) *Genetic differentiation.*

Differences in vigour between plants from mobile and stable dunes have been related to genetic differentiation within *A. arenaria* (Gray 1985). This hypothesis, however, was already rejected by Laing (1967). The results of chapter 7 showed that differences observed by Gray (1985) could have been due to the transplantation of plant-micro-organism complexes, rather than to the existence of differences in genotypes.

The present study has given a new hypothesis for relating the vigour of *A. arenaria* to burial by windblown sand. In chapter 4, it is shown by means of sterilizing the soil that sand from the root zone of *A. arenaria* contains harmful soil organisms. These organisms deform new roots (Capter 7) and reduce the root uptake-function (Chapter 4). Sand from stable, as well as from mobile dunes contains these organisms (Chapters 5, 6, and 7) they are, however, absent in sand from the beach (Chapter 6). By upward growth of *A. arenaria* and formation of roots in windblown beach sand, its vigour can be maintained by escape. As harmful organisms colonize the newly formed root zone within one growing season, plants must constantly receive windblown sand for escape in order maintaining vigour.

In stable foredunes young roots only occur in the uppermost 10-cm layer where stress from high soil temperatures and drought occurs (Baldwin and Maun 1983). Attack of the roots by harmful soil organisms results in short, strongly branched roots with few root hairs (Chapter 7). It is, therefore, concluded that attack of the root system of *Ammophila* by harmful soil organisms results in a higher susceptibility to adverse abiotic conditions such as drought.

Several terms can be used to indicate the occurrence of soil-borne diseases, e.g. 'major' and 'minor' pathogens (Salt 1979), 'self-intolerance' (Scholte and Kupers 1977), and 'soil sickness' (Hoestra 1968). These expressions have been used to indicate unexplained growth depressions of plant species that were grown repeatedly in successive crop rotations. However, the expressions 'self-intolerance' and 'soil sickness' are not specific about the possible factors involved, i.e. soil organisms, toxic compounds, or a combination. The difference between major and minor pathogens is quite artificial and may at least partly be due to insufficient knowledge of soil-borne pathogens. In the present thesis the cause of soil-borne disease of *A. arenaria* was described as being due to 'harmful soil organisms'. The use of this term unequivocally implies the involvement of soil organisms without discussing the conditions that are required for them to become pathogenic

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(Schippers et al. 1985).

Nematodes were supposed to be involved in the degeneration of *A. arenaria*, although not all growth reduction could be compensated for by the application of nematicides (chapter 5). The positive correlation between the presence of *Heterodera* (*avenae* group) and *Meloidogyne maritima* in rhizosphere sand and growth reduction of seedlings of *A. arenaria* (chapter 5) was not apparent in outdoor experiments (chapters 4 and 6). The possible interaction between nematodes and soil fungi has been examined. Nematodes and three soil fungi species (*Microdochium bolleyi*, *Fusarium culmorum*, and *F. oxysporum*) collected from roots of *A. arenaria* in the foredune, were added to pots with sterilized sand in which seedlings of *A. arenaria* were grown (greenhouse conditions see chapters 4-7). Nematodes did not significantly reduce growth of the seedlings, but *M. bolleyi* did (P<0.05; Table 1; W.H. Van der Putten and W.J.M. Van Gulik, unpublished results). Growth was reduced less by the combination of the three fungi than by *M. bolleyi* alone. However, the addition of nematodes to the pots with the mixture of fungi increased growth depression of *A. arenaria* (Table 1). These results suggest that interactions between groups of soil organisms may play an important role in the degeneration of *A. arenaria*.

Table 1. Total dry weight (mg/pot) of Ammophila arenaria seedlings that were grown in sterilized sand inoculated with soil fungi (Microdochium bolleyi, Fusarium culmorum, Fusarium oxysporum) or a mixture of these fungi with or without nematodes collected from the foredune sand. Significant differences between seedlings are indicated by different characters (P< 0.05).

		dry weight seedlings	
		without nematodes	with nematodes
0.	no fungi	608 ab	545 abc
1.	M. bolleyi	361 e	–
2.	F. culmorum	548 abc	–
3.	F. oxysporum	568 ab	–
4.	mixture of 1, 2 and 3	473 cde	339 de

Soil-borne diseases are well-known in agricultural monocultures, causing causing yield depressions in narrow rotation schemes (e.g. Domsch and Gams 1968, Scholte and Kupers 1977, Schippers et al. 1985, Haverkort 1988). In perennial crops, soil-borne pathogens cause replant problems, e.g. in pastures (Hoogerkamp 1984, Baan-Hofman and Van der Meer 1988)

and in orchards (Hoestra 1968, Catska et al. 1982, 1988, Slijkhuis and Li 1985). Replant failures in *A. arenaria* seem to be analogous to those in agricultural ecosystems. The harmful soil organisms causing replant failures in *A. arenaria* are probably the same as those initiating degeneration of this species after which it is succeeded by other plant species.

Succession in the vegetation has been related to changes in the environment (e.g. Harcombe 1980, Baldwin and Maun 1983) and to biotic interactions. The latter include interspecific competition between plant species (e.g. Werner 1976, Huiskes and Harper 1979), the occurrence of above-ground parasites (Weste 1981, Dobson and Hudson 1986, Burdon 1987, Jarosz and Burdon 1988), and changes in the below-ground community of soil micro-organisms (Webley et al. 1952, Harley 1970, Rose 1988). Only very few studies concern the involvement of harmful soil organisms in succession of natural vegetations (Oremus and Otten 1981, Oremus 1982, Maas, Oremus and Otten 1983, Zoon 1986).

The present investigation clearly indicates that the ecology of *A. arenaria* in coastal sand dunes is closely related to the occurrence of harmful soil organisms in its rhizosphere. In order to improve both knowledge of the thriving forces of natural succession and management of coastal foredunes the behaviour of harmful soil organisms in coastal ecosystems needs further study.

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SUMMARY

Introduction

This study deals with the establishment, growth, and degeneration of *Ammophila arenaria* (marram grass), a grass species that dominates the vegetation in coastal foredunes. Following natural establishment from rhizomes on high parts of the beach *A. arenaria* reduces wind velocity, which results in the accretion of windblown sand and the formation of dunes. *A. arenaria* grows vigorously in mobile dunes where fresh sand is deposited by wind, but it disappears from the vegetation when these dunes become stabilized.

Because of its ability to stabilize the sand, *A. arenaria* is often used in dunes to control erosion. It is planted according to a long practiced manual technique, using culms that are collected from stands at the foredune. When foredunes are reconstructed, however, (e.g. after storms) the establishment of *A. arenaria* is often unsuccessful, which means that erosion control can become very costly.

This study was carried out to develop new methods of establishing *A. arenaria* and to investigate the cause of the replant failures on reconstructed dunes. In addition, experiments were carried out to eludicate the relationship between the colonization of windblown sand and the vigour of *A. arenaria*.

Establishment

Two alternative methods were developed: (1) the sowing of seeds, which is rarely practiced and (2) the disk-harrowing of rhizomes, which has not previously been applied.

Experiments showed that seeds hardly germinate at constant temperatures. A high rate of germination can be achieved with fluctuating high temperatures, but at low temperatures the seeds required stratification in order to germinate. In the field the seeds germinated well if they had been sown during the winter, which was probably because of natural stratification. Seeds can be sown mechanically while afterwards the sand surface needs to be stabilized. For this purpose straw proved to be more effective for seedbed stabilization than spraid compost.

Rhizomes (i.e. vertical underground stems) were collected from the foredune by mechanical sieving of the sand. Experiments proved that the disk-harrowing of rhizomes was a useful method of establishing *A.*

arenaria provided that the sand surface was temporarily stabilized with straw or planted bundles of reed (chapter 2).

Growth

Field experiments showed that higher sowing rates and higher planting densities of rhizomes resulted in higher numbers of seedlings and primary shoots. However, after one growing season production of biomass and numbers of tillers appeared to be independent of the initial density. Application of slow-release NPK fertilizer (Osmocote, 12 to 14 months active at 21 °C) increased dry matter yield and numbers of tillers (chapter 2).

In a large-scale field experiment, rhizomes and a combination of planted culms and rhizomes produced more biomass and percentage cover than a sown stand during the first growing season. The lowest dry matter yield and percentage cover were obtained with traditionally planted culms. All plantings had been supplied with the same amount of slow-release NPK fertilizer. In the second year, however, the highest production was recorded for planted culms and for seeds in combination with compost. During these two years less than 5 per cent of the total area had to be replanted. All methods, therefore, were satisfactory in terms of sand stabilization.

The influence of uncontrolled factors was demonstrated by a 100 per cent higher production from a one-year-old stand in 1986 compared to 1987. The origin of the culms and rhizomes also influenced growth. Culms or rhizomes that had been collected from a stable dune with degenerated *A. arenaria* produced less dry matter and percentage cover than when the plant material was obtained from a mobile dune which was covered by vigorous plants (chapter 3).

Degeneration

Growth of seedlings of *A. arenaria* was strongly reduced in sand from the root zone of a foredune, when compared to growth in fresh (sea) sand. However, no differences occurred when both sand samples were sterilized prior to planting of the seedlings. In sea sand, growth was equal to that in sterilized sand. It was concluded, therefore, that the rhizosphere of *A. arenaria* contained harmful soil organisms (chapter 4).

In order to trace the nature of these organisms, biocides (bactericides, a nematicide, and fungicides) were applied to rhizosphere sand, which was planted with seedlings of *A. arenaria*. Bacteria were not supposed to be involved in the degeneration of *A. arenaria*, as bactericides did not affect plant growth. The nematicides effectively eliminated endoparasitic

nematodes (*Heterodera avenae* group, *Meloidogyne maritima*, and *Pratylenchus* sp.) and the application led to increased plant growth. Fungicides also enhanced growth, however, they also eliminated the nematodes *H. avenae* and *M. maritima*. It was concluded that nematodes were involved in the growth reduction and degeneration of *A. arenaria*, but the involvement of soil fungi could not be established unequivocally. Results of a preliminary inoculation experiment suggested that a complex of soil fungi and nematodes is responsible for the degeneration of *A. arenaria* (chapters 5 and 8).

The harmful soil organisms from a certain location reduced growth of local, as well as of foreign populations of *A. arenaria*. The growth of *Calammophila baltica* (purple, or hybrid marram grass, a sterile bastard of *A. arenaria* x *Calamagrostis epigejos*) was also reduced by harmful soil organisms, but less than *A. arenaria* (chapters 4 and 6).

In three Dutch coastal dune systems harmful soil organisms were detected in the root zones of stable, as well as of mobile foredunes (degenerating and vigorous *A. arenaria*, respectively), but not in beach sand. The relation between sand deposition by wind and vigorous growth of *A. arenaria* was explained by supposing that windblown sand, originating from the beach, enables *A. arenaria* to escape harmful soil organisms (chapter 6). However, within one year after plants had produced new roots in fresh windblown sand, the root system became colonized by harmful soil organisms (chapter 7).

If harmful organisms were present in the sand prior to root growth, root hair formation was reduced severely and the branching of the roots was stimulated (chapter 7). This deformation of the root system by harmful soil organisms is assumed to be related to the degeneration of *A. arenaria*. A reduced uptake function and a shallow placement of the root system due to attack by harmful soil organisms in stable dunes increases the susceptibility of the plants to stress of drought, high soil temperatures, and shortage of nutrients. It is concluded that the degeneration of *A. arenaria* in stable dunes is caused by a combination of harmful biotic factors and abiotic stress.

SAMENVATTING

VESTIGING, GROEI EN DEGENERATIE VAN AMMOPHILA ARENARIA IN KUSTDUINEN

Inleiding

Helm (*Ammophila arenaria* (L.) Link) is een grassoort die van nature de vegetatie van de buitenduinen domineert. Helm vestigt zich op hoge delen van het strand uit wortelstokken, stengelstukken en soms uit zaad. Door de aanwezigheid van helmplanten wordt de wind gebroken, als gevolg waarvan stuivend zand tot rust komt en duinen worden gevormd.

Helm groeit zeer goed aan de zeezijde van de buitenduinen, waar regelmatig vers strandzand door de wind wordt gedeponeerd. Meer landinwaarts, waar de aanvoer van vers zand stagneert, is helm minder vitaal dan langs het strand. Degeneratie van helm in binnenduinen is de oorzaak van het verdwijnen van de soort uit de vegetatie. Naar de oorzaak van helmdegeneratie is veel onderzoek verricht, maar een sluitende verklaring is tot op heden nog niet aangegeven.

Als gevolg van speciale eigenschappen is helm zeer geschikt om stuivend zand vast te leggen en wordt hiertoe veelvuldig aangeplant. Volgens de traditionele pootmethode worden planten met een deel van de ondergrondse stengel afgestoken en in bosjes uitgeplant. Het steken en poten van helm vindt in de wintermaanden plaats, waarna in het voorjaar knoppen op de ondergrondse stengels uitlopen en nieuwe scheuten vormen. Deze methode wordt overal ter wereld op vrijwel dezelfde wijze uitgevoerd en is al zeer oud. Op Voorne werd reeds in 1423 helm gepoot en volgens bronnen is in Engeland sinds de 15[e] eeuw al helm gebruikt voor de vastlegging van duinzand.

Problemen met de aanleg van helmbegroeiing vormden de aanleiding voor het hier beschreven onderzoek naar vestiging, groei en degeneratie van helm in kustduinen. Op Voorne zijn in het kader van de Deltawerken in de zestiger en zeventiger jaren werkzaamheden uitgevoerd in de zeereep door het waterschap De Brielse Dijkring. Na het aanplanten van helmbegroeiing sloeg deze echter moeizaam aan. De ervaring was dat drie tot vijf keer

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diende te worden gepoot voordat zich een effectief-stuifwerende
begroeiing had ontwikkeld.

De werkzaamheden op Voorne dienden slechts als voorbereiding op de
definitieve duinverzwaring, die in het kader van de Deltawerken zou
worden uitgevoerd. Problemen met de inplant van helm tijdens de
grootschalige duinverzwaring (120 ha) zou enorm kostenverhogend werken,
aangezien het eenmalig aanplanten van 1 ha met helm f20.000,- tot
f30.000,- bedraagt. Behalve het kostenaspect was er nog een tweede zorg.
Achter de zeewerende duinen van Voorne ligt een natuurgebied met
vochtige duinvalleien van internationaal unieke natuur- en
wetenschappelijke waarde, dat tegen instuivend zand beschermd diende te
worden. Problemen met de aanplant van helm na verzwaring van de
zeereep zou overstuiving van het achterliggende gebied met zich mee
kunnen brengen.

Deze twee factoren, de hoge kosten van het mislukken van de aanplant en
de schade die zou kunnen worden toegebracht aan het unieke
natuurgebied, vormden voor het waterschap De Brielse Dijkring het
argument onderzoek te laten verrichten door het Instituut voor Oecologisch
Onderzoek (IOO), afdeling Duinonderzoek "Weevers' Duin" te Oostvoorne
(april 1984 tot juli 1988). Het onderzoek werd gefinancierd door
Rijkswaterstaat, terwijl de verdere bewerking van de resultaten in dit
proefschrift door het IOO mogelijk gemaakt is.

Doelstelling

Het onderzoek diende te leiden tot methoden volgens welke snel een
effectief-stuifwerende begroeiing kan worden aangelegd op verzwaarde
zeewerende duinen. Binnen deze hoofddoelstelling kunnen drie
deeldoelstellingen onderscheiden worden:

(1) De ontwikkeling van nieuwe technieken, waardoor snel en goedkoop
begroeiing kan worden aangebracht en waardoor efficient gebruik kan
worden gemaakt van plantedelen die geschikt zijn voor vermeerdering.
Teneinde efficiënter om te gaan met het voor vermeerdering beschikbare
plantmateriaal, was het onderzoek gericht op het gebruik van zaad en
ondergrondse stengeldelen bij de aanleg van helmbegroeiing. Het zaaien
van helm wordt zelden toegepast. Stengeldelen zijn - voor zover bekend -
nog nooit gebruikt bij de aanleg van helmbegroeiing.

(2) Het testen en vergelijken van de nieuw ontwikkelde methoden met de
traditionele pootmethode op praktijkschaal.

(3) Onderzoek naar de factoren die de herinplantproblemen en de
degeneratie van helm veroorzaken.

Voor de uitvoering van het onderzoek is gebruik gemaakt van het
laboratorium, de kas, de buitenopstelling en van proefvelden. De resultaten

worden in het vervolg samengevat.

De vestiging van helm

Proeven wezen uit dat helmzaad dient te worden voorbehandeld teneinde de kieming bij lage temperaturen te bevorderen. In het veld was geen zaadvoorbehandeling nodig indien het helmzaad in de winter gezaaid werd. Hoogstwaarschijnlijk vindt tijdens de winter in het zand een natuurlijke voorbehandeling plaats. Na inzaaien (hetgeen machinaal kon worden gedaan) is het van belang het zandoppervlak te beschermen tegen verstuiving. Stro ineggen is hiertoe een beter middel dan het verspuiten van een suspensie van edelcompost.

In een helmduin bevinden zich ondergrondse stengels met daarop knoppen, die in rust zijn. Na duinafslag kunnen deze stengels op het strand terecht komen en onder gunstige omstandigheden uitlopen en nieuwe scheuten vormen. De tijdens het onderzoek ontwikkelde stengeldelenmethode is een gemechaniseerde versie van dit natuurlijke proces. Volgens de nieuwe (stengeldeel)methode worden ondergrondse stengels machinaal uit het duin verwijderd en door middel van een schijveneg gefractioneerd en tegelijkertijd ondergeploegd. Door stro in te eggen of rietbundels te poten werd het zandoppervlak gestabiliseerd. Op het buitentalud functioneerde stro beter dan riet (hoofdstuk 2).

Groei

Op een zeewerend duin werd een aantal velden aangelegd met verschillende zaaizaad- en stengeldeeldichtheden. Het aantal kiemplanten nam toe naarmate de zaaidichtheid hoger was, maar aan het eind van het groeiseizoen waren er geen significante verschillen in de droge stofopbrengst en de spruitdichtheid. Hetzelfde effect trad op bij stengeldelen. De dichtheid van stengeldelen was bepalend voor de opkomst van het aantal scheuten, maar na één groeiseizoen waren de verschillen in de droge stofproductie en uitstoeling niet significant. Langzaam werkende kunstmeststof (type 'Osmocote') had duidelijk een groter effect op de helmgroei dan de zaai- of stengeldeeldichtheid (hoofdstuk 2).

In een grootschalige praktijkproef (45 ha) zijn de verschillende pootmethoden onderling vergeleken, waarbij alle proefvakken werden voorzien van dezelfde hoeveelheid langzaam werkende kunstmeststof. Aan het eind van het eerste groeiseizoen werden de hoogste droge stofopbrengst en bodembedekking gemeten in velden met stengeldelen en in velden met een combinatie van traditioneel gepote helm en stengeldelen. De traditionele pootmethode leverde de laagste produktie op, terwijl die in velden met gezaaide helm (in combinatie met stro of met edelcompost)

intermediair was. Aan het eind van het tweede groeiseizoen werden de hoogste productie en bodembedekking gemeten in traditioneel gepote helm en in gezaaide helm met edelcompost. De laatstgenoemde methode gaf echter een nogal heterogeen resultaat en er ontstonden plaatselijk stuifplekken als gevolg van scheuren in de compostkorst (hoofdstuk 3).

Twee factoren bleken van invloed te zijn op de groei van de helmaanplant: (1) herkomst van het pootgoed en (2) overige factoren.

(1) Helmplanten en stengeldelen die verzameld waren in een stabiel duin produceerden duidelijk minder droge stof terwijl de bodembedekking geringer was dan planten afkomstig uit een mobiel duin. Deze verschillen waren in het tweede groeiseizoen nog groter dan in het eerste jaar.

(2) In 1986 was de droge stofproduktie in alle velden bijna twee keer zo hoog als in 1987. De verschillen tussen de methoden waren echter onafhankelijk van het jaar waarin ze onderzocht werden (hoofdstuk 3).

Na toepassing van de nieuwe methoden diende slechts 5 procent van het totale oppervlak opnieuw te worden beplant. Gezien het lage uitvalspercentage leidden alle gebruikte methoden tot een effectieve zandvastlegging. De conclusie is dat zowel het zaaien van helm als het ineggen van stengeldelen technisch haalbaar zijn, maar dat het zaaien van helm op geëxponeerde plekken minder kans van slagen heeft dan het gebruik van planten of stengeldelen (hoofdstuk 3).

Degeneratie

De oorzaak van slechte groei van helm in stabiele duinen werd onderzocht door kiemplanten op te kweken in zand afkomstig van (1) de wortelzone van helm en (2) in zeezand. In zeezand groeiden de planten goed, maar in duinzand werd de groei geremd. Sterilisatie van het duinzand, voorafgaand aan het planten van de zaailingen, had echter een duidelijk betere groei tot gevolg. Uit deze resultaten werd geconcludeerd, dat in de wortelzone van helm schadelijke bodemorganismen aanwezig zijn (hoofdstuk 4).

Teneinde na te gaan welke bodemorganismen schadelijk zijn voor helm werden in de kas selectieve remstoffen toegediend aan zand uit een helmduin, waarna de groei van kiemplanten bestudeerd werd. Bactericiden (bacteriënremmers) hadden geen positief effect op de groei. Nematiciden (aaltjesremmers) bevorderden de groei van de helmplanten, terwijl de behandeling tot gevolg had dat de wortels vrij bleven van aaltjes (de endoparasitaire nematoden: *Heterodera* (*avenae* groep), *Meloidogyne maritima* en *Pratylenchus* sp.). Geconcludeerd werd, dat aaltjes betrokken zijn bij de degeneratie van helmbegroeiing. Fungiciden (schimmelremmers) bevorderden eveneens de groei van helm. Omdat fungiciden echter eveneens infectie door aaltjes (*H. avenae* en *M. maritima*) remden, kon de rol van schimmels in de helmdegeneratie niet worden vastgesteld.

Desalnietemin kon uit de resultaten van een voorlopige inoculatieproef worden afgeleid dat bodemschimmels mogelijk betrokken zijn bij de helmdegeneratie, waarschijnlijk in samenwerking met de aaltjes (hoofdstukken 5 en 8).

Uit proeven met zand van de locaties Texel, Voorne en Schouwen bleek dat op al deze plekken schadelijke bodemorganismen voorkwamen. Deze bevindingen suggereren dat schadelijke bodemorganismen algemeen voorkomen langs de Nederlandse kust. Helmpopulaties van deze kustlocaties verschilden niet in gevoeligheid voor de schadelijke bodemorganismen. Ook noordse helm (*Calammophila baltica*), een steriele bastaard van helm x duinriet (*Calamagrostis epigejos*) werd in groei geremd door de schadelijke bodemorganismen, maar alleen indien planten onder semi-natuurlijke omstandigheden werden opgekweekt, hetgeen hoogstwaarschijnlijk het gevolg was van verhoogde stress-omstandigheden (hoofdstukken 4 en 6).

In een vergelijkende proef met zand afkomstig van het strand, duinen met vitale helm (mobiele duinen) en duinen met kwijnende helm (stabiele duinen) werd aangetoond dat schadelijke bodemorganismen zowel voorkomen in zand van mobiele duinen als in zand van stabiele duinen. In strandzand kwamen deze organismen niet voor (hoofdstuk 6). In duinen met verschillende hoeveelheden aangestoven zand, evenals in een jonge aanplant op zeezand, bleek dat nieuw gevormde wortels binnen één groeiseizoen worden gekoloniseerd door schadelijke bodemorganismen.

Vitaliteit van helm in mobiele duinen kan op grond van deze onderzoeksresultaten worden verklaard door aan te nemen dat de helmplanten letterlijk ontsnappen aan schadelijke bodemorganismen, als gevolg van voortdurende wortelvorming in bovenliggende lagen van vers, door de wind aangevoerd strandzand.

Indien helm werd gekweekt op zand met schadelijke organismen was de lengtegroei van de wortels geremd, de wortelvertakking gestimuleerd en de vorming van wortelharen gereduceerd. De conclusie is dat de groeiremming van helm in stabiele duinen veroorzaakt wordt doordat schadelijke bodemorganismen het wortelstelsel aantasten, zodat helm niet in staat is te overleven onder de in stabiele duinen voorkomende stressomstandigheden (droogte, schaarste aan voedingsstoffen en hoge bodemtemperaturen). De degeneratie van helm lijkt dus het gevolg te zijn van een combinatie van schadelijke biotische factoren en abiotische stress (hoofdstuk 7).

CURRICULUM VITAE

Wilhelmus Henricus van der Putten is op 12 juni 1958 geboren te Winssen (GLD). Van 1970 tot 1977 volgde hij de middelbare school aan het Canisius College te Nijmegen (resp. HAVO en Athenaeum). Van september 1977 tot september 1984 studeerde hij biologie aan de Landbouwhogeschool (nu Landbouwuniversiteit) te Wageningen en combineerde studie met de medeverantwoordelijkheid voor de ouderlijke boerderij (1976-1986). In de kandidaatsfase koos hij de orientatie "populatie" met als afstudeervakken onkruidkunde, bodemkunde en bemestingsleer en voorlichtingskunde. In het kader van de praktijktijd heeft hij gedurende 6 maanden onderzoek uitgevoerd op het FAPROCAF-project in Arequipa, Perú, naar de gevolgen van veronkruiding van lucernevelden.

In april 1984 kwam hij in dienst bij het Instituut voor Oecologisch Onderzoek (IOO), Afdeling Duinonderzoek "Weevers' Duin" te Oostvoorne. In opdracht van het waterschap De Brielse Dijkring en Rijkswaterstaat voerde hij onderzoek uit naar "Stimulering van Duinbegroeiing". De wetenschappelijke weerslag van het onderzoek is in dit proefschrift vastgelegd.

Eind 1988 aanvaardde hij een post doc aanstelling bij het IOO te Heteren, in het bijzonder voor de acquisitie en begeleiding van toepassingsgericht onderzoek.

STELLINGEN

1. De degeneratie van helm (*Ammophila arenaria*) in stabiele duinen wordt veroorzaakt door een combinatie van schadelijke bodemorganismen en abiotische stress.
    -Dit proefschrift

2. De hoge vitaliteit van helm in mobiele duinen, waar regelmatig vers zand door de wind wordt afgezet, wordt veroorzaakt doordat de planten de kans krijgen nieuwe wortels te vormen in substraat dat vrij is van schadelijke bodemorganismen. Zandsuppletie op smalle en lage stranden met aanlandige wind is daarom een effectief middel voor instandhouding van de helmbegroeiing op de zeereep.
    -Dit proefschrift

3. Het door Van Dieren (1934) en Lux (1964) gesuggereerde verband tussen ontkalking van duinzand en degeneratie van helmbegroeiing is noch correlatief (Eldred and Maun 1982), noch experimenteel aannemelijk te maken.
    -Van Dieren, J.W. (1934) Organogene Dünenbildung.
     Martinus Nijhoff, Den Haag, Holland. 304 pp.
    -Lux, H. (1964) Angew. Pflanzensoziol. 20, 5-53.
    -Eldred, R.A. and Maun, M.A. (1982) Can. J. Bot. 60, 1371-1380.
    -Dit proefschrift.

4. De rol van bodempathogenen in de vegetatiesuccessie, zoals aangetoond in duinvegetaties, is onvoldoende onderkend.
    -Oremus, P.A.I. (1982) Proefschrift Universiteit van Utrecht.
    -Zoon, F. Proefschrift in voorbereiding.
    -Dit proefschrift.

5. De rol van bodemmicro-organismen in de plantevoeding kan niet alléén worden vastgesteld op grond van inoculatie-experimenten met één of enkele organismen onder geconditioneerde omstandigheden, aangezien planten in het veld onder invloed staan van de resultante van alle in de bodem aanwezige organismen.
    -Hassouna, M.G. and Wareing, P.F. (1964) Nature 202, 467-469.
    -Ahmad, M.H. and Neckelmann, J. (1978) Z. Pflanzenernaehr.
     Bodenkd. 141, 117-121.
    -Ernst, W.H.O. et al. (1984) Acta Bot. Neerl. 33, 151-160.
    -Baas, R. Proefschrift in voorbereiding.
    -Dit proefschrift.

6. De conclusies van Gray met betrekking tot genetische differentiatie binnen de soort *Ammophila arenaria* zijn gebaseerd op een veldexperiment waarvan een verkeerde opzet het onmogelijk maakt uitspraken te doen over genetische variatie.

-Gray, A.J. (1985) Vegetatio 61, 179-188.

7. De indeling van schadelijke bodemmicro-organismen in 'major' en 'minor' pathogens is eerder een gevolg van onvoldoende kennis over de relatie tussen bodemmicro-organismen en hogere planten, dan van een tweedeling in de wijze waarop bodemmicro-organismen schade toebrengen aan planten.

-Salt, G.A. (1979) In: Soil-borne Plant Pathogens.
Ed. by B. Schippers and W. Gams p.289-312.

8. Door het benadrukken van de waarde van wegbermen als lintvormige elementen in het landschap, bestaat het gevaar dat de aanleg van autowegen als gewenste methode beschouwd wordt om natuurgebieden onderling te verbinden.

9. Toepassingsgericht onderzoek geeft meer gelegenheid tot integratie van vakgebieden dan fundamenteel onderzoek.

10. Bevordering van de welvaart in ontwikkelingslanden zonder daarbij een goed afvalpreventieplan te leveren, heeft op termijn een averechts effect op het welzijn.

11. Bij het recruteringsbeleid van het Ministerie van Defensie wordt aan de verdediging van de natie een zodanig hoge prioriteit toegekend, dat de verdediging van het land veronachtzaamd wordt.

-Uitspraak n[o]81, 28 april 1987 van de Raad van State.

12. De stelling dat stilstand achteruitgang is, impliceert ten onrechte dat beweging vooruitgang betekent.

Establishment, growth and degeneration of Ammophila arenaria
in coastal sand dunes.
W.H. van der Putten
Nijmegen, 1989