Harmful soil organisms in coastal foredunes involved in degeneration of *Ammophila arenaria* and *Calammophila baltica*

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Received April 3, 1989

VAN DER PUTTEN, W. H., and TROELSTRA, S. R. 1990. Harmful soil organisms in coastal foredunes involved in degeneration of *Ammophila arenaria* and *Calammophila baltica*. Can. J. Bot. **68**: 1560–1568.

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 were 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 dunes, 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. Degree of growth reduction by harmful soil organisms could not be related to numbers of plant parasitic nematodes.

Key words: *Ammophila arenaria*, *Calammophila baltica*, coastal sand dunes, succession, harmful soil organisms.

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Un bioessai a été utilisé pour déceler dans la zone racinaire de l'*Ammophila arenaria* la présence d'organismes nuisibles. À cette fin, trois localités ont été sélectionnées le long de la côte sablonneuse de la Hollande : Voorne, Texel et Schouwen. Des organismes nuisibles ont été retrouvés à partir des trois localités, dans le sable des dunes stables aussi bien que dans celui des dunes mobiles (avec l'*A. arenaria* dégénéré ou vigoureux, respectivement). Dans le sable de la plage, on ne retrouve cependant aucun organisme nuisible. Comme l'*A. arenaria* ne montre un développement vigoureux que lorsqu'il est régulièrement enterré par le sable soufflé à partir de la grève, les auteurs concluent que cet ensablement permet à la plante d'échapper aux organismes nuisibles du sol. Des plants de l'*Ammophila arenaria* et du *Calammophila baltica* provenant de la localité de Voorne ont été cultivés à l'extérieur en récipients, lesquels ont été remplis avec du sable de la plage, du sable de la dune mobile et du sable de la dune stable ainsi qu'avec du sable de dune stable préalablement stérilisé. La production de biomasse chez les deux espèces est plus élevée dans le stable stérilisé provenant de la dune stable suivie (par ordre décroissant) par celui de la plage et celui de la dune mobile et enfin le sable non stérilisé de la dune stable. Comparativement aux *A. arenaria*, cependant, la croissance du *C. baltica* est moins réduite et ne montre pas de mortalité. Il n'a pas été possible de relier l'effet néfaste des organismes du sol avec les nématodes phytoparasites.

Mots clés : *Ammophila arenaria*, *Calammophila baltica*, dunes de la côte, succession, effet néfaste des organismes du sol.

[Traduit par la revue]

Introduction

Ammophila arenaria (L.) Link (marram grass) is a perennial grass species occurring naturally on coastal foredune ridges in Europe and the Mediterranean (Huiskes 1979). It has been introduced in Australia and North America (Knutson 1978). *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 northwestern Europe, but is less dominant than *A. arenaria* (Westergaard 1943; Kubien 1970). In the field, *C. baltica* can be distinguished from *A. arenaria* because it 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 also planted to control erosion of coastal foredunes.

Ammophila arenaria is well known because of its ability to survive sand burial of 80–100 cm/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 plants decline soon after 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 North America (Laing 1958, 1967; Eldred and Maun 1982; Disraeli 1984; Maun 1985; 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 beneficial effects of drifting sand have been related to nutrient supply, removal of competing plant species, and avoidance of physiological ageing (Marshall 1965; Laing 1967; Eldred and Maun 1982). Recently, it was reported that the root zone of *A. arenaria* contained soil organisms that impair its growth and could be involved in its decline

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Printed in Canada / Imprimé au Canada

(Van der Putten et al. 1988). Further study showed that such harmful soil organisms colonized new roots formed in fresh, windblown sand (usually originating from the beach) within one growing season (Van der Putten et al. 1989).

These results suggest that windblown sand is beneficial for *A. arenaria*, because upward growth enables plants to escape from harmful soil organisms and therefore from degeneration (Van der Putten 1989). However, two prerequisites for this hypothesis have to be checked: (*i*) If harmful soil organisms are involved in the decline of *Ammophila*, they would be ubiquitous in randomly chosen locations with degenerating plants. (*ii*) If fresh sand enables *Ammophila* to escape from harmful soil organisms in its root zone, these organisms would be absent in the windblown sand originating from the beach.

These two assumptions will be examined, as well as the growth of *A. arenaria* populations in local and alien substrates. Moreover, the susceptibility of *A. arenaria* for the harmful soil organisms was compared with that of *C. baltica*. The interaction between harmful soil organisms and abiotic soil conditions and its possible impact on vegetation succession in coastal foredunes is discussed.

Materials and methods

Experiment 1: sand from different locations and sites

Study sites

Sampling sites were chosen at three island locations in The Netherlands: Voorne (432-62; coordinates refer to the Dutch State Survey Grid, SSG), Texel (561-109), and Schouwen (416-38). Coastal dunes of Voorne and Schouwen have a high calcium carbonate content compared with low levels in Texel dunes. At these locations, *A. arenaria* is planted frequently to protect the foredunes from sand erosion. For a long time the local wardens have almost exclusively planted locally gathered *A. arenaria* plant material.

At each location, 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 3 years before sampling to protect the foredune against erosion by the sea. At 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 a sand accretion of 10–30 cm/year. The stable dune rarely received any windblown sand.

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 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. All 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 γ irradiation (2.5 Mrad (1 rad = 10 mGy); see Oremus 1982). Treatments were carried out in a $3 \times 3 \times 2$ factorial design with four replicates. The factors were location (Voorne, Texel, and Schouwen), sand type (beach, mobile, and stable dune), and sterilization (sterilized and unsterilized).

Soil analysis

Bulk soil samples were obtained by mixing equivalent amounts of the four replicates. After drying (35°C) and sieving (2-mm mesh), these 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 H_2O or 1 M KCl. Carbonates were measured gasvolumetrically 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.

Collection of seeds

In July 1987, spikes of *A. arenaria* were collected per location from a 100×20 m^2 plot. The spikes were dried and threshed. The 1000-kernel weights of the caryopses were determined in five random samples of 100 caryopses each.

Growing and harvesting plants

Pots of 1.5 L were filled with 1950 g of sand containing 15% moisture, based on dry sand weight. Seeds were germinated in a growth chamber set at 30°C light (8 h) and 20°C dark (16 h). Uniform, 2-week-old seedlings (plumule 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 to October 1987), and illumination (Philips HLRG-400 W, 4.8 $W \cdot m^{-2}$) was supplied to keep the photoperiod at 12–14 h/day. 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 to compensate for a possible nutrient flush after sterilizing the soil. Increasing rates were applied because of increasing plant requirements (Van der Putten et al. 1988). Rates applied were (1 H = full strength solution) as follows: 25 mL ½ H (weeks 1 and 2), 25 mL 1 H (weeks 3 and 4), and 50 mL 1 H (weeks 5 and 6). Plants were harvested after 44 days. Dry weights of shoots and roots were determined after drying for 48 h at 70°C.

Experiment 2: Ammophila arenaria *populations*

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 from Texel and Schouwen populations were planted in sand from the alien locations. Sampling of sand, collection of seeds, and growing and harvesting of plants were performed as in experiment 1. Shoot to total weight ratio (SWR) was calculated as shoot dry weight divided by (shoot + root dry weight).

Treatments were carried out in a $3 \times 3 \times 2 \times 2$ factorial design with the 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: Ammophila arenaria *and* Calammophila baltica

Sampling, sand treatment, and experimental design

In May 1986, sand was collected from the beach, the mobile dune and the stable dune at Voorne (same sites as in experiments 1 and 2). At each site, sand was collected from 0–20 cm depth and within an area of 5×5 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 containing 4% moisture (based on dry weight). Additional containers were filled with 70 kg of sand from the stable dune containing 4% moisture that had been sterilized by means of γ irradiation (2.5 Mrad). Containers were planted with either *A. arenaria* or *C. baltica*. Treatments were carried out in a 4×2 factorial design with the factors sand type (beach, mobile, stable unsterilized, stable sterilized) and plant species (*A. arenaria* and *C. baltica*). Each treatment was replicated four times.

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 and sampling site	Grain size (μm)					
	<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

Collecting, growing, and harvesting 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 for 2 weeks in shallow trays filled with pure quartz sand of fine texture (Van der Putten et al. 1988). Five 2-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 μm), situated in the centre of the pot for the collection of nematodes from roots.

The containers were placed outdoors on a tiled floor in an experimental garden. Each container was supplied with 3.25 g slow-release N–P–K fertilizer (Osmocote, 24:6:6, active for 9–12 months at 21°C) to avoid possible differences in soil fertility.

Plants were grown from June 4 until November 3, 1986. The containers were supplied daily with tap water until 2 weeks after planting, after which the plants were completely dependent on natural rainfall. On July 10, plants with gauze cases were removed and the remaining pits filled with sand from the corresponding place of origin. Sand and roots in the gauze cases were examined for 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 h at 70°C and weighed. A random subsample of the fresh root material was used to determine the root length (Comair root length scanner, Commonwealth Aircraft Cooperation Ltd., Melbourne, 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).

Active nematodes were separated from the soil samples (300 mL of sand, specific volume of 0.7 $mL \cdot g^{-1}$) 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, centrifuged, and nematodes were removed by a floatation technique (Coolen and d'Herde 1972).

Plant analysis

Plant samples dried at 70°C were used for analysis. Material of each pot was analyzed separately (i.e., four replicates). In a digest of sulphuric acid and salicylic acid, N and P were determined colorimetrically. An indophenol-blue method was used for N and a molybdenum-blue method for P. K was determined by atomic absorption spectrophotometry. 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 (Fale 1951) and a calibration curve for glucose.

Data analysis

Data were analysed by means of analysis of variance (ANOVA). necessary according to Cochran's test ($P<0.05$), data were transformed to a logarithm or an arcsin(square root) to obtain homogeneit of variances. Treatment means were compared by Tukey's test (Soka and Rohlf 1981).

In experiment 2, contrasts could be calculated in the main factor location and population, since interactions of these factors with other were absent. Thus components with single degree of freedom wer obtained in the ANOVA (Sokal and Rohlf 1981). Effect of sand ste ilization was tested for each combination of sand type and populatio by pairwise *t*-tests.

Results

Soil characteristics of the sampling locations

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

The pH of all sand samples was high (≥8.4; Table 2 Organic matter, K, total N, and total P were low in all san samples. $CaCO_3$ content was obviously highest in Voorne an lowest in Texel sand (Table 2). Per location, no large diffe ences were observed in nutrient levels among the samplin sites; total N generally tended to be highest in sand from th stable site, and factors related to salinity in sand from th beach.

Experiment 1: sand from different location and sites

The effect of soil sterilization on shoot production was n the same for all sand types owing to the interaction betwee sand type and soil sterilization (Table 3). Sterilization cause an increase in shoot production only in sand from the mobi and the stable dune, and not in beach sand (Fig. 1).

At location Voorne, sterilization increased root yield si nificantly in sand from both mobile and stable dunes, but n in beach sand ($P<0.05$; Fig. 1). The same trend was observe for locations Texel and Schouwen. However, notwithstandir the significant sterilization effect in the ANOVA (Table 3), th was not significant for the individual sand types ($P>0.0$ Fig. 1).

Experiment 2: Ammophila arenaria *populations*

Soil sterilization increased shoot and root growth signif cantly in all cases according to pairwise *t*-tests ($P<0.0$ Fig. 2). Shoot yield was affected by all main factors (locatio population, sand type, and soil sterilization; Table 4). Inte action ($P<0.01$) was observed only between sand type ar soil sterilization. Calculation of contrasts showed that the si nificant location effect in the ANOVA was caused by high shoot production in sand from Texel, as compared with th in sand from Voorne and Schouwen ($P<0.05$; Table 4). Ro weight showed no significant effect of location and sand ty (Table 4).

The absence of a significant interaction between the fact population and the other factors emphasizes the generally sin ilar reaction of the three populations of *A. arenaria* wi

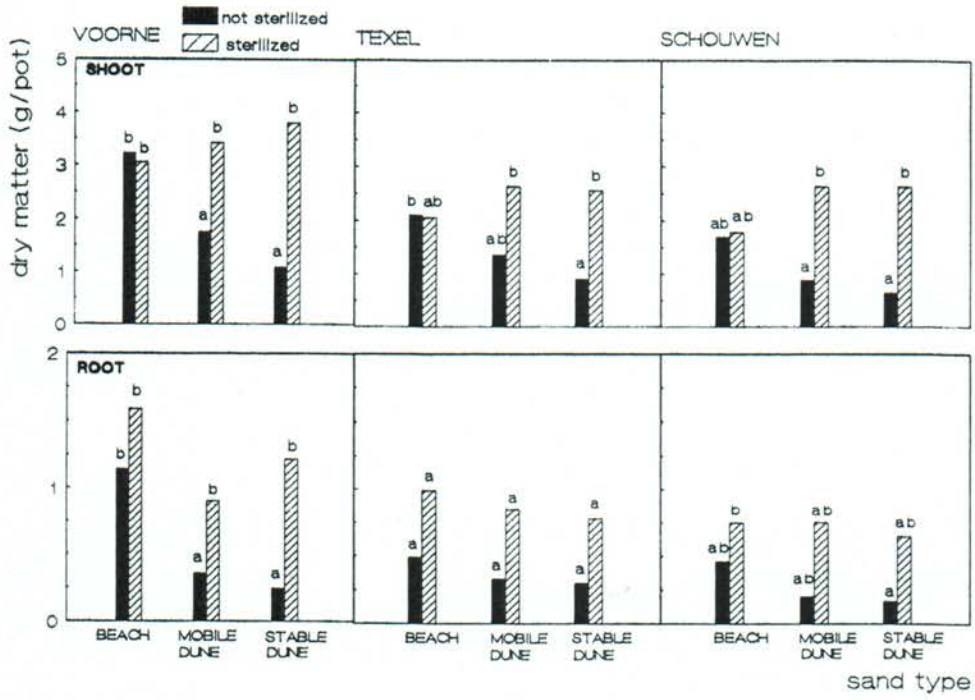

FIG. 1. Shoot and root dry matter production (g/pot) of *Ammophila arenaria* seedlings that were grown in sterilized and unsterilized sand samples. Sand was collected from three dune locations (Voorne, Texel, and Schouwen) and three sites per location (beach, mobile, and stable dunes). Within every plot, significant differences are indicated by different letters according to Tukey's test ($P<0.05$).

TABLE 2. Chemical properties of sand (0–20 cm depth) from the beach, the mobile dune, and the stable dune at Voorne, Texel, and Schouwen

Location and sampling site	pH (KCl)	$CaCO_3$ (%)	Organic Matter (%)	Total N[a]	Total P[a]	K[b]	Na[b]	Mg[b]	Cl[a]	Electroconductivity ($dS \cdot m^{-1}$)
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

[a]Measured in mg/100 g.
[b]Measured in mequiv./100 g.

TABLE 3. Degrees of freedom and *F*-values according to two-factor ANOVA (sand type and sterilization) of shoot and root dry matter of *Ammophila arenaria* grown in sand from Voorne, Texel, and Schouwen

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

NOTE: MSE, mean squares of error; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

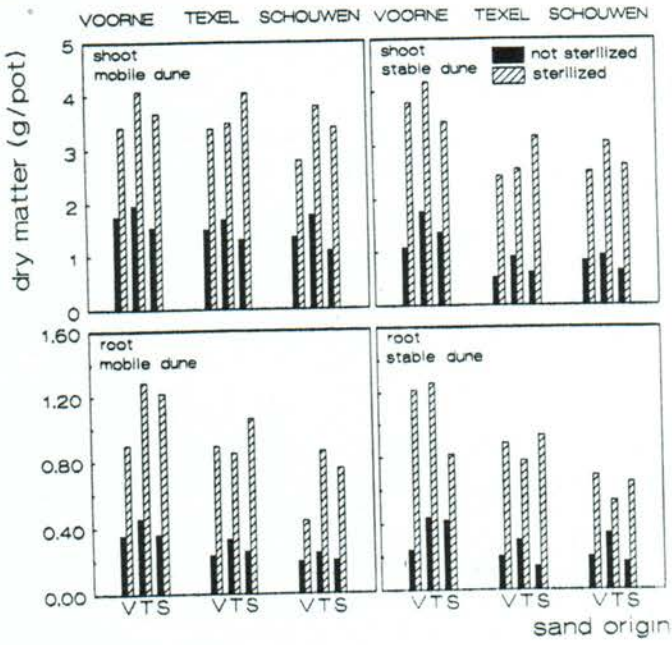

FIG. 2. Shoot and root dry matter production (g/pot) of *Ammophila arenaria* seedlings of three populations (Voorne, Texel, and Schouwen). Plants were grown in sterilized and unsterilized sand collected from the mobile and stable dunes of the original and alien locations. In all pairs of bars the dry matter yield in the sterilized sand is significantly higher ($P<0.05$) than in the unsterilized sand according to pairwise *t*-tests.

respect to different sand origins and soil sterilization (Table 4). Since there was a significant difference in dry matter production among populations ($P<0.001$; Table 4), contrasts were calculated in the ANOVA. The Voorne population 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 were partly in correspondence with the 1000-kernel weights (g) of the caryopses: Voorne 3.78*a* > Schouwen 3.36*ab* > Texel 3.28*b* (significant differences are indicated by different letters; results of 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: Ammophila arenaria *and* Calammophila baltica

Both the 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). *Calammophila baltica* produced more dry matter than *A. arenaria* ($P<0.001$). Dry yield of both plant species was significantly higher in sterilized sand than (in descending order) in beach sand and in sand from the mobile and the stable dune ($P<0.05$; Fig. 3). Differences in root length corresponded with those in root weight (data not shown).

Plants of *C. baltica* did not show any mortality in any sand type. In contrast, 6 to 44% of the cuttings of *A. arenaria* died after transplanting in sterilized and unsterilized sand, with the highest mortality in unsterilized sand from the stable dune. Shoot length, number of green tillers, and rhizomes of both

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 *Ammophila arenaria*

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

NOTE: Contrasts were calculated for the main factors location and population: V, Voorne; T, Texel; S, Schouwen; MSE, mean squares of error; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

TABLE 5. Degrees of freedom and *F*-values according to two-factor ANOVA of total, shoot, root, and rhizome dry matter of *Ammophila arenaria* and *Calammophila baltica* grown in four sand types (beach, mobile, stable unsterilized, and stable sterilized)

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 × sand	3	1.37ns	7.93***	0.06ns	1.23ns
MSE	24	0.431	0.237	0.283	0.271

NOTE: MSE, mean squares of error; ***, $P<0.001$.

plant species were lower in unsterilized sand from stable dunes compared with other treatments (Table 6).

Total uptake of N, P, and K per container by *C. baltica* was higher than by *A. arenaria* (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 roots and rhizomes revealed a similar pattern (data not shown).

Numbers of nematodes in the soil in May (before the experiment was started), and in soil and roots during the course of the experiment are listed in Tables 7 and 8, respectively. No data from beach sand and sterilized sand are presented, since only saprobiotic nematodes (i.e., not parasitizing plants) were present in soil and roots. In July and November, numbers of nematodes in soil and roots of both species were higher in containers with sand from the mobile site compared with sand from the stable site (Table 8). Compared with numbers in May (Table 7), nematode numbers of *Pratylenchus* sp. and *Tylenchorhynchus* sp. had increased 10- to 20-fold, whereas saprobiotic nematodes had increased 5- to 10-fold. Other nema-

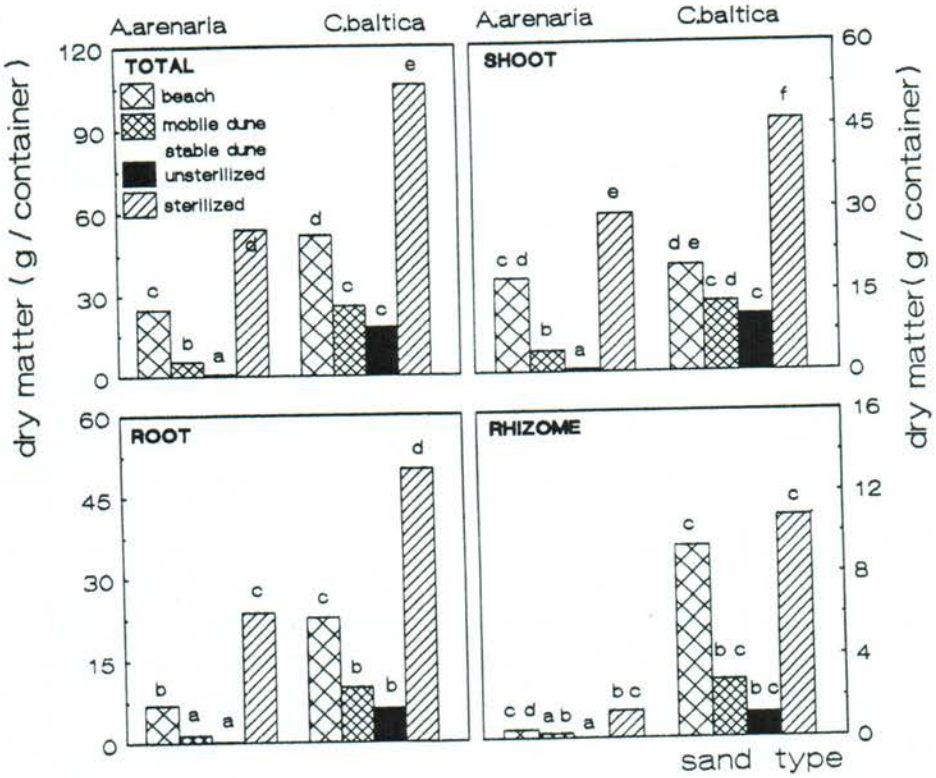

FIG. 3. Dry matter production (g/container) of cuttings of *Ammophila arenaria* and *Calammophila baltica* in sand from the beach, the mobile dune, and the stable dune, and in sterilized sand from the stable dune. Sand samples and plants originated from Voorne. Within every plot, significant differences for each measured variable are indicated by different letters according to Tukey's test ($P<0.05$).

TABLE 6. Mortality, shoot length, numbers of tillers and rhizomes, total uptake of N, P, and K per container, and concentrations of sugars and starch in the shoots of *Ammophila arenaria* and *Calammophila baltica* grown in sand from the beach, the mobile dune, the stable dune, and in sterilized sand from the stable dune

Sand type	Mortality %	Length (cm)	Tillers (no./plant)	Rhizomes (no./plant)	N (mg/pot)	P (mg/pot)	K (mg/pot)	Sugars (%)	Starch (%)
				Ammophila 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)
				Colammophila 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)

NOTE: Values in parentheses are standard deviations ($n=4$).

TABLE 7. Number of nematodes in the soil (per 100 mL; specific volume of 0.75 mL · g^{-1}; $n = 4$) from the mobile and the stable dunes, in May at Voorne

	Mobile	Stable
Pratylenchus sp.	2.7	2.7
Tylenchorhynchinae	5.0	0
Rotylenchus goodeyi	0	10
Heterodera sp.[a] (larvae in cysts)	0	25
Heterodera sp[a] (larvae in soil)	0	5.0
Saprobiotic	282	392

[a]*avenae* group.

tode species had not (or hardly) increased between May and July. *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 *Heterodera* (*avenae* group) were very low in both soil and roots.

The numbers of nematodes in replicate samples varied widely. For example, in July, the 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 more marked in the latter sand type (Fig. 3). Therefore, no positive correlation could be detected between numbers of nematodes and severity of growth reduction.

Discussion

Soil sterilization of sand samples collected from root zones of vigorous (mobile dunes) and degenerated (stable dunes) *A. arenaria* from all three foredune areas improved growth of test seedlings. Since ample nutrients had been supplied in the greenhouse (Van der Putten et al. 1988), the effect could not be related to extra release of nutrients because of soil sterilization. Therefore, reduction in plant growth in the unsterilized sand was attributed to the deleterious effect of harmful soil organisms.

Harmful soil organisms were present in the root zone of *A. arenaria* at all three locations. Hence, it can be concluded that their occurrence is quite common in coastal foredunes in The Netherlands. Harmful organisms were detected in sand from both mobile and stable dunes but were absent in beach sand (Fig. 1). Roots of *A. arenaria* colonize such windblown sand before they are colonized by harmful soil organisms (Van der Putten et al. 1989). These results therefore support the hypothesis that *A. arenaria* benefits from windblown sand by finding an escape route from harmful soil organisms (Van der Putten et al. 1988).

The harmful soil organisms were not specific to individual populations of *A. arenaria*, as seedlings from all three examined populations were reduced by harmful soil organisms of both local and alien origin. The three populations, however, produced different amounts of dry matter. Since dry yields corresponded with initial caryopsis weights, population differences can be due to population characteristics, as well as to maternal effects.

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). Although based on field observations, any experimental evidence for this suggestion is lacking.

In the outdoor experiment, growth of both *A. arenaria* and *C. baltica* was stimulated in sterilized sand from the stable dune compared with unsterilized sand from all sites. In the greenhouse (experiment 1), on the contrary, biomass production in beach sand was equal to sterilized sand from the stable dune. This difference between greenhouse and outdoor experiments could have been due to an insufficient supply of fertilizer in the latter to compensate for a nutrient flush that is released because of soil sterilization (De Nooij et al. 1986). Uptake of N and P did not exceed the amounts supplied by the fertilizer (780 mg/pot N and 195 mg/pot P, respectively). *Calammophila baltica* took up 86% of the supplied N in sterilized sand (Table 6), which is quite high taking into account that the applied fertilizer should be released in 9–12 months. However, these specifications are made for a temperature of 21°C. At higher temperatures, which occur in the surface sand layers (Baldwin and Maun 1983), release rate should increase. Nevertheless, in both experiments plants grew better in beach sand than in sand from the mobile and stable dunes.

It was shown previously that the elimination of nematodes from dune sand results in an increased growth of planted seedlings of *A. arenaria* (Van der Putten 1989). In the present experiments, however, the strongest growth reduction was observed in the sand from the stable dune that 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). Preliminary results suggests that growth reduction of *A. arenaria* may be due to synergism between nematodes and soil fungi (W. H. Van der Putten and W. J. M. Van Gulik, unpublished results).

Succession in coastal sand dunes was related to changes in the environment, i.e., changing soil fertility (Olson 1958; Lux 1969), loss of calcium carbonate (Cowles 1899; 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 to manifest than the time taken for the degeneration of *A. arenaria* (Deshmukh 1979; Wallén 1980).

By comparing the growth reduction of well-nursed test plants in the greenhouse with those grown 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). Apparently there is some interaction between the negative effects of harmful soil organisms and abiotic stress factors (Van der Putten et al. 1989). 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) dominates the foredune vegetation when *A. arenaria* and

TABLE 8. Number 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 *Ammophila arenaria* and *Calammophila baltica*

	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* group)	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 (0–10)	0	0
Heterodera (*avenae* group)	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)

NOTE: 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).

C. baltica have disappeared. Degeneration of *H. rhamnoides* can also be related to harmful soil organisms, e.g., nematodes (Oremus and Otten 1981; Maas et al. 1983; Zoon 1986). Therefore, it may be concluded that vegetation succession on coastal foredunes is influenced by abiotic factors, as well as by harmful biotic factors.

Acknowledgements

We thank Wilma van Gulik and Marianne van der Hoeven for technical assistance, Wiecher Smant, Leen Sluimer, and Margo van der Meulen for chemical analyses, and Paul Maas and Henk Brinkman for identification of nematodes. Dr. C. van Dijk and Dr. J. W. Woldendorp critically commented on this paper. This research was carried out by request of the water and civil board Brielse Dijkring and financed by the Dutch Ministry of Transport and Public Works.

AGRIOS, G. N. 1978. Plant pathology. University of Massachusetts. Academic Press, New York.

BALDWIN, K. A., and MAUN, M. A. 1983. Microenvironment of Lake Huron sand dunes. Can. J. Bot. **61**: 241–255.

CAPERTON, C. M., MARTYN, R. D., and STARR, J. L. 1986. Effects of *Fusarium* inoculum density and root-knot nematodes on wilt resistance in summer squash. Plant Dis. **70**: 207–209.

COOLEN, W. A., and D'HERDE, C. J. 1972. A method for the quantitative extraction of nematodes from plant tissue. State Agricultural Research Centre, Ghent, Belgium.

COWLES, H. C. 1899. The ecological relations of the vegetation on the sand dunes of Lake Michigan. Bot. Gaz. **27**: 95–308.

DE NOOIJ, M. P., TROELSTRA, S. R., and WAGENAAR, R. 1986. Growth reduction in *Plantago lanceolata* in relation to biotic factors in the soil environment. Oecologia (Berlin), **70**: 266–272.

DESHMUKH, I. K. 1979. Fixation, accumulation and release of energy by *Ammophila arenaria* in a sand dune succession. *In* Ecological processes in coastal environments. *Edited by* R. L. Jefferies and A. J. Davy. Blackwell Scientific Publications, London. pp. 353–362.

DISRAELI, D. J. 1984. The effect of sand deposits on the growth and morphology of *Ammophila breviligulata*. J. Ecol. **72**: 145–154.

ELDRED, R. A., and MAUN, M. A. 1982. A multivariate approach to the problem of decline in vigour of *Ammophila*. Can. J. Bot. **60**: 1371–1380.

FALES, F. W. 1951. The assimilation and degradation of carbohydrates by yeast cells. J. Biol. Chem. **193**: 113–124.

HEUKELS, H., and VAN DER MEIJDEN, R. 1984. Flora van Nederland. 20th ed. Wolters-Noordhoff, Groningen, The Netherlands.

HEWITT, E. J. 1966. Sand and water culture methods used in the study of plant nutrition. 2nd ed. Commonwealth Agricultural Bureaux. Farnham Royal, Bucks, England.

HOPE-SIMPSON, J. F., and JEFFERIES, R. L. 1966. Observations relating to vigour and debility in marram grass (*Ammophila arenaria* (L.) Link). J. Ecol. **54**: 271–275.

HUISKES, A. H. L. 1979. Biological flora of the British Isles: *Ammophila arenaria* (L.) Link (*Psamma arenaria* (L.) Roem et Schutt: *Calamagrostis arenaria* (L.) Roth). J. Ecol. **67**: 363–382.

KNUTSON, P. L. 1978. Planting guidelines for dune creation and stabilization. *In* Coastal Zone '78, Symposium on Technical, Environmental, Socioeconomic and Regulatory Aspects of Coastal Zone Management, San Francisco, CA, March 14–16, 1978. Vol. 2. American Society of Civil Engineers, New York. pp. 762–779.

KUBIEN, E. 1970. Cytological and embryological studies in *Ammophila baltica* (Flugge) Link. Acta Biol. Cracov. Ser. Bot. **13**: 99–110.

LAING, C. C. 1958. Studies in the ecology of *Ammophila breviligulata*. I. Seedling survival and its relation to population increase and dispersal. Bot. Gaz. **19**: 208–216.

——— 1967. The ecology of *Ammophila breviligulata*. II. Genetic change as a factor in population decline on stable dunes. Am. Midl. Nat. **77**: 495–500.

LUX, H. 1969. Zur Biologie des Strandhafers (*Ammophila arenaria*) und seiner technischen Anwendung im Dunenbau. *In* Exp. Pfl. Soziologie. *Edited by* R. Tüxen. The Hague, The Netherlands. pp. 138–145.

MAAS, P. W. T., OREMUS, P. A. I., and OTTEN, H. 1983. Nematodes (*Longidorus* sp. and *Tylenchorhynchus microphasmis* Loof) in growth and nodulation of sea buckthorn (*Hippophaë rhamnoides* L.). Plant Soil, **73**: 141–147.

MARSHALL, J. K. 1965. *Corynephorus canescens* (L.) P. Beauv. as a model for the *Ammophila* problem. J. Ecol. **53**: 447–465.

MAUN, M. A. 1985. Population biology of *Ammophila breviligulata* and *Calamovilfa longifolia* on Lake Huron sand dunes. I. Habitat, growth form, reproduction, and establishment. Can. J. Bot. **63**: 113–124.

MAUN, M. A., and BAYE, P. 1989. The ecology of *Ammophila breviligulata* Fern. on coastal dune systems. Crit. Rev. Aquat. Sci. **1**: 661–681.

MAUN, M. A. , and LAPIERRE, J. 1984. The effects of burial by sand on *Ammophila breviligulata*. J. Ecol. **72**: 827–839.

NOVOZAMSKY, I., HOUBA, V. J. G., TEMMINGHOFF, E., and VAN DER LEE, J. J. 1984. Determination of 'total' N and 'total' P in a single soil digest. Neth. J. Agric. Sci. **32**: 322–324.

OLSON, J. S. 1958. Rates of succession and soil changes on Southern Lake Michigan sand dunes. Bot. Gaz. **119**: 125–170.

OLSSON, H. 1974. Studies on South Swedish sand vegetation. Ph.D. thesis. Acta Phytogeogr. Suec. **60**: 1–176.

OOSTENBRINK, M. 1960. Estimating nematode populations by some selected methods. *In* Nematology. *Edited by* J. N. Sasser and W. R. Jenkins. North Carolina University Press, Chapel Hill. pp. 85–102.

OOSTING, H. J., and BILLINGS, W. D. 1942. Factors affecting vegetational zonation on coastal dunes. Ecology, **23**: 131–142.

OREMUS, P. A. I. 1982. Growth and nodulation of *Hippophaë rhamnoides* (L.) ssp. *rhamnoides* in the coastal sand dunes of The Netherlands. Ph.D. thesis, University of Utrecht, The Netherlands.

OREMUS, P. A. I., and OTTEN, H. 1981. Factors affecting growth and nodulation of *Hippophaë rhamnoides* (L.) ssp. *rhamnoides* in soils from two successional stages of dune formation. Plant Soil, **63**: 317–331.

RIHAN, J. R., and GRAY, A. J. 1985 Ecology of the hybrid Marram grass *Calammophila baltica* in Britain. Vegetatio, **61**: 203–208.

ROWE, R. C., RIEDEL, R. M., and MARTIN, M. J. 1985. Synergistic interactions between *Verticillium dahliae* and *Pratylenchus penetrans* in potato early dying disease. Phytopathology, **75**: 412–418.

SALISBURY, E. 1952. Downs and dunes: their plant life and its environment. Bell & Sons, London.

SOKAL, R. R., and ROHLF, F. J. 1981. Biometry: the principles and practice of statistics in biological research. W. H. Freeman and Co., San Francisco.

VAN DER PUTTEN, W. H. 1989. Establishment, growth and degeneration of *Ammophila arenaria* in coastal sand dunes. Ph.D. thesis, Agricultural University, Wageningen, The Netherlands.

VAN DER PUTTEN, W. H., VAN DIJK, C., and TROELSTRA, S. R. 1988. Biotic soil factors affecting the growth and development of *Ammophila arenaria*. Oecologia, **76**: 313–320.

VAN DER PUTTEN, W. H., VAN DER WERF-KLEIN BRETELER, J. T., and VAN DIJK, C. 1989. Colonization of the root zone of *Ammophila arenaria* by harmful soil organisms. Plant Soil, **120**: 213–223.

VAN DIEREN, J. W. 1934. Organogene Dünenbildung: eine geomorphologische Analyse der Dünenlandschaft der West-Friesischen Insel Terschelling mit pflanzensoziologischen Methoden. Martinus Nijhoff, The Hague, The Netherlands.

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

WESTERGAARD, M. 1943. Cyto-taxonomical studies on *Calamagrostis epigejos* (L.) Roth, *Ammophila arenaria* (L.) Link, and their hybrids (*Ammophila baltica* (Flugge) Link). Biol. Skrift. **2**: 1–66.

WILLIS, A. J., FOLKES, B. F., HOPE-SIMPSON, J. F., and YEMM, E. W. 1959. Braunton Burrows: The dune system and its vegetation. Part II. J. Ecol. **47**: 249–288.

ZOON, F. C. 1986. On the relative involvement of nematodes and other soil factors in the decline of *Hippophaë rhamnoides* L. in The Netherlands. Rev. Nematol. **9**: 314.