

Biotic soil factors affecting the growth and development of *Ammophila arenaria*

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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 significantly, 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 desiccated 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 windblown 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.

Key words: *Ammophila arenaria* – *Calammophila baltica* – Sand dunes – Soil sterilization – Soil pathogens

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 and McKenzie 1976; Brown and 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 and Gray 1985).

Both *Ammophila* and *Calammophila* are intensively planted to prevent the sand dunes from erosion. They also establish naturally from rhizome fragments and seed (Adriani and 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 co-genere *A. breviligulata* Fern. (Laing 1958; Maun and 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 and 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 and 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 and 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 pos-

sible role of soil micro-organisms in the nutrition was emphasized. The ecology of *Ammophila* has been related to the activity of Azotobacter (Ahmad and Neckelman 1978; Abdel Wahab and Wareing 1980), and the occurrence of Vesicular-Arbuscular Mycorrhizae (Nicolson 1960; Nicolson and 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 seafloor. In the Netherlands the latter is used to strengthen fore-dune ridges (Van der Putten and 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 microorganisms 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 fore-dune, covered by a vigorous *Ammophila* vegetation (Rockanje, The Netherlands, 51.25 N, 4.05 E).
- sea sand i.e. sand from the sea-floor (20–40 m below sea level) off the island of Voorne, The Netherlands (51.55 N, 4.05 E).

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°C (±3) and a day length of 16 hours by additional illumination with Philips HLRG-400 W (20000 Lux).

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°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 *Calamagrostis* 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 *Calamagrostis* 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 the 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 m²; Osmocote). There were no replicates. A frame-work of twelve compartments (four of 1 × 1 × 0.7 m³ and eight of 1 × 0.25 × 0.7 m³) 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

Table 1. Chemical properties of rhizosphere and sea sand

Sand type	pH H ₂ O	pH KCl	organic matter (%)	CaCO ₃ (%)	electrical conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$)	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

Sand type	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

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 data 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, 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 H₂O 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°C for 24 h. Total N and total 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 (fractions $> 53\text{ }\mu\text{m}$), and a pipette method (fractions $< 53\text{ }\mu\text{m}$).

Plant samples dried at 70°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 and Rohlf 1981).

Results

Chemical properties and grain size distribution of rhizosphere sand and sea sand

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 μm range (Table 2), those of the dredged sea sand was somewhat larger, ranging from 75–212 μm , and the D_{50} 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 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).

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

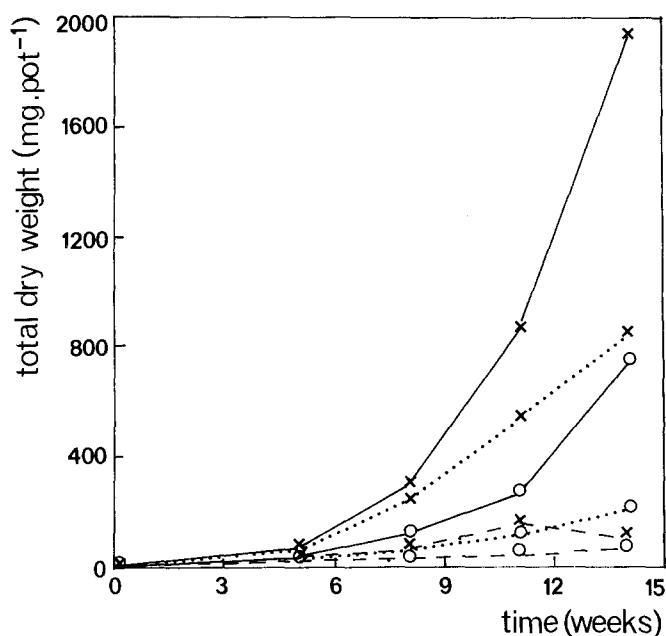


Fig. 1. Total dry weight ($\text{mg} \cdot \text{pot}^{-1}$) of *Ammophila* seedlings on rhizosphere (O) and sea (X) sand, supplied with 0 (O—) 1/8 (L···) or 1/2 H—) Hoagland NPK

the 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.

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 rhizosphere 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 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.

(2) *Ammophila* cuttings. F-values of biomass production are listed in Table 4. Irradiation affected the biomass pro-

Table 3. Total dry weight ($\text{mg} \cdot \text{pot}^{-1}$) of seedlings on five sand mixtures and three nutrient level: O 0 NPK; L 1/8 Hoagland NPK and H 1/2 Hoagland NPK (all $100 \text{ ml} \cdot \text{pot}^{-1} \cdot \text{week}^{-1}$). 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 $\arcsin(\text{square root}(x))$

harvest	rhizo-sphere (%)	sea sand (%)	nutrient (NPK) level		
			O	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			812.3***	252.6***	2
harvest			729.9***	30.0***	3
sand \times NPK			16.6***	4.6***	8
sand \times harvest			2.9**	3.1***	12
NPK \times harvest			62.4***	3.9**	6
sand \times NPK \times harvest			1.2ns	1.7*	24
mean squares of error			0.0532	0.0024	120

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

ns not significant

duction 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 statistical 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 ($P <$

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

Factor	<i>Ammophila</i> seedlings	df	<i>Ammophila</i> cuttings	df	<i>Calammophila</i> cuttings	df
sand	30.5***	1	3.53ns	1	4.01*	1
irradiation	164.7***	1	74.2***	1	8.17*	1
harvest	1431***	3	735.3***	2	538***	2
sand × irradiation	27.4***	1	6.79**	1	13.2*	1
sand × harvest	1.39ns	3	0.04ns	2	0.78ns	2
irradiation × harvest	1.38ns	3	1.42ns	2	0.67ns	2
sand × irradiation × harvest	1.11ns	3	0.26ns	2	0.60ns	2
mean squares of error	0.0325	48	0.0358	45	0.0484	47

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

ns not significant

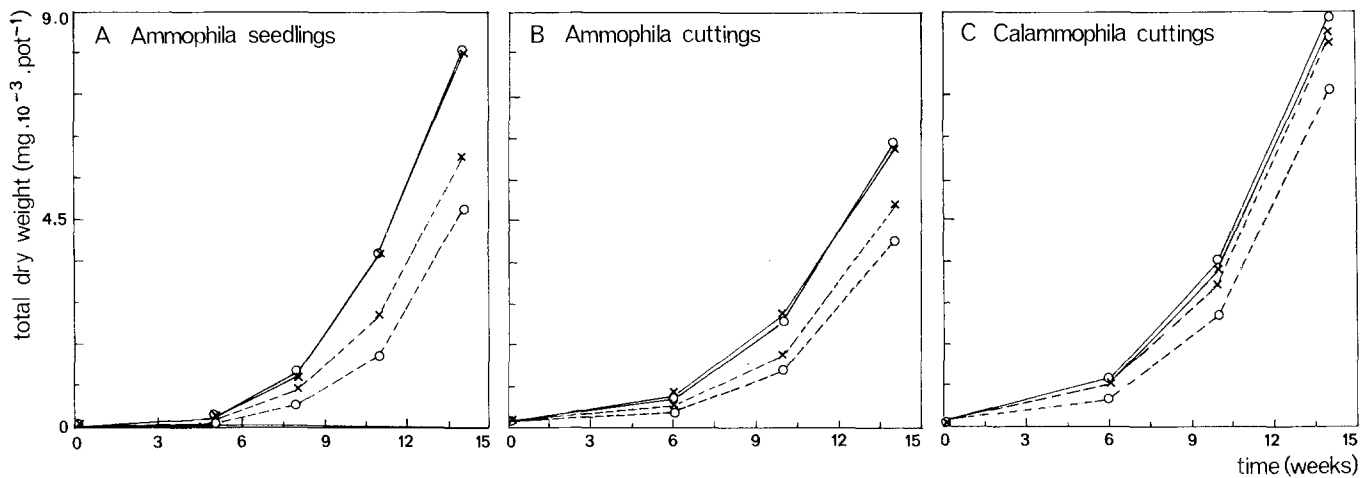


Fig. 2A–C. Total dry weight ($\text{mg} \cdot \text{pot}^{-1}$) of *Ammophila* seedlings **A**, *Ammophila* cuttings **B**, and *Calammophila* cuttings **C** on irradiated (—) and not-irradiated (---) rhizosphere (O) and sea (X) sand

Table 5. Specific root uptake rates of N ($\mu\text{mol} \cdot \text{g}^{-1} \cdot \text{day}^{-1}$), P and K ($\mu\text{eq} \cdot \text{g}^{-1} \cdot \text{day}^{-1}$) based on dry weight of *Ammophila* seedlings between $t=0$ (planting date) and $t=1$ (first harvest), and between $t=3$ (third harvest) and $t=4$ (fourth harvest) on 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	401	151	19	11	141	104
irradiated rhizosphere sand	870	65	53	3	355	49
sea sand	796	93	40	5	269	72
irradiated sea sand	821	49	41	3	297	44

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 *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 *Am-*

mophila cuttings. Irradiation did not cause any significant effect on any of the aboveground plant parameters ($P < 0.05$).

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 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.

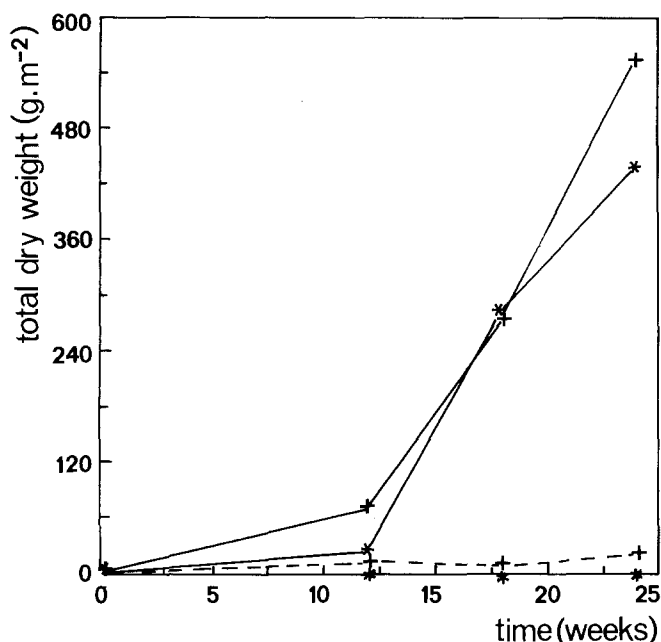


Fig. 3. Total dry weight ($\text{g} \cdot \text{m}^{-2}$) of *Ammophila* seedlings (*) and cuttings (+) on rhizosphere (---) and sea (—) sand

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 micro-organisms in the rhizosphere on the nutrition of *Ammophila* (Hassouna and Wareing 1964; Abdel Wahab 1975; Abdel Wahab and 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 (Powelson and 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 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 satisfactory 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; Marshall 1965). But this does not explain degeneration of *Ammophila* at locations where competitors are absent (Hope-Simpson and Jefferies 1966; Eldred and Maun 1982; 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 Marshall's 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. Marshall's (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 and 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 and Otten 1981; Maas et al. 1983). As plant-parasitic nematodes may also occur in the rhizosphere of *Ammophila* (Kisiel 1970; K. Kuiper, pers. communication) 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 and H. Brinkman, unpublished work).

The development of the root system of *Ammophila* on rhizosphere sand was restricted and the roots were brown. As no distinct macroscopic disease symptoms 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.

Acknowledgements. Thanks are due to Wilma van Gulik for assistance with the experiments and chemical analysis, to Henk Otten for using his soft-ware, and to Prof. Dr. L. 't Mannetje and Dr. J.W. Woldendorp for their critical comments on this manuscript.

This research was carried out by order of the Water and Civil Board De Brielse Dijkkring, and financed by the Department of Public Works (Rijkswaterstaat) of the Ministry of Transport and Public Works.

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Received October 29, 1987