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VESICULAR-ARBUSCULAR MYCORRHIZA IN DUNE VEGETATION

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SUMMARY

A field survey of the infection of dune plants by vesicular-arbuscular mycorrhiza (VAM) has been made in the coastal sand dunes of the province of North Holland, The Netherlands. Winter annual grasses and herbs are not or nearly not affected, whereas perennial plants, especially the grasses *Agrostis stolonifera* and *Calamgrostis epigeios* have a high degree of VAM infection. Seasonal changes of the infection degree generally occur; the highest infection is found in summer.

In greenhouse experiments at 20 °C infection could be induced in the dune annual *Phleum arenarium* by *Glomus fasciculatum* and *G. mosseae*. The dune annual *Aira praecox*, however, could not be infected. This result is discussed in relation to seasonal development and temperature demands of VAM.

In greenhouse experiments with *Calamagrostis epigeios*, infection by *Glomus fasciculatum* and *G. mosseae* had no positive effect on growth of, or phosphorus supply to *C. epigeios*. Both *Glomus* species cause differential translocation of phosphorus and potassium from roots to shoots of *C. epigeios*.

The ecological importance of these results is discussed in relation to the hypothesis of drought protection and nutrient translocation by VAM.

1. INTRODUCTION

Despite the extensive research of vesicular-arbuscular mycorrhiza (VAM) in field crops (DANIELS et al. 1981, RUISSEN 1982) and perennial wild plants (MEIJSTRIK 1972, VAN DIJK 1980, WHITTINGHAM & READ 1982, GAY et al. 1982) very little attention has been paid to annual wild plants (GIOVANETTI & NICOLSON 1983). But even in crops and perennials the ecological significance of this symbiosis is not clear. Plants can have a nutritional advantage, especially in relation to their phosphorus budget (ALLEN et al. 1981); however, better phosphorus supply does not in every case give rise to a better growth performance (BLACK & TINKER 1979, ALLEN et al. 1979). Generally, it has been assumed that VAM can improve the growth of plants living in marginal and less fertile environments (TINKER 1978). One of these habitats with a low nutrient supply is the coastal sand dunes, known for the occurrence of VAM spores (NICOLSON 1960, FORSTER & NICOLSON 1981, KOSKE et al. 1975, KOSKE & HALVORSON 1981, GIOVANNETTI & NICOLSON 1983). A general survey of the occurrence of VAM and its time course in annual and perennial plant species of Dutch coastal dunes was carried out, as part of a study on the ecology of dune plants (ERNST 1981, 1983a, b, ROZIJN & VAN DER WERF 1984). The possible significance of the potential for inoculation by different VAM species (JENSEN 1982) was studied in selected plant species of the dune ecosystem.

2. MATERIAL AND METHODS

2.1. Field survey

In December 1981, January, April, July, September and November 1982 and December 1983 annual dune grasses (*Aira praecox* L., *Phleum arenarium* L.), annual herbs (*Myosotis ramosissima* Roch., *Erodium cicutarium* (L.) L'Hér. *Stellaria pallida* (Dum.) Piré, *Cardamine hirsuta* L., *Erophila verna* (L.) Chevall.), perennial grasses and graminoids (*Calamagrostis epigeios* (L.) Roth., *Festuca ovina* L., *Ammophila arenaria* (L.) Link, *Agrostis stolonifera* L., *Carex arenaria* L., *Luzula campestris* (L.) DC.) and perennial herbs (*Achillea millefolium* L., *Sedum acre* L.) were investigated along a transect from the outer to the inner coastal dunes of North Holland Dune Reserve (province of North Holland, The Netherlands) between the coastal marks P.L. 48 and P.L. 49. Sand samples (c. 300 cm^3) which included a portion of the root system, were collected from the root zone of the plant species. The investigated plant communities were a *Tortulo-Phleetum, Galio-Festucetum* and a *Taraxaco-Calamagrostidetum*. Roots of the plants were thoroughly washed and after clearing with KOH and staining with trypan blue in lactophenol, examined for VAM infection according to the procedure of PHILLIPS & HAYMAN (1970). The percentage of root length containing internal mycelium including hyphae, vesicles, and arbuscules was determined on the first 30 mm from the root tip (% infection) across the field of view of a low power microscope (GIOVANNETTI & MOSSE 1980). In addition external mycelium and the presence of spores have been registered. The rooting system of dune annuals is mostly not deeper than 40 mm; incidental analysis of the entire root system gave no better result. At all collection data hyphae, chlamydospores and sporocarps were inspected in annuals and perennials.

2.2. Experiments

Calamagrostis epigeios shows an infection gradient from outer to inner coastal dunes during winter and summer periods; an experiment was set up to analyse these differences. Soil and *Calamagrostis* plants were collected from a *Calamagrostis epigeios-Festuca ovina-Luzula campestris* grassland 300 m from the sea, just behind the great white dune as well as from a similar grassland 2000 m from the sea. The latter area had been in agricultural use for the last century until 20 years ago. Since that time a vegetation with *Phleum, Aira, Erodium, Sedum, Festuca ovina* and other typical dune species has been re-established. The soil of both sites was a fine textured sand ($>$ 90% of the grains were below 300 μm) with a pH of 7.1 ± 0.1, and a very low content of nutrients (*table 1*) and humus.

The field soil was passed through a 1 mm coarse sieve to remove roots and

Table 1. Concentrations of mineral nutrients (μmol g^{-1} dry soil) of outer dune and inner dune soil, both covered with *Calamagrostis epigeios*. All values are the mean of 3 soil samples, each being a mixture of 5 subsamples. T = total concentration, being soluble in concentrated hot HCl/HNO_3, A = extractable by 1 mol NH_4-acetate (pH 7.1), W = extractable in distilled water (extractant: soil = 5:1 on weight basis), – = not determined.

Soil source		pH	K	Na	Ca	Mg	P	Fe	Mn	Zn	N
outer dune	T	–	2.8	6.7	185	27	7.70	3.40	0.75	0.25	14.0
	A	–	1.5	5.2	63	3	0.24	–	–	–	–
	W	7.2	1.2	4.9	14	1	0.05	0.03	0.08	0.02	–
inner dune	T	–	3.4	4.9	65	25	9.20	5.10	0.87	0.21	34.0
	A	–	2.8	3.8	21	2	0.57	–	–	–	–
	W	6.6	1.3	3.7	1	1	0.09	0.03	0.11	0.01	–

debris. Shoots of *C. epigeios* from outer dune and inner dune were cut, surface-sterilized and cultivated in a solution of 0.1% $Ca(NO_3)_2 \cdot 4H_2O$ for 20 days to provide material with uninfected roots, being checked for VA-infection at the start of the experiments. Plants with three leaves and new roots were transplanted in field soil (0.5 l per plant). For each soil source (outer and inner dune) 9 plants of each provenance (outer and inner dune) were used. During the experiment in the greenhouse a day/night temperature regime was given (20 °C/15 °C) with 70 ± 5% relative humidity. A light period of 12 hours per day was supplied by mercury vapour lamps with a light intensity of 75 $W.m^{-2}$. After 13 weeks the plants were harvested, checked for VA infection and analysed for the concentration of mineral nutrients.

For testing the inoculum potentials, spores of *Glomus fasciculatum* Gerdemann and Trappe were isolated by the wet sieving technique (GERDEMANN & NICOLSON 1963) from the dune soils covered by *C. epigeios*. Spores of *G. mosseae* (Nicol. and Gerd.) Gerdemann and Trappe were kindly supplied by Dr. B. Mosse, Rothamsted. Soil of the inner dune site was sterilized at 100 °C for 24 hours for use in the experiments. Shoots of *C. epigeios* were cut, surface-sterilized and cultivated in a solution of 0.1% $Ca(NO_3)_2 \cdot 4\ H_2O$ for 20 days to provide material with uninfected roots.

In each experimental unit 3 plants with new roots and three leaves were transplanted in 0.5 l sterilized sand. 50 mg of a highly enriched preparation of *G. mosseae* spores or of *G. fasciculatum* were supplied per pot and thoroughly mixed in the upper 5 cm (initial root zone). All experimental pots were watered twice a week. Each experiment was carried out with 3 replicates. At the end of the experiment the VAM infection was checked. A similar experiment with the above mentioned *Glomus* species was carried out with *Phleum arenarium* and *Aira praecox*, both sown in sterilized sand inoculated with VA. The experimental conditions were the same as in the previously described experiment. The experiments lasted 13 weeks. The plants of *P. arenarium* and *A. praecox* were harvested at the time of flowering.

Table 2. The degree of infection by VAM (% of 3 root systems for each of the sites in the transect) in dune plants during the period from december 1981 to november 1982, with additional measurements in december 1983. Not every species was present at each site.

	number of microsites	winter (november, december-april)	summer (april-september)
grasses and graminoids			
Aira praecox	5	0	0
Agrostis stolonifera	7	0 –1	0.1–30
Ammophila arenaria	4	0 –5	0.1–30
Calamagrostis epigeios	16	0.1–5	0.1–60
Carex arenaria	2	0.1	1
Festuca ovina	11	0 –5	0.1– 5
Luzula campestris	5	0 –0.1	1
Phleum arenarium	5	0	0
herbs			
Achillea millefolium	1	0	0.1
Cardamine hirsuta	3	0	0
Erophila verna	5	0	0
Erodium cicutarium	5	0 –5	0.1–10
Myosotis ramosissima	2	0 –0.1	0.1– 5
Sedum acre	3	0 –0.1	0.1
Stellaria pallida	4	0 –0.1	.1

2.3. Analytical methods

Plant parts (shoots, roots) were harvested separately, dried at 80°C and wet ashed with a mixture of $HNO_3/HClO_4$ (7:1). They were then analysed for mineral elements by standard procedures with atomic absorption spectrometry, except phosphorus which was determined as the blue molybdenum-ascorbic acid-complex (ERNST 1983a).

2.4. Statistical methods

For statistical treatments two-way anova has been used (SOKAL & ROHLF 1969) followed by Duncan's Mutiple Range test to assign significance.

3. RESULTS

3.1. Field data

All perennial grasses and graminoids in the dune area were infected by coarse and fine VAM (*table 2*). The maximum infection level was only 5% during the winter and spring and increased during the summer up to 30% in *Agrostis stolonifera* and *Ammophila arenaria* and up to 60% in *Calamagrostis epigeios*. In these species a high amount of vesicles has been observed at every collection data. Due to the seasonal variation mean values cannot be given. Annual dune grasses

Table 3. The concentration of some mineral elements (μmol g^{-1} dry weight) in leaves and roots of *Calamagrostis epigeios* from two field sites, varying in VAM infection (the most outer dune, the most inner dune). At each site five plants had been sampled at the end of september. Values per nutrient indicated by different letters are significantly different at $P < 0.01$.

	phosphorus		potassium		calcium		sodium	
dune:	outer	inner	outer	inner	outer	inner	outer	inner
leaves								
living	72.0 ± 5.5^{q}	70.7 ± 2.6^{a}	318 ± 9^{c}	363 ± 37^{d}	151 ± 12^{g}	96 ± 23^{h}	78 ± 8^{k}	101 ± 23^{l}
dead	43.2 ± 11.4^{b}	40.6 ± 8.2^{b}	101 ± 19^{e}	86 ± 15^{e}	156 ± 19^{g}	126 ± 8^{h}	23 ± 2^{m}	40 ± 14^{n}
roots	89.9 ± 21.3^{a}	48.1 ± 13.4^{b}	650 ± 52^{f}	320 ± 56^{d}	$881 \pm \pm 68^{i}$	616 ± 32^{j}	22 ± 4^{m}	7 ± 2^{o}

and the cruciferous species *E. verna* and *C. hirsuta* were completely free from infection during the whole period. *Sedum acre, Stellaria pallida* and *Achillea millefolium* were only slightly infected by coarse endophytes. *Myosotis ramosissima* showed 5% infection during the summer. In the case of the most heavily infected species, *C. epigeios,* there was a difference between the sites very near the shore (500 m) and those further away (1500–2000 m); while the latter showed a tendency towards higher infection (0.1–60.0%) at every time, the former remained lower (0.1–5%). The vesicular-arbuscular mycorrhizal fungus associated with *C. epigeios* was *G. fasciculatum* at both sites. The amount of VAM infection has no effect on the concentration of mineral nutrients, especially phosphorus in *C. epigeios* from outer- and inner dune sites (*table 3*). In contrast, in the roots of the less infected plants the concentration of P and K was twice that in the highly infected ones.

3.2. Experiments

3.2.1. Different soils

The results of the field survey do not indicate whether the difference in the accumulation of potassium and phosphorus in the roots of *C. epigeios* is due to soil, plant or VA mycorrhiza characteristics. *C. epigeios* plants of both habitats,

Table 4. Biomass production and degree of VAM infection (mean and absolute variation) of *Calamagrostis epigeios,* grown on an outer dune and an inner dune soil. The growth period was 13 weeks in a greenhouse. At each experimental unit 9 plants have been used. Values indicated by different letters are significantly different at $P < 0.01$.

soil source		biomass production (mg dry weight per plant)		per cent infection (% per 30 mm root)	
	plant source:	outer dune	inner dune	outer dune	inner dune
outer dune		246 ± 21^{a}	312 ± 45^{ab}	8.0^{c} (0.0–50.0)	1.7^{d} (0.0–10.0)
inner dune		295 ± 103^{ab}	444 ± 93^{b}	0.1^{e} (0.0– 0.1)	1.3^{d} (0.0–10.0)

Table 5. Concentration of some mineral nutrients (μmol g^{-1} dry weight) in roots and shoots of *Calamagrostis epigeios*, collected from two sites of outer dunes and inner dunes and grown on non-sterilized outer dune and inner dune soils for 13 weeks in a greenhouse. Values for each mineral nutrient indicated by different letters are significantly different at $P < 0.01$.

soil source	plant	phosphorus		potassium		calcium	
	source:	outer dune	inner dune	outer dune	inner dune	outer dune	inner dune
outer dune	shoot	29.0 ± 3.0^{a}	25.5 ± 2.8^{ab}	336 ± 82^{f}	210 ± 40^{h}	116 ± 16^{i}	138 ± 20^{i}
	root	22.7 ± 4.0^{ab}	21.2 ± 3.7^{b}	112 ± 18^{g}	106 ± 19^{g}	362 ± 156^{k}	209 ± 27^{l}
inner dune	shoot	58.3 ± 3.4^{c}	46.5 ± 2.6^{e}	288 ± 6^{f}	206 ± 34^{h}	178 ± 28^{l}	195 ± 19^{l}
	root	37.9 ± 3.2^{d}	35.8 ± 7.2^{d}	121 ± 32^{g}	112 ± 23^{g}	125 ± 5^{i}	145 ± 11^{i}

grown on non-sterilized soils, grew better in the more fertile inland soil than in the outer dune soil (*table 4*). The inner dune plants generally have a higher biomass production than the outer dune plants. The percentage of VAM infection seems to be specific for the populations. The inner dune plants remained similarly infected by VAM in both soils, whereas the outer dune plants were infected by VAM up to 50% in their own soil and almost uninfected in the other soil. In both populations the VAM fungus was again *G. fasciculatum*. Despite the differences of VAM infection in both populations there was no positive correlation between VAM infection and phosphorus uptake (*table 5*). Both plant groups took up more phosphorus when grown in the inner dune soil; apparently the nutrient conditions of the soil were more important for phosphorus supply than the infection by VAM. Due to the similar concentration of potassium and sodium in both soils there was no effect on the uptake of these elements within plant populations, but there is between plant populations. In the case of potassium, it seems that the uptake is governed by population characteristics rather than by soil supply.

Table 6. Effect of soil sterilization (inner dune sand) on biomass production and phosphorus concentration of *Calamagrostis epigeios* (inner dune population) after 13 weeks growth in a greenhouse. The experiment is based on 9 plants per treatment. Different letters indicate significant difference at $P < 0.05$.

		Biomass	phosphorus concentration (μmol g^{-1} dry weight)
non-sterilized	shoot	189 ± 42^{a}	46.5 ± 2.6^{d}
	root	255 ± 56^{b}	35.8 ± 7.2^{e}
	total	444 ± 93^{c}	
sterilized	shoot	165 ± 61^{a}	59.6 ± 2^{d}
	root	187 ± 91^{b}	32.2 ± 5.3^{e}
	total	349 ± 147^{c}	

Table 7. Effect of *Glomus* species (*G. mosseae* and *G. fasciculatum*) on growth and nutrient concentration (μmol g^{-1} dry weight) of plants from an inner dune population of *Calamagrostis epigeios*, grown for 13 weeks in a sterilized inner dune soil in a greenhouse. Results with different letters indicate significant differences at $P < 0.01$.

addition of	biomass production (mg per plant)	phosphorus		potassium		calcium	
		shoot	root	shoot	root	shoot	root
none	750 ± 177^a	75.5 ± 5.9^b	39.9 ± 4.7^c	258 ± 19^e	89 ± 38^g	12.2 ± 2.7^h	20.6 ± 5.4^i
G. mosseae	679 ± 104^a	73.3 ± 14.4^b	44.7 ± 4.1^c	286 ± 46^e	93 ± 2^g	10.8 ± 1.9^h	19.7 ± 1.0^i
G. fasciculatum	693 ± 153^a	53.7 ± 2.4^d	37.2 ± 1.8^c	192 ± 10^f	137 ± 22^g	12.7 ± 0.5^h	21.7 ± 3.2^i

3.2.2. Different *Glomus* species

Annual grasses: Similar to the field observation, vesicular-arbuscular mycorrhiza was not found in the roots of *Aira praecox* in the greenhouse, not even after addition of *Glomus mosseae* and *G. fasciculatum* spores to the soil. *Phleum arenarium* on the other hand, could be infected by both *Glomus* species in the greenhouse, by *G. mosseae* more (up to 5%) than by *G. fasciculatum* (up to 0.1%).

Calamagrostis epigeios: Sterilization of soils can result in a flush of nutrients. Therefore an experiment was set up to compare growth and phosphorus concentration of plants grown in a sterilized and non-sterilized inner dune soil (*table 6*). Biomass production was not stimulated by soil sterilization. The phosphorus concentration of roots was the same independent of the treatment, whereas that of the shoots tends to higher values in sterilized soil ($0.2 < P < 0.01$).

For the following experiment only the inner dune population was grown on sterilized inner dune soil. The biomass production of plants infected by one of the *Glomus* species is similar to that of non-mycorrhiza plants (*table 7*). On the sterilized soil growth of plants infected by *Glomus* species was not significantly ($P < 0.05$) different from the control series. The degree of infection by *G. mosseae* was around 5%, but less than 1% by *G. fasciculatum*, in accordance with the inoculum potential of the host plants (DANIELS et al. 1981). Although no significant difference was found in the total amount of nutrients in the plants of all series, there were differences in the translocation between roots and shoots due to varied treatments. The phosphorus concentration of shoots of plants infected by *G. fasciculatum* was lower than in the other experimental groups. The same plants accumulated potassium in the roots to a higher degree and showed a slightly diminished translocation to the shoots. The concentration of calcium, magnesium, iron and zinc in roots and shoots of *C. epigeios* was not affected by the two *Glomus* species (Mg, Fe, Zn are not shown in *table 7*).

4. DISCUSSION

In contrast to most annual cereals, dune annuals have nearly not been infected by VAM in the field. In the case of the Brassicaceae *Erophila verna* and *Cardamine hirsuta* this result is in agreement with the general non-mycorrhizal behaviour of this family (PENDLETON & SMITH 1983). The weak infection of *Stellaria pallida* in this study and that of *Silene* species in the mediterranean sand dunes (GIOVANETTI & NICOLSON 1983) may be a warning against a rapid generalisation of dates, because this family was thought to be non-mycorrhizal (HIRREL et al. 1978, PENDLETON & SMITH 1983). The absence or low degree of infection of other dune annuals may be explained by their growth period in autumn, winter and spring, when the VA mycorrhizal development is low due to their sensitivity to and delayed spore germination at low temperature (SANDERS et al. 1977, BLACK & TINKER 1979, TOMMERUP 1983). The VAM infection of *Phleum arenarium* in the greenhouse (at 20 °C) gives some further evidence for this argument.

In the warmer climate of the mediterranean sand dunes GIOVANNETTI & NICOLSON (1983) found a low degree of VAM infection in two annual dune grasses *Lagurus ovatus* and *Vulpia ligustica*. However, no information has been presented about sampling time and soil afertility. Some further dune annuals with a strong vegetative growth during late spring and early summer (ERNST 1983a, b) i.e. *Erodium cicutarium* and *Myosotis ramosissima* give further evidence, that the low temperature during winter and spring may be responsible for the non-mycorrhizal status of real winter annuals. In contrast, the lack of VAM infection in *Aira praecox*, even at 20 °C, may indicate a general resistance mechanism against VAM infection in this annual dune grass. WHITTINGHAM & READ (1982) have stated that VAM may be of considerable benefit to seedlings by increasing their resistance to frost and drought. Although the phosphorus content of dune soils is low (ERNST 1981) and winter annuals are exposed to frost and drought (ERNST 1983b), this hypothesis can be rejected for wild winter annual grasses, and perhaps also for winter cereals (BLACK & TINKER 1979) as well as other winter annual herbs (GAY et al. 1982).

The high percentage of VAM infection of the perennial grasses *A. stolonifera*, *A. arenaria* and *C. epigeios* is consistent with percentages reported for these and other perennial grassess in dune habitats (GIOVANETTI & NICOLSON 1983, FORSTER & NICOLSON 1981, KOSKE & HALVORSON 1981, NICOLSON & JOHNSTON 1979). The ecological effect on the growth performance of *C. epigeios* was at least not positive; this is in agreement with some other observations in grasses (SPARLING & TINKER 1978. FORSTER & NICOLSON 1981). This suggests that the dune population of *C. epigeios* may have such an efficient exploitation of the labile pool of soil phosphates that VAM cannot supply additional phosphate. A high internal use of phosphorus (ERNST 1983a) and photosynthates (ERNST, unpubl.) by dune winter annuals may be another explanation of a lack of VAM, because only a high root exudation of reducing sugars in relation to the P-status of the plant enables VAM infection (RATNAYAKE et al. 1978, GRAHAM et al. 1981).

The results presented in this paper confirm that the inoculum potential of the *Glomus* species differs (DANIELS et al. 1981, JENSEN 1982), and that a high degree of infection is time-dependent. The metabolic demand of the VAM species will also influence growth and mineral nutrition, especially translocation processes of mineral nutrients, in their hosts. Therefore, it remains doubtful if interspecific nutrient transfer through VAM (WHITTINGHAM & READ 1982) without identification of the VAM species will help to understand its ecological aspects. Perhaps effects of VAM infection of wild plants may have the same low ecological importance as *Rhizobium*-N_2-fixation for wild legumes (VINCENT 1974), both in contrast to cultivated plants.

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