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

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

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

CHAPTER 1
GENERAL INTRODUCTION

Coastal foredunes: function, morphogenesis and stabilization

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

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

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

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

Sand accumulation and the ecological response of Ammophila arenaria

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

Many explanations have been suggested for the widely-reported decline in vigour of *A. arenaria* and *A. breviligulata* (generally summarized by Marshall (1965) and Laing (1967)). According to one hypothesis, the decline is caused by nutrient deficiency, and soil accretion is considered to provide nutrients. However, the plant vigour is less stimulated by fertilizers than by burial with sand, in spite of the low amounts of nutrients in the new sand layers (Salisbury 1952, Willis 1965, 1989). According to a second hypothesis, *Ammophila* species are outcompeted after dune stabilization (Huiskes 1979). However, degeneration also occurs in the absence of competing plant species (Hope-Simpson and Jefferies 1966, Disraeli 1984). According to a third hypothesis, nodal root production and the uptake of nutrients and water is inhibited when dunes become stabilized. The efficiency of old roots declines with age and in stabilized dunes old roots cannot be replaced by new ones due to morphological constraints (Marshall 1965, Willis 1965, Wallén 1980). Formation of new roots was found to be inhibited when dead leaves accumulate at the base of the shoots (Laing 1967). However, the positive response of shoots to sand accretion can not be explained by the replacement of degenerating roots or by the impaired uptake capacity of the root system, since vigorous growth was evident directly after emergence from deep burial even before new nodal root systems had developed (Baye 1990). Recently, these findings led to a fourth hypothesis: when plants are buried by sand, their physiological activity increases (Yuan et al. 1993), which, in turn, leads to vigorous growth (Baye 1990). Finally, a fifth hypothesis has been put forward by Van der Putten et al. (1988), who concluded that the decline of *A. arenaria* may be due to the occurrence of harmful soil organisms in its root zone. In beach sand, the natural source of the deposited sand, no harmful soil organisms were found (Van der Putten and Troelstra 1990). Nevertheless, within one growing season, harmful soil organisms could be detected in the newly formed root layer. Apparently by the formation of new roots following emergence of the plants from burial with beach sand, the plants may temporarily escape from harmful soil organisms in their root zone by upward growth (Van der Putten et al. 1989). When no sand accumulates, new roots become immediately infected by the soil organisms, so that plant growth becomes inhibited. In the inner dunes degenerated stands regain their vigour when they are subjected to burial by fresh windblown sand, as occurs along the accumulating edges of blowouts (Van Dieren 1934, Willis 1989). In these cases, the windblown sand does not originate from the beach, but from the soil profile of existing dunes. This sand has already been colonized by *A. arenaria* roots. It is not understood why *A. arenaria* under these conditions regains its vigour, as this sand once or still contained harmful soil organisms prior to becoming windborne.

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

Pathogenic soil-organisms in natural ecosystems

Plant roots are associated with different types of soil organisms, such as nematodes, fungi, bacteria and insects. Associations with soil organisms can be either beneficial or harmful to plants. The fitness-reducing effects of soil organisms on wild plants have, untill now, not received much attention (Newman 1978, Sewell 1981, Alexander 1990, Harper 1990). Although the presence of soil-borne diseases in natural vegetations is rather inconspicuous, their impact on plant populations is not (Sewell 1981, Bever 1994, Dobson and Crawley 1994). Infections by soil-borne diseases may affect the rate of growth of plants and their ability to withstand competition (Van der Putten et al. 1993, Bever 1994, Dobson and Crawley 1994). The extent to which soil-borne diseases reduce the plants' fitness and their interaction with other biotic and abiotic factors are, however, poorly understood (Alexander 1990).

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

Objective of the present study

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

Outline of the thesis

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Zoon, F.C., Troelstra, S.R. and Maas, P.W.Th. 1993. Ecology of the plant-feeding nematode fauna associated with sea buckthorn (*Hippophaë rhamnoides* L. ssp. *rhamnoides*) in different stages of dune succession. Fundamental and Applied *Nematology* **16**: 247-258.

SUMMARY

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

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

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

A. arenaria plants were buried with sand to test the hypothesis that upward growth following sand accretion enables plants to escape from infections by soil organisms (chapters 4 and 5). If plants in a degenerated field stand or potted plants grown in sterilized soil were covered with sand, the stems elongated and new shoots and roots were formed in the deposited sand layer (chapter 4). This response to burial, based on physiological mechanisms within the plant tissue, enabled the plants to emerge from the deposited sand and escape temporarily from plant-pathogenic fungi and nematodes (chapters 4 and 5).

After burial with sand, fungi immediately colonized the added soil where they probably grew on the buried non-functional leaves that surround the stems. Plant-parasitic nematodes migrated only after roots had been formed which were infected soon after being formed (chapter 5). If sand is deposited in spring, nematodes migrate directly after new roots have been formed. However, if sand is deposited in autumn, *A. arenaria* may escape from rapid infection by plant-parasitic nematodes due to the plants' ability to grow and form new roots during winter while the nematodes are largely inactive. During the summer period, plants continue to form roots (chapter 5). As a result of this prolonged formation of new roots, numbers of pathogens and parasites will increase. Without a renewed burial of the plants by sand, increasing populations of pathogens and parasites will eventually lead to their degeneration.

Degenerated stands of *A. arenaria* rejuvenate when sand is deposited on the vegetation. This phenomenon is observed along the accumulating edges of blowouts. This deposited sand is not fresh beach sand but originates from foredunes (chapter 6). Unlike sand from the beach, sand from the coastal foredunes is usually infested by soil-borne pathogens and parasites. When *A. arenaria* plants were buried with sand from its own root zone, they responded less vigorous than when plants were buried with pathogen-free sand (chapter 4). Therefore, it is hypothesized that during the proces of sand movement by wind the pathogens and parasites will, in some way, disappear. Field measurements in blowouts and experiments in a wind-tunnel demonstrated that numbers of plant-parasitic nematodes and, to a lesser extent, numbers of fungal propagules were reduced during aeolian transport. The nematodes and fungi may have been killed by scouring of sand particles and are sifted from the sand fraction. However, fungi were also transported attached to the soil particles (chapter 6).

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

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

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