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published in

Nematologia Mediterranea
2005

document version

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citation for published version (APA)

Schreck Reis, C., Freitas, H., & Van der Putten, W. H. (2005). Plant-parasitic nematodes associated with *Ammophila arenaria* (L.) Link in Portuguese coastal sand dunes. *Nematologia Mediterranea*, 33, 11-18. <http://journals.fcla.edu/nemamedi/article/view/86819>

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PLANT-PARASITIC NEMATODES ASSOCIATED WITH *AMMOPHILA ARENARIA* (L.) LINK IN PORTUGUESE COASTAL SAND DUNES

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Summary. Portuguese sand dunes extend over a total length of 450 Km and include more than half of the country's coastline. In the mobile part of the Portuguese sand dunes, *Ammophila arenaria* (L.) Link (Marram Grass) is the dominant and, often, the only naturally occurring plant species. The unrivalled contribution of *A. arenaria* to sand stabilization and dune formation is related to its extensive root and rhizome system and its vertical growth, stimulated by sand deposition. Because work on plant parasitic nematodes in dunes has mostly been done in more northerly temperate climate regions, little information on the warmer and drier climate regions is available. Therefore, in order to enhance knowledge on nematodes found in dunes in such regions, we present results of a survey of plant parasitic nematodes in the roots and rhizosphere of *A. arenaria* in two dune systems along the Portuguese coast. In the most northern population, the number and diversity of nematodes was higher than in the south of the country, and *A. arenaria* plants were more vigorous. Only two of the three endoparasitic nematode genera known to parasitize *A. arenaria* in north-western Europe were present in the Portuguese dunes.

Ammophila arenaria (L.) Link, Marram Grass, is an important sand-stabilizing plant species in dune ecosystems and frequently is the only dominant species present in open areas of mobile dunes. The wide distribution of *A. arenaria* all over the European coast, including Portugal, under distinct climatic conditions, reflects considerable ecological amplitude (Huiskes, 1979). Even though coastal sand dunes, with *A. arenaria* as the dominant species, cover more than half of the Portuguese coast (Cruz, 1984), no study has been performed on nematode communities and their population dynamics of this plant in such relatively warm and dry climate regions.

Although nematodes are the most abundant metazoan phylum on earth with respect to species richness and abundance (Wall *et al.*, 2002), studies on this important group of species have been performed only recently in sand dune systems. Differences in nematode community structure were found along a dune succession (Goralczyk, 1998) and also between different stations of a coastal dune system (Orselli and Vinciguerra, 2000). Studies with *A. arenaria* (Van der Putten and Troelstra, 1990; De Rooij-Van der Goes *et al.*, 1995; Van der Stoel *et al.*, 2002) and *A. breviligulata* Fern. (Seliskar and Huettel, 1993; Little and Maun, 1997) found correlations with the presence of nematodes and the decline of the plant, although correlations were not always very strong (Van der Putten and Troelstra, 1990; Van der Stoel *et al.*, 2002).

The present study was aimed at identifying the genera of plant-parasitic nematodes present in the roots and rhizosphere sand of *A. arenaria* in Portuguese coastal sand dunes. As previous work with this plant species was done with northern and southern populations on

the Portuguese coast, and differences were found regarding plant growth, flowering, population density and diversity (Schreck Reis and Freitas, 2003), we assessed the presence and abundance of nematode taxa in the same sites. The possible relations between nematodes and plant growth parameters could be an important help in understanding the population dynamics of *A. arenaria* along the Portuguese coast. Climate conditions were taken into consideration, and possible interactions of nematode presence and climate conditions were analysed. The results obtained were then compared with those from north-western European dunes, where *A. arenaria* is also the dominant species and responsible for sand fixation and dune formation, and the occurrence and ecology of dune nematodes have been more extensively studied (De Rooij-Van der Goes, 1995; De Rooij-Van der Goes *et al.*, 1995; Van der Stoel *et al.*, 2002).

MATERIALS AND METHODS

This study was conducted in the coastal dunes of two Natural Reserves: São Jacinto Dunes, in the north, and Sado Estuary, on the southwest coast of Portugal. In both sites, *A. arenaria* is the dominant species. However, the two dune systems represent different stages of conservation. The northern one is a protected area with a well-preserved mobile and fixed dune ecosystem, which supports characteristic natural vegetation (Neto, 1993). At the southern site, where human disturbance makes the ecosystem more vulnerable, *A. arenaria* is present in several small patches, not forming a continuous foredune.

Every two months from May 2001 to March 2002,

samples of *A. arenaria* roots and rhizosphere sand were collected from stands with vigorous plants. At each date, at each site, ten replicate sampling positions were chosen at random along a 200 meter long and four meter wide stretch, parallel to the coastline. From each position, about 2 kg of sand and *A. arenaria* roots were collected from the upper 20 cm root layer.

In the laboratory, roots were separated from the sand using a 0.4-cm-mesh sieve. After gentle homogenization, the sand was sub-divided for different purposes: about 250 g of sand were used for nutrient analysis and 70 g was weighed and dried to constant weight at 70 °C, for moisture content determination. For the extraction of living nematodes, 500 g of each sand sample were processed by the tray method described by Whitehead and Hemming (1965). After 72 hours, the nematodes were collected in water, killed, fixed with a solution of 4% formalin at 90 °C and stored in cold formalin until identification and quantification. The root samples were weighed fresh and chopped into pieces of 1 cm prior to extraction of nematodes by the Baermann funnel method (Southey, 1986). Nematodes from roots were stored and counted as for the sand samples.

The total numbers of nematodes extracted from root and sand samples were counted separately. A binocular microscope (40-100×magnification) was used and all the plant parasitic nematodes were identified to genus using the identification keys of Bongers (1988) and Brzeski (1998).

Temperature and precipitation data for the two sites were obtained from the stations of the National Meteorological Institute that were nearest to the study field sites. At the start of the survey, the sand from both the north and south site was analysed chemically using five replicate samples from each site. The pH was measured potentiometrically in a 1:2 (w/v) suspension of water. Organic matter was determined by weight loss after ignition at 430 °C for 24 hours. Total N was determined by the Kjeldahl method and total P was measured colorimetrically using the sodium bicarbonate method (Olsen and Sommers, 1982). An extraction with ammonium acetate was performed for the quantification of exchangeable cations by atomic absorption spectrophotometry.

The two dune systems studied and the sampling dates were used as independent variables for statistical tests using Statistica 5.1 for Windows. One-way analysis of variance was preceded by Bartlett's test to determine if there was homogeneity in the data. If not, the log (x+1) transformation was performed. When parametric tests were not allowed, non-parametric correspondent analysis (Kruskal-Wallis Anova) was applied. The nematode data were also analysed using the plant parasite index, which corresponds to the maturity index and is exclusively based on plant parasitic nematodes (Bongers, 1990), the Shannon-Weaver diversity index, and the Pielou evenness index (Magurran, 1988).

RESULTS

In both systems the temperature pattern was very similar, with slightly higher values, of about 0.6 °C on average, in the south (Fig. 1). The southern site was significantly ($P < 0.0001$) warmer throughout the year. From September to December there was hardly any rain at the northern site, which is not usual. Nevertheless, on almost all sampling occasions the northern site had higher soil moisture content ($P < 0.0001$; Fig. 2A). November was the only month with higher soil moisture content in the south than in the north, which is consistent with the precipitation data (Fig. 1). Soil moisture of both sites was lowest in July, and highest in January (north) and March (south).

Root weight was different between sites and during the course of sampling ($P < 0.0001$). The amount of roots per kg of sand was higher in the northern site ($P < 0.040$), and differences between the sites were slight-

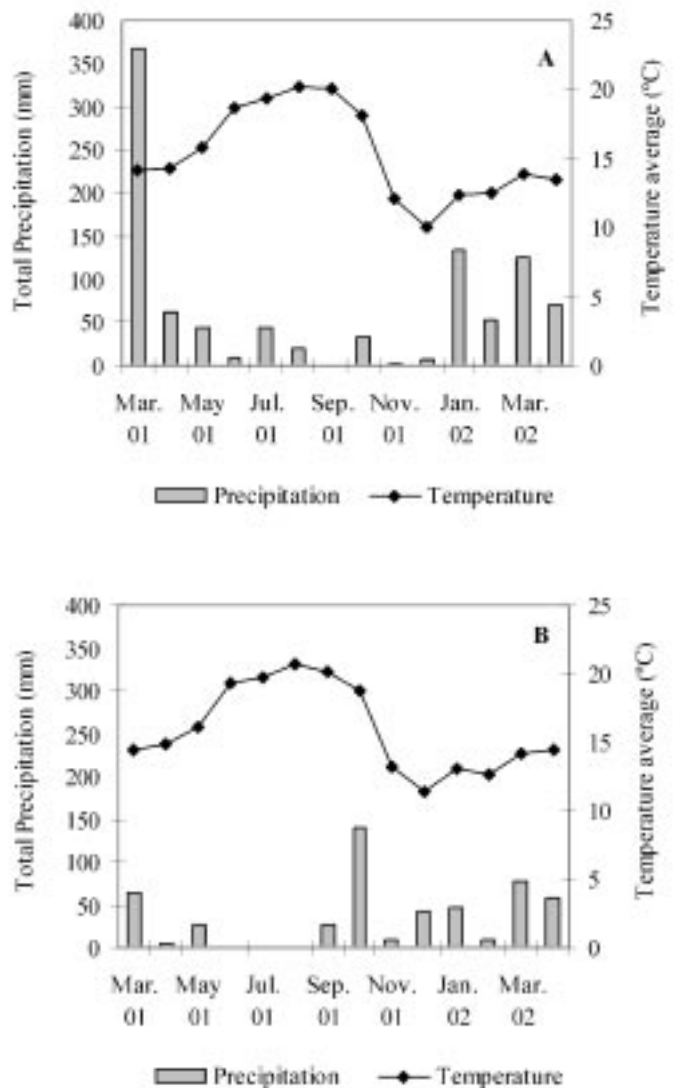


Fig. 1. Monthly values of total precipitation and average air temperature in the north (A) and south (B) systems between March 2001 and April 2002.

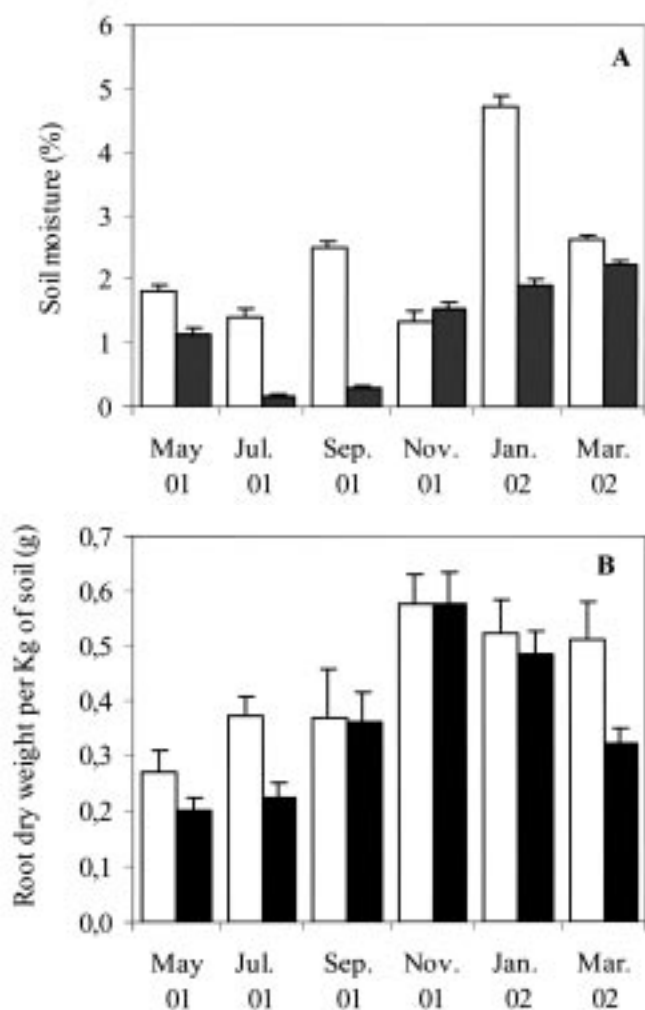


Fig. 2. Soil moisture (A) and root dry weight (B) in north (□) and south (■) populations between May 2001 and March 2002.

test in autumn and winter months (Fig. 2B). At both sites, *A. arenaria* had more root mass per soil mass in the cooler months.

The chemical properties of the rhizosphere sand of the two study sites were quite similar (Table I). The southern site had higher organic matter ($P < 0.007$), sodium ($P < 0.010$) and calcium ($P < 0.0001$) contents than the northern site.

Total numbers of plant parasitic and non-parasitic nematodes (Fig. 3) were greater per unit weight in the roots (including the ones attached to the roots) than in the sand ($P < 0.0001$, for both groups of nematodes). The numbers of nematodes in roots and sand were lower in the southern site than in the north ($P < 0.0001$ for plant parasitic and $P < 0.014$ for non-parasitic nematodes).

More plant parasitic nematodes were present in root samples from the northern site ($P < 0.001$, Fig. 3) and numbers of nematodes were greatest in spring and summer ($P < 0.016$). No significant differences were found between sites in the numbers of non-parasitic nematodes in roots (Fig. 3), although the variations that occurred through the year, higher in summer for the northern

Table I. Chemical properties of rhizosphere sand samples collected in May 2001, from the north and south localities.

| Chemical property | North | South |
|---------------------------------------|----------------------|----------------------|
| pH | 8.21 ± 0.06^a | 8.25 ± 0.06^a |
| Organic Matter (%) | 0.12 ± 0.005^a | 0.15 ± 0.005^b |
| Total Nitrogen (%) | 0.002 ± 0.0002^a | 0.002 ± 0.0005^a |
| Total Phosphorous ($\mu\text{g/g}$) | 3.55 ± 0.08^a | 3.69 ± 0.14^a |
| Sodium (mg/g) | 0.04 ± 0.003^a | 0.21 ± 0.05^b |
| Potassium ($\mu\text{g/g}$) | 10.40 ± 0.59^a | 9.78 ± 1.41^a |
| Magnesium ($\mu\text{g/g}$) | 7.56 ± 0.76^a | 7.88 ± 0.81^a |
| Calcium (mg/g) | 0.71 ± 0.04^a | 2.64 ± 0.04^b |

Values in each row followed by the same letters are not significantly different at $P < 0.05$ according to Tukey's test.

populations and higher in winter for the southern populations, were considered significant ($P < 0.006$).

The number of plant parasitic nematodes present in soil samples (Fig. 3) was quite similar in the northern and southern populations, and no significant variations were found through the year. Non-parasitic nematodes in soil (Fig. 3) also failed to show significant differences during the sampling period, but the northern site presented higher values than the south ($P < 0.0001$) throughout the year, except in November.

The northern sand dunes had greater numbers and a greater diversity of plant parasitic nematodes than the southern dunes (Table II). All ten genera of nematodes were present in the soil samples of the northern site. In the roots, four of the ten genera were not found in both northern and southern samples, three of these being the

Table II. Percentage contribution by each genus or family to the total nematode community found in root and soil samples in the north and south populations.

| Nematode genus or family | Roots | | Sand | |
|--------------------------|-------|-------|-------|-------|
| | North | South | North | South |
| <i>Criconema</i> | 0 | 0 | 5.26 | 0 |
| <i>Ditylenchus</i> | 0.04 | 0.14 | 0.42 | 4.50 |
| <i>Filenchus</i> | 4.68 | 10.52 | 8.74 | 57.39 |
| <i>Helicotylenchus</i> | 83.93 | 14.89 | 72.11 | 5.67 |
| Heteroderidae | 0 | 0 | 0.42 | 0 |
| <i>Longidorus</i> | 0.09 | 0.41 | 1.47 | 0.21 |
| <i>Meloidogyne</i> | 0 | 0 | 4.21 | 3.43 |
| <i>Paratylenchus</i> | 0 | 0.14 | 3.47 | 0.43 |
| <i>Pratylenchus</i> | 11.08 | 73.91 | 3.16 | 28.48 |
| <i>Rotylenchus</i> | 0.18 | 0 | 0.74 | 0 |

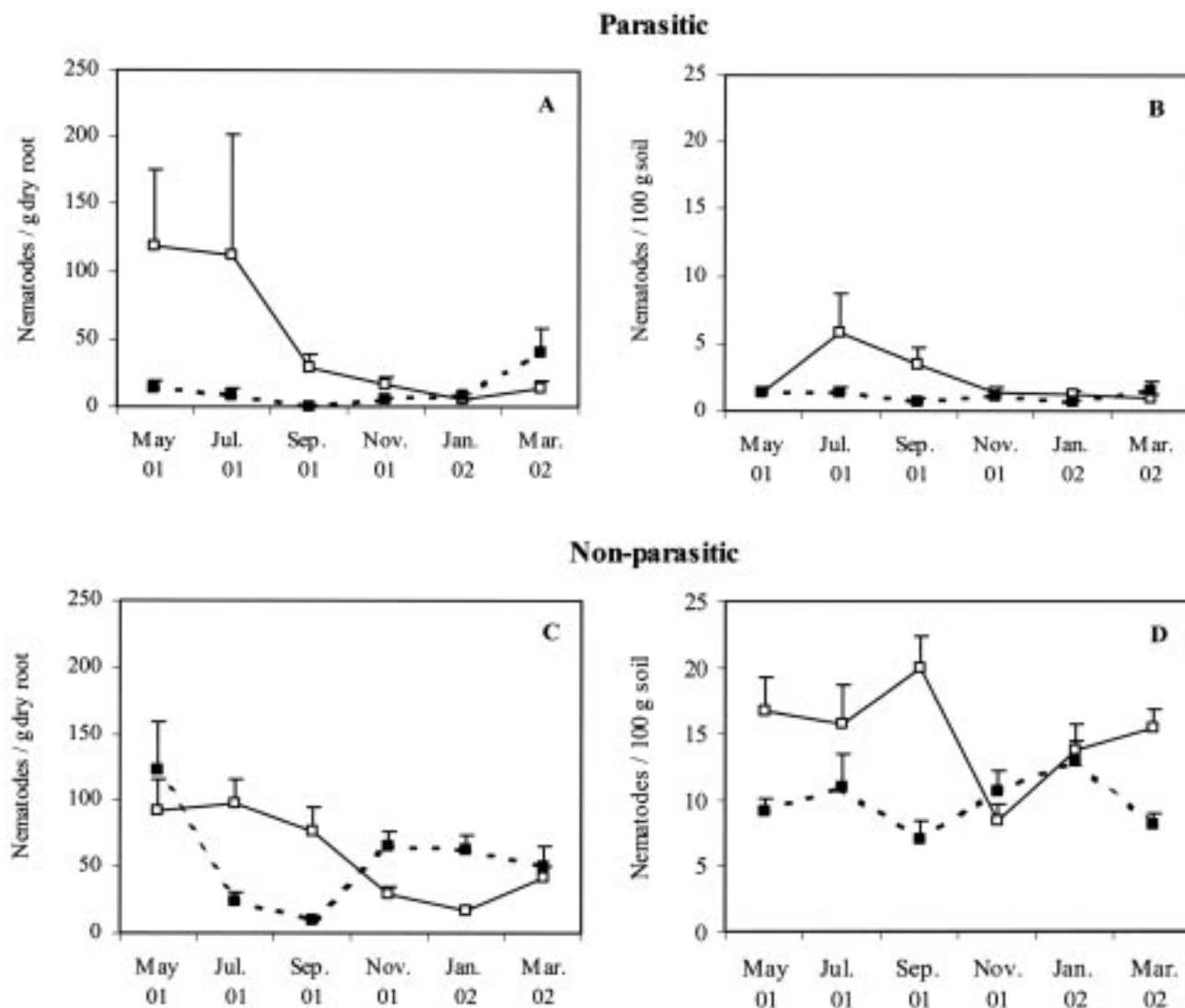


Fig. 3. Number of plant parasitic nematodes in roots (A) and soil (B) samples and number of non-parasitic nematodes in roots (C) and soil (D) samples from north (—□—) and south (-■-) populations between May 2001 and March 2002.

same at the two sites. *Filenchus*, *Helicotylenchus* and *Pratylenchus* were the most abundant genera and they were present in almost every sample. *Rotylenchus* occurred in both sand and root samples, but only in the north. *Meloidogyne* was found in soil samples from both sites, although not very often. *Paratylenchus* was present in greatest numbers in the soil samples from the northern site and was not present in the root samples from this same site. The less representative genera (a Heteroderidae genus and *Criconema*) were only present in the sand of the northern dunes. Finally, *Ditylenchus* and *Longidorus* nematodes were identified in roots and sand from both the northern and the southern dunes, but in very few samples.

The abundance of nematodes differed considerably between populations as well as within populations between sample dates (Fig. 4). On average, more nematodes of *Filenchus* and *Helicotylenchus* were found in the roots of northern dunes, and for *Pratylenchus* and *Filenchus* in the soil of southern dunes. In general, for

all three of these genera, more nematodes were found in the root than in the soil samples.

Helicotylenchus reached the highest density of all nematodes in roots of the northern population and was notably different from the southern population, not only in root samples ($P < 0.0001$, Fig. 4A) but also in the soil ($P < 0.0001$, Fig. 4B). Although *Helicotylenchus* reached its greatest abundance in summer, no significant differences between months were found during the period of study. *Pratylenchus* showed a relatively low density in soil and the patterns of distribution in the northern and southern dune systems were similar. The highest peaks of *Pratylenchus* were found in spring, both in root ($P < 0.003$, Fig. 4C) and soil ($P < 0.001$, Fig. 4D) samples. *Filenchus* was the least abundant in the root samples (Fig. 4E), at both study sites. The soil of the southern site contained more ($P < 0.0001$) *Filenchus* than soil of the northern site (Fig. 4F). There were no temporal differences.

The ecological analysis of the nematode data by

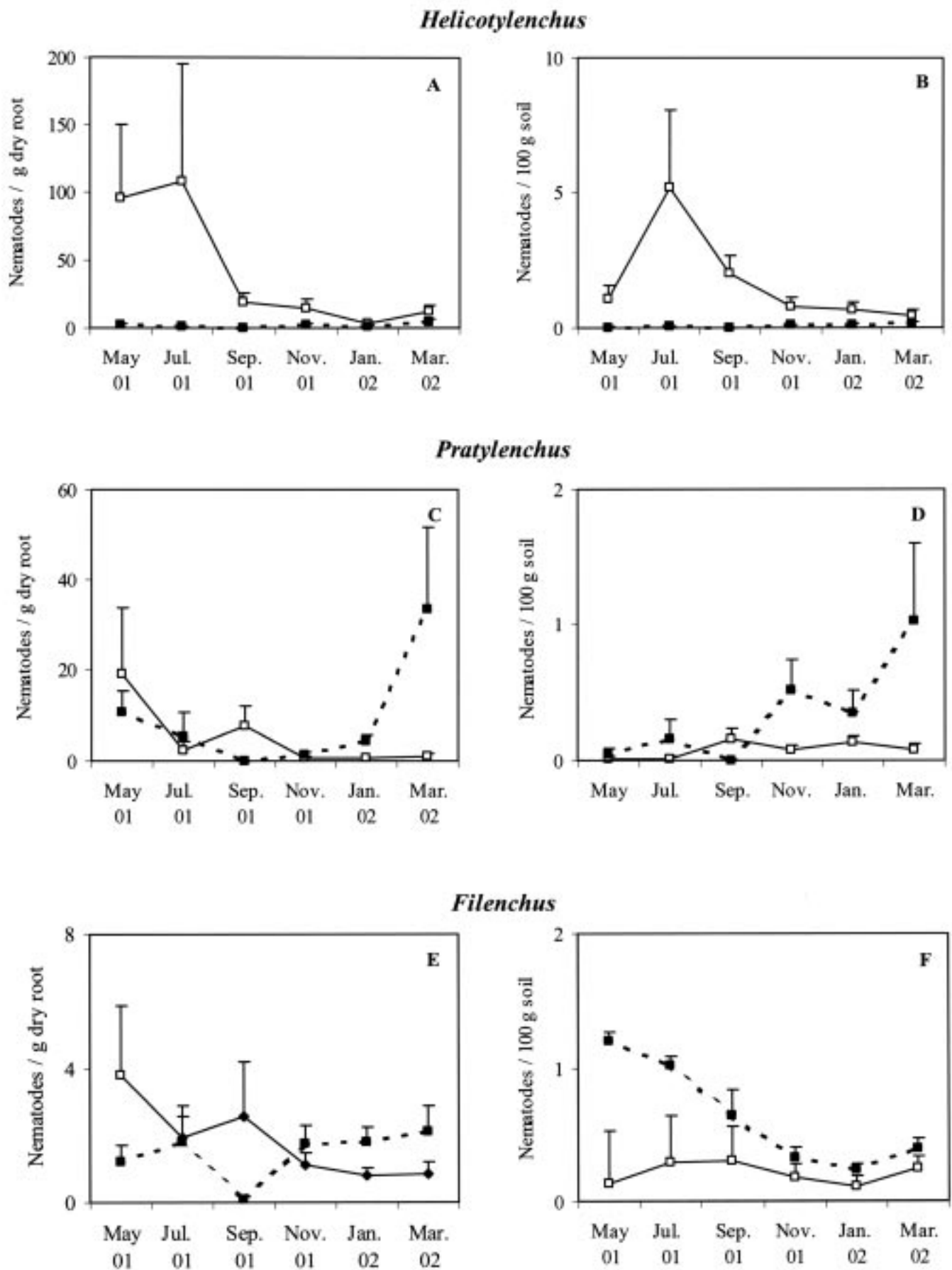


Fig. 4. Number of *Helicotylenchus* in root (A) and soil (B) samples *Pratylenchus* in root (C) and soil (D) and *Filenchus* in root (E) and soil (F) samples from north (—□—) and south (---■---) populations between May 2001 and March 2002. (Note that scales on the y-axes differ among subplots.)

Table III. Mean values (\pm standard deviation) for plant parasite, diversity and evenness indexes for root and soil samples from north and south populations.

| | | Plant Parasite Index | Shannon-Weaver Diversity Index | Pielou Evenness Index |
|------|-------|----------------------|--------------------------------|-----------------------|
| Root | North | 2.93 \pm 0.05 | 0.58 \pm 0.23 | 0.56 \pm 0.31 |
| | South | 2.80 \pm 0.11 | 0.81 \pm 0.32 | 0.68 \pm 0.16 |
| Soil | North | 2.86 \pm 0.10 | 1.15 \pm 0.40 | 0.62 \pm 0.23 |
| | South | 2.39 \pm 0.34 | 0.79 \pm 0.45 | 0.53 \pm 0.32 |

means of univariate indices (Table III) revealed a low diversity since the populations were dominated by few genera of nematodes. The plant parasite index demonstrated higher values in the northern dunes than in the southern ones, in both root ($P < 0.028$) and soil samples ($P < 0.010$).

DISCUSSION

Portuguese dunes are influenced by a transition of climates, ranging from Atlantic in the north to Mediterranean in the south. Although differences in annual average of temperature are not considerable, the average of annual precipitation is usually two times higher in the north than in the south (Ferreira *et al.*, 2000). However, the winter of 2001 was somewhat atypical in terms of amount of rain. Nevertheless, in spite of the lower rainfall in the northern dunes during four months of the fieldwork, the soil moisture content did not reflect this trend, probably because the sand from the southern system has coarser particles (Schreck Reis and Freitas, 2003), which influenced water drainage. Therefore, the lower rainfall in the north during our study may only have had limited effects on our comparison of nematode dynamics and diversity between northern and southern dunes.

The root mass per kg of soil was similar to the amount found in stands of *Ammophila arenaria* in northwestern European dunes in The Netherlands (Van der Stoel *et al.*, 2002). In Portugal, there were fewer roots in summer months and more in winter, which is the inverse pattern of north-western European dunes. The northern Portuguese site had some more roots than the southern Portuguese site, which is due to the higher plant density and lower plant diversity in the north compared to the south (Schreck Reis and Freitas, 2003). The root mass in the Portuguese samples did not reach as low values in warmer months nor as high values in cooler months as the populations in the north-western dunes of Europe, probably because of the relatively mild climate conditions.

Nematodes were present in significantly smaller numbers in the Portuguese *Ammophila* than in *Ammophila* from The Netherlands (Van der Putten and

Troelstra, 1990; De Rooij-Van der Goes *et al.*, 1995; Van der Putten and Peters, 1997; Van der Stoel *et al.*, 2002). Nevertheless, differences were also evident, in many cases, between the northern and the southern dunes in Portugal. In the northern dunes, where *A. arenaria* is denser and appears healthier (Schreck Reis and Freitas, 2003), more nematodes were collected. The diversity of nematode genera was higher in the sand from the northern dunes than in sand from the south. However, there was no statistical difference in root mass between the northern and southern dunes and, therefore, nematode diversity was certainly more influenced by abiotic factors (e.g. drought, high temperatures, or coarser sand in the southern than in the northern dunes) than by biotic factors.

Heterodera, *Meloidogyne* and *Pratylenchus* are the dominant nematodes in the roots of *A. arenaria* in the Netherlands (Van der Putten *et al.*, 1990). The first genus was not identified in Portugal, and the second one was present in very low numbers, and even absent from one site. *Pratylenchus*, although present in both root and soil samples in the two dune systems, is found in smaller quantities when compared not only with the results from The Netherlands (De Rooij-Van der Goes *et al.*, 1995) but also with the congener species, *A. breviligulata*, in Canada (Little and Maun, 1997).

Besides these three nematode genera, *Filenchus* was a quite common taxon in both Portugal and The Netherlands, although, once more, densities were higher in the dunes in The Netherlands than in Portugal (Van der Stoel *et al.*, 2002). Both *Pratylenchus* and *Filenchus* were present in greater numbers in the sand of the southern dunes of Portugal. Further experimental studies are required to determine if there is any relationship with the less vigorous performance of *A. arenaria* plants in the dunes from the southern population.

One particular nematode genus, *Helicotylenchus*, was the most abundant in Portugal, especially in the northern dunes, but it was not the dominant genus in the dunes of The Netherlands (Van der Stoel *et al.*, 2002). This nematode genus is associated with both species of *Ammophila*, and has been found in small numbers in *A. arenaria* in New Zealand (Yeates, 1967, 1968) and in higher numbers in the congener *A. breviligulata* in North America (Seliskar and Huettel, 1993). It may

be that *Helicotylenchus* prefers warmer climates, but it might also be that this genus performs better when other dominant (endoparasitic) nematodes are absent.

Nematode diversity, calculated by the Shannon-Weaver index, was rather small for the samples of the Portuguese coast when compared to other studies in dune systems (Orselli and Vinciguerra, 2000; Wall *et al.*, 2002), which could be explained by the few nematode genera that dominate the Portuguese coastal dunes, or simply because in this study only the plant parasitic nematodes were taken into consideration. However, similar results were found concerning the homogeneity in genus distribution, given by the Pielou index. The only consistent differences between the two dune systems in our study were a higher plant parasite index in the better-preserved system in the north of Portugal.

Further studies are required to evaluate the cause of the observed differences, not only between the two dune systems in Portugal but also with other countries, and their possible impact on the ecology of *A. arenaria*. It would also be interesting to identify the nematodes to species level, not only because more than one species for each genus can be present, but also because the species of *Helicotylenchus* found in Portugal is different from those found in north-western and northern Europe (G. Karssen and H. Duyts, pers. comm.). Controlled indoor experiments might help to understand the influence on particular nematode species of climatic conditions and how interactions between these factors work on the development of *A. arenaria* in its natural range of occurrence.

ACKNOWLEDGMENTS

We thank Henk Duyts, from the Netherlands Institute of Ecology, for his help in identifying nematodes, Professor Susana Santos and Professor Isabel Abrantes, from the Department of Zoology of the University of Coimbra, for use of their laboratory facilities and for help in extracting and identifying nematodes, and all the colleagues who helped with field work.

This work was supported by a PhD grant (BD/970/2000) to CSR from the Portuguese Foundation for Science and Technology (FCT), by the project POCTI/BSE/42395/2001 from FCT to HF and by the EU-EcoTrain project (RTN2-2001-0464) to HF and WvdP.

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Accepted for publication on 10 December 2004.