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Interaction between a fungal endophyte and root herbivores of *Ammophila arenaria*

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Plant mutualists

Summary

The effect of an endophytic fungus (*Acremonium strictum*) on plant-growth related parameters of marram grass (*Ammophila arenaria*), and its potential as a protective agent against root herbivores (*Pratylenchus dunensis* and *Pratylenchus penetrans*, root-lesion nematodes) was investigated in two inoculation experiments under different conditions. *Acremonium strictum*-inoculated plants showed increased plant development in terms of root biomass in the first experiment and increased number of tillers in the second experiment and biomass was less suppressed by nematodes than the *Acremonium strictum*-free plants. In neither experiment did *Acremonium strictum* reduce multiplication of root herbivores. On the contrary, *Acremonium strictum*-inoculated plants seemed to increase herbivore multiplication. Plants infected with *P. penetrans* benefitted more from the endophytic fungus than those with *P. dunensis* in terms of total biomass. The effect of *Acremonium strictum* on interspecific competition was also analyzed by plant inoculation with both nematode species. In *Acremonium strictum*-free plants with mixed nematode inoculum, the total number of nematodes, compared to numbers observed in one-species inoculation, was less than expected, suggesting that interspecific competition took place. In *Acremonium strictum*-inoculated plants no interspecific competition was observed. Plants inoculated with *P. dunensis*, *P. penetrans* and *Acremonium strictum* showed decreased total biomass compared to *Acremonium strictum*-free plants inoculated with the same nematodes. The implications of increased tillering and root growth of plants with *Acremonium* endophytes are discussed in relation to the sand stabilizing role of *Ammophila arenaria* in coastal dunes.

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Zusammenfassung

Der Effekt eines endophytischen Pilzes (*Acremonium strictum*) auf Wachstumsparameter des Dünengrases (*Ammophila arenaria*), und sein Potential als schützender Wirkstoff gegen Wurzelherbivore (*Pratylenchus dunensis* und *Pratylenchus penetrans*) wurde in zwei Inokulationsexperimenten bei unterschiedlichen Bedingungen untersucht. Endophyten-inokulierte Pflanzen in Experiment 1 zeigten gesteigertes Pflanzenwachstum bezüglich der Wurzelbiomasse und der Anzahl der Schösslinge in Experiment 2. Weiterhin zeigten sie weniger Schaden durch Nematoden als die Endophyten-freien Pflanzen.

In keinem der Experimente reduzierten die Endophyten die Vermehrung der Wurzelherbivoren. Im Gegenteil, Endophyten-inokulierte Pflanzen scheinen die Herbivoren-Vermehrung zu steigern. Hinsichtlich der absoluten Biomasse profitierten Pflanzen, die mit *P. penetrans* infiziert waren, mehr vom endophytischen Pilz als jene, die mit *P. dunensis* infiziert waren. Der Effekt von *Acremonium strictum* auf die interspezifische Konkurrenz wurde untersucht, indem die Pflanzen mit beiden Nematodenarten inokuliert wurden. In Endophyten-freien Pflanzen mit gemischtem Nematoden-Inokulum war die absolute Anzahl der Nematoden im Vergleich mit der Anzahl, die für Ein-Arten-Inokulationen beobachtet wurde, geringer als erwartet, was darauf hindeutet, dass interspezifischer Wettbewerb stattgefunden hat. Pflanzen, die sowohl mit *P. dunensis* als auch mit *P. penetrans* und dem endophytischen Pilz inokuliert waren, wiesen eine geringere absolute Biomasse auf als Endophyten-freie Pflanzen die mit beiden Nematodenarten inokuliert worden waren. Die Implikationen einer Zunahme an Schösslingen und eines gesteigerten Wurzelwachstums der Pflanzen mit *Acremonium*-Endophyten werden in Hinblick auf die sandstabilisierende Rolle von *Ammophila arenaria* in Küstendünen diskutiert.

Introduction

Endophytic bacteria and fungi take part in plant protection against herbivores and modify the competing abilities of plants in relation to different environmental stresses. Therefore endophytes may have strong bottom-up effects in trophic webs. In grasses, a high diversity of fungal endophytes can be found in roots (Vandenkoornhuyse, Baldau, Leyval, Straczek, & Young, 2002) and shoots (Schulz & Boyle, 2005). Traditionally, fungal endophytes have been considered plant mutualists mainly because they reduce herbivory via production of mycotoxins (e.g. alkaloids) or reduce drought stress. These mutualistic interactions are mostly attributed to the highly specialized *Neotyphodium* spp. but there are also many other endophytes whose interactions with plants vary from latent pathogenic to mutualistic (Schulz & Boyle, 2005).

In habitats with regular sand deposition such as coastal sand dunes, burial imposes a strong physiological constraint on plant establishment (Maun, 1998). *Ammophila arenaria* is a perennial grass that dominates foredunes of the Atlantic and the Mediterranean coast lines (Tutin et al., 1980) and has been introduced in different areas of the world for sand-fixation purposes and coastal management. *Ammophila arenaria* needs regular sand-deposition in order to maintain vigorous growth since it allows the plant to escape temporarily from soil pathogens, that otherwise would accumulate in the rhizosphere, by developing new roots in a fresh layer of sand (de Rooij-van der Goes, van der Putten, & Peters, 1995). *Ammophila arenaria* harbours different symbiotic organisms: vesicular–arbuscular mycorrhizal fungi (Kowichuk, de Souza, & van Veen, 2002) and nitrogen-fixing bacteria (Dalton et al., 2004), which enhance nutrient uptake by the plant. In Europe, no endophytic fungi for *Ammophila arenaria* have been reported until recently. Hol, Nash, & Cook (2005) isolated different *Acremonium* spp. from *Ammophila arenaria* from The Netherlands, United Kingdom and Portugal. Unlike the widely researched and more specialized *Neotyphodium* spp., the genus *Acremonium* comprises a diverse group of unspecialized fungi that can be found on different host plants. The nature of the association between *Acremonium* spp. and *Ammophila arenaria* is unknown; it could be mutualistic, pathogenic, or neutral. The question whether these plant symbionts affect plant performance directly or act as mediators of plant–herbivore interaction by reducing herbivore damage or multiplication remains unanswered thus far. Previous studies with

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other host plants suggested that *Acremonium* species could act as plant–herbivore mediators with negative effects on larval growth rate and mortality (Jallow, Dugassa-Gobena, & Vidal, 2003; Raps & Vidal, 1998).

Root-feeding nematodes are part of the pathogen complex that occurs in the rhizosphere of *Ammophila arenaria*. *Pratylenchus* species (root-lesion nematodes) penetrate, multiply, feed and move within the root cortex of the host resulting in necrotic lesions and promoting fungal infections (Back, Haydock, & Jenkinson, 2002). This nematode genus has a wide distribution along the Atlantic and Mediterranean coasts in Europe and members of this genus are the first nematodes species to colonize *Ammophila arenaria* roots (van der Stoel, van der Putten, & Duyts, 2002). Different species with a different degree of specificity (host range) have been reported in *Ammophila arenaria*. *Pratylenchus dunensis* only occurs in foredunes where *Ammophila arenaria* is abundant (de la Peña, Moens, van Aelst, & Karssen, 2006). *Pratylenchus penetrans* is a very polyphagous species occurring at high densities in later stages of dune succession (Zoon, Troelstra, & Maas, 1993). It is a key factor in the die-out of the North-American *Ammophila breviligulata* (Seliskar & Huettel, 1993) and is also a serious pest on economically important crops (Duncan & Moens, 2006).

Several mechanisms have been recognized to explain the regulation of population densities of root-feeding nematodes in coastal dunes, including bottom-up (van der Stoel, 2001), top-down (de Rooij-van der Goes, 1995) and horizontal factors (Brinkman, 2004). Only recently the contribution of plant mutualists, i.e. arbuscular mycorrhizal fungi, to nematode control in *Ammophila arenaria* has been studied (de la Peña, Rodríguez-Echeverría, van der Putten, Freitas, & Moens, 2006). The potential of fungal endophytes as a control agent of root-feeding nematodes has never been explored in coastal dunes although fungal endophytes are well known for affecting nematodes (Cook & Lewis, 2001).

In this paper, we test whether *Acremonium strictum*, an endophytic fungus isolated from the grass *Ammophila arenaria*, affects plant growth in terms of biomass and number of leaves and tillers. In addition, we explore whether this endosymbiont acts as a plant–herbivore mediator affecting the tolerance or resistance to two root-feeding nematode species. Tolerance is measured as a decrease of biomass reduction caused by nematodes; resistance is measured as a reduction in nematode multiplication. Finally, the effect of inoculation with *Acremonium strictum* on competition between nematodes is examined measuring nematode multiplication.

Material and methods

Seedling establishment

Seeds were collected in August 2003 from *Ammophila arenaria* growing in dunes in Oostvoorne, The Netherlands (51°52N, 04°04E). Seeds were heat treated at 57 °C for 15 min prior to germination. This heat treatment is known to kill *Neotyphodium*-like endophytes (Siegel, Latch, & Johnson, 1987), but effects on other endophytic organisms are not proven. Seeds were germinated on 2 mm glass beads. Two weeks after germination seedlings were transferred to 600 cm^3 pots containing 550 cm^3 autoclaved (121 °C for 2 h) dune sand.

Sand was collected in the foredunes of Het Zwin Nature Reserve, Belgium, from the top 30 cm of a freshly deposited sand dune with vigorous *Ammophila arenaria*.

Endophyte isolation and maintenance

The endophytic fungus was isolated from an *Ammophila arenaria* stem collected in Oostvoorne, The Netherlands. The ITS sequence of the isolate showed high homology with *Acremonium strictum* (Hol et al., 2005) and morphological characters further confirmed the identity of the isolate as belonging to the *Acremonium strictum* complex. The isolate is deposited in the public collection of the Fungal Biodiversity Centre, The Netherlands (Accession: CBS 118929). The isolate was maintained on full strength potato dextrose agar plates (PDA) at 25 °C in the dark.

Nematode cultures and inoculation

P. dunensis was isolated from samples of *Ammophila arenaria* collected in Oostvoorne, The Netherlands in October 2003; *P. penetrans* was isolated from *Zea mays* in Zandhoven, Belgium. The two nematode species were cultured on *Ammophila arenaria* (Oostvoorne, NL) growing in autoclaved sand in 20 dm^3 PVC pots for 12 months prior to experimental set-up. Plants were fertilized monthly with 500 ml of Hoagland's solution (Hewitt, 1966) and the water content was weekly adjusted to 5–10%. Before experimental set-up nematode identity was confirmed using morphology and rDNA ITS region sequencing.

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Nematode inocula were harvested from the cultures using the modified mistifier technique (Seinhorst, 1950). Roots were cut into 1 cm pieces and placed in a funnel over a cotton filter. The funnels were placed in a mist chamber at 20 °C and the obtained nematode suspension was tapped off every 24 h during one week to collect nematodes for inoculation. Nematodes were kept in water at 8 °C until use.

Two inoculation experiments were conducted. In experiment 1, nematodes were first inoculated to *Ammophila arenaria* seedlings and later *Acremonium strictum* was applied to the stem when the seedlings were sufficiently developed to survive the cut in the stem. In experiment 2, plants were first inoculated with the *Acremonium strictum* via a root dip method and later with nematodes; the latter experiment is closer to the field situation, in which *Ammophila arenaria* plants would already contain endophytes when the root-feeding nematodes reach the plant roots.

Experiment 1

Fifty-six 3-week-old *Ammophila arenaria* seedlings were planted separately in 600 cm^3 plastic pots filled with autoclaved dune soil. The experiment compared six treatments: C: plants in sterilized soil, no organisms added ($n = 14$), E: plants inoculated with *Acremonium strictum* ($n = 14$), P: plants+100 *P. penetrans* ($n = 7$), PE: plants+100 *P. penetrans* and *Acremonium strictum* ($n = 7$), D: plants+100 *P. dunensis* ($n = 7$), DE: plants+100 *P. dunensis* and *Acremonium strictum* ($n = 7$). In the nematode treatments, 100 mobile nematodes were added to the pots nine days after planting. Twenty-six days after planting half of the plants were inoculated with *Acremonium strictum*. The other plants were inoculated with PDA homogenate only. Inoculation of the fungus was done by inserting a homogenate of PDA and fungus in a superficial cut made by a sterile razor blade in the coleoptile. The homogenate contained both mycelium and conidia at a density of 5×10^5 CFU/ml. Plants were placed in a randomized block design with seven blocks and eight pots per block (2*C*, 2*E*, 1*P*, 1PE, 1*D*, 1DE) on the bench of a greenhouse. All pots received 60 and 40 ml half strength Hoagland's solution 16 and 26 days after planting, respectively. Plants received tap water when necessary. The experiment was run in the greenhouse for 13 weeks (May–August 2005) under ambient light conditions and $25 \pm 5/20 \pm 5$ °C mean day/night temperatures.

Experiment 2

Forty 3-week-old seedlings of *Ammophila arenaria* were dipped for 3 h in a suspension of mycelium and conidia (1×10^5 CFU/ml) of *Acremonium strictum*, PDA and sterilized water. Forty other seedlings were placed in a similar suspension but without the fungus. After the root dip, all seedlings were planted in 600 cm^3 pots filled with autoclaved dune soil. Eight treatments were compared, with ten replicates each: control (C), Plants+*Acremonium strictum* (E), Plants+100 *P. penetrans* (P), Plants+100 *P. penetrans* on *Acremonium strictum* plants (PE), Plants+100 *P. dunensis* (D), Plants+100 *P. dunensis* on *Acremonium strictum* plants (DE), Plants+50 *P. penetrans* and 50 *P. dunensis* (PD) and plants+50 *P. penetrans*+50 *P. dunensis* on *Acremonium strictum* plants (PDE). Twenty-one days after planting the seedlings, nematodes were added to the pots in the nematode treatments. Twenty pots received *P. penetrans*, 20 pots *P. dunensis*, and 20 pots received the mixture of *P. penetrans* and *P. dunensis*. Plants were placed in a randomized block design with 10 blocks and 8 pots per block, one of each treatment, in a climate chamber under 16/8 h day/night artificial illumination (250 $\mu mol\, m^{-2}\, h^{-1}$) at a regime of $24 \pm 1/18 \pm 1$ °C and 70% humidity. All pots received 50 ml Hoagland's solution seven days after planting. Plants were further watered three times a week to 10% soil moisture. After 13 weeks plants were harvested and nematodes extracted.

Data collection

At harvest, the number of leaves and tillers were counted and the length of the longest tiller was measured. Sand was stored at 4 °C for three weeks until the extraction of nematodes. After the roots had been washed, the fresh weight of roots and shoots was determined. The roots were divided into two parts: one part for nematode extraction and the other to estimate the water content. A random fraction of approx. 1 g (fresh weight) was collected for nematode extraction. Roots were kept in moist tissue at 4 °C until extraction. The remaining plant material was dried at 70 °C. For the shoots: (i) the lower 3 cm of the basal stem part was used to isolate *Acremonium strictum* (in experiment 1), (ii) the remainder was dried at 70 °C during 3 days. The percentage moisture in the shoot was calculated based on the difference between fresh weight and dry weight divided by fresh weight and multiplied by 100. Fungal extraction was done after surface sterilization [70% MeOH (30 s) and

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1.3% NaClO (10 min)] of the basal stem part by placing stem pieces on full strength PDA. After three weeks, plates were examined for presence of *Acremonium strictum*. Morphological characters were used to compare the isolated fungi with the original strain inoculated. Nematodes were extracted from roots and soil by centrifugal flotation (Hendrickx, 1995). Mobile nematodes in any developmental stage present in the final eluted suspension were counted.

Data analysis

Experiment 1: Differences in dry weight of shoot and root biomass were tested with two-way ANOVA, after checking the assumptions for normality and homogeneity of variance. The analysis was carried out with 'endophyte' (2 levels) and 'nematode' (2 levels) as main factors, followed by a comparison of the two nematode species. When variables did not meet the ANOVA assumptions (number of tillers, leaves, height), then effects of endophyte and nematodes were tested with the Friedman test, followed by Wilcoxon signed rank test. All plants that were inoculated with *Acremonium strictum* were included in the analysis as E+. Unsuccessful isolation of *Acremonium strictum* was not taken as evidence for absence of *Acremonium strictum*, since the success of fungal isolation can vary between consecutive tissue pieces (Christensen, Bennett, & Schmid, 2002). Five plants (2C and 3E) died during the experiment and were excluded from further analysis. The effect of *Acremonium strictum* on numbers of nematodes (square root transformed) per g root was tested in a two-way ANOVA (main factors 'endophyte' and 'nematode species' with root biomass as covariate.

Experiment 2: Differences in dry weight of shoot and root biomass (sqrt transformed) and moisture levels in the shoot were tested with three-way ANOVA, after checking the assumptions for normality and homogeneity of variance. Main treatments were 'endophyte', '*P. penetrans*' and '*P. dunensis*'. Transformation of nematode numbers did not help to meet ANOVA assumptions. Pairwise comparisons between *Acremonium strictum*-inoculated and *Acremonium strictum*-free plants per nematode treatment were made with paired *t*-tests. To test whether *Acremonium strictum* affects nematode competition, nematode numbers were calculated in plants with and without *Acremonium strictum*-inoculation. Competition was defined as a lower number of nematodes in the combined inoculation than expected from the observed numbers for the individual species. For each block the expected number was calculated as $0.5D+0.5P$ with D: *dunensis* and P: *penetrans*.

For all tests SPSS 13.0 was used.

Results

Experiment 1. Inoculation of *Ammophila arenaria* stems with *Acremonium strictum*

Acremonium strictum was successfully re-isolated from 16 out of the surviving 25 plants that had been inoculated. Shoot biomass was significantly reduced by nematodes ($F_{1,41} = 17.597$,

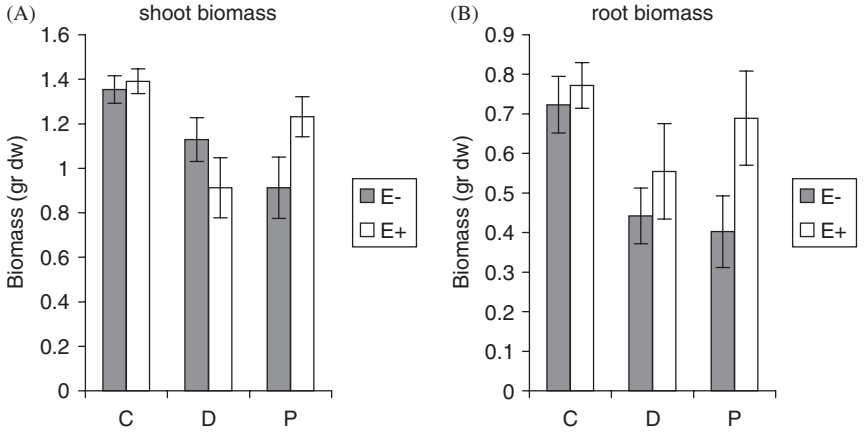

Figure 1. The effect of shoot inoculation of *Ammophila arenaria* with *Acremonium strictum* and the nematode species *Pratylenchus penetrans* or *Pratylenchus dunensis* on (A) shoot and (B) root biomass of *Ammophila arenaria* (experiment 1; mean ± SE). E+: *Acremonium strictum* inoculation; E−: no *Acremonium strictum* inoculation; C: no nematodes; D: *P. dunensis*; P: *P. penetrans*.

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Table 1. The effect of the nematodes *Pratylenchus dunensis* and *Pratylenchus penetrans* on plant parameters of *Ammophila arenaria* plants inoculated at the stems with the endophyte *Acremonium strictum*

	Control (no nematodes)		*P. dunensis*		*P. penetrans*		X^2	P
	E−	E+	E−	E+	E−	E+		
Number[a]	12	11	7	7	7	7		
Height (cm)	86(2)a	96(6)a	92(6)a	82(3)a	84(4)a	79(7)a	8.93	0.115
Leaves (N)	10.3(0.9)ab	10.5(0.8)a	7(0.8)bc	6(0.9)c	7(0.9)bc	9(1.2)ab	13.199	0.022
Tillers (N)	2.8(0.3)a	2.8(0.3)a	1.7(0.3)a	1.3(0.3)a	2.3(0.5)a	2.4(0.5)a	10.156	0.071
Nematodes (N)			171(25)a	165(41)a	159(26)a	446(128)a	5.229	0.156

E− : no *Acremonium strictum*; E+: *Acremonium strictum* inoculation. The total number of nematodes in the root system at the end of the experiment (Nematodes) are shown as well. In a row, different letters indicate significant differences between treatments. Friedman test ($n = 7$, df $= 5$), followed by Wilcoxon tests for pairwise comparisons. Averages with standard errors (in parentheses) are given.

[a]Number of plants per treatment. E+ plants are all plants inoculated with *Acremonium strictum*, including those from which no *Acremonium strictum* could be reisolated at the end of the experiment.

$P<0.001$), but not affected by *Acremonium strictum* inoculation ($F_{1,41} = 0.287$, $P = 0.595$; Fig. 1A). When looking at the nematode treatments only, there was a significant interaction between nematode species and *Acremonium strictum* ($F_{1,18} = 4.416$, $P = 0.05$). Plants with *P. penetrans* had increased shoot biomass with *Acremonium strictum*, while the opposite applied to *P. dunensis*. The reduction in biomass was associated with fewer leaves per plant; plant height was not significantly affected by nematodes or *Acremonium strictum* (Table 1). Root biomass was reduced by nematodes ($F_{1,41} = 11.329$, $P = 0.002$) but tended to be increased by *Acremonium strictum* inoculation ($F_{1,41} = 3.191$, $P = 0.081$; Fig. 1B). The *Acremonium strictum* effect on root biomass was most pronounced in the presence of nematodes, but this was not significantly different (Fig. 1B). Nematodes reduced the root biomass by 0.34 ± 0.09 g in the absence of *Acremonium strictum* while the reduction was only 0.14 ± 0.10 g in the presence of *Acremonium strictum* (paired *t*-test, $n = 14$, $P = 0.12$). The total number of nematodes in the roots varied with a weak interaction between *Acremonium strictum* and nematode species, even after including root biomass as a covariate ($F_{1,23} = 3.581$, $P = 0.071$). Plants inoculated with *Acremonium strictum* and *P. penetrans* had on average nearly three times as many nematodes in the root system then plants inoculated with *Acremonium strictum* and *P. dunensis* (Table 1). Plants inoculated with *P. penetrans* produced a significantly greater total biomass when inoculated with *Acremonium strictum*; this was not the case for *P. dunensis* (Fig. 2).

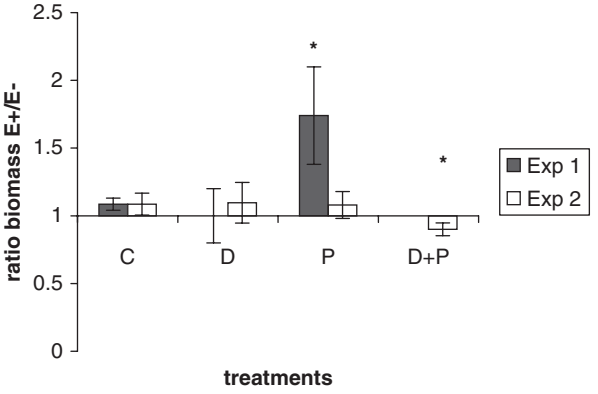

Figure 2. Ratio of the total biomass of *Acremonium strictum* infected plants divided by the total biomass of *Acremonium strictum*-free *Ammophila arenaria* inoculated with *Pratylenchus* spp. (mean ± SE). Exp 1: *Acremonium strictum* inoculation of *Ammophila arenaria* stems; Exp 2: *Acremonium strictum* inoculation of *Ammophila arenaria* roots. C: control; D: *P. dunensis*; P: *P. penetrans*; D+P: *P. dunensis* and *P. penetrans*. Asterisks indicate a significant difference between E+ and E− plants ($P<0.05$, *t*-test).

Experiment 2. Inoculation of *Ammophila arenaria* roots with *Acremonium strictum*

Neither shoot nor root biomasses were significantly affected by *Acremonium strictum* or nematodes (Fig. 3A and 3B). *Acremonium strictum* inoculation had a significant effect on other plant parameters. First, the moisture content of the shoots of *Acremonium strictum*-inoculated plants was less than that in non-inoculated plants ($F_{1,72} = 6.315$, $P = 0.014$), regardless of the presence of nematodes (Table 2). Second,

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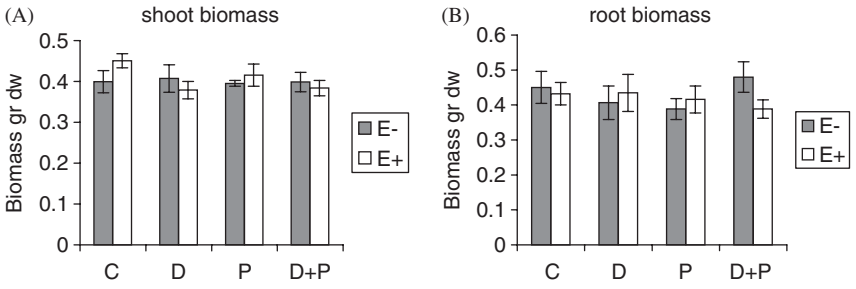

Figure 3. The effect of root inoculation of *Ammophila arenaria* with *Acremonium strictum* and the nematode species *Pratylenchus penetrans* or *Pratylenchus dunensis* on (A) shoot and (B) root biomass of *Ammophila arenaria* (experiment 2; mean ± SE). E+: *Acremonium strictum* inoculation; E−: no *Acremonium strictum* inoculation; C: no nematodes; P: *P. penetrans*; D: *P. dunensis*; D+P: *P. dunensis* and *P. penetrans*.

Acremonium strictum inoculation tended to increase plant tillering; plants with *Acremonium strictum* had on average 2.9 tillers per pot, while control plants had 2.6 tiller per pot ($Z = -1.941$, $n = 40$, $P = 0.052$; Table 2). The total number of *Pratylenchus* spp. extracted from *Ammophila arenaria* differed significantly between the nematode species (Table 2), but was not affected by endophyte inoculation of the plant. In the absence of *Acremonium strictum* the number of *Pratylenchus* in plants inoculated with a mixture of species deviated from the expected numbers calculated on basis of the individual species (expected = 0.5∗numbers in D+0.5∗numbers in P). In *Acremonium strictum*-free plants, an average of 4301 nematodes per g root were expected, but only 2172 were found (paired *t*-test, $n = 10$, $P = 0.051$, Fig. 4). In the presence of the endophyte, the calculated expected number of nematodes per g root was not different from the observed number (2659 per g: paired *t*-test, $n = 10$, $P > 0.10$). Therefore, the endophyte status of the plant interacts with competition between *Pratylenchus* spp.

Inoculation of plants with *Acremonium strictum* had a negative effect on plant biomass only when they were inoculated also with both *P. dunensis* and *P. penetrans* (Fig. 2).

Discussion

For the first time it is shown that the fungal endophyte *Acremonium strictum*, isolated from *Ammophila arenaria*, had a positive effect on the growth of his host plant: root biomass was increased in the first experiment and the number of tillers per plant in the second experiment. *Acremonium strictum*-infected plants further showed a lower water content than *Acremonium strictum*-free plants which is most likely the result of increased transpiration since root biomass was increased and the total water applied did not differ between the *Acremonium strictum* treatments (data not shown). The effect on the transpiration of *Ammophila arenaria* might be of major importance since water availability is a limiting factor in the establishment, seedling survival and leaf photosynthetic rate in several dune colonizing plant species (Alessio, De Lillis, Brugnoli, & Lauteri, 2004).

The increased root growth of *Acremonium strictum*-infected plants, especially in the presence of nematodes, might have consequences for dune stabilization. Faster root growth means faster spread and thus more retention of sand; in combination with increased tillering this allows faster clonal spread. The enhancement of clonal properties of the host plant might have consequences in the competition ability against other dune grasses. However, in the longer term the stimulation of root herbivores by *Acremonium strictum* could lead to a negative soil feedback.

The effects of both *Acremonium strictum* and *Pratylenchus* spp. on plant growth and nematode multiplication differed between the experiments. This disparity can be attributed to the different experimental conditions in each experiment that probably affected the fungi–host plant symbiosis and subsequent interactions with herbivores. In the first experiment plants were inoculated with nematodes after 4 weeks and *Acremonium strictum* was inoculated later in the aboveground parts of the plant. In contrast, in the second experiment plants were 6 weeks old when they were inoculated with nematodes and *Acremonium strictum* was most likely already inside the roots. Furthermore the climatic conditions were much more controlled in the second experiment. From these variable results between the two experiments we conclude that the effect of *Acremonium strictum* might depend on the order of inoculation, the plant part

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Table 2. The effect of the nematodes *Pratylenchus dunensis* and *Pratylenchus penetrans* (alone or in combination) on plant parameters of *Ammophila arenaria* plants inoculated at the roots with the endophyte *Acremonium strictum*

	Control (no nematodes)		*P. dunensis*		*P. penetrans*		*P. dunensis*+*P. penetrans*		*Z*	*P*
	E−	*E*+	*E*−	*E*+	*E*−	*E*+	*E*−	*E*+		
Number	10	10	10	10	10	10	10	10		
Height (cm)	38(2)a	41(2)a	36(2)a	35(2)a	37(1)a	39(2)a	37(2)a	36(2)a	5.389	0.613
Leaves (N)	6(0.7)a	6.8(0.6)a	5.8(0.7)a	6.7(0.7)a	6.1(0.6)a	7(1.2)a	6.8(1.0)a	6.8(0.4)a	4.655	0.702
Tillers (N)	2.4(0.3)a	2.9(0.2)a	2.5(0.3)a	2.9(0.2)a	2.4(0.2)a	3(0.5)a	2.8(0.4)a	2.8(0.1)a	5.012	0.659
Moisture shoot (%)	64(1.1)b	61(0.4)a	64(1.6)b	63(0.9)a	65(0.7)b	63(0.9)a	64(0.7)b	63(0.7)a	6.315[a]	0.014
Nematodes (N)			2320(360)a	1738(421)a	128(27)c	148(26)c	852(154)b	802(266)b	37.829	<0.001

E−: no *Acremonium strictum*; *E*+: *Acremonium strictum* inoculation. The total number of nematodes in the root system at the end of the experiment (Nematodes) are shown as well. Within rows, different letters indicate significant differences between treatments. Friedman test ($n = 10$, df = 8), followed by Wilcoxon tests for pairwise comparisons. Averages with standard errors (between brackets) are presented.

[a]Based on ANOVA, not Friedman.

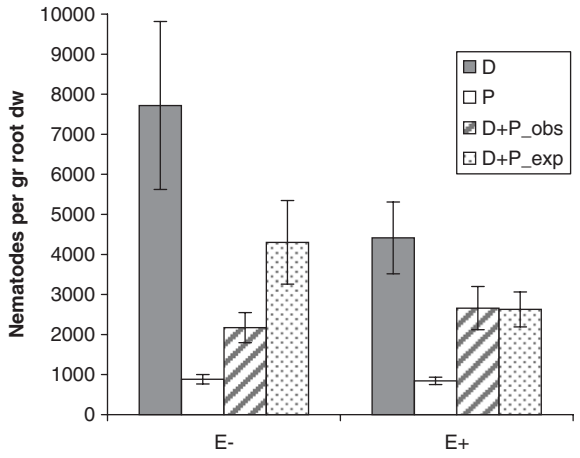

Figure 4. Nematode numbers (mean ± SE) in the roots of *Ammophila arenaria* inoculated with *Pratylenchus dunensis* (100 individuals; D), *Pratylenchus penetrans* (100 individuals; P), or *P. dunensis* (50 individuals)+*P. penetrans* (50 individuals) (D+P_obs) (experiment 2). Expected number is calculated as 0.5∗D+0.5∗*P* (D+P_exp).

that is inoculated or on climatic conditions. Further studies including field experiments are needed to evaluate the role of *Acremonium strictum* in the ecology of *Ammophila arenaria*.

Increase in resistance of *Ammophila arenaria* to *Pratylenchus* spp. was not found in any of the experiments established. The presence of *Acremonium strictum* might be even beneficial for the nematodes due to the greater root biomass of endophyte-infected plants enhancing bottom-up effects (increase in resources). *Acremonium strictum* inoculation might also change plant quality, since the positive effect on nematode numbers was still present after correcting for root biomass.

Our results show interspecific competition between *P. dunensis* and *P. penetrans* and differences in multiplication potential. This is the first time that competition between two nematode species within the same genus (*Pratylenchus*) has been explored. Competition was dependent on endophyte status of the plant. The effect of interspecific competition between species of different root-feeding nematode genera in *Ammophila arenaria* has recently been analyzed, indicating that *Pratylenchus* is a good competitor when compared to other endoparasitic nematodes (Brinkman, Duyts, & van der Putten, 2005).

The fact that in our experiments *Acremonium strictum* did not reduce *Pratylenchus* spp. numbers does not automatically imply that *Acremonium strictum* would be unimportant for nematode control in *Ammophila arenaria*. More nematode species with different parasitic strategies need to be tested; previous studies have shown that

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Acremonium strictum is a common parasite of nematode eggs in sedentary root feeding nematodes (Nigh, Thomason, & Vangundy, 1980; Verdejo-Lucas, Ornat, Sorribas, & Stchiegel, 2002).

In the past decade, the dynamics and feedback processes between soil-biota and the aboveground plant communities in coastal dunes have been extensively studied, either from a conservational point of view or as a model for multi-trophic interactions in soil. Our results point to *Acremonium strictum* as a new player in this complex and therefore the positive or negative effects of fungal endophytes on the plant and its symbionts needs to be further explored in order to get a more comprehensive view.

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