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Growth responses of maritime sand dune plant species to arbuscular mycorrhizal fungi

MARIUSZ TADYCH and JANUSZ BŁASZKOWSKI

Department of Plant Pathology, Agricultural Academy in Szczecin
Słowackiego 17, PL-71-434 Szczecin, Poland

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In a pot experiment conducted in a greenhouse, the response of 6 plant species dominating in the succession of vegetation of a deflation hollow of the Łeba Bar to inoculation with arbuscular mycorrhizal fungi (AMF) was investigated. The inoculum was a mixture of soil, roots and spores of 5 species of AMF with the dominant species *Glomus aggregatum*. Except for *Corynephorus canescens* and *Festuca rubra* subsp. *arenaria*, both the growth and the dry matter of above-ground parts of plants of *Agrostis stolonifera*, *Ammophila arenaria*, *Corynephorus canescens*, *Juncus articulatus* and *J. balticus* inoculated with AMF were higher than those growing in soils lacking infection propagules of these fungi. Inoculation with AMF decreased the dry matter of root: shoot ratios in 5 plant species. This property was not determined in *Festuca rubra* subsp. *arenaria* due to the death of all control plants. The level of mycorrhizal infection was low and did not correlate with the growth responses found. The high growth reaction of *Juncus* spp. to AMF found in this study suggests that the opinion of non-mycotrophy or low dependence of plants of *Juncaceae* on AMF was based on results of investigations of plants growing in wet sites known to inhibit the formation of mycorrhizae.

Key words: arbuscular mycorrhiza, dune plants, *Glomales*, mycorrhizal dependence.

INTRODUCTION

About 80% of plants of the Earth associate with arbuscular mycorrhizal fungi (AMF) of the order *Glomales* (*Zygomycota*; G i a n i n a z z i and G i a n i n a z z i-P e a r s o n 1986). Sites especially favouring AMF are dune soils, mainly due to their low content of phosphorous (B ł a s z k o w s k i 1993b; K o s k e 1987).

Plants associated with AMF are known to have enhanced inorganic nutrition (C o o p e r 1984), greater rates of photosynthesis (A l l e n et al. 1981) and improved resistance to drought (N e l s o n 1987) and pathogens

(Schönbeck 1978). Inoculation of plants with AMF increased their growth and vigour (Pfleger and Linderman 1994; Smith and Read 1997). However, most of the investigations dealt with cultivated plants, whose species and even cultivars of the same species differed in their response to mycorrhizal infections (Azcon and Ocampo 1981; Saif 1987). Of dune plants, responses to inoculation with AMF have been recognized in only 9 species, including *Ammophila arenaria*, *A. breviligulata* and 7 plant species colonizing tropical dunes of the Gulf of Mexico (Corkidi and Rincón 1997; Gemma and Koske 1989, 1997; Nicolson and Johnston 1979).

The unique areas of the Łeba Bar are deflation hollows (Ostrowski and Symonides 1994). The consistent occurrence of AM in plants dominating in the succession in these hollows (Tadych and Błaszkowski in press; Błaszkowski 1995) suggests that the plants are dependent on AMF. However, the degree of their dependence is unknown.

The soils of initial successional stages of vegetation of the Łeba Bar deflation hollows either have no or contain very low numbers of infection propagules of AMF (Tadych and Błaszkowski in press). Therefore, recognition of the degree of dependence of plants initiating and dominating in the succession of the deflation hollows may help in their stabilization through either planting non-mycorrhizal plants, plants of a low mycorrhizal dependence or introduction of AMF.

The aim of this paper was to determine the influence of a mixture of AMF on growth of 6 plant species dominating in the succession of vegetation of the 12 deflation hollow of the Łeba Bar.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse of the Agricultural Academy in Szczecin in 1996. Pots of a diameter of 7 cm and a height of 8 cm were filled with a mixture of soil and roots coming from a many-species pot culture of AMF whose host plant was *Plantago lanceolata*. The culture was established based on a mixture of soil samples collected from the 12 deflation hollow considered. The spore abundance in 10 g dry soil of the culture was 739. The spores represented 5 species of AMF, i.e., *Acaulospora koskei* Błaszk., *Glomus aggregatum* Schenck et Smith emend. Koske, *G. corymbiforme* Błaszk., an undescribed *Glomus* 107 and *Scutellospora persica* (Koske et Walker) Walker et Sanders. The fungus dominating in the inoculum was *G. aggregatum*. Control pots were filled with the same mixture of soil and roots earlier autoclaved 3 times for 2 h at 24-h intervals. To equalize non-mycorrhizal microflora, 50 ml of filtered washing from a living culture was added to each control pot.

About 20–50 seeds of *Agrostis stolonifera*, *Ammophila arenaria*, *Corynephorus canescens*, *Festuca rubra* subsp. *arenaria*, *Juncus articulatus* and *J. balticus* were seeded at a depth of 0.3–0.5 cm. After 2 weeks, the plant density in each pot was thinned to 5, remaining seedlings of equal height. Plants were watered 3 times a week, using 10 ml of tap water per pot. Plants were harvested 25 weeks after sowing.

The growth rate of plants was measured at 2-week intervals beginning 11 weeks after sowing. After harvest, shoots and roots were dried at 105°C for 16 h and then weighted. Levels of mycorrhizal infections were determined based on 1–1.5 cm root fragments stained in 0.05% trypan blue (Phillips and Hayman 1970). The percent length of roots with arbuscules, vesicles and intramatrical hyphae was calculated on the basis of 50 root fragments examined under a light microscope (McGonigle et al. 1990).

RESULTS

Except for *Corynephorus canescens* and *Festuca rubra* subsp. *arenaria*, inoculation with AMF of the other four plant species increased both their growth rate in the study period and height determined in the last term of measurement (Fig. 1). The highest differences in the growth between mycorrhizal and non-mycorrhizal plants appeared in *Agrostis stolonifera*, *Juncus articulatus* and *J. balticus*. The lower average height of plants of *Corynephorus canescens* found beginning from the 7th measurement resulted from the death of some plants. The height and rate of growth of *Festuca rubra* subsp. *arenaria* were not determined due to the death of control plants before the first term of measurement.

Dry weight of shoots. The highest increases in dry weight of shoots (Fig. 2) were found in mycorrhizal *Juncus articulatus* (by 5533.33%), *Agrostis stolonifera* (1610.64%) and *Juncus balticus* (1040.00%).

Dry weight of roots. Compared with control plants (Fig. 3), inoculation with AMF decreased the dry weight of roots of *Corynephorus canescens* (by 82.25%), *Ammophila arenaria* (49.51%) and *Agrostis stolonifera* (22.51%), but increased it in *Juncus articulatus* (536.36%) and *J. balticus* (144.09%). The dry weights of shoots and roots of *Festuca rubra* subsp. *arenaria* growing in pots without AMF were not determined due to the death of plants.

Dry matter of root:shoot ratio. Inoculation with AMF most decreased the dry matter of root:shoot ratio (Fig. 4) in *Agrostis stolonifera* (1:22.08) and *Juncus articulatus* (1:8.05). Lower decreases of this parameter occurred in *J. balticus* (1:4.67), *Corynephorus canescens* (1:3.68) and *Ammophila arenaria* (1:2.26).

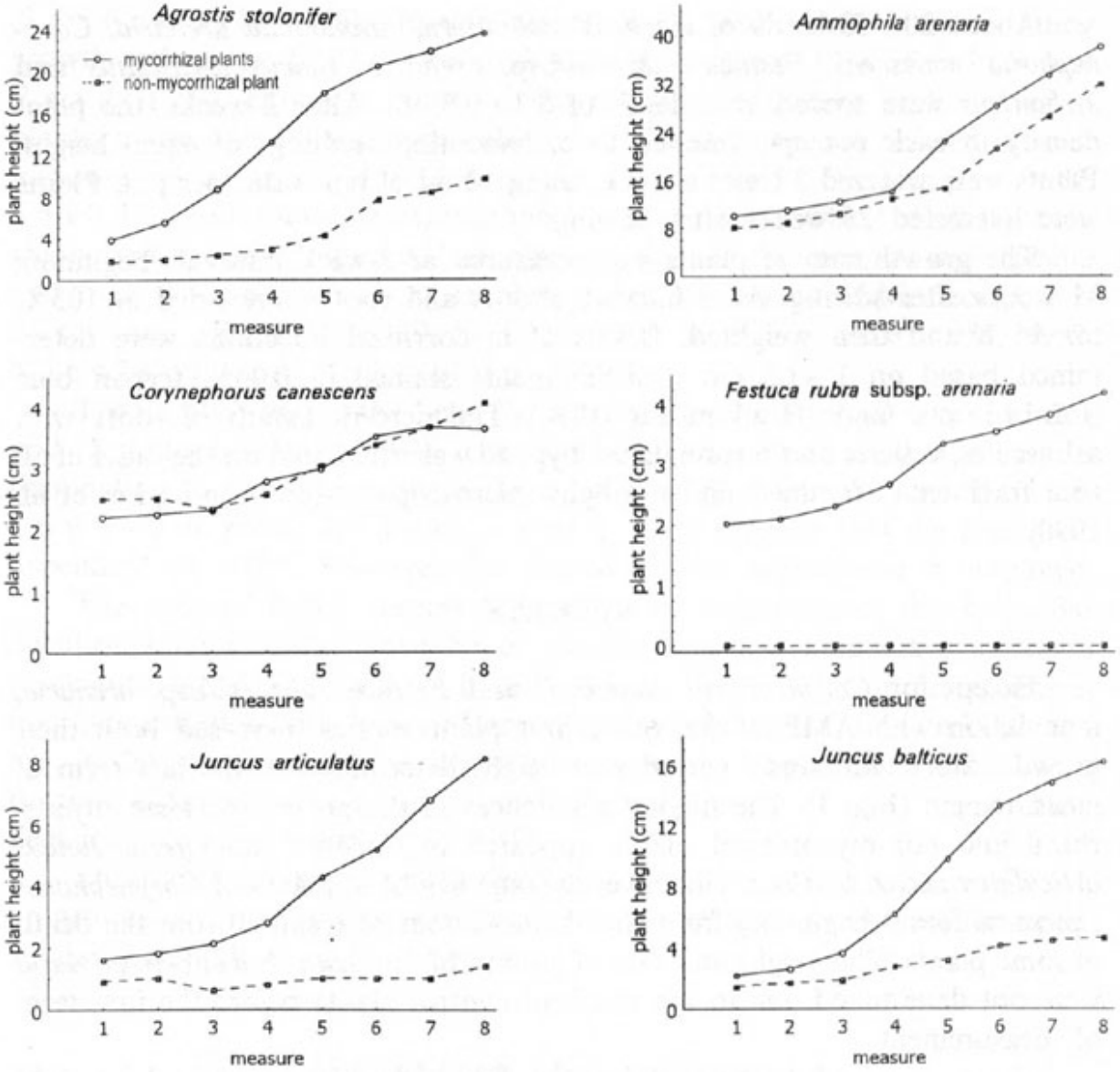

Fig. 1. The influence of AMF on growth of 6 plant species

Arbuscular mycorrhizae

A r b u s c u l e s. The highest values of the percent of root length with arbuscules (Fig. 5) were found in *Ammophila arenaria* and *Juncus articulatus*, and lowest in *Corynephorus canescens* and *Juncus balticus.*

V e s i c l e s. The highest values of root colonization by vesicles (Fig. 5) were found in *Agrostis stolonifera*, *Ammophila arenaria* and *Juncus articulatus.* Only control plants of *Juncus articulatus* harboured a very low level of colonization by vesicles.

H y p h a e. Plants having the highest root infections with intramatrical hyphae (Fig. 5) were *Juncus articulatus* and *Ammophila arenaria.* Very low infections with intramatrical hyphae were found in *Juncus articulatus* coming from control plots.

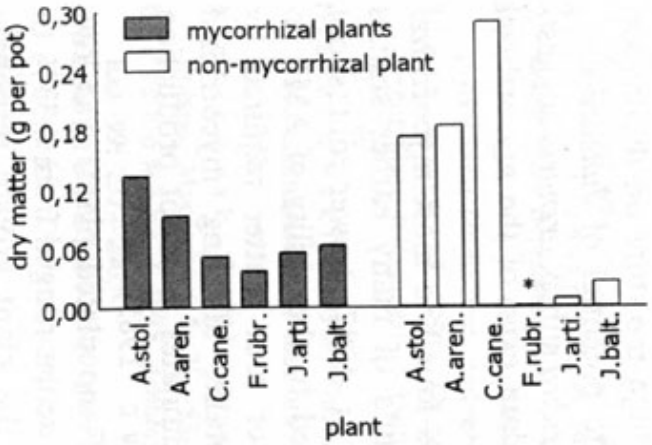

Fig. 2. The influence of AMF on the dry matter of shoots of 6 plant species (*not determined due to the death of plants)

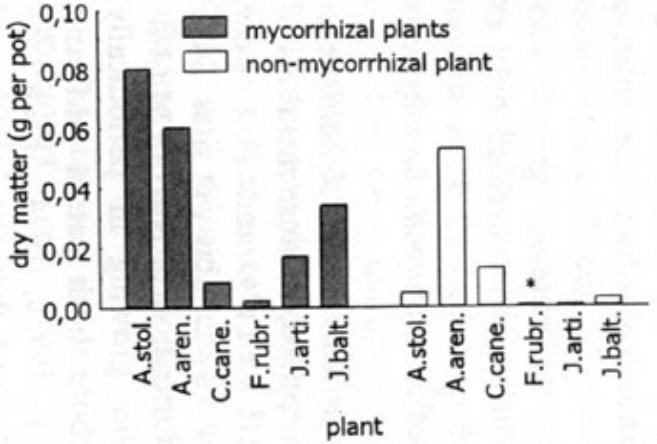

Fig. 3. The influence of AMF on the dry matter of roots of 6 plant species (*not determined due to the death of plants)

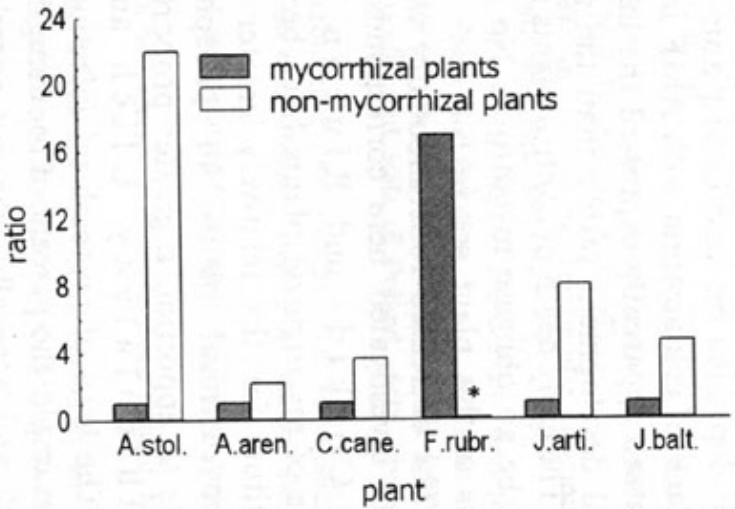

Fig. 4. The influence of AMF on the dry matter shoot : root ratio of 6 plant species (*not determined due to the death of plants)

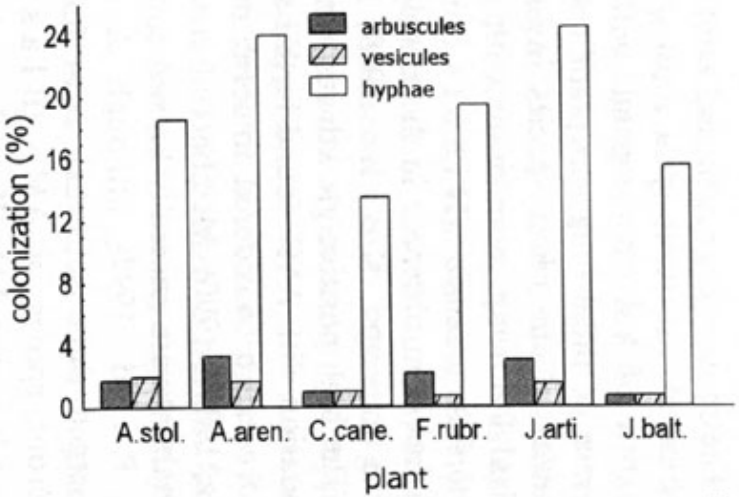

Fig. 5. Percent of root length with arbuscular mycorrhizae in 6 plant species

DISCUSSION

The increased growth and content of dry matter of above-ground plant parts found in this study in most of the dune plant species inoculated with a mixture of AMF corresponds with many literature reports of the positive influence of these fungi on plant condition (Smith and Read 1997). However, of the plant species investigated here, an increased growth and survival following inoculation with AMF has earlier been revealed only in *Ammophila arenaria* (Nicolson and Johnston 1979). A similar effect has been observed in the closely related species *Ammophila breviligulata* (Gemma and Koske 1989, 1997).

The high positive growth response of *Juncus articulatus* and *J. balticus* to inoculation with AMF found in this study was surprised, because members of *Juncaceae* are considered to form rarely AM (Harley and Harley 1987a, 1987b, 1990). Members of *Juncaceae* essentially inhabit wet sites. Such wet habitats are generally known to inhibit the formation of AM (Smith and Read 1997), although *J. conglomeratus* growing in periodically submerged dune soils has been found to harbour both extent AM and numerous spores of AMF (Błaszkowski 1993a, 1994; Błaszkowski unpubl.). Koske et al. (1985) showed that the infection of *Equisetum* species, plants commonly considered to be non-mycorrhizal, by AMF depended on associated plants and/or soil moisture conditions. Hence, the lack of colonization with AMF in species of *Juncaceae* mentioned in the literature apparently expressed the influence of high moisture on the mycorrhizal development rather than the mycotrophic potential of *Juncaceae*.

The early death of control plants of *Festuca rubra* subsp. *arenaria* suggests it to be an obligate mycotroph. No literature data exist of the mycorrhizal status of this plant species.

The decreased root:shoot dry weight ratios found in most mycorrhizal plants investigated here corresponds with results of many earlier studies (e.g., Corkidi and Rincón 1997; Saif 1987). Lower root:shoot ratios of mycorrhizal plants have been attributed to the ability of AMF to substitute for the relatively greater amounts of root matter required by non-mycorrhizal plants for phosphorous uptake, allowing mycorrhizal plants to apportion a greater proportion of assimilates to shoot production (Bethlenfalvay, Ulich and Brown 1985).

The level of mycorrhizal infections in AMF-inoculated plants was low. For example, the percent of root length with arbuscules ranged from 0.33% to 3.33% and generally was not correlated with the plant growth responses found. Arbuscules are the main sites of exchange of nutrients between an AM fungus and a host plant (Bonfante-Fasolo 1984) and, thereby, their presence could be considered the most important measure of the amount

of exchange and functionality of an AM symbiosis (Claperton and Reid 1992). The lack of correlation between the level of mycorrhizal infection and the degree of enhancement of plant growth parameters has many times been found in other investigations on mycorrhizal dependence of different cultivated and uncultivated plants (Schubert and Hayman 1986; Hayman and Tavares 1985). Gemma and Koske (1989) found improved growth of *Ammophila breviligulata* in response to inoculation in the field when colonization extent was as low as 1.5%. Boyetchko and Tewari (1990), Graham et al. (1982) and Hetrick et al. (1985) concluded that plant growth improvement is dependent on a minimum level of root colonization and degree of growth improvement is more correlated with the extent of extramatrical hyphae. Unfortunately, the amount of extramatrical hyphae associated with roots of the plant species investigated here was not determined. According to Saif (1987) and Manjunath and Habte (1991), other important factors affecting mycorrhizal dependence are root fibrosity and root geometry.

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Wpływ arbuskularnych grzybów mikoryzowych na wzrost nadmorskich roślin wydmowych

Streszczenie

W doświadczeniu wazonowym przeprowadzonym w szklarni zbadano reakcję 6 gatunków roślin dominujących w sukcesji pola deflacyjnego Mierzei Łebskiej na inokulację arbuskularnymi grzybami mikoryzowymi (AGM). Inokulum była mieszanina gleby, korzeni i zarodników 5 gatunków AGM z gatunkiem dominującym *Glomus aggregatum*. Z wyjątkiem *Corynephorus canescens* i *Festuca rubra* subsp. *arenaria*, zarówno wzrost jak i sucha masa części nadziemnych roślin *Agrostis stolonifera*, *Ammophila arenaria*, *Juncus articulatus* i *J. balticus* zainokulowanych AGM były wyższe niż u roślin rosnących w glebach bez AGM. Inokulacja obniżyła stosunek suchej masy korzeni do pędów u 5 gatunków roślin. Własności tej nie określono u *F. rubra* subsp. *arenaria* z powodu zamarcia roślin kontrolnych. Poziom infekcji mikoryzowej był niski i nie korelował z stwierdzonymi zmianami wzrostu. Silne zwiększenie wzrostu *Juncus* spp. zainokulowanych AGM sugeruje, że opinia o niemikotroficzności lub niskim uzależnieniu przedstawicieli *Juncaceae* od AGM została oparta na wynikach badań roślin rosnących w stanowiskach wilgotnych, znanych z tego, że tłumią formowanie mikoryz.