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Effects of the Aqueous Extract from *Artemisia campestris* ssp. *caudata* on Mycorrhizal Fungi Colonization and Growth of Sand Dune Grasses

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We used the aqueous extract from *Artemisia campestris* ssp. *caudata* to investigate its effects on the colonization of sand dune grass roots by mycorrhizal fungi and seedling growth. The percent colonization decreased with higher extract concentrations, and growth of three grass species was inhibited. Colonization by mycorrhizal fungi was more sensitive to the extract than was seedling growth, and no significant differences in the latter were found between the mycorrhizal and non-mycorrhizal treatments.

Keywords: aqueous extract, *Artemisia campestris* ssp. *caudata*, colonization, mycorrhizal fungi, sand dune grass

Artemisia campestris ssp. *caudata* is a biennial or monocarpic perennial plant (Stairs, 1986) that originated in Asia and is now distributed circumborealy in North America, extending south to Florida and Arizona (Gleason and Cronquist, 1963). It occurs in all Canadian provinces and territories except Prince Edward Island (Scoggan, 1979), and is prevalent on the open sand dunes, slacks, and transition zones within the Lake Huron sand dune complex in Ontario (Stairs, 1986). In particular, this important species is associated with *Calamovilfa longifolia*, *Andropogon scoparius*, *Agropyron psammophilum*, *Arctostaphylos uva-ursi*, *Sorghastrum nutans*, and many other herbaceous species found in the Pinery and Ipperwish Provincial Parks along the southeastern shore of Lake Huron (Baldwin and Maun, 1983).

Plants of the genus *Artemisia* have allelopathic potential (Yun, 1991). And Yun and Choi (2002) have reported that an aqueous extract prepared from *Artemisia princeps* var. *orientalis* inhibits the seedling growth of mycorrhizae-inoculated plants. The unique mycorrhizal relationship between fungus and plant enhances the uptake of water and mineral nutrients (Smith and Daft, 1978). However, plant allelochemicals can adversely affect the normal functioning of symbiotic fungi associated with root systems (Olsen et al., 1971), thus reducing their beneficial effects (Yun and Choi, 2002). Likewise, *A. campestris* ssp. *caudata* has allelopathic potential in that the field distribution of test plants shows differential responses in seed germination and the elongation of roots and shoots. Although the dominant plant species are not affected, those that do not occur within the *A. campestris* ssp. *caudata*-dominated microhabitat are inhibited (Yun and Maun, 1997).

The purpose of this study was to examine the influence of aqueous extracts of *A. campestris* ssp. *caudata* on both mycorrhizal fungi colonization and subsequent plant growth of three sand dune grasses.

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MATERIALS AND METHODS

Plants, Aqueous Extract, and Mycorrhizal Fungi

Aqueous extracts were made from the shoots and roots of *A. campestris* ssp. *caudata*. To do so, fresh, intact plants were cut into 1-cm-long pieces. Afterward, 1000 mL of distilled water was added to 200 g of plant material at 20°C, and the resultant aqueous extract was filtered through a sieve after 24 h. This extract, considered a 100% stock solution, was then diluted to a 50% extract with distilled water. We examined the effects of the extract on the colonization of mycorrhizal fungi and the growth of three sand dune grasses, *A. psammophilum*, *Elymus canadensis*, and *Panicum virgatum*. Their seeds were pre-germinated in Petri dishes before the seedlings were transplanted to plastic pots containing sterilized soil. When the plants were two months old, they were inoculated with mycorrhizal fungi according to the procedures of Little and Maun (1996) and Yun and Maun (1997). This involved first growing corn seedlings in soil containing mycorrhizal fungi for two months, then confirming the presence of that fungi. The corn roots that had been colonized were washed, divided into 5-g batches, and buried 5 cm deep in newly prepared 10-cm-diam. plastic pots. The sand dune grass seedlings were then transplanted to these treated pots.

Cultivation of Three Sand Dune Grasses with Aqueous *Artemisia* Extracts

After plants of the three grass species were inoculated with mycorrhizal fungi, they were grown in a greenhouse maintained at 22°C for 8 h (day) and 18°C for 16 h (night). Although they were watered daily, no supplemental nutrients were applied. These plants were reared for an additional two months to ensure that their roots had been colonized by the mycorrhizal fungi. They were then exposed to the aqueous extract treatments over a 30-d period that entailed adding 40 mL of 50 or 100% solutions every 2 d. The control and non-mycorrhizal plants received only distilled water, and six replications were used.

Estimation of Mycorrhizal Colonization and Plant Growth

The grass plants were removed from their pots and washed carefully to rid them of sand before root samples were taken to determine the degree of mycorrhizal colonization. This was quantified with a staining procedure according to the method of Brundrett et al. (1984). The percent colonization was then calculated by the method of magnified intersections (McGonigle et al., 1990). The growth parameters used here included measurements of shoot and root lengths, leaf area, and seedling dry weights for each treatment.

RESULTS

Effect of Aqueous Extracts on Mycorrhizal Fungi Colonization

Aqueous extracts of *A. campestris* ssp. *caudata* are able to inhibit the colonization of mycorrhizal fungi in host-plant roots (Yun and Maun, 1997). In the current study, treatment with such extracts hindered all vesicular and hyphal colonization, except for the latter in *P. virgatum* (Table 1). Likewise, the vesicular mycorrhizal colonization of *E. canadensis* roots was significantly diminished with increasing extract concentrations.

Effect of Aqueous Extracts on Sand Dune Grass Growth

Mycorrhizal inoculation had no statistically significant effect on shoot elongation. For example, the shoots of *A. psammophilum* and *E. canadensis* were longer from non-mycorrhizal seedlings than from mycorrhizal plants. Moreover, shoot lengths from the colonized seedlings were reduced as the extract concentration increased (Table 2).

In contrast, root elongation was decreased significantly by extract treatment, but mycorrhizal seedlings had longer roots than the non-mycorrhizal plants (Table 3). This was beneficial to our three grass species because it offset the inhibitory effect of the *Artemisia* extract. For *E. canadensis*, the decline in root elongation that resulted from application of the 100% extract was more pronounced than the observed decrease in shoot elongation (Table 2, 3).

These aqueous extracts did not cause any statistically significant difference in the seedling dry weights of non-mycorrhizal versus mycorrhizal seedlings, but any reduction in

Table 1. Percent colonization (%, mean ± SE) by arbuscular mycorrhizal fungi in roots of potted *A. psammophilum*, *E. canadensis*, and *P. virgatum* plants grown under greenhouse conditions and exposed to 0, 50, or 100% concentrations of aqueous extracts from *A. campestris* ssp. *caudata*. Data for colonization by hyphae and vesicles and percent uncolonized (UNC) roots were determined according to the method of McGonigle et al. (1990).

Test species	*Agropyron psammophilum*			*Elymus canadensis*			*Panicum virgatum*		
	UNC	Vesicle	Hyphae	UNC	Vesicle	Hyphae	UNC	Vesicle	Hyphae
Extract concentration (%)									
0	44.3 ± 3.21b	22.3 ± 1.53a	33.3 ± 2.06a	42.4 ± 2.50b	51.8 ± 2.48a	5.8 ± 1.07a	54.3 ± 2.91b	28.9 ± 3.36a	16.4 ± 1.79a
50	69.8 ± 3.84a	12.5 ± 2.34b	17.8 ± 2.90b	68.0 ± 3.01a	24.9 ± 2.48b	7.1 ± 1.44a	71.8 ± 3.53a	15.1 ± 1.09b	13.3 ± 3.03a
100	68.0 ± 5.42a	11.6 ± 2.71b	20.5 ± 4.14b	72.5 ± 2.29a	20.4 ± 4.16b	7.2 ± 2.37a	71.8 ± 2.63a	19.8 ± 1.79ab	8.6 ± 2.18b

Note: Values followed by the same letter within a column are not significantly different at $p < 0.05$, based on Tukey's tests.

Table 2. Shoot lengths (mm, mean ± SE) for potted plants of *A. psammophilum*, *E. canadensis*, and *P. virgatum* that were inoculated by mycorrhizal fungi and exposed to 0, 50, or 100% concentrations of *A. campestris* ssp. *caudata* aqueous extracts.

	Non-mycorrhizal	Mycorrhizal		
Extract concentration (%)	0	0	50	100
Test species				
Agropyron psammophilum	293.3 ± 11.30a	275.0 ± 4.65a	239.2 ± 5.54b	240.0 ± 11.33b
Elymus canadensis	295.0 ± 28.48a	286.7 ± 14.64a	235.8 ± 18.18ab	224.2 ± 23.39b
Panicum virgatum	238.3 ± 9.10ab	248.3 ± 9.10a	221.7 ± 4.77bc	213.3 ± 2.11c

Note: Values followed by the same letter within a row are not significantly different at $p < 0.05$, based on Tukey's tests.

Table 3. Root lengths (mm, mean ± SE) for potted plants of *A. psammophilum*, *E. canadensis*, and *P. virgatum* that were inoculated by mycorrhizal fungi and exposed to 0, 50, or 100% concentrations of *A. campestris* ssp. *caudata* aqueous extracts.

	Non-mycorrhizal	Mycorrhizal		
Extract concentration (%)	0	0	50	100
Test species				
Agropyron psammophilum	217.5 ± 17.50a	223.3 ± 14.12b	206.7 ± 20.11b	173.3 ± 22.90b
Elymus canadensis	243.3 ± 36.66a	258.3 ± 8.33a	226.7 ± 13.33a	123.3 ± 29.85b
Panicum virgatum	262.5 ± 28.03a	266.7 ± 35.27a	220.0 ± 19.82a	218.3 ± 23.00a

Note: Values followed by the same within a row are not significantly different at $p < 0.05$, based on Tukey's tests.

Table 4. Dry weights (g, mean ± SE) for potted plants of *A. psammophilum*, *E. canadensis*, and *P. virgatum* that were inoculated by mycorrhizal fungi and exposed to 0, 50, or 100% concentrations of *A. campestris* ssp. *caudata* aqueous extracts.

	Non-mycorrhizal	Mycorrhizal		
Extract concentration (%)	0	0	50	100
Test species				
Agropyron psammophilum	0.132 ± 0.025a	0.142 ± 0.015a	1.159 ± 0.030a	0.139 ± 0.022a
Elymus canadensis	0.105 ± 0.014a	0.119 ± 0.012a	0.095 ± 0.008a	0.092 ± 0.012a
Panicum virgatum	0.183 ± 0.034a	0.192 ± 0.010a	0.142 ± 0.018ab	0.107 ± 0.012b

Note: Values followed by the same letter within a row are not significantly different at $p < 0.05$, based on Tukey's tests.

Table 5. Leaf areas (cm^2, mean ± SE) for potted plants of *A. psammophilum*, *E. canadensis*, and *P. virgatum* that were inoculated by mycorrhizal fungi and exposed to 0, 50, or 100% concentrations of *A. campestris* ssp. *caudata* aqueous extracts

	Non-mycorrhizal	Mycorrhizal		
Extract concentration (%)	0	0	50	100
Test species				
Agropyron psammophilum	11.61± 0.08a	9.93 ± 1.16a	12.49 ± 1.61a	10.14 ± 1.20a
Elymus canadensis	8.11 ± 0.65ab	9.52 ± 1.21a	7.35 ± 0.69ab	6.02 ± 0.82b
Panicum virgatum	11.46 ± 1.63ab	12.67 ± 0.91a	10.45 ± 1.26b	9.02 ± 1.64b

Note: Values followed by the same letter within a row are not significantly different at $p < 0.05$, based on Tukey's tests.

those weights was proportional to the extract concentration tested. In fact, mycorrhizal fungi mitigated the injurious impact of this extract on plant dry weights for *E. canadensis*.

Leaf area measurements from plants of *E. canadensis* and *P. virgatum* revealed that this parameter was markedly inhibited in proportion to the extract concentration applied. For example, the leaf areas from two of our three test species were increased by mycorrhizal fungi associations (at 0% extract), while that of *A. psammophilum* showed a greater area value when a 50% extract solution was used compared with the mycorrhizal plants and non-mycorrhizal controls (Table 5).

DISCUSSION

The allelopathic potential of *A. campestris* ssp. *caudata* is most likely an important trait that aids in its survival under harsh habitats (Yun and Maun, 1997), probably because of its levels of either phenolic acids or other water-extractable allelochemicals (Halligan, 1976; Friedman et al., 1977; Hoffman and Hazlett, 1977; Groves and Anderson, 1981; Yun, 1991; Kil and Yun, 1992; Yun and Maun, 1997).

Such relationships between these phenolic compounds and arbuscular mycorrhizae (AM) are widespread in the plant kingdom, with plants and mycorrhizal fungi having co-evolved to form valuable symbioses. Therefore, it is likely that micromolar concentrations of these compounds can stimulate the growth of AM fungi and promote root colonization. Host growth and colonization rates, however, are inhibited when higher concentrations of phenolics are applied to the substrate (Fries et al., 1997).

The shoot and root growth of mycorrhizal plants exposed to the *Artemisia* extract differed from that of the non-mycorrhizal control, suggesting that the fungi mitigated the inhibitory effect of that aqueous solution. In contrast to earlier experiments by Yun and Maun (1997), in which the extract was applied to non-mycorrhizal plants, the current study involved inoculated seedlings instead. Here, treatment with *Artemisia* inhibited fungal colonization in our three test species, contributing to a decline in their growth. Nilsson et al. (1993) have stated that the spread of mycorrhizal infection and nutrient uptake by roots and mycorrhizae are more sensitive to such extracts than are the processes of seed germination and radicle formation. Previously, shoot and root elongation in mycorrhizal seedlings receiving that extract was shown to be less inhibited than in extract-treated non-mycorrhizal seedlings (Yun and Maun, 1997). However, plants of the three species tested here were not exposed to the aqueous extract until after they had grown for an additional two months. In fact, Rho and Kil (1986) have demonstrated that the development of species under examination can be more severely inhibited when they are treated with extracts of pitch pine at an early growth stage; i.e., seedling elongation is less influenced in the sequence, seed > 1-leaf > 3 leaves > 5 leaves.

Here, the particular concentration of extract (50% vs 100%) did not cause significant differences in root colonization by the mycorrhizal fungi but it did markedly affect root lengths, dry weights, and leaf areas for all three sand dune grasses. Moreover, Wacker et al. (1990) have indicated that the reduced growth of mycorrhizal asparagus plants at higher ferulic acid concentrations may be associated with a decrease in the symbiotic effectiveness of *Glomus fasciculatum* due to a decline in the rate of colonization. That is, plants infected with vesicular-arbuscular mycorrhizae produce less biomass when the concentration of ferulic acid is increased. Nonetheless, that decline in asparagus biomass is not a direct result because the growth of non-mycorrhizal stems was not altered by any concentration of ferulic acid tested in that research.

In conclusion, we have demonstrated here that the aqueous extract from *A. campestris* ssp. *caudata* inhibits colonization by mycorrhizal fungi and causes reduced growth in the

seedlings of three sand dune grasses.

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