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SHORT NOTE

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Mycorrhizal status of the endangered species *Astragalus applegatei* Peck as determined from a soil bioassay

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Abstract The mycorrhizal status of *Astragalus applegatei* Peck is reported for the first time on plants from a greenhouse soil bioassay. Seedlings were grown in a potting mix inoculated with soil collected near *A. applegatei* plants in nature. Plants were also grown in non-inoculated potting mix. Only plants from the native soil inoculation survived. Abundant colonization of VAM fungi was found in all 15 plants analyzed from the native soil treatment, and chlamydospores produced by *Glomus* spp. were observed. Mycorrhizal colonization was estimated to be 23% of total fine root length after 6 weeks and 53% after 14 weeks. Our results provide ecologically important information for conservation and restoration efforts underway to recover populations of this endangered species.

Key words Vesicular-arbuscular mycorrhizae · *Glomus* · Conservation biology

Introduction

Astragalus applegatei Peck is a caulescent perennial legume (Fabaceae) endemic to moist alkaline meadows in southern Klamath County, Oregon (Barneby 1964). Listed as endangered by the US Fish and Wildlife Service (58 FR 40551, 1993) and the State of Oregon (OAR 603-73-070, 1995), this species is threatened by habitat loss, competition with invasive exotic weeds, herbivory by butterfly larvae, seed loss to pre-dispersal predation, and small population sizes (Gisler and Meinke 1997; USFWS 1997).

Astragalus is considered the largest genus of vascular plants (Mabberley 1993; Sanderson and Liston 1995) and is widely distributed throughout the temperate regions of the Northern Hemisphere and in temperate South America. About 400 species occur in North America, with Oregon containing 74 taxa (species and varieties). Of these, 27 are of conservation concern, and 8 are state endemics, including *Astragalus applegatei* (Liston 1997).

As VAM can improve fitness of plants (Allen 1991; Perry 1994; Smith and Read 1997), knowledge of mycorrhizae has assumed particular importance for ecological restoration, preservation, and maintenance of endangered species. Members of the genus *Astragalus* have been shown to be both VAM and non-mycorrhizal in different situations (Trappe 1981; O'Dell and Trappe 1992), but no information is available about *A. applegatei*. Information on the mycorrhizal status of plants of special concern may be crucial for the implementation of successful conservation strategies. In this paper, we report the mycorrhizal status of *A. applegatei* seedlings from a soil bioassay, as part of an ongoing study of the species propagation and the effect of mycorrhizal inoculation on growth and reproduction.

Materials and methods

Immediately following seed germination on moist filter paper, 120 *A. applegatei* seedlings were individually placed into 10-cm-diameter pots in an Oregon State University greenhouse. Pots were randomly partitioned into two treatments: (1) native soil inoculation, and (2) control (no inoculum). The native soil inoculum consisted of screened soil (3-mm mesh size) collected from the immediate vicinity (within 0.5 m) of *A. applegatei* individuals occurring in a natural population. The soil inoculation treatment involved placement of approximately 15 ml of soil into the top 1 cm of pots that had been filled with 200 ml of sterilized potting mix consisting of equal amounts of perlite and peat. A similar volume of sterilized soil was not added to the controls because

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our goal was to investigate soil inoculum effects on mycorrhiza formation. Determination of "fertilization" effects from the 7% by volume addition of soil was not an objective of the investigation. Plants were grown with a 12-h photoperiod, approximately 75 cm beneath a 1::1 mixture of 40 W extended spectrum and 34 W standard florescent lights. Plants were irrigated as needed, but received no fertilization to avoid variability in nutrient availability and potential fertilizer effects on mycorrhiza formation.

Mycorrhizal assessment was performed at 6 weeks and 14 weeks after seed germination. To conserve plant material, the first harvest included only two complete root systems per treatment. Only plants from the native soil treatment survived for the second harvest. Late in the study, half of the surviving plants were fertilized to alleviate stress symptoms, and the second harvest was drawn from the remaining non-fertilized plants. To increase sampling while conserving plant material for recovery efforts, the second harvest consisted of two complete root systems and 11 plants that were sub-sampled. Each sub-sample included 2–3 lateral roots.

Roots were thoroughly washed, cleared, and stained as described by Cazares and Trappe (1993). VAM colonization percentage was measured using the gridline intersection method for vesicles, arbuscules, chlamydospores, or non-septate hyphae with any of the previous structures present (Giovannetti and Mosse 1980). Only fine roots (less than 2 mm in diameter) were considered in the quantification because wider roots were not colonized.

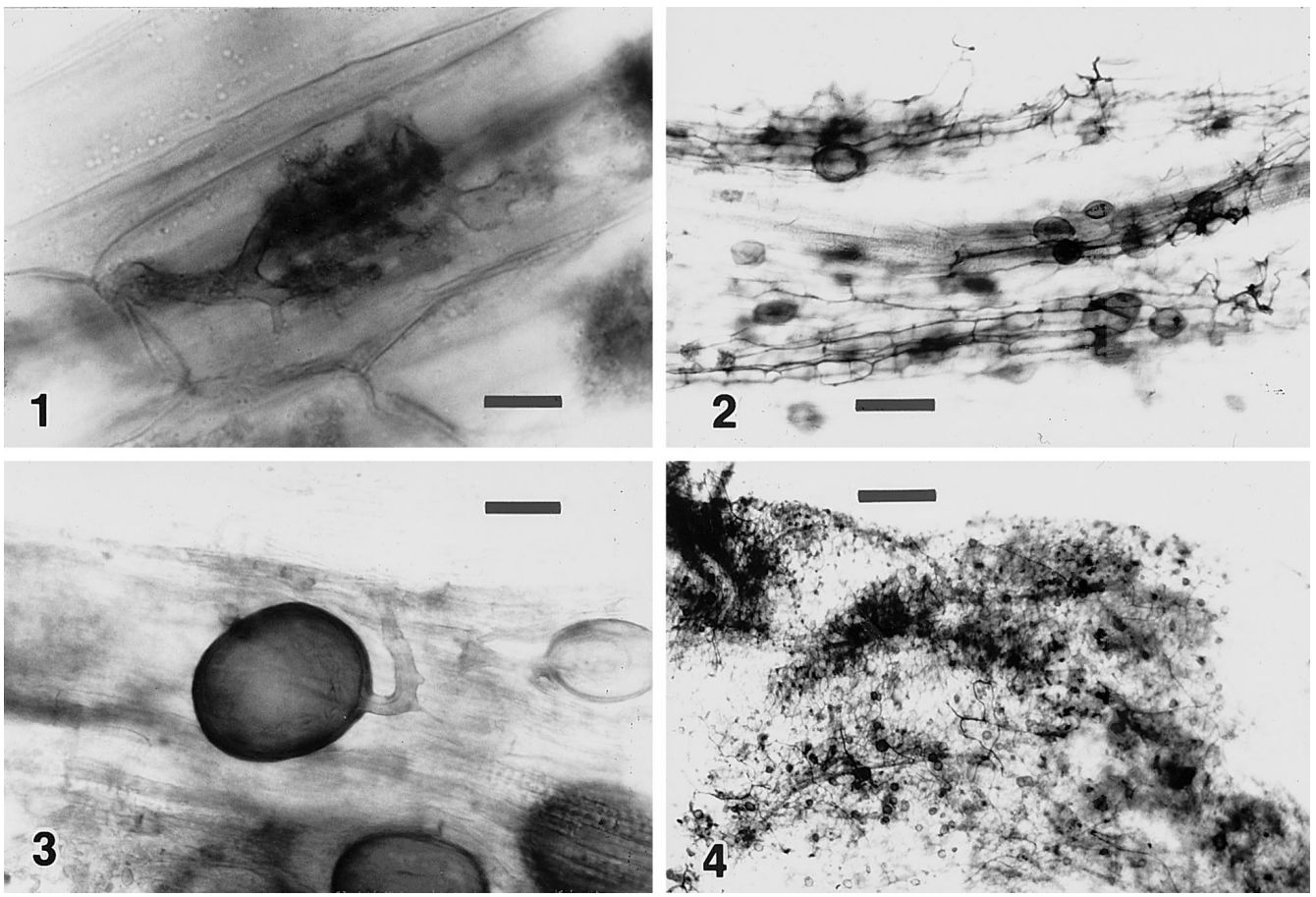
Results

After 6 weeks growth, seedlings from the control treatment lacked mycorrhizae as well as nodules. Root systems from the native soil treatment had 23% of the total fine root length colonized with VAM. Arbuscules (Fig. 1), vesicles (Fig. 2), non-septate hyphae, and chlamydospores (Fig. 3) were observed.

Only plants from the native soil treatment survived (97%) for the second harvest. All 13 seedlings displayed VAM and were nodulated. The same VAM structures as previously noted were found. The colonization was estimated to be 53% of total fine root length.

The chlamydospores observed outside the root system were those typical of the genus *Glomus* (Fig. 3), characterized by being single, round, and with contents at maturity separated from attached hyphae by a septum or occluded by spore wall thickening (Gerdemann and Trappe 1974). We also noted hyphae and chlamydospores growing on the surface of some nodules (Fig. 4).

Fig. 1 Arbuscule in a cortex cell of *Astragalus applegatei*. Nomarski optics; *bar* 4 μm. **Fig. 2** Fine root colonized by non-septate hyphae with vesicles. Nomarski optics; *bar* 70 μm. **Fig. 3** Chlamydospores typical of the genus *Glomus* growing on the surface of a fine root. Spore characteristics indicate close affinity to *Glomus deserticola* Trappe, Bloss, and Menge. Nomarski optics; *bar* 10 μm **Fig. 4** *A. applegatei* root nodule with hyphae and chlamydospores growing on the surface. Nomarski optics; *bar* 175 μm



Discussion

This report presents the first assessment of the mycorrhizal status of the rare species *A. applegatei*. Low seed production in nature limited the availability of new plants for ongoing and future research programs and necessitated the small sample of plants used. Non-destructive subsampling methods preserved study plants for future transplanting and population enhancement efforts.

VAM colonization on *A. applegatei* roots from this soil bioassay suggests that natural populations may also be mycorrhizal. Complete mortality among the non-inoculated plants may indicate that this rare species is evolutionarily dependent (Trappe and Luoma 1992) on mycorrhizal symbiosis and other interactions for survival, although more research is needed to address this hypothesis. As the soil inoculum included not only mycorrhizal fungi but other soil microorganisms such as nodulating bacteria, we can not differentiate among their effects. There was also a potential soil "fertilization" effect but the small amount of soil added leads us to discount the possibility that any such difference could account for the disparity in survival.

All plant genera growing in association with *A. applegatei* are characteristically VAM. Therefore, we expect that the general VAM inoculum potential is high in the native habitat of *A. applegatei*. It is also possible that the presence of *Rhizobium* may be required for the initiation of the mycorrhizal symbiosis (Trappe 1979). *Rhizobium* spp. have been shown to enhance spore germination and mycelial growth of VAM fungi and influence root colonization by the fungi (Azcon-Aguilar et al. 1986; Azcon-Aguilar and Barea 1991).

Despite these uncertainties, we have shown evidence of the effectiveness of native soil as an inoculum source, and the mycorrhizal capabilities of *A. applegatei*. Future research in the mycorrhizal ecology of *A. applegatei* should compare our results with samples from healthy individuals growing in their natural environment using non-destructive root and soil sampling techniques (Allen and MacMahon 1988). Restoration and conservation programs for this species should include an assessment of the mycorrhizal status of the plant community and the availability of mycorrhizal propagules in habitats to be managed for those plants.

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