Mycorrhiza (1999) 8:225–228

SHORT NOTE

Michael F. Allen · Louise M. Egerton-Warburton
Edith B. Allen · Ola Kårén

Mycorrhizae in *Adenostoma fasciculatum* Hook. & Arn.: a combination of unusual ecto- and endo-forms

Accepted: 5 September 1998

Abstract The mycorrhizal status of *Adenostoma fasciculatum*, the dominant shrub in California chaparral, has been unclear. In two typical, nearly monospecific stands, *A. fasciculatum* was found to have arbuscules and intercellular hyphae. Antisera detected hyphae of the arbuscular mycorrhizal (AM) fungal genera *Acaulospora, Glomus,* and *Scutellospora*, although we found only spores of *Glomus*. Some roots had partial sheaths and inter- and intracellular septate fungi without indications of root necrosis. Ectomycorrhizal root tips were also found, including *Cenococcum* and other unknown taxa. Sporocarps of EM fungi including species of *Rhizopogon, Pisolithus, Balsamia, Laccaria, Hygrophorus,* and *Cortinarius* were found in the stand, with no other EM or arbutoid mycorrhizal plants nearby. These observations indicate that *A. fasciculatum* forms mycorrhizae with AM, septate, and EM fungi, but often fails to form easily recognizable mycorrhizal structures.

Key words *Adenostoma fasciculatum* · Ectomycorrhizae · Arbuscular mycorrhizae · Chaparral

Introduction

Mycorrhizal associations traditionally have separated into distinct types based on the morphology of the mycorrhizal structure attached to the plant. They have been characterized as arbuscular mycorrhizal (AM) for the glomalean-plant associations, ectomycorrhizal (EM) if a mantle and hartig net is present, and so on (Molina et al. 1992). However, inconsistencies occasionally appear that do not fit these characteristics. Unusual structures that may be mycorrhizal have been postulated based on a lack of necrotic response in the infected roots (see Allen 1996) or on the presence of known mycorrhizal fungi linked to a particular plant.

Adenostoma fasciculatum is the dominant shrub of the southern California chaparral comprising the vast majority of the plant cover of these ecosystems and providing most of the net primary productivity (Cooper 1922). It resprouts or reseeds following fire and may form almost monospecific stands with a thick litter layer. Its root system is very coarse and, therefore, would be expected to form mycorrhizae. It is a member of the Rosaceae, species of which form both AM and EM. Yet the mycorrhizal status of *A. fasciculatum* remains unclear. Cooper (1922) reported it as forming EM-type mycorrhizae, after observation of a partial sheathing structure. Kummerow and Borth (1986) suggested that it was ericoid (in the sense that we now use arbutoid) because of the same characteristics. In both reports, a hartig net was not distinguishable. T. Horton and T. Parker found EM fungi in stands of *A. fasciculatum,* but other EM or arbutoid mycorrhizal plants were nearby (Horton 1992). We found AM in a related plant, *A. sparsifolium* (Allen 1991), but failed to find convincing structures in *A. fasciculatum.* The goal of this present effort was to determine the mycorrhizal status of this plant.

Materials and methods

From 1995 through 1998, we undertook surveys of *A. fasciculatum* in nearly monospecific stands. The first site was at the Lake Skinner Reserve (33°37′, 117°2′) in southwestern Riverside County at an altitude of 420 m. The second was conducted at the Sky Oaks Ecological Reserve which is in northern San Diego County, at an altitude of 1500 m. The low average annual precipitation (400 mm, predominantly in winter) and moderate temperatures (35/14 °C summer, 18/3 °C winter) are indicative of a Mediterranean-type climate (Allen et al. 1995). The soils are a lithic haploxerol with relatively high P and low N (Allen et al. 1996; Padgett et al. 1998). At Lake Skinner, a 40×30 m patch of *A. fasciculatum* was surrounded by converted Mediterranean annual grassland and coastal sage scrub vegetation, both AM vegetation

M.F. Allen (✉) · L.M. Egerton-Warburton · E.B. Allen
O. Kårén
Center for Conservation Biology, University of California, Riverside, CA 92521–0124, USA
e-mail: michael.allen@ucr.edu, Fax: +1-909-787-4437

types (Allen et al. 1996). Much of the site burned in the 1993 fires, but the stand of *A. fasciculatum* surveyed has not burned since the 1930s. Further description of the vegetation can be found in Westec Services Inc. (1988). At the Sky Oaks site, roots were taken from two stands, a young stand which regenerated from old *A. fasciculatum* plants sprouting after a prescribed fire in 1993, and an adjacent 70-year-old stand. Further details on this stand can be found in Allen et al. (1996).

We looked for mycorrhizae using direct microscopy. Soil cores were taken about mid-canopy from all sites. Roots were wet-sieved (with a mesh-size of 0.5 mm), placed in water and carefully rinsed to remove soil particles. Roots were observed for sheathing formation under a dissecting microscope (×7–40 magnification). Short-roots were considered ectomycorrhizal when they were covered by a fungal mantle and hartig Net. To check for the presence of a hartig Net, cryotome sections (10–15 um thick longitudinal views) of root tips were prepared and studied microscopically (×250–1000 magnification). Other fine roots were separated and cleared in 10% KOH overnight at room temperature, then heated to just below boiling in hydrogen peroxide and stained using trypan blue (Kormanik et al. 1980). An additional set of roots were sent to R. L. Peterson. In those materials, roots were cleared and stained (Brundrett et al. 1994) and photographed using a Leitz Orthoplan microscope with differential interference contrast objectives.

Fine root segments were also evaluated for attached AM external hyphae using direct immunofluorescence. Root segments were incubated for 12 h in fluorescein isothiocyanate-labeled antisera of *Glomus deserticola, Acaulospora laevis, Scutellospora calospora* or *Gigaspora margarita*. Spores for preparation of the antisera were provided by INVAM. The antisera were raised against whole spore fractions of each species following the protocol described in Friese and Allen (1990). Reciprocal tests using spores and antisera of all genera indicated no detectable cross-reactions. Roots were scored on the presence or absence of fluorescing hyphae after incubation in the individual antisera.

Sporocarps of all fungi fruiting in this patch were collected as could be found and identified. Glomales spores were extracted from soil using differential centrifugation (Ianson and Allen 1986) to reduce loss of small-spored species and separate spores from high organic matter soils.

Results

At the Lake Skinner site, roots forming AM were found. Internal arbuscules and hyphae could be observed (Fig. 1a), but no vesicles were present in the February samples. By March, vesicles were present but no arbuscules could be found. Immunofluorescent assessments indicated an abundance of *Acaulospora* on the roots (>75% of samples), while *Glomus* was detected on 25% of roots and *Scutellospora* on only 15% of root segments. *Gigaspora* was not detected in any root sample in the current study. Spores of AM fungi were relatively abundant (a mean of 38 spores per 10 g soil), and all were *Glomus* spp.

Although we observed partial sheaths on many roots (Fig. 1b) in 1996 and 1997, no roots were completely encased, and we found no well-developed hartig net. However, intercellular septate fungi were found abundantly in the cortex (Fig. 1c) and occasionally penetrating individual cortical cells (Fig. 1d), appearing very similar to dark-septate mycorrhizae.

At the Sky Oaks site, we found no distinctive mycorrhizae in either 1996 or 1997. However, in 1998, all cores containing live roots (16 of 18) contained EM short roots (Fig. 2a). In both old and young plants, an average of 70% of the short-roots were ectomycorrhizal. Eight ectomycorrhizal types could be distinguished on the basis of mantle type and texture (Agerer 1987–93). One of these (Fig. 2b) was identical with descriptions of mycorrhizae formed by *Cenococcum geophilum* Fr. and *Picea engelmannii* (Parry) Engelm. (Harniman and Durall 1996), and *C. geophilum* and *P. abies* (L) Karst. (Agerer 1987–97; Gronbach 1988) and

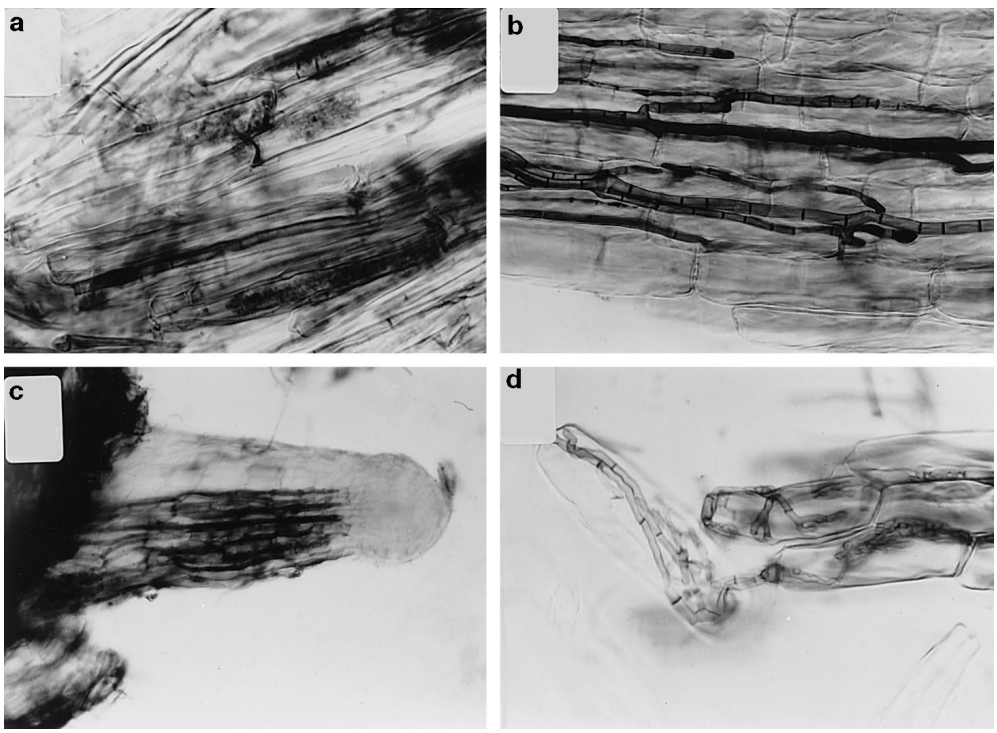

Fig. 1 Mycorrhizal structures in roots of *Adenostona fasciculatum* in 1996 including **a** arbuscules, **b** partial sheathing on a new root branch, **c** intercellular septate hyphae and **d** intracellular septate hyphae. Photographs provided by R.L. Peterson, University of Guelph

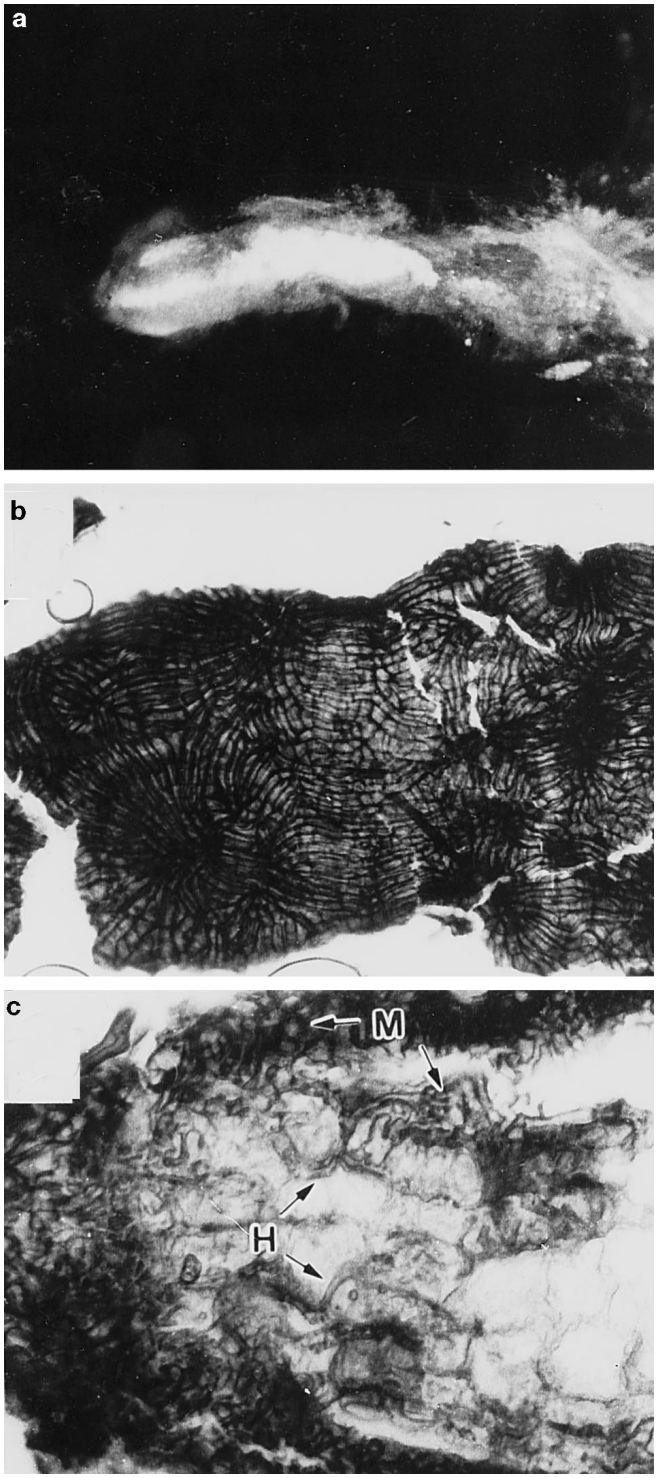

Fig. 2 Ectomycorrhizae in roots of *A. fasciculatum* in 1998 including **a** mycorrhizal short-root where the root is partly colonized by white, hydrophobic hyphae with clamp connections, **b** a longitudinal section of a fungal mantle of a *Cenococcum geophilum* ectomycorrhiza (showing a star-shaped net synenchyma), and **c** a longitudinal section of the outer cortical cells of a *C. geophilum* ectomycorrhiza. *Arrows labeled H* show intercellular hyphae forming a hartig Net, *arrows M* show parts of the inner fungal mantle

occurred in seven of the cores. The microscopic observations of longitudinal sections of cryotome-sectioned mycorrhizae of several types confirmed that they possessed a well-developed mantle (Fig. 2b) and that a hartig Net was present (Fig. 2c).

Several EM fungi were found in the patch in February and March of 1996 at the Lake Skinner site. These fungi included a new species, *Rhizopogon mengei* (nom.iden) Allen, Trappe, and Horton (in preparation). *Pisolithus tinctorius, Balsamia vulgaris,* and *Laccaria bicolor*, a sesquitoid *Hygrophorus* sp., and *Cortinarius* spp were also found. No fruiting has yet been observed in the Sky Oaks site.

Discussion

Assessing mycorrhizae for most plants has been relatively straight-forward based on commonly accepted structures such as vesicles, arbuscules, hartig net, and mantle. However, occasionally an important species forms mycorrhizae infrequently, or with uncharacteristic structures, or in uncharacteristic forms. This appears frequently to be the case with *A. fasciculatum.* Because of its unclear mycorrhizal structures, previous notes on its mycorrhizae have been hard to interpret.

We have clearly demonstrated that this species forms AM as the roots contain arbuscules, coils and internal hyphae. In addition, spores and antiserum tests confirm the presence of *Acaulospora*, *Glomus, Gigaspora,* and *Scutellospora*.

A. fasciculatum also forms EM, although not always forming either a distinct mantle or hartig net. In the Lake Skinner samples, the presence of several EM fungi, with no other potential host plants nearby demonstrates some form of relationship. One fungus, *Rhizopogon mengei*, has been found at several sites across California and always in association with *A. fasciculatum* (Allen, Trappe and Horton, unpublished results). This occurs despite the presumed relationship between *Rhizopogon* and conifers. Interestingly, the *C. geophilum* is generally found in this region with conifers. The formation of an arbutoid-like mycorrhiza with a septate fungus is interesting. Molina et al. (1992) consider arbutoid mycorrhizae to be a form of EM. We found EM at Sky Oaks during the 1998 growing season which we had previously been unable to see. Likewise, the appearance of an apparent dark-septate-like mycorrhiza deserves further investigation from direct isolation and study and molecular characterization of the fungi from the field.

The macrofungi found forming EM do so with a wide range of plant species (e.g., Molina et al. 1992). This supports the tentative observations of Cooper (1922) and Kummerow and Borth (1986) that only a partial sheathing form of mycorrhiza was present during most years. However, distinctive EM can be found during some years. This variation in time and among sites means that in arid regions extensive sampling may be necessary to understand mycorrhizal dynamics.

Acknowledgements We thank John Klironomos, Matthias Rillig and Steven Barlow for their help with observations. Photographs of intracellular mycorrhizae were provided by R.L. Peterson, University of Guelph. This research was conducted under funding from the US Department of Agriculture, Ecosystems Research Program to E.A. and the US Department of Energy, Program for Ecosystem Research to M.A.

References

Agerer R (1987–1997) Colour atlas of ectomycorrhizae. 1st–7th edn. Einhorn, Schwäbisch Gmünd, Germany

Allen MF (1991) The ecology of mycorrhizae. Cambridge University Press, Cambridge

Allen MF (1996) The ecology of arbuscular mycorrhizae: a look back into the 20th century and a peek into the 21st. Mycol Res 100:769–728

Allen MF, Morris SJ, Edwards FE, Allen EB (1995). Microbe-plant interactions in Mediterranean-type habitats: shifts in fungal symbiotic and saprophytic functioning in response to global change. In: Moreno JM, Oechel WC (eds) Global change and Mediterranean-type ecosystems, Springer, New York, pp 287–305

Allen MF, Figueroa C, Weinbaum BS, Barlow SB, Allen EB (1996) Differential production of oxalates by mycorrhizal fungi in arid ecosystems. Biol Fertil Soils 22:287–292

Brundrett M, Melville L, Peterson L (1994) Practical methods in mycorrhizal research. Mycologue, Waterloo, Canada

Cooper WS (1922) The broad-schlerophyll vegetation of California. Carnegie Institute Publication 319:1–124

Friese CF, Allen MF (1990) Tracking the fates of exotic and local VA mycorrhizal fungi: methods and patterns. Agric Ecosyst Environ 34:87–96

Gronbach E (1988) Characterizierung und Identifizierung von Ektomykorrhizen in einem Fichtenbestand mit Untersuchungen zur Merkmalsvariabilität in saueren beregneten Flächen. Bibl Mycol 125:1–217

Harniman SMK, Durall DM (1996) *Cenococcum geophilum* Fr. + *Picea engelmannii* (Parry) Engelm., CDE10. In: Goodman DM, Durall DM, Trofymow JA, Berch SM (eds) Concise descriptions of North American ectomycorrhizae. Mycologue Publications and Canada-BC Forest Resource Development Agreement, Canadian Forest Service, Victoria, BC pp. CDE1.1–CDE1.4

Horton, TR (1992) *Pseudotsuga menziesii* invasion into stands of chaparral. MA thesis, San Francisco State University, San Francisco

Ianson DC, Allen MF (1986) The effects of soil texture on extraction of vesicular-arbuscular mycorrhizal fungal spores from arid sites. Mycologia 78:164–168

Kormanik PP, Bryan WC, Schultz RC (1980) Procedures and equipment for staining large numbers of plant roots for endomycorrhizal assay. Can J Microbiol 26:536–538

Kummerow J, Borth W (1986) Mycorrhizal associations in chaparral. Fremontia 16:11–13

Molina R, Massicotte H, Trappe J (1992) Specificity phenomena in mycorrhizal symbiosis: community-ecological consequences and practical implications. In: MF Allen (ed) Mycorrhizal functioning. Chapman & Hall, New York pp 357–423

Padgett PE, Allen EB, Bytnerowicz A, Minnich RA (1998) Evaluation of a gradient in atmospheric nitrogen deposition in coastal sage scrub ecosystems of southern California. Atmos Environ (in press)

Westec Services Inc. (1988) Reservoir studies: biological resources investigation, Riverside County, California. Report prepared for Metropolitan Water District, Los Angeles, Calif