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# *Paris*-type mycorrhizas in *Smilax aspera* L. growing in a Mediterranean scherophyllous wood

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**Abstract** An unusual *Paris*-type mycorrhiza is described in *Smilax aspera* L., an evergreen climbing plant of Mediterranean sclerophyllous woods. Wild plants were sampled from a protected area inside the Regional Natural Park Migliarino-S.Rossore-Massaciuccoli, on the northwestern coast of Italy, near Pisa. Mycorrhizas formed by *S. aspera* were identified as a variation of *Paris*-type arbuscular mycorrhizas. Detailed observations on stained roots and on fresh root sections showed that, after forming the appressorium, the fungus colonized the root by penetrating individual cells, growing intracellularly from cell to cell, and producing many coils and terminal arbuscules. *S. aspera* seedlings inoculated with the arbuscular mycorrhizal fungi *Glomus mosseae* and *G. viscosum*, which are known to form *Arum*-type mycorrhizas in many plant species, produced the same *Paris*-type-like mycorrhizas found in nature. This confirms that the type of arbuscular mycorrhizal infection is largely governed by the plant host genotype. Plants of *S. aspera* inoculated with *G. mosseae* and *G. viscosum* had larger growth increments than uninoculated plants. Thus *Paris*-type mycorrhizas produce growth responses comparable to those of *Arum*-type mycorrhizas.

**Key words** Arbuscular mycorrhizas · *Smilax aspera* · *Glomus mosseae* · *Glomus viscosum* · *Paris*-type mycorrhizas

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## Introduction

Mycorrhizas constitute the absorbing root system in approximately 90% of land plants (Smith and Read 1997). Although different mycorrhizal types have evolved to prominence in diverse land plant ecosystems, Mediterranean biomes are characterized by the occurrence of all known types of mycorrhizal symbioses (Read 1991a, b). Recent studies confirmed the occurrence of ericoid, arbutoid, orchidoid, ecto- and arbuscular mycorrhizas in different plant species growing in undisturbed Mediterranean areas (Matosevic 1996). Nevertheless, there is little information about the mycorrhizal status of the majority of Mediterranean plant species (Lansac et al. 1995; Requena et al. 1996).

Two classes of arbuscular mycorrhizas (AM) were described by Gallaud (1905): *Arum*-type and *Paris*-type. The *Arum*-type is the best known and is characterized by rapid spread of the fungus via the apoplastic space between cortical cells of the root parenchyma. Vesicles, when present, can be intercellular or intracellular and arbuscules are terminal on intracellular hyphal branches (Smith and Smith 1997). The *Paris*-type is characterized by the absence of intercellular hyphae. In this type, the fungus develops symplastically, spreading directly from cell to cell within the cortex and forming many intracellular hyphal coils and intercalary arbuscules along the coils ("compound" arbuscules). Although *Paris*-type mycorrhizas frequently occur in the plant kingdom, (Smith and Smith 1997), most literature data involve the *Arum*-type mycorrhizas, which are widely distributed among herbaceous plant species.

*Smilax aspera* (Liliaceae) is an evergreen liana-like shrub, commonly found in the Mediterranean scrub, and in Italy it is a typical component of scherophyllous woods (*Quercetalia ilicis* Br. Bl.). In the present work, we investigated the mycorrhizal status of *S. aspera* plants growing in their natural habitat and

showed that this species develops an unusual kind of mycorrhizal infection, resembling the *Paris*-type, whose importance in nature has been recently stressed (Smith and Smith 1997). In addition, we inoculated *S. aspera* seedlings with *Glomus viscosum* and *G. mosseae*, two fungi capable of forming *Arum*-type arbuscular mycorrhizas, to examine whether the kind of root colonization is dependent on the plant genome. The growth effects of the two fungi producing *Paris*-type mycorrhizas in *S. aspera* were also assessed.

## Materials and methods

### Sampling area and sample collection

*S. aspera* (Liliaceae) was sampled in the "Macchia lucchese" in north Tuscany (approximately 10°17′ W; 43°49′ N), at an undisturbed site inside the Migliarino-S.Rossore-Massaciuccoli Natural Park. The site consists of a succession of dunes and backdune depressions parallel to the coastline, characterized by a well-preserved beach vegetation along the seashore backed by forest and marsh formations. In addition to *S. aspera*, other important plant species typical of Mediterranean scrub were present which are hosts to different mycorrhizal types: *Quercus ilex* L. (ectomycorrhizas), *Alnus glutinosa* (both ecto- and endomycorrhizas), *Arbutus unedo* (arbutoid mycorrhizas), *Erica arborea* (ericoid mycorrhizas), *Laurus nobilis* (arbuscular mycorrhizas), *Cephalantera rubra* (orchid mycorrhizas) (Maremmani, unpublished results). Replicate samples (15) of *S. aspera* plants, collected with roots and rhizospheric soil, were placed in polyethylene bags and stored at 4 °C upon return to the laboratory until processed.

### Plant material

Ripe fruits of *S. aspera* were collected in October 1996. Seeds were maintained at 4 °C for 4 months to relieve seed dormancy, scarified with 96% $H_2SO_4$ and rinsed for 24 h in distilled water. Seeds were germinated in 20-ml plastic pots containing sterile sand. After 70 days, seedlings were transplanted into 1-l pots with a 2 : 1 v/v mixture of sterile peat and sand. Plants were kept under greenhouse conditions, with a day/night temperature of 20/25 °C and natural photoperiod from May to September 1997.

### Fungal material

The inoculum consisted of soil containing spores, external mycelium and root fragments obtained from pot cultures of *G. viscosum* Nicolson (Banque Européenne des Glomales, BEG 27) and *G. mosseae* Gerdemann and Trappe (BEG 12), with 10 g of pot culture soil in each plant pot.

### Experimental design

After assessing the mycorrhizal status of wild plants, experimental inoculations with *G. mosseae* and *G. viscosum* was carried out. Three different trials were set up: (A) plants inoculated with *G. viscosum*, (B) plants inoculated with *G. mosseae*, (C) controls. Twenty replicate plants were used. At harvest, 6 months after transplanting, height, fresh and dry weight of leaves and stem numbers were assessed.

The mycorrhizal status of native plants was assessed on the basis of morphological and cytological features of the fungus-root association. The root system of each plant was thoroughly washed in running tap water to remove attached soil debris and examined with a Wild (Leica) dissecting microscope. Selected root pieces were sectioned with a Leitz-Kryostat 1720 freezing microtome and sections 10- to 30-μm thick were mounted in lactic acid and observed under a Reichert-Jung (Wien, Austria) Polyvar light microscope. Root samples were stained with trypan blue (Phillips and Hayman 1970) and percentage root colonization was estimated by the gridline intersect method (Giovannetti and Mosse 1980). Plant growth data were analysed by ANOVA and means for experimentally inoculated plants were separated by Tukey's multiple range test.

## Results

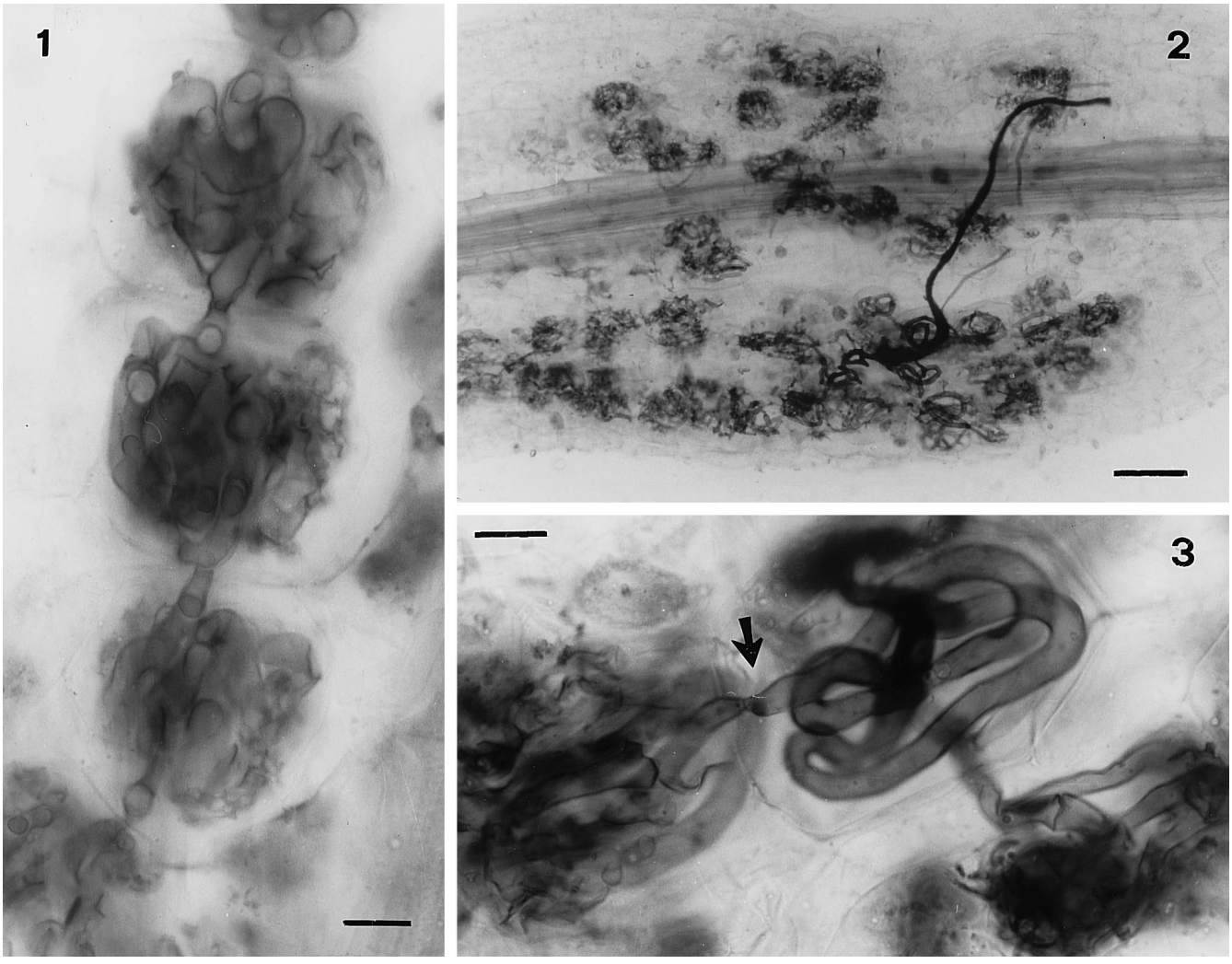
The root systems of wild plants of *S. aspera* showed $32.8 \pm 3.2\%$ infection ($n = 15$). The mycorrhizal colonization was characterized by high numbers of appressoria and infection points along the root surface. From each penetration point, hyphae spread from cell to cell and no intercellular hyphae were detected (Figs. 1, 2). In all samples, many coils formed by hyphae penetrating adjacent cells were observed (Fig. 3). Arbuscules were always simple and terminal, formed in cells otherwise free of hyphae, as branches from coils penetrating the cell wall of adjacent cortical cells to form the arbuscular trunk (Fig. 4). The inability of fungal hyphae to spread intercellularly along the longitudinal axis conferred a patchy appearance to the infection pattern when roots were observed at low magnification (Fig. 5).

Plants of *S. aspera* inoculated with *G. mosseae* and *G. viscosum* showed the same mycorrhizal morphology as that detected in native plants. No morphological differences were observed between the two endophytes tested.

In pot trials, mycorrhizal plants showed higher fresh and dry weights and higher leaf and shoot numbers than controls (Table 1). Differences in shoot height were visible as early as 6 weeks after inoculation.

**Table 1** Growth parameters measured after mycorrhizal inoculation of *Smilax aspera* L. plants with the arbuscular mycorrhizal fungi *Glomus mosseae* and *G. viscosum*. Values in columns followed by the same letters are not significantly different from each other ($P = 0.05$, Tukey's multiple range test)

| Treatment | Fresh wt. (g) | Dry wt. (g) | Number of stems | Number of leaves | Stem length (cm) |
|---|---|---|---|---|---|
| Control | 0.475a | 0.082a | 1.5a | 6.5a | 7.27a |
| *Glomus mosseae* | 4.442b | 0.677b | 3.0b | 20.5b | 12.25b |
| *Glomus viscosum* | 4.750b | 0.737b | 3.7b | 21.7b | 15.70b |




**Figs. 1–3** Light micrographs showing *Paris*-type-like mycorrhizas of *Smilax aspera* in nature and synthesized in the laboratory

**Fig. 1** Hyphae of the mycorrhizal symbiont spreading from cell to cell and forming coils in individual cells; *bar* = 15 μm

**Fig. 2** Infection point showing many coils and the absence of intercellular hyphae; *bar* = 100 μm

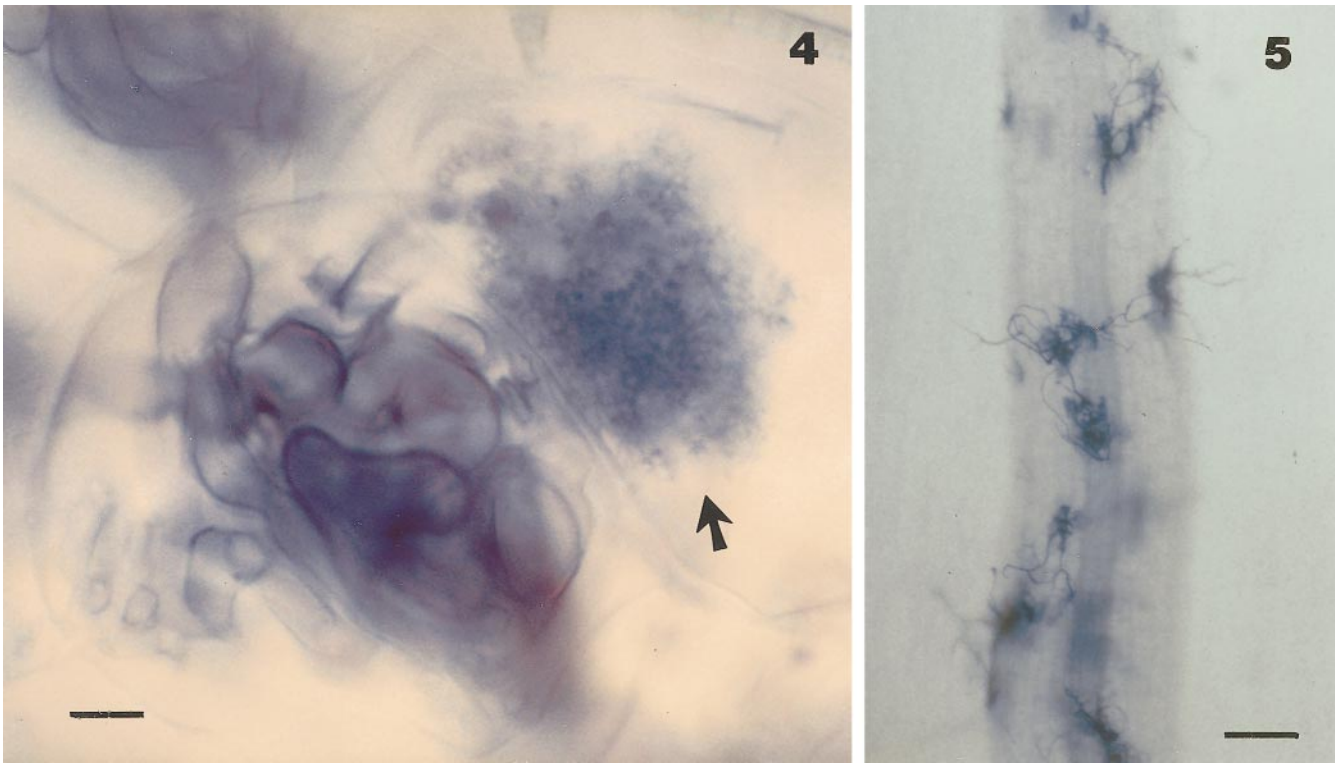
**Fig. 3** Coils formed by hyphae penetrating adjacent cells. Hyphal spread from cell to cell is evident (*arrow*); *bar* = 12 μm

## Discussion

The results of this work show that *S. aspera* in nature establishes arbuscular mycorrhizal symbioses of the *Paris*-type and can be colonized by arbuscular mycorrhizal fungi such as *G. mosseae* and *G. viscosum*, which in experimental conditions produce mycorrhizas with the same morphology observed in nature. *S. aspera* also responds to mycorrhizal inoculation by growth increments.

Mycorrhizal symbioses are a key component in the maintenance of plant biodiversity in natural ecosystems (Read 1991a). Studies on the mycorrhizal status of plants growing in undisturbed areas are fundamental for improving knowledge of mycorrhizal biology and diversity and for revegetation programs. Accordingly, the undisturbed site inside the Migliarino-S.Rossore-Massaciuccoli Regional Natural Park represents an ideal sampling area for this type of study because of its richness in vegetation types (Arrigoni 1990) and, in particular, in mycorrhizal types, a characteristic of Mediterranean biomes (Read 1991a; Matosevic 1996). This is further confirmed by our findings on the occurrence of a variation of *Paris*-type mycorrhizas in *S. aspera* growing in the Park.

*Smilax aspera* belongs to the family Liliaceae (Tutin et al. 1980), a very interesting class of Angiosperm with species producing both *Arum*- and *Paris*-type mycorrhizas (Gallaud 1905; Bonfante Fasolo and Scannerini 1977; McGee 1986; Brundett and Kendrick 1990; Smith and Smith 1997). The mycorrhizal colonization of *S. aspera* observed resembles the typical *Paris*-type series in the entirely intracellular spread of the hyphae with extensive formation of coils, while arbuscules are always simple and terminal and never inter-




**Figs. 4, 5** Light micrographs showing mycorrhizal structures in the cortex of *S. aspera*

**Fig. 4** Arbuscule formed as the final step of the colonization process (*arrow*); *bar* = 7.5 μm

**Fig. 5** Patchy appearance of the mycorrhizal infection pattern due to the absence of intercellular fungal spread; *bar* = 300 μm

calary, born laterally along the coils (Gallaud 1905). This variation of the *Paris*-type mycorrhiza has been described also by Gerdemann (1965) in *Liriodendron tulipifera* (Magnoliaceae) inoculated with *Endogone fasciculata*, where arbuscules were formed in free cells as terminal branches from coils present in adjacent cells.

Families with genera showing both types of arbuscular mycorrhizas are listed in many reports, and a deeper investigation of mycorrhizal diversity could be useful for understanding the reasons for such morphological variability. In this regard, *S. aspera* represents a good field of studies for investigations on the characteristics of *Arum*- and *Paris*-type mycorrhizal structures and for testing the hypothesis of the control exerted on mycorrhizal structures by the host plant genome (Smith and Smith 1997).

When in symbiosis with the most commonly used experimental plants (*Allium*, *Trifolium*, *Fragaria*), *G. mosseae* and *G. viscosum* are able to form *Arum*-type mycorrhizas. Our results clearly demonstrate the susceptibility of *S. aspera* to the infection by *G. mosseae* and *G. viscosum*, with formation of arbuscular mycorrhizas of the *Paris*-type. This evidence is consistent with previous observations (Barrett 1958; Gerdemann 1965; Jacquelinet-Jeanmougin and Gianinazzi-Pearson 1983), and confirms that the formation of *Paris*- or *Arum*-type arbuscular mycorrhizas is primarily under the genetic control of the host plant (Barrett 1958; Smith and Smith 1997). In particular, Brundrett and Kendrick (1990) suggested that the form of spread of the fungus is controlled by the plant, whereas the type of arbuscule is characteristic of the fungus. Our experimental results on inoculation of *S. aspera* with known simbionts confirm Brundrett and Kendrick's conclusions.

Only few experimental studies have been carried out on growth responses of *Paris*-type mycorrhizas (Gerdemann 1965; McGee 1985; Smith and Read 1997). Our data show that growth increments obtained during *Paris*-type colonization of *S. aspera* are comparable with those reported in many works on *Arum*-type mycorrhizas (Smith and Read 1997), suggesting similar benefits for the host. Further studies on the physiology of *Paris*-type mycorrhizas could lead to a better understanding of the role played by the two structures, intracellular coils and arbuscules, as sites for nutrient transfer.

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