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Root Structures and Mycorrhiza of the Achlorophyllous *Voyria obconica* Progel (Gentianaceae)

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Abstract

The genus *Voyira* comprises 19 achlorophyllous, mycotrophic species with reduced cormi. Roots of *Voyria obconica* are up to 1 cm long, 1–1.5 mm thick, succulent, brittle and radiate from the shoot base, forming a star-shaped root system. In cross section the central cylinder consists of up to 10 central vessels, surrounded by some parenchymatous cells, 5 to 7 strands of phloem and a pericycle. The cell walls of the anatomically inconspicuous endodermis are characterised by a faint suberin lamella. The cortex is divided into an inner cortex, with 3 to 5 layers of longitudinally elongated cells and a multilayered outer cortex, comprising isodiametric cells. The 2–3 cell layers of the dermal tissue also show a faint suberin lamella within their thickened cell walls. Non-parasitic, achlorophyllous plants need symbiotic interactions with mycorrhizal fungi. In *V. obconica* the exclusively intracellular hyphae of a single mycorrhizal fungus grows after penetration of the dermal tissue straight towards the inner cortex. Within the inner cortex the hyphae proceed parallel to the central cylinder. Branches of these straight inner cortex hyphae then colonize the outer cortex, where they form coils, swell, and eventually degenerate to amorphous clumps. Similarities and differences in root structure and mycorrhiza to the closely related *Voyria tenella* are elucidated. Arguments are given to call this association a special form of a *Paris*-type arbuscular mycorrhiza. The ecological significance of the revealed mycorrhizal compartmentation is discussed.

Keywords: *Voyria*, Gentianaceae, root anatomy, root morphology, mycorrhiza, myco-heterotrophy

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1. Introduction

Achlorophyllous plants must obtain their carbon supply from other organisms since vascular plants are unable to metabolise dead organic material. Achlorophyllous plants must therefore be either parasitic (Weber, 1993) or myco-heterotrophic (Leake, 1994). The exclusively achlorophyllous genus *Voyria* Aublet (Gentianaceae), comprising 18 neotropical and one palaeotropical species (Maas and Ruyters, 1986), belongs to the latter group (Imhof and Weber, 1997; Imhof, 1997; Imhof, 1999a). *Voyria obconica* is a rarely collected species, only known from seven localities in eastern Brazil (H. Maas, pers comm.). It shares many features with the more widespread *V. tenella*, e.g. the single flowered stem, stem anatomy, as well as fruit and seed characters (Maas and Ruyters, 1986). Both species belong to the more advanced representatives within the genus, based on morphological (Maas and Ruyters, 1986; Bouman and Devente, 1986; Bouman and Louis, 1989; Imhof et al., 1994), anatomical (Svedelius, 1902; Solereder, 1908; Welle, 1986; Imhof and Weber, 1997; Imhof, 1997), embryological (Johow, 1885, 1889; Oehler, 1927) and ecological data (Imhof et al., 1994). Only Johow (1889) did some research on *V. obconica*, solely providing a drawing of its habitus. It has been shown before, that gentians have a *Paris*-type arbuscular mycorrhiza (Jacquelinet-Jeanmougin and Gianinazzi-Pearson, 1983; Weber, 1984; McGee, 1985; Kühn and Weber, 1986; Demuth et al., 1989; Demuth and Weber, 1990). Investigations on the myco-heterotrophic members of the family revealed that they also have an arbuscular mycorrhiza (AM). However, with increasing morphological reduction they also showed gradual alterations of the mycorrhizal pattern (Imhof and Weber, 1997; Imhof, 1999a), so far ending in the mycorrhizal pattern of *V. tenella*. Hence, the advanced phylogenetic position of *V. tenella* seems also be reflected by its mycorrhiza, where straight, intracellular hyphae keep their definite shape in the inner root cortex, but the coiled derivates of them become degenerated in the outer cortex (Imhof, 1997). In analogy to the fungus cultivating leaf-cutter ants, this mycorrhizal pattern has been called an 'intraradical fungus garden'. Analogous to the ants, *Voyria tenella* also cultivates the fungal hyphae in its inner root cortex, and only uses branches of these inner cortex hyphae sent into the outer cortex for its carbon supply, thus achieving a sustained benefit of the endophyte. Other myco-heterotrophic plants (Imhof, 1998; Imhof, 1999b; Imhof, 1999c) were also shown to have mycorrhizal pattern deviating from all the well-known types of mycorrhiza (see Smith and Read, 1997), as well as being different from each other. Hence, investigating the mycorrhizal structures of myco-heterotrophic plants seems to be most rewarding to the search for extraordinary mycorrhizas. The present paper describes root morphology, anatomy and mycotrophy of *V. obconica* as the most likely species to share the so far unique mycorrhizal

pattern of *V. tenella* and discusses the ecological significance of the revealed features.

2. Materials and Methods

Alcohol material of *Voyria obconica* Progel was supplied by the Herbarium of the University of Utrecht. It was collected 1992 by P.J.M. Maas/Utrecht (s.n.) in Reserva Florestal da Companhia Vale do Rio Doce (CVRD), Linares, State Espírito Santo, Brazil ('at the path behind the garden').

After dehydration in an ascending ethanol-series, roots were embedded in Unicryl™ (British Biocell Int.). Sections of 2–5 μm were cut, stained with Toluidine Blue (1 g Toluidine Blue O + 1 g Sodium Tetraborate in 100 ml distilled H_2O) and mounted in Corbit-Balsam. Suberin was detected on Unicryl™ sections by using a saturated solution of Sudan IV in 90% ethanol, mixed 1:1 with glycerol, and differentiated with diluted glycerol (Gerlach, 1984).

Samples for scanning electron microscopy were dehydrated through ethylene glycol monoethyl ether, washed twice in acetone, dried by the 'critical point method' (Anderson, 1951), and coated with gold in a Balzers Union Sputter Coater.

3. Results

Roots of *V. obconica* reach up to 1 cm in length, are 1–1.5 mm thick, succulent, brittle, hairless and bluntly tapering. They form a star-shaped root system, seemingly radiating from the shoot base (Fig. 1), but due to the densely clumped roots, their actual origin could not be determined with certainty. Depressions and bumps shape the root surface, probably due to the restrictions of the rhizosphere during growth. Foreign organic material often occur in these depressions.

In cross section, the root consists of up to 10 central vessels, surrounded by parenchymatous cells, and 5 to 7 strands of phloem in a radial arrangement around the xylem. Only Sudan IV treatment reveals a weak suberin lamella in the endodermis, more pronounced in its inner periclinal and anticlinal cell walls. A pericycle is present between the phloem strands and the endodermis. The cortex is divided into an inner cortex with 3 to 5 layers of longitudinally elongated cells (20–30 μm radially, up to 200 μm longitudinally), and a multilayered outer cortex, consisting of isodiametric cells (30–60 μm in diameter). Except for the fungal material, all cortex cells lack visible content. The 2–3 cell layers of the dermal tissue also show a faint suberin lamella
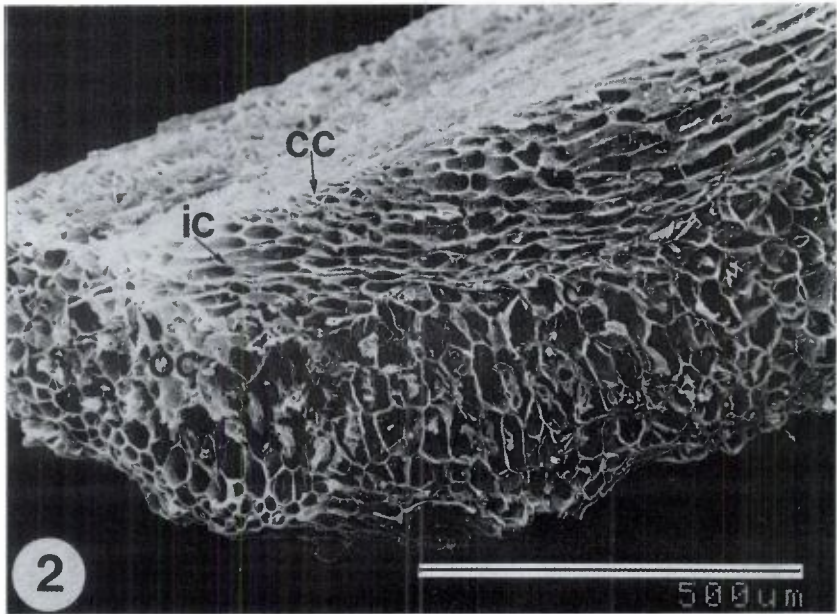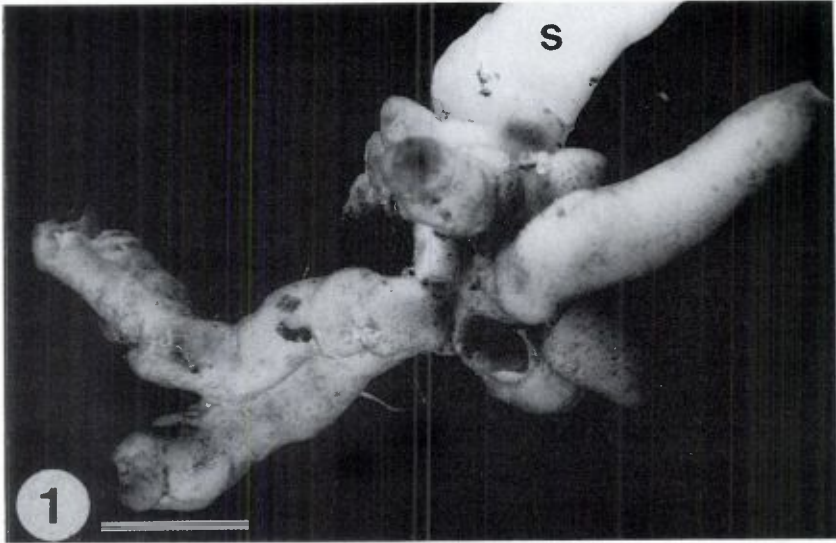
Figure 1. Star-shaped root system of *Voyria obconica*. s = shoot. Scale bar = 2 mm.
Figure 2. SEM micrograph of an inner look on a root of *V. obconica*, showing the central
            cylinder (cc), the inner cortex (ic), and the outer cortex (oc). Note the degenerated
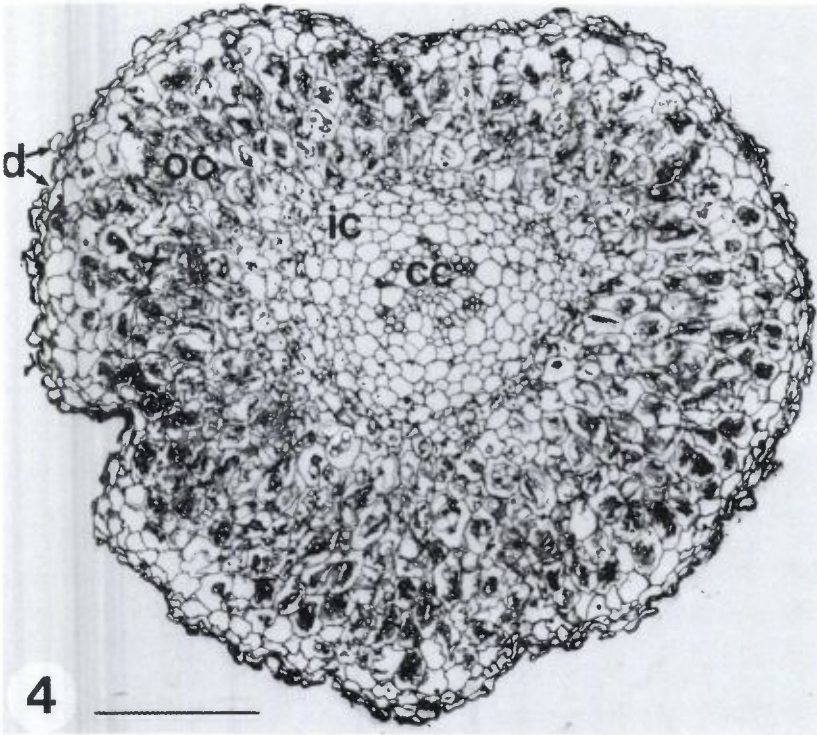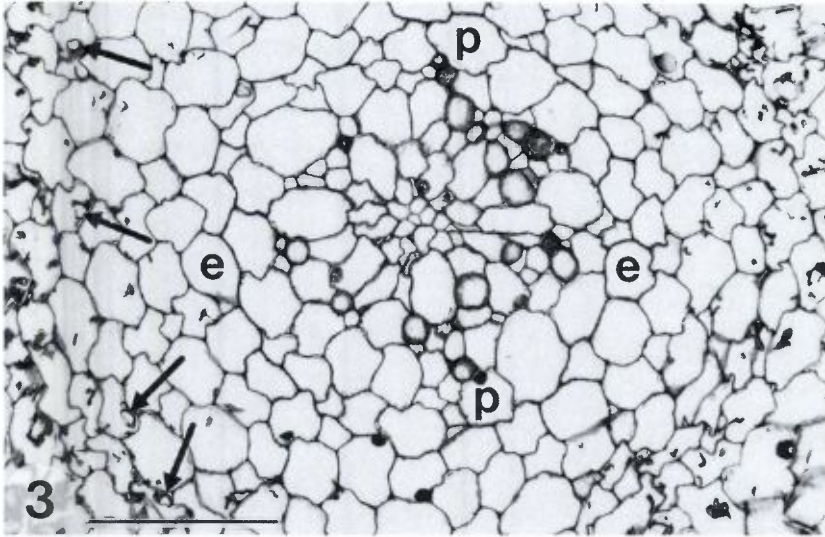   hyphal clumps in the outer cortex cells. Scale bar = 500 μm.

Figure 3. Cross section of the central cylinder of *V. obconica*. e = endodermis, p = pericycle; arrows = straight hyphae in the inner cortex. Scale bar = 100 μm.

Figure 4. Cross section of a root of *V. obconica*. cc = central cylinder, ic = inner cortex, oc = outer cortex, d = 2–3 layered dermal tissue, partly scaling off. Scale bar = 250 μm.
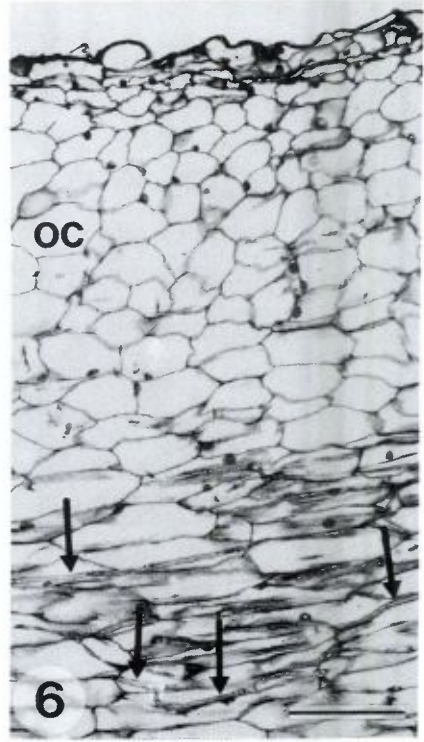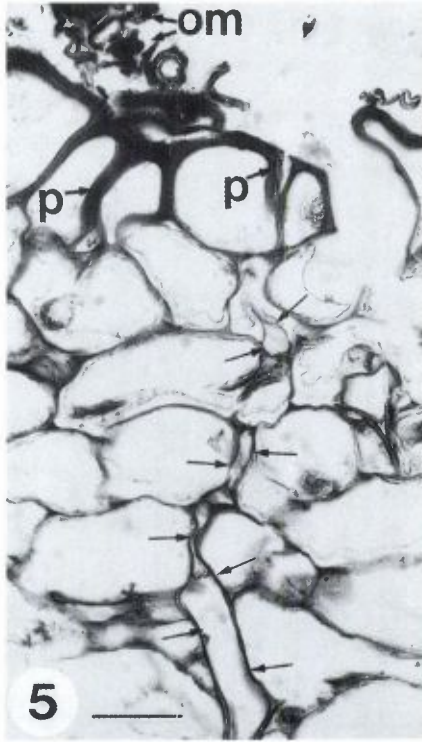
Figure 5. Fungal penetrations (p) close to organic material (om) attached to a root of
           V. obconica. The penetrating hypha directly grows towards the inner cortex
           (small arrows). Scale bar = 20 µm.
Figure 6. Tangential section close to a root tip of *V. obconica*. The outer cortex (oc) is not
           yet colonized, whereas straight hyphae (arrows) grow within the inner cortex.
           Scale bar = 100 µm.

within their thickened cell walls, suggesting an exodermis. Cells of the
outermost layer can scale off (Figs. 2–4).

Only where organic material is attached to the root surface, infections by the
mycorrhizal fungus ocurred. The hyphae penetrate the epidermal tissue as
well as the cell walls in the cortex without generating particular defense
reactions. No appressoria were found. The intraradical mycelium is mostly
aseptate and entirely colonizes the cortex intracellularly from cell to cell.
After penetration, up to 10 µm thick hyphae grow straight towards the inner
cortex (Fig. 5). In the inner cortex, but preferentially in its outer parts (see Figs.
3 and 4), the hyhpae are between 4 and 7 µm thick, straight, and follow the
central cylinder longitudinally (Figs. 6 and 7), appearing as small circles in
cross section (see Fig. 3). Eventually, the endodermis is a barrier to the fungus.
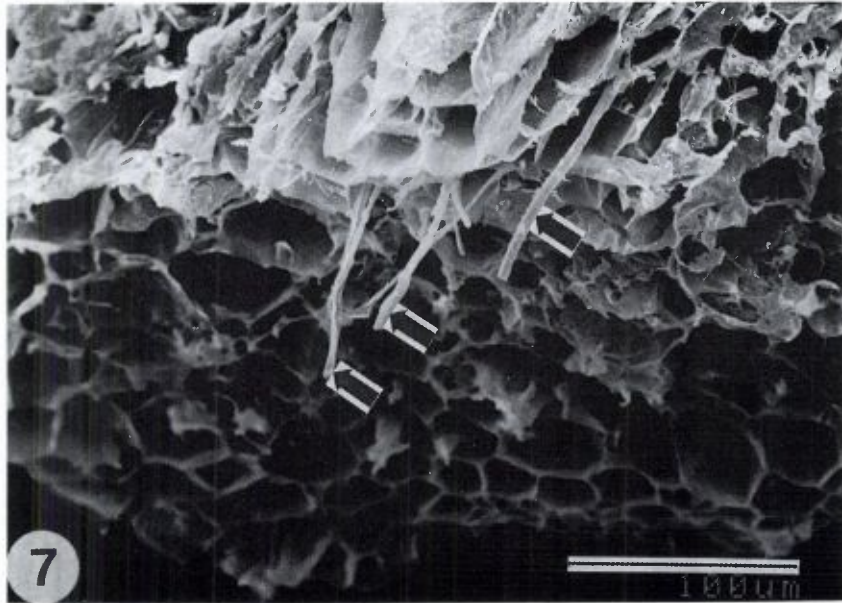
Figure 7. Close-up SEM micrograph of Fig. 2 focussing on the straight hyphae of the inner cortex hyphae (arrows) exserting. Scale bar = 100 μm.

Ramifications of the inner cortex hyphae colonized the outer cortex, where the hyphae form dense coils, swell up to irregular bladders and eventually degrade to amorphous clumps (see Figs. 2 and 4). Close to the root tip, little to no colonization of the outer cortex was observed, but the straight hyphae in the inner cortex were already present (see Fig. 6). Serial sectioning ascertained, that all fungal structures were interconnected and, thus, belong to a single fungus.

4. Discussion

Due to the rarity of *V. obconica*, the material studied was limited and, hence, measurements must be regarded with caution. However, anatomical features are rather constant within a species, and the new data can be compared with information on the closely related *V. tenella* (Imhof et al., 1994; Imhof, 1997), thus providing a more secure base for interpretation.

The reduction line within the genus *Voyria* also relates to root morphology. *Voyria truncata* has an extensive root system, comprising up to 2 mm thick, runnerlike roots (Imhof et al., 1994; Imhof and Weber, 1997). The root system of

Voyria aphylla is a miniature version of *V. truncata* in both the root diameter as well as the extent of the root system, with the tendency to concentrate the development of new roots close to the shoot bases (Imhof, 1999a). The morningstar-like root system of *V. tenella* is easily conceived as a further reduction of the roots and contraction of the root system (Imhof et al., 1994; Imhof, 1997). The general habit of *V. obconica* looks very much alike to *V. tenella* (compare Imhof et al., 1994). Also the central cylinder, except for the pericycle which is lacking in *V. tenella*, the dermal tissue and the size of the outer cortex cells are very similar. Nevertheless, the above-ground parts of *V. obconica* tend to be slightly bigger in size than those of *V. tenella* (see Maas and Ruyters, 1986). Concordantly, the root diameter of 1–1.5 mm was also larger (0.5–1.0 mm in *V. tenella*), and the root system looked more stout than the one of *V. tenella*, due to the more slender roots of the latter. The division of the cortex into two distinct layers appeared more pronounced in *V. obconica* than in *V. tenella*, since the cells of the inner cortex of *V. obconica* were longitudinally more elongated (up to 120 µm in *V. tenella*), and the inner cortex consisted of more cell layers than in *V. tenella* (1–3 cell layers). Although both species are most morphologically and anatomically reduced within their genus (see Introduction), the presence of the pericycle layer in *V. obconica* and the generally larger dimensions of the plants suggest that *V. tenella* is more advanced than *V. obconica*, and therewith, possibly, the most advanced gentian of all.

The mycorrhizal structures of *V. obconica* are identical to those in *V. tenella*. There is good evidence for *V. tenella* to have an arbuscular mycorrhiza (see Imhof, 1997), but the limited material of *V. obconica* did not yield as much information. However, all so far investigated members of the Gentianales not only showed AM, but their mycorrhizas can be arranged in a line of evolutionary progress, ending in the mycorrhizal pattern of *V. tenella* (Weber et al. 1995; Imhof, 1999a). Moreover, considering the close taxonomic affinity of the two sister species, comparing the hyphal dimensions in *V. obconica* to other AM plant species (see Bonfante-Fasolo, 1984), and knowing that coiled hyphae and hyphal swellings have long since been observed in *Paris*-type arbuscular mycorrhizas (e.g. Schlicht, 1889; Neumann, 1934; Gay et al., 1982; Kühn and Weber, 1986; Demuth and Weber, 1990), the most obvious conclusion is to call the mycorrhiza of *V. obconica* a special *Paris*-type AM. The exclusively intracellular, straight hyphae in the inner cortex must not be compared to the intercellular hyhpae of the *Arum*-type AM. The latter stay in the apoplast of the corteces, and, unless to form arbuscules, start to coil as soon as the hyphae penetrate the cells, as it happens in intermediate forms between *Arum* and *Paris*-type AM (see Smith and Smith, 1997). The uncoiled, integer shape of the hyphae in the inner cortex of *V. obconica* are likely to be a sign of prolonged survival, and suggest an innercortical rather than outercortical or external

spread of the mycorrhizal colonization. In fact, close to the root tip, in other words in younger tissue, the outer cortex is not yet infected, but the inner cortex already is colonized by straight hyphae (see Fig. 6).

It seems to be a widespread feature in myco-heterotrophic plants, and, therefore, is likely to have ecological significance, that their mycorrhizal organs are partitioned in regions where the hyphae keep a definite shape and others where they become amorphous. This compartmentation of mycorrhizas has gone to extreme in *Afrothismia winkleri*: a single fungus attains four different appearances in five different parts of its root/rhizome structure (Imhof, 1999c). But also the three cortex layers with distinct mycorrhizal morphologies in roots of *Triuris hyalina* (Imhof, 1998), as well as the different hyphal growth in the inner and outer corteces of *V. tenella* and *V. obconica* are further examples for mycorrhizal compartmentation within roots. In general, any function needs a definite structural base, and, in particular, special functions require an attuned structure. This does not necessarily mean that the different hyphal morphologies indicate different functions. But considering the reduced root surface diminishing the probability to become infected by an appropriate fungus, in combination with the indispensable need for an efficient mycorrhiza (see also Imhof, 1999b), the different hyphal morphologies in *V. obconica* and *V. tenella* are yet likely to be signs of different purposes, and are interpreted to serve for a sustained use of the endophyte (see Introduction).

Direct hyphal bridges between myco-heterotrophic plant roots and roots of neighboring plants have been described (Weber, 1979; Imhof and Weber, 1997; Imhof, 1999a; Imhof, 1999b; Imhof, 1999c), and they have given valuable hints to the identity of the mycorrhizal fungus. The material studied in the present investigation did not include entire neighboring roots connected to the roots of *V. obconica*, but the organic material always attached where fungal infections ocurred is a strong indication for a likewise fungal acquisition. And, like the explanation for the special mycorrhizal pattern, the concentrated fungal potential of neighboring mycorrhizal roots used as a source for infection also helps to compensate the disadvantage of a minimal root surface.

Acknowledgements

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