GOVERNMENT OF CANADA
SUBMISSION TO SBSTTA

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BIODIVERSITY OF MYCORRHIZAL FUNGI

1. Introduction

Only in the last few decades have botanists and mycologists realized that most terrestrial plants live in symbiosis with soil fungi (Mosse, 1956). The term *mycorrhiza*, created to reflect this reality, comes to us, moreover, from the combination of two words, one Greek *mikès* (fungus) and the other Latin *rhiza* (roots). It therefore basically designates the symbiotic association between fungi and plant roots. Among the types of mycorrhizae observed in nature, one is found on the vast majority of cultivated plants, the arbuscular mycorrhiza, which lives in association with approximately 85% of herbaceous plants. This means therefore that in the plant world, mycorrhizal symbiosis is the rule rather than the exception.

Four hundred million years ago, when the continents were virtually deserted, plants and fungi formed symbiotic systems. Plants used solar energy to grow, while fungi specialized in absorbing nutrients from the soil. Thanks to the complementary role and function of these organisms, a wide variety of terrestrial plants emerged over the course of subsequent geological eras. Today, arbuscular mycorrhizal fungi (AMF) are found under all climates and in all ecosystems, irregardless of the type of soil, vegetation or growing conditions.

A symbiosis refers to an association of living organisms that benefits both partners, enabling them to survive, grow and reproduce more effectively. AMF, which are microscopic soil fungi, simultaneously colonize the roots and their rhizosphere and spread out over several centimetres in the form of ramified filaments. This filamentous network, found inside as well as outside the roots, gives the plant access to a greater quantity of water and soil minerals required for its nutrition (Figure

1). In return, the plant provides the fungus with sugars, amino acids and vitamins essential to its growth (Harley and Smith, 1983). As a result of its improved nourishment, a mycorrhiza-colonized plant has better growth. It bears abundant fruit and, above all, acquires increased resistance to environmental stresses such as drought, cold and root pathogens (Sylvie and Williams, 1992).

Given that the majority of cultivated plants used for human and animal food purposes are colonized by mycorrhizae, we can consider utilizing this symbiosis for the benefit of agriculture, by selecting the best plant-fungus combinations (Abbot and Robson, 1991). It would then be possible to promote healthier cropping systems and to reduce the use of chemical inputs (pesticides, fertilizers), while ensuring crop profitability and environmental quality. The AMF biodiversity study project was developed in this perspective through inventories of indigenous and agricultural soils (Dalpé, 1989; Dalpé et al., 1986; Hamel, 1994,96,97; Bâ et al., 1996; Diop et al., 1997), the development of germplasm banks, taxonomic data bases, descriptions of fungi (Dalpé 1995; Koske, 1986,92) and computerized taxonomic documents (Dalpé and Séguin, in preparation).

The Eastern Cereal and Oilseed Research Centre of Agriculture and Agri-Food Canada (ECORC) is currently the only Canadian establishment that has and maintains an AMF reference collection available to industry partners, government agencies, universities or private producers. All services relating to the supply and identification of strains and consultation services are currently offered at this Centre. Many collaborative research agreements with companies that manufacture natural fertilizers, mycorrhizal inocula and geotextiles are in progress, and other agreements in the areas of micropropagation and the production of liquid fertilizers are currently being negotiated.

Most of the work under way focussing on mycorrhizae, whether at the university, industry or government levels, is aimed primarily at:

-conducting an inventory of AMF in indigenous and agricultural soils;
-understanding the biological and physiological processes of this symbiosis;
-using the symbiotic potential of these soil microorganisms for agricultural development.

However, despite the repeated experimental demonstration of the benefits of using mycorrhizae in agriculture, this biotechnology remains badly known and under-utilized, except by the scientific community and a few leading-edge industries. To succeed in getting national and international bodies to adopt this technology, it is important to persevere in acquiring more knowledge, primarily in the areas of biodiversity and the rational, efficient use of this biodiversity.

2. *Arbuscular mycorrhizae*

2.1 *Biology and taxonomy*

Morphology and nutrition: AMF develop a major network of microscopic filaments in the soil (Figure 2). When filaments of these fungal organisms come into contact with a young root, they thread their way between the cortical cells and quickly propagate, forming intracellular arbuscules and, in some cases, intercellular vesicles (Figure 3). Arbuscular fungi received their designation from the tree-like structures that are differentiated in the roots. Spores are also differentiated in the soil and in the roots. They act as reserve and propagation organs and as a reference structure for species identification (Figure 4). Thus far we have no serious clue as to the sexuality of AMF. Their association with the Zygomycetes is based on the similarity of their spores to the spores of other known representatives of this class.

The close contact created between the plant and fungus through the filamentous network allows the exchange of nutrients for the survival and growth of the two partners. First, the wide dispersal of the fungus in the soil through its large filament network gives the fungus access to a much larger volume of soil than the root system alone. The fungal filaments act more or less as a pump, supplying the root with a supplement of water and mineral salts to which it would not normally have access. In return, the fungus receives from the plant metabolized nutrients that it is unable to synthesize itself, such as sugars, amino acids and secondary metabolites (Figure 1).

Benefits of symbiosis: The colonized plant is better nourished and better adapted to its environment (Figures 10, 11, 12). It obtains increased protection against environmental stresses (Sylvia and Williams, 1992), including drought (Subramanian et al., 1995), cold (Charest et al., 1993; Paradis et al., 1995), salinity (Davis and Young, 1985) and pollution (Leyval et al., 1994; Shetty et al., 1995). In addition, symbiosis tends to reduce the incidence of root diseases and minimizes the harmful effects of certain pathogenic agents (Dehne, 1982; St-Arnaud et al., 1995). By and large, the growth and health of colonized plants is improved. At the same time they obtain increased protection against environmental conditions detrimental to their survival.

Taxonomy: The AMF belong to a very old category of fungus, the Zygomycetes, and have been recently regrouped in a single order, the Glomales (Morton and Benny, 1990), which include all species capable of living in symbiosis with plants. The bulk of known species belong to the family Glomaceae (Pirozynski and Dalpé, 1989), which includes the genera *Glomus* and *Sclerocystis*. The AMF are currently considered to consist of approximately 160 species belonging to 3 families and 6 genera and have a worldwide distribution. The bulk of known species have been described over the last two decades, which indicates the increased interest in these organisms and also the difficulty inherent in their taxonomic treatment. In fact, the greatest difficulty is that the entire taxonomy of these organisms is currently based on the morphological characters of the spores. They are single-celled structures, of generally globoid shape, with thick walls made of several layers of different textures, connected to the filamentous network by a suspensor hypha of varied morphology (Figure 4). Since the morphological characters are reduced and often variable depending on the maturity of the spores studied, ultrastructural studies (Figures 6, 7) serve to support observations made previously in optic microscopy. In addition, although still exploratory, molecular approaches can be used to detect, with the help of specific probes and only for some species, the qualitative and quantitative presence of a fungus directly in its substrate (Moutoglis, 1997).

Culture: A major obstacle that slows work on biodiversity and the utilization of these soil microorganisms is their obligate symbiont status. These fungi can grow only in the presence of a living plant, which means that their propagation requires the use of pot cultures, either in greenhouses or in growth chambers (Figures 8, 9). To overcome this barrier, an *in vitro* culture method on excised roots (Figure 5) was developed, but only half a dozen strains are currently available in monoaxenic culture. This fairly recent methodology, though, represents a very promising avenue for the development of studies on biodiversity, taxonomy, ontogeny of spores, and verification of the mycorrhizal potential of the isolated AMF strains. One last point, more positive, is that the AMF are not specific in the choice of their partner plant, which means that the same fungus can be grown on a large number of plant species.

2.2 *Biodiversity and agricultural potential*

The fact that colonized plants are better able to obtain their nourishment in the soil and resist environmental stresses gives fungal symbionts a biofertilizing and crop protection role. In agriculture, the increased uptake of soil minerals by colonized plants means that it is possible to consider reducing applications of fertilizers and pesticides substantially and still obtain equivalent or even higher crop yields (Abbott and Robson, 1991). Through appropriate management of mycorrhizae in agriculture, it is also possible to maintain soil quality and sustainability while protecting the environment over the long term and reducing the costs of production (Figures 9, 13).

3. *General objectives of the research project*

ECORC's expertise on AMF relates at present to the fungus resources study of the biological resources program. The general objectives of the project may be summarized as follows:

- to improve and diversify the collection of mycorrhizal cultures;
- to evaluate the mycorrhizal potential of strains on a variety of cultivated plants;
- to develop a reference grid of the best plant-fungus partners capable of promoting optimum growth of colonized plants in an underfertilized environment.

The main subobjectives are:

- to conduct an inventory of agricultural and indigenous soils, so as to isolate, characterize and identify new strains, diversify the sources of inocula, and determine the most effective ones for crop production and protection;
- to propagate and evaluate their mycorrhizal potential in an underfertilized environment;
- to develop cryopreservation techniques for long-term preservation of strains;
- to produce and publicize educational software, computerized taxonomic keys and video documents in order to train and inform people involved in mycorrhizae research and development;
- to develop micropropagation techniques so that pure high-quality cultures may be obtained.

4. Methodologies used

Isolation of strains: by sieving and sucrose gradient method followed by extraction of fungus spores under the stereomicroscope and their characterization in optical and electron microscopy with cytochemical tools.

Propagation of fungus strains: carried out in growth chambers on living plants and through micropropagation on roots transformed with *Agrobacterium tumefaciens*.

Characterization of strains: by using optical microscopy to evaluate the morphological characters that distinguish different species, by using transmission and scanning electron microscopy to describe newly observed species, and by using histochemical tools to identify useful characters that will make it easier to distinguish species in an applied working environment.

Long-term storage: with cryopreservation techniques such as lyophilization, low-temperature incubation and liquid nitrogen.

Evaluation of mycorrhizal potential: carried out in greenhouses on various cultivated plants; (Biomass, maturation, photosynthesis, sugar, protein, and mineral content are the physiological and biochemical parameters used to evaluate plant reaction to mycorrhizae.)

Illustrated and computerized identification keys: in optical microscopy, using an image capture system, high-quality illustrations from material developed in the germplasm bank and from the observation of AMF cultivated in vivo and in vitro.

5. Products and technologies under development

- Inventory of the diversity of AMF in a temperate environment, for both agricultural and indigenous soils
- Bank of fungus strains available for research and for industry
- Evaluation grid showing the potential of AMF strains in various crops
- Methodology for practical evaluation of the benefits of symbiosis in crop production
- Methodology for the processing and propagation of strains in in vitro culture
- Computerized document, video to communicate the basic notions of AMF taxonomy and illustrating the species of fungi most frequently observed in agricultural environments
- Visual key for the identification of species covered in the video document

6. Research update

The work is currently being pursued as follows: the inventory of mycorrhizal species in agricultural and indigenous soils; the culture and propagation of strains selected from these inventories; evaluation of the mycorrhizal potential of these same species for the development of endomycorrhizae as crop protection and biofertilizing agents in order to ensure the sustainability of agricultural ecosystems while maintaining crop yields and quality.

The fields, research possibilities and overall results obtained in the present study on mycorrhizae are summarized in the following table:

BIODIVERSITY		
Inventories	Agricultural soils	Hamel et al., 1996 (fallow land, Quebec)
		Dalpé et al., 1986 (fruit trees, Quebec)
		Bâ et at., 1996 (corn, Burkina Faso)
		Diop et al., 1997 (acacias, Senegal)
	Indigenous soils	Dalpé, 1989 (riparian dunes, Eastern Canada)
		Dalpé and Aiken, 1992, 97 (Canadian Arctic)
		Dalpé, 1993, 97 (riparian dunes, Great Lakes)
Taxonomic tools	Herbarium	250 reference specimens
	Germplasm bank	120 reference strains
	Data bases	photographic, taxonomic, distribution
	Biochemistry of lipids	Grandmougin et al. (95, 97), Sancholle and Dalpé, (92, 93)
	Ultrastructure	11 species treated
	Descriptions	Fungi Canadenses (6 species described)
		3 newly listed species in the Great Lakes and Canadian Arctic regions

EXPLOITING THE BIODIVERSITY OF MYCORRHIZAL FUNGI			
Mycorrhizal potential	Greenhouses	Wheat	Cold resistance (Paradis et al., 1995)
		Corn	Evaluation of potential of AMF strains (Boucher et al., 1997)
		Turfgrass	Fertilization (Boudreault et al., 1997)
	Fields	Trees	Survival in an urban environment (agreement with industries)
		Corn	Crop yields
			Reduction of fertilization
			Survival of strains
		Turfgrass	Benefits, grass seeding (industries)

			Comparative effect of various strains of fungi
			Compatibility with natural fertilizers (industries)
Long-term storage	Viability	Comparative analysis of technologies available	
		Establishment of a cryopreserved germplasm bank	

7. *Impact of mycorrhiza research data on decision-making in agriculture*

The main objective of collaborative research on AMF biodiversity is to contribute to the adoption of mycorrhiza biotechnology in agriculture, in order to gradually integrate this technology with conventional agricultural practices. Over the medium term, therefore, work can be done to restore soil quality and prolong if not ensure soil survival.

It has been shown that appropriate management of mycorrhizae in agriculture allows a substantial reduction in the use of chemicals, thus lessening the level of pollution of surface waters, reducing farm work, maintenance and costs of production, while maintaining yields at their highest levels. The objective in maintaining and developing a high-quality germplasm bank is to support and accelerate the use of mycorrhizae as biofertilizing agents in agriculture and in turn contribute to the development of a healthy and sustainable environment.

Using reference grids to optimize the pairing of fungus and plant partners will demonstrate (with supporting data) the benefits of mycorrhization to improve soil quality, crop protection and yields, not to mention the value added to foods and the reduced use of pesticides and fertilizers.

The diagram below, showing the advantages and benefits of adopting mycorrhizae in agriculture, allows us to better visualize the scope of this phenomenon at the crop level and, in turn, the impact of its long-term adoption on the quality of life.

FIELD	AVANTAGES	BENEFITS
Plant physiology	Improvement in nutrition	Reduced use of fertilizers (15-25% or more)
	Tolerance of drought stress	Cultivation of arid soils or soils unfit for agriculture
	Resistance to low temperatures	Diversity of crops in inhospitable areas
Plant morphology	Transformation of root architecture	Adaptation to stress, increased resistance to erosion, fixation of soils
		Improved production with certain root crops
Plant community	Microorganism diversity in the subsoil	Reestablishment of soil microflora
		Improvement of soil quality, compost quality
	Survival of partners	Improvement in yields
		Better acclimatization at transplanting

		Diversity of plant cover
Agriculture	Plant production	Increase in aerial and/or root biomass
	Stress resistance	Drought
		Cold
		Pollution
	Resistance to pathogens	Crop protection
		Reduced use of pesticides
		Improvement in product quality
		Improvement in animal, plant and human health
	Value added to product	Increased synthesis of primary or secondary metabolites
		Crop earliness

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Legends

Figure 1:	Diagram of nutrient exchanges of mycorrhizal symbiosis
Figure 2:	Spore-carrying mycorrhizal filaments, along a root (1mm = 10:m)
Figure 3:	Section of root colonized with vesicles (v), arbuscules (a) and hyphae (h) (1mm = 2μm)
Figure 4:	Spore of *Glomus coronatum* species attached to its suspensor hypha (1mm = 5μm)
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Figure 6:	Spore of *Glomus intraradices* in scanning electron microscopy (1mm = 2μm)
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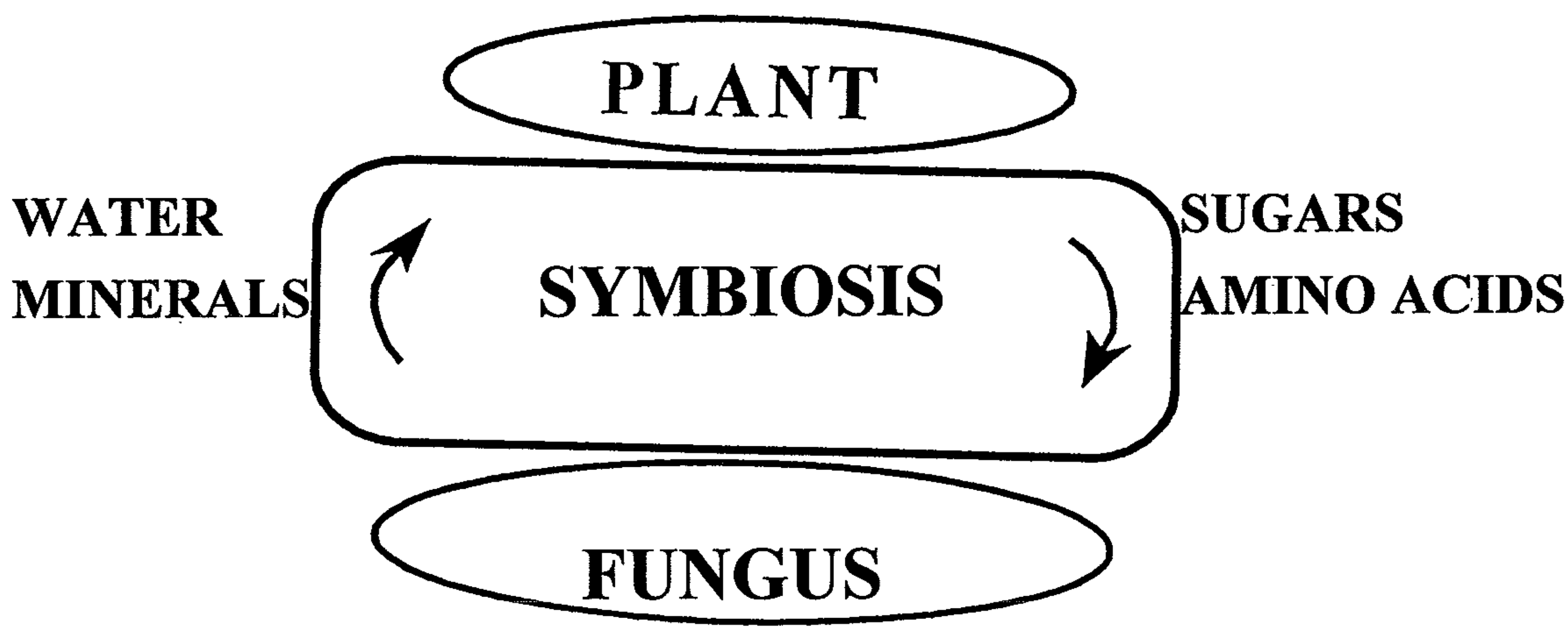
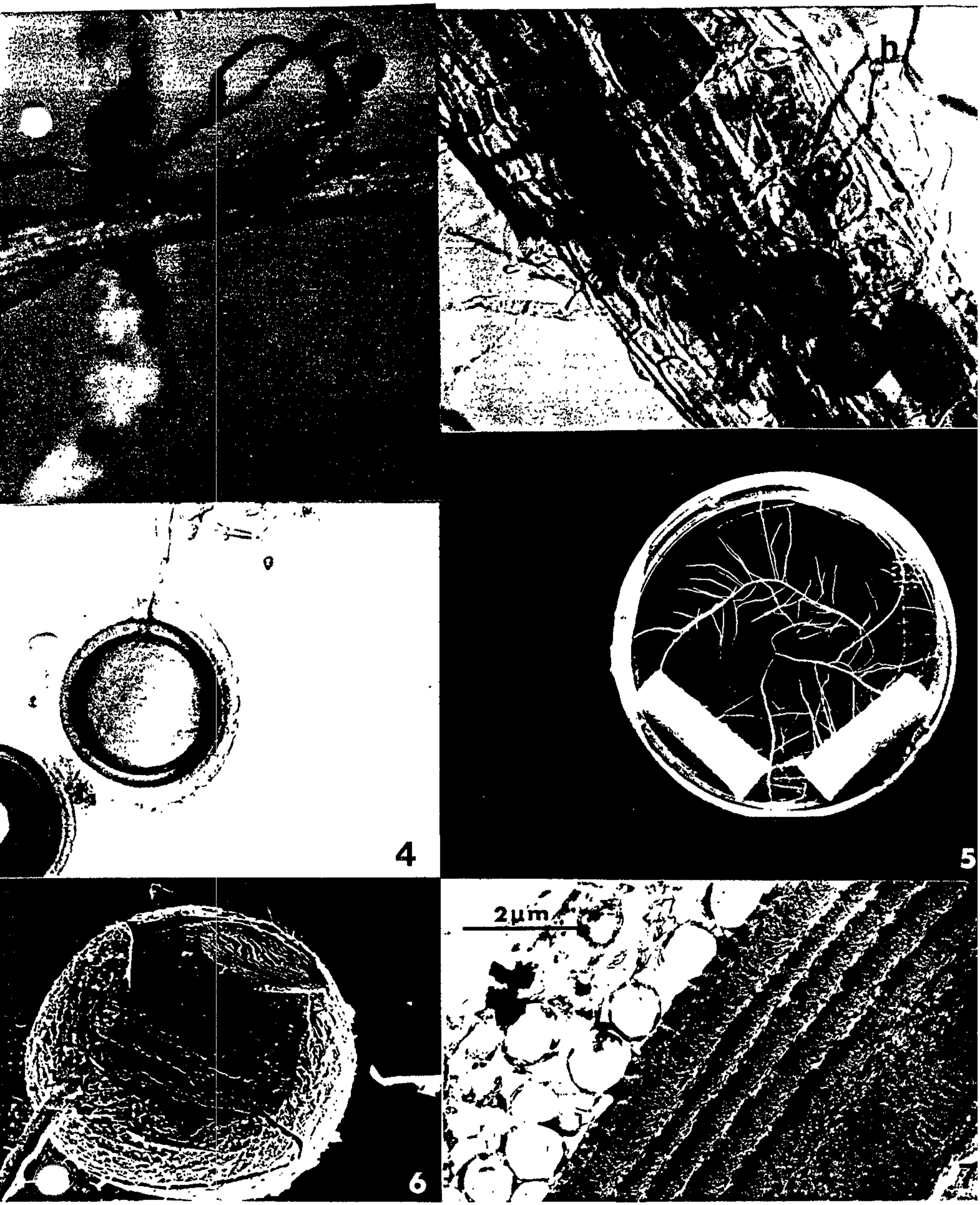
Figure 8:	Potted culture of AMF from ECORC germplasm bank
Figure 9:	AMF culture in screened greenhouses for propagation of strains
Figure 10:	Comparative analysis of biomass yields of radicchio (*Cichorium intybus*) cultivated in the presence of various species of AMF
Figure 11:	Comparative growth after 12 weeks of onion (*Allium cepa*) without mycorrhizal colonization or with colonization by *Glomus intraradices*
Figure 12:	Comparative analysis of biomass yields of the ash (*Fraxinus americana*) cultivated in the presence of various species of AMF
Figure 13:	Experimentation in the field of the benefits of mycorrhization of turf grasses

FIGURE 1

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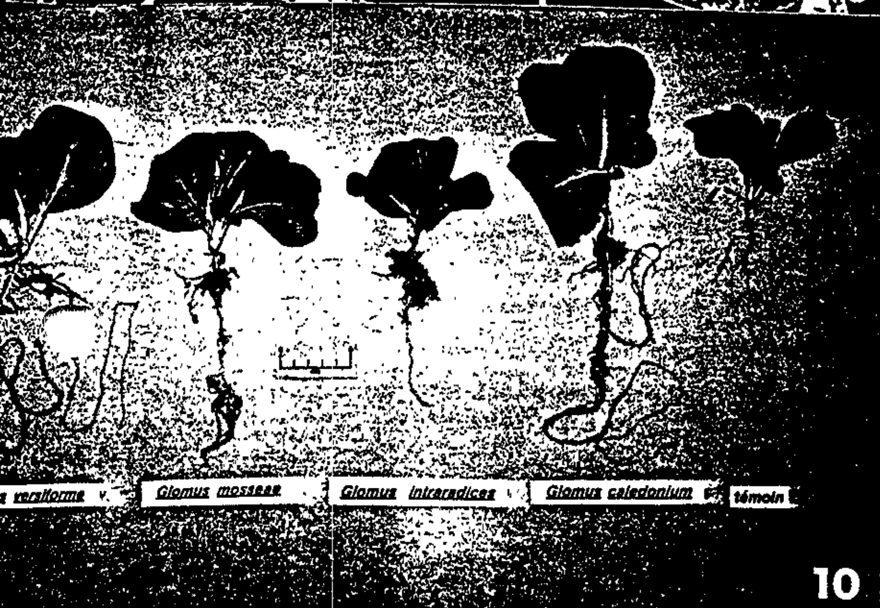
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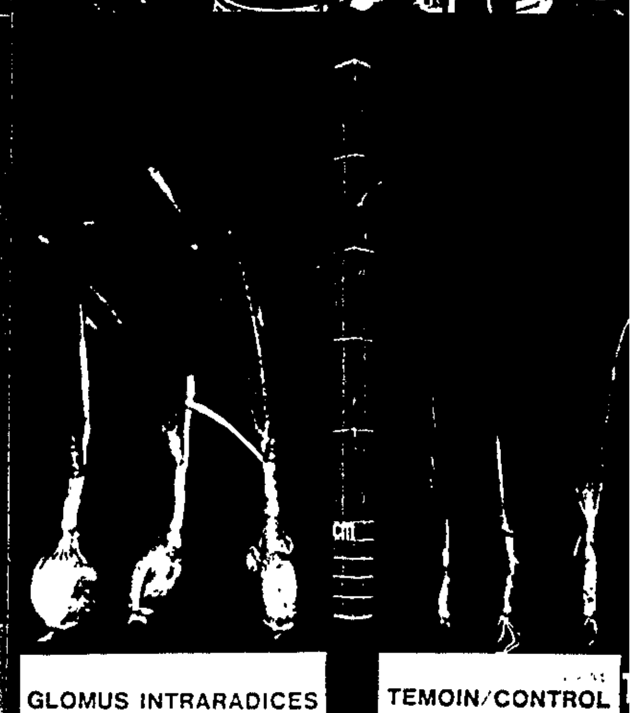
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