Chapter 3: FUNGI AND THEIR HABITAT.

3.1. Principle life forms.

Fungi are heterotrophs. They are unable to manufacture their own food as photosynthetic organisms can and therefore depend on other organisms for their nutrition. Fungi obtain the carbon and energy necessary for their growth from dead or living organic substances through extracellular digestion typically by secreting enzymes into the environment and absorbing the nutrients produced.

Over time, fungi have thrived in many different conditions in aquatic and terrestrial environments. They live under snow, in very hot conditions, in sweet and salty water, in soil, on wood, excrements, dunes and sand beaches, on bryophytes, and many other places.

Classification of fungi according to nutrition

| Saprophytic | Parasitic | Symbiotic |

a).- Saprophytic.

Saprophytic fungi feed on dead or decomposing organic material (sapros = putrefied and phyto = plant). They are the most common fungi and they are essential in the process of humification through fermentation and mineralization of plant remnants, (RAMELLI, A. & BARTOLI, A., 1971).

Fungi can decompose any type of natural organic material and, thanks to their intervention, close the cycle of organic material as this material is transformed into mineral elements that plants need. This cycle is essential in the maintenance of life.

Cycle of degradation of organic material
Sometimes, the distinction between parasitic and saprophytic fungi is not obvious. Some fungi are even classified as semi saprophytic or semi parasitic; which means that they can be saprophytic or parasitic depending on the environmental conditions. An example is *Kuehneromyces mutabilis*, a very efficient fungus which becomes parasitic when it comes in contact with a fragile organism (such as a damaged tree trunk).

**b).- Parasites.**

Parasitic fungi live or colonise animals, plants and other fungi living on their expenses, inflict diseases or even death. Fungi represent 90% of plant parasites and destroy 15% of the world plant production every year. Thanks to the high number of enzymes, toxins and antibiotics that they produce, they are able to overcome the defences of the attacked organisms.

**c).- Symbiotic or mycorrhizal.**

The mycelium feeds on organic compounds in the soil substrate through decomposition or through particular associations with plants (trees, grasses, ferns, algae…). The relation between fungi and the plant roots constitute a particular type of symbiosis called mycorrhizal symbiosis.

Through mycorrhizal symbiosis the fungus obtains soluble sugars, from the plant roots; in exchange it enables the plant to increase the intake of some mineral elements (e.g. phosphorus) and its capacity to retain water.

One particular mycorrhizal species often associates with various plant species some species however are host specific. *Sepultaria sunmeriana* presents an example, it associates exclusively with cedar; *Boletus elegans*, associates with larch and the genus *Leccinum* associates with most birch species. *Amanita muscaria* is one of the non-host specific or “cosmopolitan” species. It can be found under a variety of trees including pines, birch, chestnut, cistus.

**3.2. Rhizosphere.**

The rhizosphere is a micro-zone at the root-soil interface that is under the influence of the plant root. The chemical and biological characteristics of the rhizosphere are apparent in a portion of only 1 mm thick.

<table>
<thead>
<tr>
<th>Two characteristics of the rhizosphere stand out:</th>
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<tbody>
<tr>
<td>- The presence of various organisms in higher density than in the “normal” soil.</td>
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<td>- Stability of the soil particles through the mechanical action of the roots and the binding action of exudates discharged by soil organisms.</td>
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In the below-ground environment plant roots and a large variety of micro-organisms (even more numerous in the surroundings of the roots) compete for resources. In relation to the interaction between plants, competition for space, water and food, grows with an increase in proximity between their roots. It has been observed that the interactions between the plants and the other organisms are of great importance in the rhizosphere.

In addition to the products emitted by under-ground micro-organisms, plants also discharge various substances through their roots. Some of these released products have the function to attract and trigger the establishment of colonies of bacteria and symbiotic fungi favourable to the plants.

Within the group of rhizosphere micro-organisms the mycorrhizal fungi are a key component though the regulation of the formation and role of their symbiotic association is influenced by other microbial groups which are important in recycling nutrients and for plant nutrition. Reciprocally, mycorrhizal associations change the quality and quantity of plant exudates and introduce physical and chemical modifications that in return affect the microbial communities of the rhizosphere creating a new equilibrium.

Effect of rhizosphere interactions:

- Activation of propagule germination
- Creation of access points
- Development of associations
3.3. Mycorrhizal associations.

Mycorrhizal fungi form very advanced mutualistic associations (symbioses) with plant roots. The members of this association include fungi from the Basidiomycetes, Ascomycetes and Zygomycetes and the majority of the vascular plants. Very often the mycorrhizal fungus is not able to live without its symbiotic partner.

a). Types of mycorrhizal associations.

According to their structure and morphology mycorrhizae are classified into two groups: ectotrophic and endotrophic mycorrhizae.

The first group is composed of mycorrhizal fungi that surround the root with hyphae forming a hyphal “mantle”. The growth of hyphae within the epidermis of the root is intercellular and forms the so-called network of Hartig.

The endomycorrhizal fungi do not form a mantle around the root but the hyphae penetrate through the epidermis cells. Presently, it has been found necessary to modify this classification and to divide the endomycorrhizal species into the following groups.
Without a doubt, the most common association is the vesicular-arbuscular type (VA). They can be found in all climates and they associate with most of the agriculturally and industrially interesting plants.

**b) Host plants.**

Field investigations showed that plants with mycorrhizal associations are predominant in the majority of natural ecosystems of the world. Trees and plants with ECM associations are important in the majority of habitats.

**3.4. Mycorrhizal fungi.**

The members of the fungal kingdom obtain their nutrients in many ways, decomposition of organic substrate, predation, parasitism, and the participation in mutualistic association. Many soil-dwelling fungi are saprophytic with the ability to digest organic substrates of distinctive levels of complexity, (some enduring very low levels of organic and inorganic substrates). Mycorrhizal fungi are an important component of the soil microflora of many ecosystems, but generally they have limited saprophytic abilities.

It is thought that populations of mycorrhizal fungi occupied the same habitats during millions of years, slowly adapting to the climatic changes. It appears that some of these fungi are distributed world-wide.

The mycorrhizal association relies on three elements: the fungi, plants, and soil, maintaining a balance that should be taken into account when studying these symbiotic relationships.
It is easier to influence or manage ECM associations in plantation-silviculture than to manipulate VAM associations in other systems of cultivation.

The reasons are:

1. The presence of VAM fungi in practically all soil types
2. The capacity of many ECM (not VAM) fungi to be cultivated in sterile cultures
3. Conventional manipulative practices tie in with fungal inoculation in tree nurseries
4. The specificity between host trees and many ECM fungi

3.5. Structure and development of mycorrhizal roots.

It is necessary to understand the structure of non-mycorrhizal root before examining in detail any change made by mycorrhizal associations. The anatomical characteristics of roots also have the potential to control mycorrhizal development.

The distinction between primary and secondary roots as well as various parts of the root is important as they have different functions. The different parts vary in their growth-cycle, vitality period, structural characteristics, etc. as well as in their capacity to obtain water and nutrients, or support mycorrhizal associations.

a) Ectomycorrhizal associations

The ectomycorrhizal associations (ECM) are mutualistic associations between superior fungi and gymnosperms and angiosperms plants. They are systems made up of mycelium with mycorrhizal roots and reproductive or storage structures. The mycorrhizal roots are characterized by a mantle and a Hartig-net; however, sometimes these structures are not well developed.

Ectomycorrhizae are formed where the host-root and associating fungi grow in close proximity and where the climatic conditions are favourable.
1. The hyphae make contact, recognise and adhere to the cells of the epidermis close to the apex of lateral roots.

2. Mycelia proliferate on the root surface and grow to form the mantle.

3. Hyphae penetrate through the epidermis cells and form a network (Hartig-net).

4. Most of the mycorrhizal activity occurs in the youngest cells of root-tips of fine-roots. The hypha of the Hartig-net that can invade deeper, ageing in the older regions of the root-tip.

5. The mantle generally remains on the roots a long time after the associations have ceased their activity.

The ECM roots can be recognized by their exterior and interior hyphal characteristics that are typical of some distinctive fungi. These differences are expressed in the growth of the root and the models of division.

**b) Vesicular-arbuscular associations.**

The VAM associations are hyphae, spores and auxiliary structures formed in the soil, and of hyphae, arbuscules and vesicles formed in the roots. These structures can be used to differentiate and identify fungi, quantify and propagate these associations.

![Distinctive types of mycorrhizae](image1.jpg)
1. The association starts when hyphae respond to the presence of a root growing towards them, by establishing contact and growing along its surface.

2. The penetration of the root occurs when one or more hyphae form an appression between the adjacent cells of the epidermis and penetrate them.

3. One or more hyphae succeed in penetrating the hypodermis and split up in the outer cortex.

4. The hyphae (aseptate) extend in both directions from the point of penetration to form a colony.

5. The arbuscules grow within the cells of the cortex from the sub-apical branches of the internal hyphae.

6. The vesicles are used for storing reserves.

7. In posterior phases, the arbuscules collapse.

Roots with VAM associations look superficially similar to roots without them. The morphology of mycorrhizal associations varies according to the fungal species and is influenced by the root anatomy.

The mycelial system of fungi can produce various structures. Some of them work as organs for storage, dispersion and survival of the fungi in the soil.

Studying ECM fungi in proximity to a host-plant does not ensure that there is an association.

c) Dualistic associations.

Plants from the same genus generally have the same mycorrhizal type (ECM, VAM, etc., or no mycorrhiza) and these associations are generally also maintained in a family. This narrow correlation between plant phylogeny and the mycorrhizal associates has been observed in families with ECM, as well as in non-mycorrhizal species, though there are many exceptions.

Up to this point, not enough is known to be able to predict without doubt which type or types of mycorrhizal associations can occur on a chosen plant, or what could be the functional signification of different associations. Variation in the root structure of many shrubs and trees can simply be an adaptation to environmental conditions.
d) Facultative associations and non mycorrhizal plants

The concept supporting the fact that plants have various levels of dependency on mycorrhizal associations is more and more accepted. Detailed observations of plants in natural ecosystems show differences of intensity and consistence in the mycorrhizal formation.

<table>
<thead>
<tr>
<th>Mycorrhizal plants – compulsory / obligate</th>
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<tbody>
<tr>
<td>It regroups the plants that cannot survive when reaching the stage of reproductive maturity without being associated with underground mycorrhizal fungi of their natural habitats.</td>
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<table>
<thead>
<tr>
<th>Mycorrhizal plants - facultative</th>
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<tbody>
<tr>
<td>Grouping plants that benefit of the associations when growing in less fertile soils.</td>
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<table>
<thead>
<tr>
<th>Non mycorrhizal plants</th>
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<tr>
<td>They have roots that consistently resist the colonisation of the mycorrhizal fungi, at least when they are young and healthy.</td>
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</table>

These observations confirm that the intrinsic properties of roots can restrain mycorrhizal formation. The nutritive levels and other properties of the soil and the dynamics of mycorrhizal organs of propagation can also reduce the mycorrhizal formation, but generally they do not obstruct it completely.

In natural ecosystems, plants with facultative mycorrhizal associations or roots without mycorrhizae are more common in dry humid or cold habitats where the plant productivity is limited by the environmental conditions of the soil, or in disturbed habitats where the inoculation of mycorrhizal fungi is limited.

### 3.6. Effects of arbuscular mycorrhizae on plants.

- Encourage growth.
- Resistance to environmental factors.
- Improve the phosphorous nutrition.
- Increase the nitrogen nutrition.
- Tolerance to calcium and oligo elements.
- Increase water absorption.
- Phytosanitary, protection against parasites.
3.7. Mycorrhizal applications.

An important part of the present research focuses on studying the potential of mycorrhizal associations in sylviculture, agriculture, horticulture. Another aim is to ascertain new edible fungi.

The manipulation of mycorrhizal associations in order to increase plant productivity in plantations, or to help the reestablishment of plants after a great environmental alteration on restoration sites, is the objective of many research projects.

<table>
<thead>
<tr>
<th>Mycorrhizal trees of economic interest can be found in the following families:</th>
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<tbody>
<tr>
<td>Pinaceae</td>
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<td>Fagaceae</td>
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<td>Tiliaceae</td>
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<tr>
<td>Betulaceae</td>
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<td>Salicaceae</td>
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<tr>
<td>Rosaceae</td>
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<tr>
<td>Juglandaceae</td>
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<tr>
<td>Mimosaceae</td>
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<tr>
<td>Ulmaceae</td>
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<tr>
<td>Ericaceae</td>
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</table>

In all these families ectomycorrhizae are permanently present. They sometimes host endomycorrhizae, which prevail mainly in the first months or years of the tree getting replaced by ectomycorrhizae at a later stage.

<table>
<thead>
<tr>
<th>Examples of some of the main genera of mycorrhizal fungi</th>
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<tbody>
<tr>
<td><strong>Ectomycorrhizae</strong></td>
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<tr>
<td>Amanita</td>
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<td>Boletus</td>
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<tr>
<td>Cortinarius</td>
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<tr>
<td>Paxillus</td>
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<tr>
<td>Russula</td>
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<tr>
<td>Rhizopogon</td>
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<tr>
<td>Phallus</td>
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<tr>
<td>Pisolitus</td>
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<tr>
<td>Laccaria</td>
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<tr>
<td>Scleroderma</td>
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<tr>
<td>Tuber</td>
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<tr>
<td>Suillus</td>
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<tr>
<td>Lactarius</td>
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The majority of forests depend on ectomycorrhizae. By occupying poor soils the fungus helps the plant through adaptation and nutrition.

Controlled mycorrhizal inoculation in nurseries enables foresters to reforest land with plants already mycorrhized. The main objective is to improve tree establishment and growth and sometimes to produce carpophores. The mycorrhizae reduce the mortality by limiting the negative effects of relocation, by accelerating the initial growth and the total carbon production of the forest. In adult forest populations, it is impossible, with the present knowledge, to significantly and permanently modify the balance between the mycorrhizal flora and the root.

### Plant nurture systems

- **Bare root**: This is the most simple and economical way when conditions are good.
- **Plants in pots**: Mostly used when conditions are poor.

### Advantages and disadvantages of natural inoculation

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
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</thead>
<tbody>
<tr>
<td>The inoculation does not require any particular equipment or technique</td>
<td>Difficult to obtain pure inoculate</td>
</tr>
<tr>
<td>Insignificant cost</td>
<td>Genetic instability</td>
</tr>
<tr>
<td>Insignificant cost</td>
<td>Risks of pathogenic infections</td>
</tr>
</tbody>
</table>

### Diagram of mycorrhizal inoculation

- **Plants obtained by micro-propagation**
- **Mix of peat and coconut fibre with controlled application of fertilizer**
- **Use of natural inoculates**
- **The same as in the phase of root growth and implantation**
- **Mycorrhized plant with optimal development**
When the necessary quantity of inoculum is obtained, it can be used for the mycorrhizal inoculation.

<table>
<thead>
<tr>
<th>Inoculation techniques in nurseries</th>
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<tbody>
<tr>
<td><strong>Soil disinfection</strong></td>
</tr>
<tr>
<td><strong>Application of the inoculate</strong></td>
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<tr>
<td><strong>Management required</strong></td>
</tr>
</tbody>
</table>

**Factors that affect the development and activity of the mycorrhizae.**

- Light affects mycorrhizal infections. In dark conditions the infection rate decreases drastically. The production of spores decreases of 80%.
- Low temperatures have the same effect.
- Application of nitrogen and phosphoric fertilizers.
- Soil fertility which is linked with the root growth:
  - Rapid growth does not improve mycorrhizal infection.
  - Slow growth does improve mycorrhizal infection.
- Presence of auxin and ethylene (vegetal hormones) helps the formation of mycorrhizae.
- Interaction with other micro organisms of the rhizosphere.
- Azotobacter has a positive influence.
- Pseudomonas has a positive influence.
- Pesticides have a negative influence.
Hydroponic cultures do not give good results because of practical complications.

Aeroponic cultures give better results.

It is difficult to produce a high quality inoculum in huge amounts which is easily applicable and transportable.

Roots are treated and divided into pieces of 1 – 2 mm and used as quality inoculum. Problems:
- They require technology.
- Specialised labour force.
- High price.

Inoculum production has various limitations

Necessary requirements for inocula

Technical improvement in the production stage

Registration of the inoculum

Control of quality

Transfer of technologies -> viable forms of inoculum exploitation by SMEs.

Native fungi.

Natural and profitable substrates.

Ecological criteria.

Bio security.

Bioethics.

Follow the norms of each country.

Use of specific protocols.

Guarantee the protection of ecosystems.