

## A study of the macrofungal community in the beech forest of Altube (Basque Country, Northern Spain)

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**Résumé** – Un total de cinq parcelles, chacune de 800 m<sup>2</sup>, ont été délimitées afin d'étudier la diversité des macromycètes dans la forêt atlantique et acidophile de hêtre (*Saxifraga hirsutae*-*Fagetum sylvaticae*) d'Altube (pays Basque). Au total 125 espèces ont été trouvées : 119 Basidiomycota et 6 Ascomycota ; 70 d'entre elles sont mycorrhiziennes, 32 saprophytes lignicoles, 19 saprophytes humicoles et 4 parasites. La forêt de hêtre d'Altube est caractérisée par un nombre élevé d'espèces généralistes telles que des *Amanita rubescens*, *Cortinarius cinnamomeus*, *Megacollybia platyphylla*, *Rhodocollybia butyracea*, ou *Russula cyanoxantha*, et d'espèces acidophiles comme *Amanita citrina*, *Cortinarius purpurascens*, *Craterellus tubaeformis*, *Lactarius chrysorrheus*, *Mycena pura*, *Russula densifolia* ou *R. nigricans* ; alors que les espèces mycorrhiziennes présentant une préférence pour ces écosystèmes n'étaient pas très fréquentes, comme *Lactarius blennius*, *Cortinarius cinnabarinus*, *Hebeloma radicosum*, *Lactarius camphoratus*, *Russula densifolia*, *R. nobilis* ou *Tricholoma ustale*. *Xerula radicata* et *Oudemansiella mucida*, liées au *Fagus*, étaient fréquentes dans cette forêt, alors que *Marasmius alliaceus* et *Mycena crocata*, au contraire étaient absentes ou très rares. L'aspect des espèces est discuté en liaison avec les différents écosystèmes et la nature des sols. Les précipitations et la température minimum pendant la saison de croissance semble avoir une influence sur la production de carpophores mais les facteurs microclimatiques et d'autres variables doivent être pris en considération de la même manière.

### Forêt atlantique / Macromycètes / Mycoenologie / groupes trophiques

**Abstract** – A total of five plots, each 800 m<sup>2</sup>, were delimited in order to study the macrofungal diversity in the managed atlantic, acidophilous beech forest (*Saxifraga hirsutae*-*Fagetum sylvaticae*) of Altube (Basque Country). A total of 125 species of macrofungi were found, 119 Basidiomycota and 6 Ascomycota, 70 of them mycorrhizal species, 32 lignicolous saprotrophs, 19 humicolous saprotrophs and 4 parasitic. Beech forest of Altube is characterized by a high number of generalist species such as *Amanita rubescens*, *Cortinarius cinnamomeus*, *Megacollybia platyphylla*, *Rhodocollybia butyracea*, or *Russula cyanoxantha* and acidophilous species like *Amanita citrina*, *Cortinarius purpurascens*, *Craterellus tubaeformis*, *Lactarius chrysorrheus*, *Mycena pura*, *Russula densifolia* or *R. nigricans*; whereas mycorrhizal species with preference for those ecosystems were not very frequent or abundant, such as *Lactarius blennius*, *Cortinarius cinnabarinus*, *Hebeloma radicosum*, *Lactarius camphoratus*, *Russula densifolia*, *R. nobilis* or *Tricholoma ustale*. The species *Xerula radicata* and *Oudemansiella mucida*, both associated with *Fagus*, were frequent and abundant in this forest, but *Marasmius alliaceus* and *Mycena crocata*, on the contrary were absent or very scarce. The appearance of species is discussed and has also been related to different ecosystems and soil conditions. Precipitation and minimum temperature during the growth season seems to have an influence on sporocarp production but microclimatological factors and other variables must be considered in the same way.

### Beech forest / macrofungal fructification/ mycoenology/ trophic groups

## INTRODUCTION

Beech forests are widespread and represent the potential natural vegetation of many areas of the lowlands of NW and NC Europe (up to S Sweden) and the mountains of C, S, and E Europe (Ellenberg 1996, Jahn 1991). The Southwestern boundary of the beech forest is in the North of the Iberian Peninsula; in consequence, the beech forests of the Basque Country are in this S-W limit of the distribution, mainly above 500-600m. The acidophilous beech forest of the *Saxifraga hirsutae-Fagetum sylvaticae* community is the most common in the Basque Country, and it is one of the (sub)natural woodland vegetation types of temperate Europe with European Community interest (9120; Annex I of the 92/43/EEC Habitats Directive).

Conservation and protection of biodiversity has become one of the main tasks for future forest management. The first step in the conservation and management of natural life is the knowledge of all the components of the ecosystem. Fungi play a fundamental role in forest ecosystems (Christensen, 1989; Bruns *et al.*, 2002), but a decrease in sporocarp production of many fungi, or changes in species composition of macrofungi in temperate forest, has been observed in many parts of Europe (Arnolds, 1989, 1991; Fellner, 1993; Rühling & Tyler, 1990). Those changes in macrofungal communities have been attributed to different reasons, such as habitat destruction, soil acidification or eutrophication by atmospheric pollution (Arnolds, 1989; Fellner, 1993).

Fungi are a large group of very diverse species, which makes it difficult to study all the fungi together, since the methodology used in each case is very different. In this study, only the epigeous macrofungi were considered; i.e. those fungi that develop an aboveground conspicuous fruit body.

The studies of macrofungal communities together with chorological studies are essential for the knowledge of the fungal composition of ecosystems. They can be considered the first step in all the processes involved in the conservation and management of macrofungi. Macrofungal community research is based on fruit bodies; the qualitative and quantitative occurrence of sporocarps is studied. As suggested by Arnolds (1981, 1988) this approach has some advantages and some limitations. Among them can be mentioned the omission of hypogeous sporocarps in many studies or the fact that the number of sporocarps is not necessarily representative of the abundance of vegetative mycelium. Nevertheless, this kind of study is, for the present, the best way to study macrofungi in a biocoenological context and may provide valuable information on the ecology of individual species and contribute, as mentioned above, to the knowledge of the biodiversity of an ecosystem.

The study of macrofungal communities started mainly with Haas in 1932, and until now a lot of research has been developed (Bohus & Babos, 1967; Lisiewska, 1974; Arnolds, 1981, 1988, 1989; Jansen, 1981; De Dominicis & Barluzzi, 1983; Barluzzi *et al.*, 1987; Perini *et al.*, 1989; Tyler, 1989, 1992; Keizer, 1994; Adamczyk, 1995, 1996; Salerni *et al.*, 2001; Richard *et al.*, 2004). Most of these works have been carried out in the north of Europe. However, in the Iberian Peninsula these types of studies are scarce (Losa Quintana, 1974; Losa Quintana *et al.*, 1980; García Bona, 1978, 1982; Ruiz Ferro *et al.*, 1993; Sánchez *et al.*, 1995). In the Basque Country, even though mycological societies are very popular, studies on macrofungal communities are isolated (Salcedo *et al.*, 1998). Chorological data on macrofungi are, on the contrary, numerous but have been very scattered until now. Recently all those data have been compiled by Salcedo (2003).

Most of the mycocoenological research on beech forests has been conducted in Northern Europe (Lisiewska, 1974; Tyler, 1984; Arnolds *et al.*, 1994; Keizer, 1994; Adamczyk, 1995, 1996), but more meridional information is necessary for the best understanding of macrofungi communities in those ecosystems. This study has been the first assay of this type of work done in the Basque Country. The main aim of the research was to study the macrofungal community of the beech forest of Altube (Basque Country) that belongs to the *Saxifrago hirsutae-Fagetum sylvaticae* community, and add new data to the knowledge of the diversity of macrofungi of the before mentioned ecosystem.

## MATERIALS AND METHODS

### Study area

This study was conducted in the beech forest of Altube, which is on the border of the provinces of Biscay and Alava on the northern slope of the watershed mountains of the Basque Country (Northern Spain). It has around 3500 ha and is next to the Natural Park of Gorbea. It is located in the Atlantic European province, with a temperate oceanic bioclimate, that means mild winters and warm summers (Berastegi *et al.*, 1997). The annual mean temperature is around 11°C and the precipitation around 1655 mm.

The potential vegetation corresponds to an oligotrophic beech forest that belongs to the association *Saxifrago hirsutae-Fagetum sylvaticae* Br.-Bl. *em.* Rivas-Martínez, Bascos, T.E. Díaz, Fernández-González and Loidi (Rivas-Martínez *et al.*, 2002). Nevertheless, it must be assumed that the considered forest was planted and then naturally developed, so it is a seminatural forest where the average age of the trees is 50-60 years. Dead wood in managed stands typically consists only of small twigs and branches and short stumps (Kruys *et al.*, 1999), which become the main wood resource in the studied beech forest consequence of continued removal of wood (small clearcuts, wood for fire). However, coarse woody debris (CWD) and fine wood debris are of vital importance to wood-decaying fungi (Nordén *et al.*, 2004, Vasiliaskas *et al.*, 2004).

### Sampling design

In order to register the variability of mycocoenoses growing in a plant community and in accordance with Arnolds (1992) five permanent plots (20 m × 40 m) were delimited in the spring of 1995, all of them chosen randomly. The plot size used in previous works carried out in beech forests vary from 400 m<sup>2</sup> (Lisiewska, 1974; Adamczyk, 1995) to 1000 m<sup>2</sup> (Gyosheva, 1994). After a first estimation, 800 m<sup>2</sup> plot size was chosen for this beech forest. One of the plots (control plot), however, became 1600 m<sup>2</sup> since we decided to determine the representative sampling area for macrofungi in beech forest. In that plot the sampling area was gradually duplicated starting from 25 m<sup>2</sup> until 1600 m<sup>2</sup> (Fig. 1).

All the plots were located on the northwestern slope at different altitudes; plots 1, 2 and 3 at a height of 800-850 m and plots 4 and 5 at 450 m. The number of trees and stumps, diameter of trees, herb and moss cover and pH (KCl) were measured in each plot (Table 1). The pH was measured in four soil samples taken randomly at 10 cm depth in each plot and then the average

was calculate for each. The pH is moderately to highly acidic ranging from 3.73 to 4.45. The structure of the stands is very simple with an only 50-60 year-old trees homogeneous layer. Nevertheless the structure of plots 1, 2 and 3 differ from plots 4 and 5. The former have higher tree and stump density, and a smaller diameter of trees than plots 4 and 5. Forest floor is completely bare of herb and mosses in all plots, but plot 5 has a notable layer of fallen leaves covering the soil. The presence of oak is notable around plot 4 and in plot 5, both located in the upper limit of the distribution of the oak forests of *Quercus robur* L.

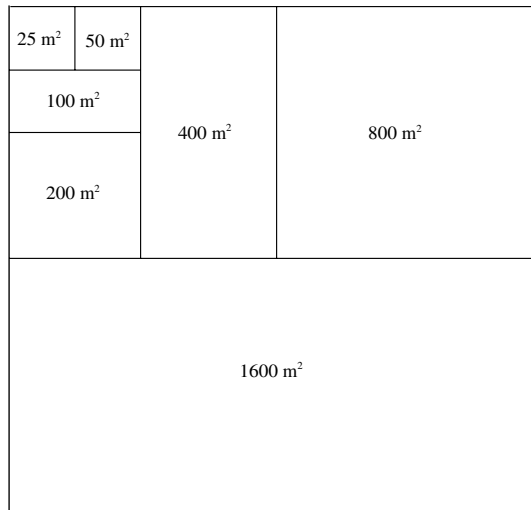


Fig. 1. Control plot. Gradual duplication of the sampling area.

Table 1. Environmental and structural parameters of the studied plots.

| Altitude<br>(m) | N° of<br>trees | Moss and<br>Herb cover<br>(%) | N° of<br>stumps | PH<br>(KCl) | Tree-diameter (cm)  |                          |                    |                      |                 |      |
|-----------------|----------------|-------------------------------|-----------------|-------------|---------------------|--------------------------|--------------------|----------------------|-----------------|------|
|                 |                |                               |                 |             | <i>F. sylvatica</i> | <i>Fagus</i><br>seedling | <i>C. monogyna</i> | <i>I. aquifolium</i> | <i>Q. robur</i> |      |
| Plot 1          | 850            | 55                            | 0               | 28          | 3.97 ± 0.22         | 23.18                    |                    |                      |                 |      |
| Plot 2          | 850            | 39                            | 0               | 22          | 4.01 ± 0.04         | 25.13                    |                    |                      |                 |      |
| Plot 3          | 850            | 39                            | 0               | 23          | 4.12 ± 0.22         | 27.36                    |                    |                      |                 |      |
| Plot 4          | 450            | 21                            | 5               | 2           | 4.02 ± 0.25         | 52.07                    |                    | 3                    | 16              |      |
| Plot 5          | 450            | 33                            | 30              | 1           | 4.14 ± 0.15         | 56.67                    | 3.5                | 10.67                | 3.29            | 46.4 |

### Sampling and data analysis

Macrofungi are defined as those fungi forming reproductive structures, which are individually visible to the naked eye, that is, larger than about 1 mm (Arnolds, 1981). This limit is not consistently applied in other works (Villeneuve *et al.*, 1991) and in this study, were mainly considered those species with epigeous fruitbodies bigger than 1cm. Within the Basidiomycota all morphological groups, excluding most of the fully resupinate corticioid fungi, were included; stromatic Ascomycota were excluded, with the exception of species easy to recognize.

The sampling was carried out from September to December during 1995 and 1996, that is, in the period of maximum fructification of macrofungi in our

territory. The plots were visited weekly and on each visit the macrofungal species were recorded and counted. Although some data shown that removal of carpophores does not adversely affect the future production (Egli *et al.*, 2006) we wanted to alter the ecosystems as little as possible. Carpophores were only removed for herbarium collection and the correct identification of some species. In order not to count them twice, we marked each one with half a toothpick. General works, Moser (1983, 1986), Courtecuisse and Duhem (1994), Breitenbach and Kränzlin (1995), and other more specific ones, Merlo *et al.* (1980), Maas Geesteranus (1992), Bas *et al.* (1988-1995) were used for identification. For the abbreviation of the authors' names we have followed Brummitt & Powell (1992).

After two years of sampling the number of species and carpophores, the Shannon diversity index ( $H'$ ) were determined for each plot (Table 2).

$$H = -\sum_{i=1}^n p_i \ln p_i$$

Species abundance (A) and frequency (Fr) were calculated as well (Table 2). Abundance was considered as the total number of fruit bodies counted after two years, and frequency as the sum of presence of a species in each plot. Those macrofungi whose carpophores are uncountable were recorded with "+". All taxa were assigned to a trophic group: mycorrhizal species (M), humicolous saprotrophs (Sh), lignicolous saprotrophs (Sw) and parasitic (P). Pearson's  $\chi^2$  was conducted to see differences in trophic groups between plots.

To see the influence of the climatology on the fructification of macrofungi, maximum temperature, minimum temperature and precipitation data were obtained from the Agriculture School of Murgia (5 km from the sampling area and at a height of 600 m).

## RESULTS AND DISCUSSION

Sampling of the control plot demonstrated that the number of species did not increase above 800 m<sup>2</sup> and most of the species were recorded in an area of 400 m<sup>2</sup> (Fig. 2). Attending to growth patterns of the fungi we consider that an area of 800 m<sup>2</sup> is optimum for sampling macromycetes in beech forests.

Many works pointed out that a period of 3-5 years, or even 2, are enough to register a reasonably complete list of the macrofungal species in a forest ecosystem (Kalamees, 1968; Richardson, 1970; Arnolds, 1988; Perini *et al.*, 1989; Vogt *et al.*, 1992). Nevertheless, both number of years and frequency of visits must be taken into account in this kind of studies (Arnolds, 1981). Arnolds (1992) estimates that sampling of 500-1000 m<sup>2</sup> plots visited every fortnight during three years, once per month during four years or once every two months during 5 years are quite enough to establish the fungal composition of a forest ecosystem. Other studies reflect that only 54% of the total species registered in 13 years were recorded after four years of sampling (Barkman, 1976). In longer studies it has been proved that new species appear after 21 years of monitoring (Straatsma *et al.*, 2001).

Table 2. Synthesis of mycocoenological surveys.

|     | <i>Plot number</i>   | <i>1</i> | <i>2</i> | <i>3</i> | <i>4</i> | <i>5</i> | <i>A</i> | <i>Fr</i> |
|-----|--|----------|----------|----------|----------|----------|----------|-----------|
|     | Altitude   | 850      | 850      | 850      | 450      | 450      |          |           |
|     | H'   | 3.17     | 3.56     | 3.14     | 3.85     | 4.08     |          |           |
| TG  | Number of species  | 32       | 46       | 34       | 61       | 78       |          |           |
|     | <i>Plot number</i>   | <i>1</i> | <i>2</i> | <i>3</i> | <i>4</i> | <i>5</i> | <i>A</i> | <i>Fr</i> |
| M   | <i>Amanita rubescens</i> (Pers. : Fr.) Gray                    | 6        | 8        | 5        | 28       | 1        | 48       | 5         |
| M   | <i>Cortinarius livido-ochraceus</i> (Berk.) Berk.              | 10       | 16       | 45       | 1        | 1        | 73       | 5         |
| Sh  | <i>Gymnopus peronatus</i> (Bolton) Antonín, Halling & Noordel. | 11       | 11       | 41       | 21       | 37       | 121      | 5         |
| Sw  | <i>Hypholoma fasciculare</i> (Huds.) Quél.                     | 45       | 6        | 82       | 8        | 3        | 144      | 5         |
| Sh  | <i>Mycena pura</i> (Pers. : Fr.) P.Kumm.                       | 19       | 6        | 2        | 97       | 48       | 172      | 5         |
| Sh  | <i>Rhodocollybia butyracea</i> (Bull.) Lennox                  | 78       | 126      | 11       | 22       | 46       | 283      | 5         |
| M   | <i>Russula cyanoxantha</i> (Schaeff.) Fr.                      | 8        | 15       | 9        | 4        | 1        | 37       | 5         |
| M   | <i>Russula densifolia</i> Gillet                               | 2        | 1        | 1        | 1        | 1        | 6        | 5         |
| M   | <i>Russula nigricans</i> (Bull.) Fr.                           | 23       | 3        | 12       | 27       | 6        | 71       | 5         |
| Sw  | <i>Xerula radicata</i> (Rehlan. : Fr.) Dörfelt                 | 60       | 49       | 74       | 35       | 13       | 231      | 5         |
| M   | <i>Amanita citrina</i> (Schaeff.) Pers.                        | 1        | 3        |          | 3        | 40       | 47       | 4         |
| Sh  | <i>Mutinus caninus</i> (Huds. : Pers.) Fr.                     | 1        | 9        |          | 2        | 1        | 13       | 4         |
| P   | <i>Oudemansiella mucida</i> (Schrad. : Fr.) Höhn.              | 4        | 20       |          | 25       | 36       | 85       | 4         |
| M   | <i>Russula ochroleuca</i> Pers.                                |          | 2        | 114      | 1        | 2        | 119      | 4         |
| Sw  | <i>Stereum hirsutum</i> (Willd. : Fr.) Gray                    | +        | +        |          | +        | +        | +        | 4         |
| Sw  | <i>Stereum ochraceoflavum</i> (Schwein.) Ellis                 | +        | +        | +        | +        |          | +        | 4         |
| Sw  | <i>Stereum ostrea</i> (Nees : Fr.) Fr.                         |          | +        | +        | +        | +        | +        | 4         |
| M   | <i>Xerocomus chrysenteron</i> (Bull.) Ouél.                    | 13       |          | 1        | 22       | 1        | 37       | 4         |
| M   | <i>Cortinarius cinnamomeus</i> (L. : Fr.) Fr.                  |          |          | 4        | 17       | 66       | 87       | 3         |
| M   | <i>Cortinarius infractus</i> (Pers. : Fr.) Fr.                 |          | 6        |          | 5        | 4        | 15       | 3         |
| M   | <i>Cortinarius purpurascens</i> Fr.                            |          | 3        |          | 1        | 3        | 7        | 3         |
| M   | <i>Cortinarius stillatitius</i> Fr.                            |          |          | 2        | 1        | 4        | 7        | 3         |
| Sh  | <i>Gymnopus dryophilus</i> (Bull.) Murrill                     | 1        | 3        |          |          | 1        | 5        | 3         |
| Sw? | <i>Hebeloma radicosum</i> (Bull. : Fr.) Ricken                 |          | 10       |          | 1        | 7        | 18       | 3         |
| M   | <i>Laccaria amethystina</i> (Huds.) Cooke                      |          |          | 2        | 1        | 15       | 18       | 3         |
| M   | <i>Laccaria laccata</i> var. <i>pallidifolia</i> (Peck) Peck   |          |          | 3        | 9        | 14       | 26       | 3         |
| Sw  | <i>Megacollybia platyphylla</i> (Pers. : Fr.) Kotl. & Pouzar   |          |          | 1        | 1        | 5        | 7        | 3         |
| Sh  | <i>Psathyrella</i> sp.   | 20       |          | 2        | 10       |          | 32       | 3         |
| M   | <i>Russula decipiens</i> (Singer) Kühner & Romagn.             |          | 4        |          | 1        | 1        | 6        | 3         |
| M   | <i>Russula lepida</i> Fr.                                      | 1        | 3        | 1        |          |          | 5        | 3         |
| M   | <i>Russula nobilis</i> Velen.                                  |          | 1        |          | 49       | 23       | 73       | 3         |
| M   | <i>Xerocomus badius</i> (Fr. : Fr.) Gilb.                      |          | 1        |          | 1        | 1        | 3        | 3         |
| M   | <i>Amanita phalloides</i> (Vaill. : Fr.) Link                  |          |          |          | 2        | 1        | 3        | 2         |
| P   | <i>Armillaria mellea</i> (Vahl : Fr.) P.Kumm.                  |          | 37       |          | 5        |          | 42       | 2         |
| Sw  | <i>Bjerkandera adusta</i> (Willd. : Fr.) P.Karst.              |          | +        | +        |          |          | 0        | 2         |
| M   | <i>Boletus edulis</i> Bull. : Fr.                              |          | 1        | 1        |          |          | 2        | 2         |
| Sw  | <i>Calocera cornea</i> (Batsch : Fr.) Fr.                      |          | +        |          |          | +        | +        | 2         |
| M   | <i>Clavulina cristata</i> (Holmsk. : Fr.) J.Schröt.            |          |          |          |          | +        | +        | 2         |
| Sh  | <i>Clitocybe nebularis</i> (Batsch : Fr.) P.Kumm.              |          |          |          | 14       | 6        | 20       | 2         |
| M   | <i>Cortinarius duracinus</i> Fr.                               |          |          | 1        | 1        |          | 2        | 2         |

Table 2. Synthesis of mycocoenological surveys.

|    | Plot number  | 1 | 2 | 3 | 4  | 5   | A   | Fr |
|----|--|---|---|---|----|-----|-----|----|
| M  | <i>Cortinarius hinnuleus</i> (With.) Fr.                   | 2 | 3 |   |    |     | 5   | 2  |
| M  | <i>Craterellus cornucopioides</i> (L. : Fr.) Pers.         |   |   |   | 37 | 102 | 139 | 2  |
| M  | <i>Craterellus tubaeformis</i> (Fr.) Quéf.                 |   |   | 1 |    | 1   | 2   | 2  |
| Sw | <i>Crepidotus variabilis</i> (Pers. : Fr.) P.Kumm.         |   |   |   | +  | +   | +   | 2  |
| M  | <i>Entoloma nidorosum</i> (Fr.) Quéf.                      |   |   | 1 |    | 1   | 2   | 2  |
| Sw | <i>Exidia thuretiana</i> (Lév.) Fr.                        |   |   |   | +  | +   | +   | 2  |
| M  | <i>Hydnum repandum</i> L. : Fr.                            |   | 5 |   |    | 7   | 12  | 2  |
| Sw | <i>Hymenoscyphus calyculus</i> (Sowerby : Fr.) W.Phillips  | + | + |   |    |     | +   | 2  |
| M  | <i>Lactarius blennius</i> (Fr.) Fr.                        |   |   |   | 7  | 5   | 12  | 2  |
| M  | <i>Lactarius piperatus</i> (L. : Fr.) Pers.                |   |   | 1 |    | 1   | 2   | 2  |
| M  | <i>Lactarius vellereus</i> (Fr. : Fr.) Fr.                 |   |   | 1 |    | 3   | 4   | 2  |
| Sh | <i>Lepista flaccida</i> (Sowerby : Fr.) Pat.               |   |   |   | 30 | 1   | 31  | 2  |
| Sh | <i>Lycoperdon perlatum</i> Pers. : Pers.                   |   |   |   | 6  | 30  | 36  | 2  |
| Sw | <i>Mycena polygramma</i> (Bull. : Fr.) Gray                |   |   | 6 |    | 30  | 36  | 2  |
| Sh | <i>Mycena</i> sp.  |   |   | 2 |    | 2   | 4   | 2  |
| Sw | <i>Plicaturopsis crispa</i> (Pers. : Fr.) D.A.Reid         | + | + |   |    |     | +   | 2  |
| Sw | <i>Pluteus cervinus</i> (Schaeff. : Fr.) P.Kumm.           | 1 | 1 |   |    |     | 2   | 2  |
| M  | <i>Rozites caperata</i> (Pers. : Fr.) P.Karst.             | 4 | 6 |   |    |     | 10  | 2  |
| M  | <i>Russula foetens</i> Pers. : Fr                          | 1 |   |   |    | 9   | 10  | 2  |
| M  | <i>Russula krombholzii</i> Shaffer                         |   |   |   | 4  | 5   | 9   | 2  |
| Sw | <i>Trametes hirsuta</i> (Wulfen : Fr.) Pilát               |   | + |   | +  |     | +   | 2  |
| Sw | <i>Trametes versicolor</i> (L. : Fr.) Pilát                | + | + |   |    |     | +   | 2  |
| M  | <i>Tricholoma sciodes</i> (Pers.) C.Martín                 | 3 |   |   |    | 2   | 5   | 2  |
| M  | <i>Tricholoma sulphureum</i> (Bull. : Fr.) P.Kumm.         |   |   |   | 2  | 89  | 91  | 2  |
| M  | <i>Tricholoma ustale</i> (Fr. : Fr.) P.Kumm.               |   |   |   | 5  | 4   | 9   | 2  |
| Sw | <i>Xylaria hypoxylon</i> (L. : Fr.) Grev.                  | + | + |   |    |     | +   | 2  |
| Sh | <i>Agaricus sylvicola</i> (Vittad.) Peck                   |   |   |   |    | 2   | 2   | 1  |
| M  | <i>Amanita pantherina</i> (DC. : Fr.) Krombh.              |   |   |   |    | 4   | 4   | 1  |
| M  | <i>Amanita porphyria</i> (Alb. & Schwein. : Fr.) Mlady     |   |   |   |    | 3   | 3   | 1  |
| M  | <i>Amanita spissa</i> (Fr.) P.Kumm.                        |   |   |   | 8  |     | 8   | 1  |
| M  | <i>Amanita vaginata</i> (Bull. : Fr.) Vittad.              |   |   |   |    | 1   | 1   | 1  |
| Sw | <i>Bisporrella citrina</i> (Batsch : Fr.) Korf & C.W.Carp. |   |   | + |    |     | +   | 1  |
| M  | <i>Boletus erythropus</i> Fr.                              |   |   |   | 1  |     | 1   | 1  |
| M  | <i>Boletus pinophilus</i> Pilát & Dermek                   |   | 1 |   |    |     | 1   | 1  |
| M  | <i>Cantharellus pallens</i> Pilát                          |   |   | 5 |    |     | 5   | 1  |
| M  | <i>Clavulinopsis helvola</i> (Pers. : Fr.) Corner          |   |   |   |    | +   | +   | 1  |
| Sh | <i>Clitocybe gibba</i> (Pers. : Fr.) P.Kumm.               |   |   |   |    | 58  | 58  | 1  |
| Sw | <i>Coprinus micaceus</i> (Bull. : Fr.) Fr.                 |   | 4 |   |    |     | 4   | 1  |
| M  | <i>Cortinarius anserinus</i> (Vel.) Rob.Henry              |   |   |   | 1  |     | 1   | 1  |
| M  | <i>Cortinarius caesiocyaneus</i> Britzelm.                 |   |   |   |    | 5   | 5   | 1  |
| M  | <i>Cortinarius cinnabarinus</i> Fr.                        |   |   |   |    | 9   | 9   | 1  |
| M  | <i>Cortinarius cyanites</i> Fr.                            |   |   |   |    | 17  | 17  | 1  |
| M  | <i>Cortinarius fulmineus</i> Fr.                           |   |   |   |    | 9   | 9   | 1  |
| M  | <i>Cortinarius multiformis</i> (Fr.) Fr.                   |   |   |   |    | 3   | 3   | 1  |
| M  | <i>Cortinarius trivialis</i> J.E.Lange                     |   |   |   |    | 1   | 1   | 1  |
| Sh | <i>Cystoderma jasonis</i> (Cooke & Massal.) Harmaja        |   |   |   | 3  |     | 3   | 1  |

Table 2. Synthesis of mycocoenological surveys.

|    | Plot number   | 1 | 2 | 3 | 4  | 5  | A  | Fr |
|----|---|---|---|---|----|----|----|----|
| Sw | <i>Dasyscyphus niveus</i> (Hedw. : Fr.) Sacc.                     |   | + |   |    |    | +  | 1  |
| Sw | <i>Datronia mollis</i> (Sommerf. : Fr.) Donk                      | + |   |   |    |    | +  | 1  |
| Sw | <i>Exidia glandulosa</i> (Bull. : Fr.) Fr.                        |   |   |   | +  |    | +  | 1  |
| P  | <i>Gymnopus fusipes</i> (Bull.) Gray.                             |   |   |   | 10 |    | 10 | 1  |
| M  | <i>Hebeloma crustuliniforme</i> (Bull.) Quéf.                     |   | 2 |   |    |    | 2  | 1  |
| M  | <i>Hebeloma sinapizans</i> (Paulet : Fr.) Gillet                  |   |   |   |    | 5  | 5  | 1  |
| M  | <i>Hydnum rufescens</i> Pers. : Fr.                               |   |   |   |    | 15 | 15 | 1  |
| Sh | <i>Hygrophoropsis aurantiaca</i> (Wulfen : Fr.) Maire             |   |   | 1 |    |    | 1  | 1  |
| M  | <i>Hygrophorus eburneus</i> (Bull. : Fr.) Fr.                     |   |   |   |    | 17 | 17 | 1  |
| Sw | <i>Hypoxylon fragiforme</i> (Pers. : Fr.) J.Kickx                 |   | + |   |    |    | +  | 1  |
| M  | <i>Lactarius acerrimus</i> Britzelm.                              |   |   |   |    | 2  | 2  | 1  |
| M  | <i>Lactarius acris</i> (Bolton : Fr.) Gray                        |   |   |   |    | 2  | 2  | 1  |
| M  | <i>Lactarius aurantiacus</i> (Pers. : Fr.) Gray                   |   |   |   | 5  |    | 5  | 1  |
| M  | <i>Lactarius camphoratus</i> (Bull.) Fr.                          |   |   |   | 4  |    | 4  | 1  |
| M  | <i>Lactarius chrysorrheus</i> Fr.                                 |   |   |   |    | 41 | 41 | 1  |
| M  | <i>Lactarius hepaticus</i> Plowr.                                 |   |   |   | 3  |    | 3  | 1  |
| M  | <i>Lactarius quietus</i> (Fr.) Fr.                                |   |   |   |    | 1  | 1  | 1  |
| M  | <i>Leccinum scabrum</i> (Bull. : Fr.) Gray                        |   |   |   |    | 3  | 3  | 1  |
| Sh | <i>Lepiota clypeolaria</i> (Bull.) Quéf.                          |   |   |   |    | 6  | 6  | 1  |
| M  | <i>Lepista nuda</i> (Bull. : Fr.) Cooke                           |   |   |   |    | 20 | 20 | 1  |
| Sh | <i>Lepista panaeolus</i> (Fr.) P.Karst.                           |   |   |   |    | 1  | 1  | 1  |
| Sh | <i>Mycena abramsii</i> (Murrill) Murrill                          | 3 |   |   |    |    | 3  | 1  |
| Sw | <i>Mycena crocata</i> (Schrad. : Fr.) P.Kumm.                     |   |   |   | 1  |    | 1  | 1  |
| P  | <i>Nyctalis parasitica</i> (Bull. : Fr.) Singer                   |   |   |   | 3  |    | 3  | 1  |
| Sh | <i>Otidea alutacea</i> (Pers. : Fr.) Masee                        |   |   |   |    | 1  | 1  | 1  |
| Sw | <i>Panellus stipticus</i> (Bull. : Fr.) P.Karst.                  |   | + |   |    |    | +  | 1  |
| Sw | <i>Phaeomarasmius erinaceus</i> (Pers. : Fr.) Scherff. ex Romagn. | 1 |   |   |    |    | 1  | 1  |
| Sw | <i>Phanerochaete sanguinea</i> (Fr.) Pouzar                       |   | + |   |    |    | +  | 1  |
| Sw | <i>Pleurotus dryinus</i> (Pers. : Fr.) P.Kumm.                    |   |   | 1 |    |    | 1  | 1  |
| Sw | <i>Pluteus</i> sp.  |   |   |   | 1  |    | 1  | 1  |
| M  | <i>Ramaria flavescens</i> (Schaeff.) Petersen                     |   |   |   | +  |    | +  | 1  |
| M  | <i>Russula olivacea</i> (Schaeff.) Pers.                          |   |   |   |    | 2  | 2  | 1  |
| M  | <i>Sebacina incrustans</i> (Pers. : Fr.) Tul.                     |   |   |   | +  |    | +  | 1  |
| Sh | <i>Tephroclybe rancida</i> (Fr.) Donk                             |   |   |   |    | 1  | 1  | 1  |
| Sw | <i>Trametes gibbosa</i> (Pers. : Fr.) Fr.                         |   | + |   |    |    | +  | 1  |
| M  | <i>Tricholoma saponaceum</i> (Fr. : Fr.) P.Kumm.                  |   |   |   | 3  |    | 3  | 1  |
| M  | <i>Tricholoma sejunctum</i> (Sowerby : Fr.) Quéf.                 |   |   |   | 2  |    | 2  | 1  |
| M  | <i>Tricholoma ustaloides</i> Romagn.                              |   |   |   |    | 15 | 15 | 1  |
| Sw | <i>Tulasnella violea</i> (Quéf.) Bourdot & Galzin                 | + |   |   |    |    | +  | 1  |

Note: Those macrofungi whose carpophores are uncountable are recorded with “+”

**Trophic groups (TG):** mycorrhizal species (M), parasitic (P), humicolous saprotrophs (Sh), lignicolous saprotrophs (Sw)

A: Abundance, Fr: Frequency, H' : Shannon diversity index



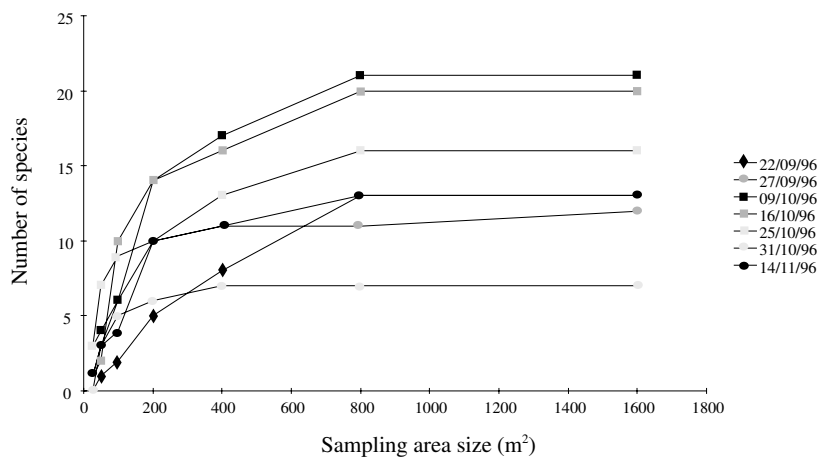


Fig. 2. Accumulation curve of macrofungal species per sampling day in the control plot.

During the study the plots were visited 16-17 times and 125 macrofungal species were found (Table 2): 119 are Basidiomycota species and 6 Ascomycota. Research of mycoenological studies in beech forests demonstrates that macrofungal species number in this kind of ecosystem is considerably higher than the 125 species we found (Lisiewska 1974, Arnolds *et al.* 1994, Keizer 1994, Adamczyk 1996). The research has been conducted in more than one kind of beech forest and over different soil types. It is known that mycoenoses of *Fagus* forest on different soil types have a widely different composition, so it makes the total number of species to be higher than when only one beech community is considered, as occurs in our case. Data from Adamczyk (1996) or Lisiewska (1974) researches reveal similar results to ours when comparison is made with each forest type instead of with the overall results. In both cases species number per forest was less than 155, in spite of the number of plot and sampling years being higher than in our study. Similar studies conducted over 2 years reveal similar results (Mihál & Bučinová, 2005). Other *Fagus* forests of the territory have a much more diverse mycoflora (Olariaga, pers. com.), but the observation has been done in areas with higher amount of dead wood, where all corticioid Basidiomycota have been considered. Taking into account that the beech forest of Altube is a managed and structurally very simple ecosystem, we can conclude that this forest is considerably diverse, even more than some European ones, although we know that more years are necessary to complete the knowledge of the macromycetes growing in this beech forest.

The number of species per plot varied between 32 and 78 species. Plot 4 (61 species) and plot 5 (78 species) were richer in the number of species than plot 1 (32 species), plot 2 (46 species) or plot 3 (34 species) (Table 2). Plot structure influences macrofungal community and positive correlation has been found between the number of tree species and mycorrhizal species (Laganá *et al.*, 1999; Ferris *et al.*, 2000). Ecotones are considered to be more diverse ecosystems since species of the different ecosystems grow together. Plots 4 and 5 were, in fact, on the limit or ecotone of the beech forest and oak forest, and this could be the reason for the higher number of species registered in those two plots.

Altitude is another factor to consider since mycorrhizal species production decreases with elevation (Laganá *et al.*, 1999; Kernaghan & Harper, 2001). It has been pointed out that an altitude of 700 m could be a limit for species decreasing (Laganá *et al.*, 1999). In our study plots 1, 2 and 3, all above 700 m, have, in fact, less richness in the number of species than plots 4 and 5, which are at a height of 450 m. Nevertheless, we cannot conclude in this case that altitude is the main reason for those differences since other factors, such as tree diversity, might have an influence on fungal diversity.

Only ten species appeared in all the plots (Table 2), *Amanita rubescens*, *Cortinarius livido-ochraceus*, *Gymnopus peronatus*, *Hypholoma fasciculare*, *Mycena pura*, *Rhodocollybia butyracea*, *Russula cyanoxantha*, *R. densifolia*, *R. nigricans* and *Xerula radicata*, and another 8 species appeared in four of the five plots (Table 2). Half of the species only appeared in one of the plots, and most of them were found in plot 4 or plot 5 (Table 2). It seems to be recognised that concrete edaphical preference and host specificity in many fungi exists (Bohus & Babos, 1967; Tyler, 1985; Villeneuve *et al.*, 1991). Both factors must be taken into account when mycocoenological communities are analysed.

The mycocoenoses of the beech forest of Atube is characterized by mainly generalist species or growing on broadleaf forest like *Amanita rubescens*, *Cortinarius cinnamomeus*, *Megacollybia platyphylla*, *Rhodocollybia butyracea*, or *Russula cyanoxantha* and acidophilous species such as *Amanita citrina*, *Cortinarius purpurascens*, *Craterellus tubaeformis*, *Lactarius chrysorrheus*, *Mycena pura*, *Russula densifolia* or *R. nigricans* (Thoen 1970, Tyler 1985, Courtecuisse & Duhem 1994). The presence of mycorrhizal species associated to *Fagus* is limited to few species. In this context, *Lactarius blennius* that is considered to be restricted to beech forests, only fructified in two plots and produced a total of 12 carpophores. *Cortinarius cinnabarinus*, *Hebeloma radicosum*, *Lactarius camphoratus*, *Russula densifolia*, *R. nobilis* or *Tricholoma ustale* have preference for beech forest (Smârda, 1972; Tyler, 1992; Courtecuisse Duhem, 1994). Only *Russula densifolia* fructified in all the plots but it produced 6 carpophores, whereas *Russula nobilis* produced 73, most of them in two plots. The rest of the mentioned species only appeared in two or one plot, and with low abundance (Table 2). It is therefore surprising the presence of *Cortinarius anserinus*, *C. multiformis*, *Hygrophorus eburneus* and *Lactarius acris*, all of them calcicolous species and restricted to plot 5. Some researches point out that fungal species are closely related to tree species irrespective of edaphic conditions (Nantel & Neumann, 1992), and that could be the reason why those species grow in a moderately to highly acidic soil, since being mycorrhizal species they have high affinity with *Fagus*.

Host specificity of ectomycorrhizal fungi seems to be more apparent on a local or regional scale than on a territorial or continental scale (Tyler, 1992). Average environmental differences are likely to change the relative competitive power and distribution of the fungi (Tyler, 1989; Rühling & Tyler, 1990). *R. densifolia* and *T. ustale* for example, are abundant in evergreen oak forest in our territory. In the same way *Craterellus cornucopioides* presents high affinity with hornbeam (Tyler, 1992), but in our territory we found it growing with both *Fagus* and *Quercus*. On the contrary, *Lactarius chrysorrheus* is characterized as a quercicolous species and we have only found it with oak species. In the forest of Atube it fructified in plot five quite abundantly, probably due to the presence of *Quercus robur*.

*Xerula radicata* and *Oudemansiella mucida*, both species growing on wood, are very common in *Fagus* forests, and they appeared abundantly in most

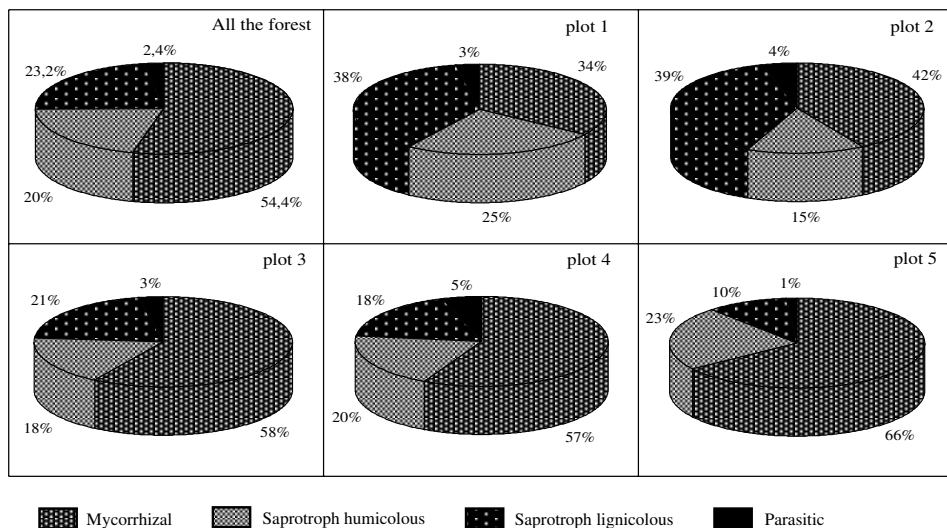


Fig. 3. Percentage of the different fungal trophic groups in the studied plots.

of the plots. Other species that show high preference for beech forests were missing or were very scarce, such as *Marasmius alliaceus* and *Mycena crocata* (Smârda, 1972; Tyler, 1992). *Marasmius alliaceus* and *Mycena crocata* both grow in less acid mull soils (Tyler, 1985), which could explain their absence.

There were 39 species in common with those considered to be characteristic species of beech forests, at least in Poland (Lisiewska, 1974). Among them, *Gymnopus peronatus*, *Lactarius blennius*, *Oudemansiella mucida*, *Russula cyanoxantha*, *R. nigricans*, or *Xerula radicata* can be pointed out. In comparison with the results of Arnolds *et al.* (1994) in the Netherlands, we have found 38 species in common; 13 of them were species that appeared in more than three plots, such as *Amanita citrina*, *A. rubescens*, *Cortinarius livido-ochraceus*, *Russula nigricans*, *R. ochroleuca* or *Xerula radicata*. The same species were also found in roadside verges planted with beech in the Netherlands, but most of them are species with broad ecology and not restricted to beech forests. We did not find any species considered to be endangered and registered in the preliminary Red List of European species (Ing 1993), among the 33 threatened fungi in Europe (Dahlberg & Croneborg, 2006) or among the recent compiled preliminary Red List of the Basque Country and Cantabria (Salcedo, 2008).

From the total species of macrofungi that we found, 70 (56%) were mycorrhizal fungi, 32 (26%) were lignicolous saprotrophs, 19 (15%) humicolous saprotrophs and 4 (3%) were parasitic (Fig. 3). A decrease in sporocarp production of many fungi, or changes in species composition of macrofungi in temperate forest, has been observed in many parts of Europe (Arnolds, 1989, 1991; Fellner, 1993; Rühling & Tyler, 1990). Atmospheric pollution is one of the reasons of the decrease (Arnolds 1989, Fellner 1993). Fellner (1993) noticed that as consequence of air pollution ectomycorrhizal species fructification decrease, whereas lignicolous species increase their production. He considers that the conservation level of a forest can be measured by the proportions of the trophic groups of macrofungi. In this context he establishes that forest deterioration can

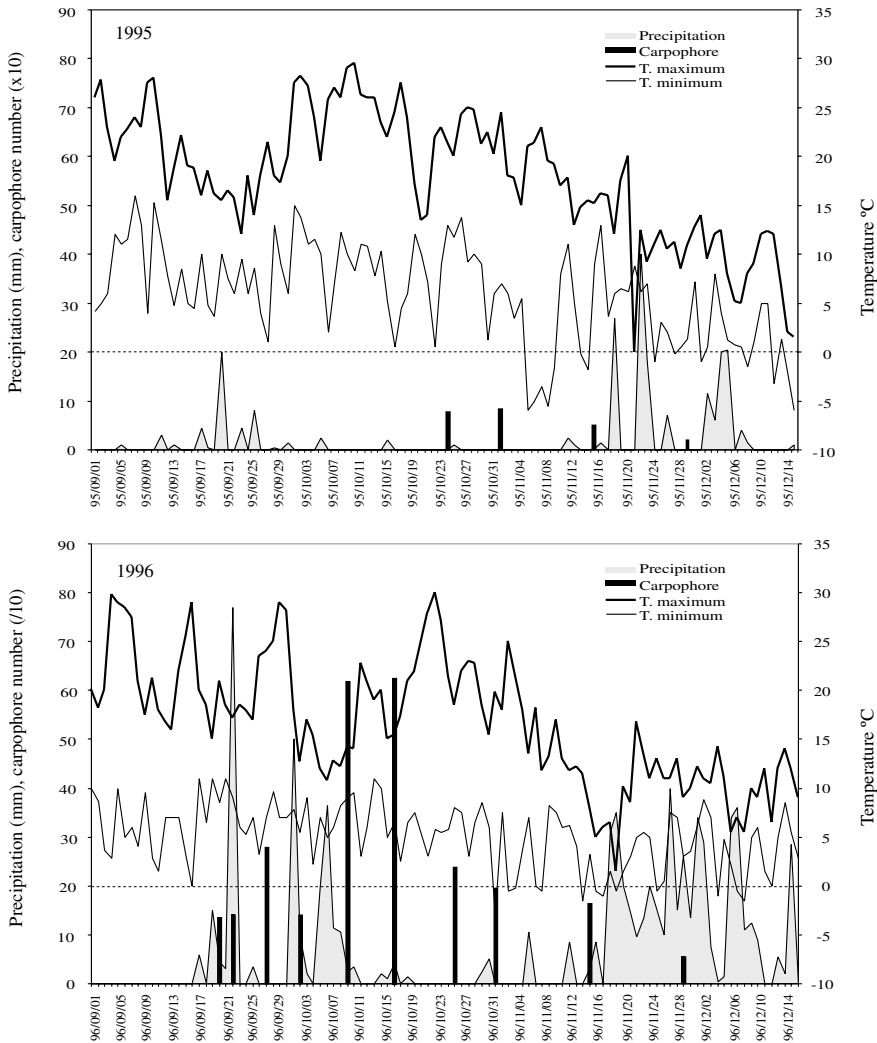


Fig. 4. Daily minimum and maximum temperature, rainfall and number of carpophores in the studied period.

be considered acute when the ectomycorrhizal species constantly contribute less than 40% of the total number of macromycetes while lignicolous species, as a rule, contribute more than 40%. Proportions of these two trophic groups in plots 1 and 2 were near to this data (Fig. 3).

Proportion of trophic groups depends firstly on the forest structure, and secondly on the fungal groups considered in the study. The same methodology was used in all the plots, but the proportions of the trophic groups varied depending from plot to plot (Fig. 3), and statistical differences ( $p = 0.023$ ) were found. The proportion of lignicolous and mycorrhizal species in plots 1 and 2 were close to

those established by Fellner for a deteriorated forest. Environmental conditions with respect to air pollution can be assumed to be similar in all the plots. So we cannot relate high contribution of lignicolous species and low of ectomycorrhizal only to a deteriorated situation. In fact, more coarse woody debris (CWD) was available in those plots, which could be responsible for the high proportion of lignicolous species in plots 1 and 2. So care must be taken if macrofungal species are used to determine the health of a particular forest.

Ecological and climatological conditions such as, humidity, temperature, light, wind, soil have an influence on the fructification of macrofungal species (Friedrich, 1940; Barkman, 1976; Thoen, 1976; Eveling *et al.*, 1990; Arnolds, 1991; Ohenoja, 1993; Trudell & Edmonds, 2004). Precipitation and temperature during the growing period determine sporocarp production (Thoen, 1976), which makes it very different from year to year. That was what happened from 1995 to 1996. The autumn of the first year was extremely dry in our territory. Only 211.7 mm were registered from September 1<sup>st</sup> to December 15<sup>th</sup>; whereas 776.5 mm were registered in 1996 during the same period. Besides, most of the rain in 1995 fell from the middle of November onwards, after the first frosts. In 1995, there was little production of species in the plots, and when the carpophores appeared, the number was very low (Fig. 4). The only two species that appeared in 1995 were *Cortinarius livido-ochraceus* and *Xerula radicata*. In 1996, on the contrary, sporocarp production was considerably high (Fig. 4).

Precipitation during the fructification period seems to determine sporocarp production, but autumn night frosts have a considerable impact on fungi growing in moist places (Lange, 1984). In fact, sporocarp production started with the first rains and drastically disappeared after the middle of November, when the first frosts were registered in our area, even though the rain continued (Fig. 4). Most of the carpophores found after the middle of November were found in plots 4 and 5; just the plots that are situated at a height of 450 m and where the temperatures would be milder than at 850 m.

Sporocarp production is suggested to be high after a period of high temperature, thus supporting the idea that a hot summer is invariably followed by a prolific autumn (Wilkins & Patrick, 1940). Otherwise, mycorrhizal yields seem to correlate more significantly with precipitation over the whole period from January to September than with the proportion received during the growth season (Ohenoja, 1993). Horak and Rölling (1988) came to the conclusion that the best autumn yields were obtained after a damp spring, dry early and midsummer period and rainy autumn. However, Arnolds (1988) did not find meteorological factors to be of any great significance for fungal yields on heaths, but was inclined to place more weight on the interaction with other micro-organism, the fauna and the production of plant litter. Fungal production still remains without a solution, but we can conclude that precipitation and minimum temperature are two factors which influence the macrofungal fructification. We only registered climatological variables at the time of sporocarp production, so more research is necessary on this subject.

## CONCLUSION

In conclusion we can point out that many plots of 800 m<sup>2</sup> are suitable for the study of macromycetes community in beech forests. In spite of the complete diversity of a beech forest not being reflected, comparison made with other single

beech forests shows that 125 species is quite a high number of species. Among the reported species none appear in the preliminary European Red List of threatened macrofungi or among the recent compiled preliminary Red List of the territory.

Beech forest of Altube is characterized by a high number of generalist species such as *Amanita rubescens*, *Cortinarius cinnamomeus*, *Megacollybia platyphylla* *Rhodocollybia butyracea*, or *Russula cyanoxantha* and acidophilous species like *Amanita citrina*, *Cortinarius purpurascens*, *Craterellus tubaeformis*, *Lactarius chrysorrhoeus*, *Mycena pura*, *Russula densifolia* or *R. nigricans*; whereas mycorrhizal species with preference for those ecosystems were not very frequent or abundant, such as *Lactarius blennius*, *Cortinarius cinnabarinus*, *Hebeloma radicosum*, *Lactarius camphoratus*, *Russula densifolia*, *R. nobilis* or *Tricholoma ustale*. The species *Xerula radicata* and *Oudemansiella mucida*, both associated with *Fagus*, were frequent and abundant in this forest, but *Marasmius alliaceus* and *Mycena crocata*, on the contrary were absent or very scarce.

Finally, and in accordance with our results, we consider that precipitation and minimum temperatures during the fructification season have an influence on the fructification of macrofungi, but microclimatological factors and other variables of the different stands determine in the same way the fructification of the different species.

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