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CURRICULUM CROP PROTECTION - CICLE XXII

Department of Land Use and Agro-Forestry Systems – Plant Pathology

Linden tree (*Tilia vulgaris* Hayne) *decline in urban environment*

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Declaration

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgment has been made in the text.

January 15th, 2010 Claudia Alzetta

A copy of the thesis will be available at *http://paduaresearch.cab.unipd.it/*

To my sons Davide and Luca,
young beloved trees

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Riassunto

In ambiente urbano gli alberi vengono sottoposti a molteplici fattori di disturbo e di stress che possono portare al loro deperimento. A livello radicale il deperimento dell'albero si associa spesso ad importanti variazioni della composizione della comunità ectomicorrizica (ECM) che viene pesantemente influenzata da fattori biotici e abiotici. Fino ad oggi non sono noti studi che abbiano trattato tale argomento in ambiente urbano.

A livello della chioma, i primi sintomi aspecifici del deperimento sono la trasparenza e l'ingiallimento delle foglie. Quest'ultimo è associato ai contenuti fogliari di clorofilla e di azoto, utili e oggettivi indicatori diagnostici della salute della pianta e della sua *performance* fisiologica.

Con questo studio si è voluta indagare la condizione di deperimento di piante mature di tiglio intermedio (*Tilia vulgaris* Hayne) in ambiente urbano. Sedici tigli adulti situati nella città di Padova e rappresentanti due classi di deperimento (lievemente e gravemente deperente) sono stati selezionati e studiati al fine di verificare se la comunità ECM dei loro apparati radicali fosse influenzata da fattori ambientali. I funghi appartenenti alla comunità ECM sono stati descritti ed identificati tramite analisi morfologiche e molecolari. In seguito si è analizzata la composizione della comunità ECM in relazione ai diversi siti di impianto, alla classe di deperimento ed alle principali caratteristiche del suolo. La struttura della comunità è risultata fortemente connessa alle variabili ambientali, ed alcune specie fungine sono risultate significativamente associate a specifiche caratteristiche del suolo.

La seconda parte dello studio è stata dedicata alla valutazione di un misuratore portatile di clorofilla (Minolta Chlorophyll Meter SPAD-502) per la diagnosi dello stato di salute dei tigli. Trattandosi di piante adulte e quindi di notevole altezza, si è dapprima identificato il punto ottimale di campionamento e sono quindi state individuate le equazioni di regressione utili a spiegare la relazione tra i contenuti fogliari di clorofilla ed azoto (quantificati tramite tradizionali analisi chimiche) ed i valori di SPAD. E' stato inoltre condotta un'ulteriore analisi della comunità ECM per verificare se la sua variabilità possa essere associata a variazioni del contenuto fogliare di clorofilla e del grado di deperimento dell'albero. L'indagine ha dimostrato che sia la clorofilla fogliare che la classe di deperimento influenzano la struttura della comunità ECM.

I risultati ottenuti possono essere d’aiuto nella comprensione delle associazioni tra la struttura della comunità ectomicorrizica e l’efficienza fotosintetica ed evidenziano l’importanza che l’ambiente sotterraneo riveste per gli alberi in città.

Summary

Trees growing in urban environment undergo a large number of threats and stresses that often translate into decline.

At root level decline is associated with a significant change in the ectomycorrhizal (ECM) community composition which can be strongly influenced by abiotic and biotic factors. Studies on the subject regarding urban trees are so far lacking.

At crown level, the first and non specific symptoms of decline are crown transparency and leaf yellowing. The latter is linked to foliar chlorophyll and nitrogen content that can be useful and objective diagnostic indicators of the health and physiological performance of a plant.

The present study inquired into decline expressions in mature linden trees rooted in urban environment.

To verify if ECM community in urban linden trees (*Tilia vulgaris* Hayne) is affected by environment features, 16 mature linden trees located in the city of Padova (northern Italy) and representing two visually assessed decline classes (moderately and severely declining) were selected and studied.

The community of the ectomycorrhizal fungi was explored and ECM fungi were identified by morphotyping and molecular analysis.

The analysis of the ECM community composition in relation to the main soil features, site location and tree decline class revealed that the ECM community structure was strongly connected with the environmental variables. A few fungal species were significantly associated to specific soil properties.

A second study evaluated an hand held chlorophyll meter (Minolta Chlorophyll Meter SPAD-502) as a diagnostic tool for linden trees health. Further to the detection of the best sampling point position in the mature trees crown, significant regression equations were established between both foliar N and Chl contents, obtained by chemicals analyses, and SPAD values. SPAD meter resulted to be an effective tool for quickly assessing foliar N and Chl, and consequently health status, in mature linden tree in urban environment.

Besides, the ECM community was investigated in order to verify whether its variability could be associated to variations in foliar chlorophyll content and in tree decline. The study

demonstrated that both foliar Chl content and decline classes had influence on the ECM community structure.

These results can help in understanding the associations between ECM community structure and photosynthetic efficiency and they highlight the importance of the belowground environment for urban trees.

Chapter 1

General introduction

Introduction

Urban vegetation and its management can significantly influence human health and environmental quality in cities. Trees balance the urban microclimate by reducing the "heat island" effect (Alcoforado et al. 2009), by protecting surfaces from direct sunlight, by capturing dusts, by increasing the biodiversity. In addition, trees sequester carbon and reduce the impact of rainwater, and foster citizens' wellbeing thanks to their positive influence on physical and mental health. Thus, adequate vegetation designs and management practices are essential to sustain human and environmental well-being. To this end, urban forest managers need accurate information on the urban forest resource, on its changes and on the ecosystem services it provides.

Information on urban forest structure (e.g. number of trees, species, composition, tree health) is essential to improve urban forest management and enhance the ecosystem services provided by trees and other vegetation. Since urban trees management requires pondered resource allocations, it would be of major interest to understand the different performances of different trees genus in order to highlight existing biological tolerance to extreme urban conditions. However, urban forestry is a still-developing research field. Recent reviews have identify that among the themes to be prioritized within future urban forestry research in Europe, tree stresses and urban forest management have a top position (Konijnendijk et al. 2000; Konijnendijk et al. 2007).

Relationships between ectomycorrhizal community diversity and soil characteristics

Soil is a critical medium that directly affects the health and structure of trees growing in both woodland and urban landscapes. Ectomycorrhizal (ECM) symbiosis is a basic interface between soil and roots. ECM fungi, forming a fungal tissue surrounding the host fine roots, mediate the adsorption of soil nutrients and water, while they receive from the plants carbon as photosynthate (Smith & Read 1997).

There is a considerable published research in the scientific literature evaluating the biological, physiological and ecological significance of ectomycorrhizae to the survival, growth, development and health of many species of crop plants and of forest tree species

(Smith & Read 1997). Knowledge about this symbiosis is critical to our understanding of plants growth and ecology. However, due to complexities of the urban environment, little is known about the attributes and variation of urban biogeochemical processes, and still less about the community ecology of ectomycorrhizae of urban trees (Timonen & Kauppinen 2008).

The main characters of the composition of ECM fungal community are host plant species and edaphic factors (Gerhing et al. 1998; Kernaghan & Harper 2001). Within each forest or plantation, the spatial variation of ECM fungi is very high and most fungal species show aggregated distributions (Gardes & Bruns 1996). A study on pinyon trees showed one or a few ECM fungal taxa to dominate single trees, and the dominant fungi varied between trees (Gerhing et al. 1998). On the other hand, ECM fungal structure is strongly depending on the nutrient status of the soil (Scattolin et al. 2008a; Scattolin et al. 2008 b) and it is well known that environmental variables, especially soil characteristics, are closely linked one to each other and changes in one of them may influence the others. For example, variations in ECM community were ascribed to variation in soil nitrogen content (Peter et al. 2001) or to different substrate qualities (Urban et al. 2008), organic layers and mineral horizons (Tedersoo et al. 2003). A significant step forward in understanding ECM fungal community structure is to establish studies that allow estimation of the relative importance of different mechanisms that create this diversity. A possible hypothesis is based on niche differentiation between coexisting species and it states that a disturbance leads to a predictable sequence of species replacing each other (Dahlberg 2001). For instance, an intermediate range of disturbance would allow a range of species to coexist and lead to a high species richness. At the same time, significant associations among ECM species may occur because species with similar requirements towards soil resources tend to occupy similar sites (Koide et al. 2005).

A better cognition of the functional diversity of mycorrhizal symbiosis in the poorly studied urban environment will improve the understanding of trees responses to environmental and climatic perturbations and will be of great importance to future applications in urban forest management.

Urban trees vitality assessment

During their lifetime, urban trees are subjected to plenty of environmental stresses that can cause tree decline. Initial symptoms of tree decline are generally evident as leaves yellowing, often used as a visible index to assess tree vitality.

The vitality of a plant is a theoretical concept. It may be defined as "the ability to grow under the present conditions " (Shigo 1990) and it is one of the most important indicators of trees and forests conditions. As vitality cannot be directly measured, other features can instead be useful to describe it and field practical methods are of great importance in health tree monitoring. Field methods may range from crown foliage or transparency assessments (Eichorn et al. 2004), to foliar nutrient content analysis (Kopinga & van den Burg 1995), to chlorophyll fluorescence (Lichtenthaler & Miehé 1997) and many others. Unfortunately most of them are time consuming, or need expensive and complex tools to be performed, or are unreliable because subjective. The use of hand-held chlorophyll absorbance meters may be of great interest when the investigation of the physiological status of trees vegetation is needed, since they are fast, easy to use, and cheaper than other optical methods. The Minolta Chlorophyll Meter SPAD-502 calculates a numerical value which is proportional to the amount of chlorophyll present in the leaf. Once the relation between SPAD index and chlorophyll content has been established by standard chemical techniques, SPAD can provide a fast and non-destructive assay of various photosynthetic pigments.

As foliar concentration of pigments, most notably the chlorophylls and carotenoids, are affected by a variety of stress factors (Ögren 1990; Maki & Colombo 2001; Neufeld et al. 2006), SPAD index can also provide a useful tool to assess the plant stress degree (Peñuelas & Filella 1998; Percival et al. 2006).

There is a lack of published research in the scientific literature dealing with the testing of this tool on mature urban trees. Nevertheless this application is intriguing, since it could be a remarkable help in detecting different classes of tree decline, a goal of difficult achievement by the traditional visual assessment.

Thesis structure and aim

The thesis, mainly founded by the Municipality of Padova, consists of two chapters describing the composition of the ectomycorrhizal community in urban linden trees (*Tilia vulgaris* Hayne) plantations (chapter 2) and its relationship both with soil properties and qualitative environment variables (chapter 3). Their aim is to verify if the tips vitality and the composition of the ECM consortium in mature urban linden trees growing in two different sites (roadside and park side sites) and at two decline levels (moderately and strongly declining) can be associated to main soil properties.

Chapter 4, after determining the mathematical relationship between SPAD-502 readings and both the foliar chlorophyll content and total foliar nitrogen content in linden trees in urban environment, evaluates the possibility of using foliar chlorophyll meter readings as a diagnostic tool for mature linden trees health, and presents how ECM community variability can be associated to variations in chlorophyll content and to different classes of tree health decline.

Chapters 3 and 4 are based on papers being processed for an international peer-reviewed journal. A general discussion follows.

References

ALCOFORADO MJ, ANDRADE H, LOPES A, VASCONCELOS J (2009) Application of climatic guidelines to urban planning – the example of Lisbon (Portugal). Landsc Urban Plann 90:56-65

DAHLBERG A (2001) Community ecology of ectomycorrhizal fungi: an advancing interdisciplinary field. New Phytologist 150:555-562

EICHORN J, SZEPESI A, FERRETTI M, DURRANT D, ROSKAMS P (2004),Visual Assessment of Crown Condition. In: UN/ECE, Manual on Methods and Criteria for Harmonized Sampling, Assessment, Monitoring and Analysis of the Effects of Air Pollution on Forests. Federal Research Centre for Forestry and Forest Products, Hamburg

GARDES M, BRUNS T D (1996) Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest. Above- and below-ground views. Can J Bot 74:1572-1583

GEHRING CA, THEIMER TC, WHITHAM TG, KEIM P (1998) Ectomycorrhizal fungal community structure of pinyon pines growing in two environmental extremes. Ecology 79:1562–1572

KERNAGHAN G & HARPER KA (2001) Community structure of ectomycorrhizal fungi across an alpine/subalpine ecotone. Ecography 24:181-188

KOIDE RT, XU B, SHARDA J, LEKBERG Y, OSTIGUY N (2005) Evidence of species interactions within an ectomycorrhizal fungal community. New Phytologist 165:305-316

KONIJNENDIJK C, RANDRUP TB, NILSSON K (2000) Urban forestry research in Europe: an overview. Journal of Arboriculture 26(3):152-161

KONIJNENDIJK C, BUSSE NIELSEN A, SCHIPPERIJN J, ROSENBLAD Y, SANDER H, SARV M, MÄKINEN K, TYRVÄINEN, DONIS J, GUNDERSEN V, AKERLUND U, GUSTAVSSON R (2007) Assessment of urban forestry research and research needs in Nordic and Baltic countries. Urban Forestry & Urban Greening 6: 297–309

KOPINGA J & VAN DEN BURG J (1995) Using soil and foliar analysis to diagnose the nutritional status of urban trees. Journal of Arboriculture 21:17-23

LICHTENTHALER H K, MIEHÉ JA (1997) Fluorescence imaging as a diagnostic tool for plant stress. Trends in Plant Science 2-8:316-320

MAKI DS, COLOMBO SJ (2001) Early detection of the effects of warm storage on conifer seedlings using physiological tests. Forest Ecology & Management 154:237-249

NEUFELD HS, CHAPPELKA AH, SOMERS GL, BURKEY KO, DAVISON AW, FINKELSTEIN PL (2006) Visible foliar injury caused by ozone alters the relationship between SPAD meter readings and chlorophyll concentrations in cutleaf coneflower. Photosynthesis Research 87:281-286

ÖGREN E (1990) Evaluation of chlorophyll fluorescence as a probe for drought stress in willow leaves. Plant Physiol 93:1280-1285

PEÑUELAS J, FILELLA I (1998) Visible and near-infrared reflectance techniques for diagnosing plant physiological status. Trends in Plant Science 3:151-156

PERCIVAL G C, KEARY I P, AL-HABSI S (2006) An assessment of the drought tolerance of *Fraxinus* genotypes for urban landscape plantings. Urban Forestry & Urban Greening 5:17-27

PETER M, AYER F, EGLI S (2001) Nitrogen addition in a Norway spruce stand altered macromycete sporocarp production and below-ground ectomycorrhizal species composition. New Phytologist 149:311-325

SCATTOLIN L, MONTECCHIO L, AGERER R (2008a) The ectomycorrhizal community structure in high mountain Norway spruce stands. Trees 22:13-22

SCATTOLIN L, MONTECCHIO L, MOSCA E, AGERER R (2008b) Vertical distribution of the ectomycorrhizal community in the top soil of Norway spruce stands. Eur J Forest Res 127:347-357

SHIGO A (1990) A new tree biology. Bernhard Thalacker Verlag, Braunschweig

SMITH SE, READ DJ (eds) (1997) Mycorrhizal Symbiosis. Academic Press, London

TEDERSOO L, KÕLJALG U, HALLENBERG N, LARSSON K-H (2003) Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. New Phytologist 159:153-165

TIMONEN S, KAUPPINEN P (2008) Mycorrhizal colonisation patterns of *Tilia* trees in street, nursery and forest habitats in southern Finland. Urban Forestry & Urban Greening 7:265-276

URBAN A, PUSCHENREITER M, STRAUSS J, GORFER M (2008) Diversity and structure of ectomycorrhizal and co-associated fungal communities in serpentine soil. Mycorrhiza 18:339-354

Chapter 2

The composition of the ectomycorrhizal community in urban linden trees (*Tilia vulgaris* Hayne)

Introduction

In order to study the relationship between ECM community and environmental variables it is necessary to achieve a complete description of the composition of the ECM consortium; the first step towards it, is characterization and identification of mycorrhizal fungi linked to different plant hosts.

Ectomycorrhizae have traditionally been identified by morphotyping, i.e. the description of peculiar morphological traits together with the recognition of anatomical characters (Agerer 1991; Agerer 1987-2008; Agerer and Rambold, 2004-2009).

Anyway, morphological analyses alone are often insufficient to identify fungal species, both because many species have not yet been described and because ECM belonging to the same genus often share similar morphological and anatomical features.

Since the 1990s new DNA-based methods have been developed to gain fungi identification (Gardes & Bruns 1993; Horton & Bruns 2001; Landeweert et al. 2003; Tedersoo et al. 2003; Tedersoo et al. 2006) and PCR based techniques are nowadays largely employed thanks to their specificity. As a result the combination of microscopic investigations with DNA-based analyses is nowadays considered to be the most effective way to obtain valuable information on ECM community structure (Jakucs & Erös-Honti 2008).

The world total number of ECM fungi species may only be estimated, since the use of molecular analysis keeps on increasing the number of known taxa (Urban et al. 2003; Tedersoo et al., 2005; Erös-Honti & Jakucs 2009; Tedersoo et al. 2009).

Up to now, only few of the conservative estimate of 5.000-6.000 fungal species forming ECM (Agerer 2006; Taylor & Alexander 2005) have been investigated by anatomical studies as well.

Ectomycorrhizae and the genus *Tilia* spp.

Up to 2005, only 6 ECM had been described as hosted by the genus *Tilia* spp. (De Roman et. al. 2005), all of them coming from material collected in Europe.

In 2008 Timonen & Kauppinen reported an updated list (including the 6 ECM cited above) in which 28 ECM species were recorded associating with *Tilia* spp. in Europe or North America.

Moreover Timonen & Kauppinen (2008) related 10 more ECM species described in Finland, and 5 more species and/or genera found in their study on mycorrhizal status of *Tilia vulgaris* Hayne and *Tilia cordata* Mill. growing in different habitats in Finland.

It must be pointed out that De Roman et al. (2005) have referred only to ECM of which morphological descriptions were reported, explicitly excluding those cited in papers dealing with molecular techniques alone. On the other hand, Timonen & Kauppinen didn't explain whether their sources (some of which may not be accessed) referred to morphologically described ECM or not.

Recently Tedersoo et al.(2003; 2006) undertook studies on mixed forests with *Tilia cordata* Mill. among the dominating tree species with the aim of describing the community composition of ECM fungi. Both studies reported very rich ECM communities but they didn't highlight the association between each morphotype and the host tree species.

From the aforementioned published sources it may be concluded that up to now 43 ectomycorrhizal fungi have been discovered to associate with the genus *Tilia* spp., regardless the host tree species: 39 morphotypes have been identified to species level, 3 to genus level, 1 to sub-family level.

Of them all, only one (Tiliaerhiza sebaciniodes + *Tilia*) is described in the *Colour atlas of ectomycorrhizae (*Agerer R 1987-2008), and only two (*Tiliaerhiza sebaciniodes* + *Tilia* and *Russula delica* Fr. + *Tilia*) appear in www.deemy.de (Agerer & Rambold 2004-2009)

Mycorrhizal colonization of *Tilia* ssp. trees in urban environment

The previously cited studies always dealt with forest stands or with inoculated plantlets, apart from Timonen & Kauppinen (2008) who also took into account street trees. In their study root samples at the street sites were taken from trees comprised of *Tilia vulgaris* Hayne stems rooted to *Tilia cordata* Mill roots and their mycorrhizal population was compared to the one of Tilia spp. nursery trees and of Tilia *cordata* Mill. forest trees.

The study highlighted how, despite common morphotypes, the *Tilia* spp. roots in the street, nursery and forest habitats harboured rather dissimilar ectomycorrhizal fungi. Anyway, street, nursery and forest didn't house the same tree species. In this study 12 different ECM morphotypes were observed, and most of them comprised of more than one *Tilia* spp.-

ectomycorrhizal fungus combination. As a consequence the study doesn't supply any kind of description of the single tree-ECM fungus combination.

Nielsen and Rasmussen (1999) compared mycorrhizal population of *Tilia cordata* Mill. nurseries seedlings, nurseries young plants, old forest trees and young street trees. In total 37 morphotypes were found, but of only four of them a very brief description was given, and the fungi forming them were not further characterised.

Materials and methods

In this study 16 linden trees *(Tilia vulgaris* Hayne) rooted in the city of Padova were analyzed and ECM fungi were identified by a combination of morphotyping and molecular analysis. A detailed description of the study site selection and sampling collection is given in Chapter 3.

Ectomycorrhizal anatomotypes were classified morphologically according to Agerer (1991) and using the available literature (Pigott 1982; Agerer, 1987-2008; Cairney & Chambers 1999; Urban et al. 2003; Liu & Hall 2004; Douhan & Rizzo 2005; Agerer & Rambold 2004-2009; De Roman et al. 2005; Tedersoo et al. 2005; CABI Bioscience et al. 2007-2009; Bidartondo & Read 2008; Jakucs & Erös-Honti 2008; Erös-Honti & Jakucs 2009).

Among all the methods now available for molecular analyses a quick one was employed that allows rDna amplification directly from very small portions of mycorrhizal tissue (Iotti & Zambonelli, 2006). Molecular analysis was carried on fresh tips, immediately after morphotyping, and on frozen tips (-80 °C).

ECM manipulation for molecular analyses was carried out in sterile conditions.

Selected and previously washed tips of each morphotype were put in Petrie dishes under a stereomicroscope (x20). The mantle was firstly cleaned from surrounding hyphae to prevent PCR contamination with rhizoplane fungi. A very small mantle fragment (approximately 0.02-0.03 mm^2) was removed from the tip using a fine needle, and it was transferred to the PCR tube containing 20 µl of sterile water.

The universal primers ITS1 and ITS4, commonly used in ECM community studies (Bruns & Shefferson 2004; Koide et al. 2007; Diedhiou et al. 2009), were used to amplify the ITS-1, 5.8S and ITS-2 regions of the fungal nuclear ribosomal DNA.

PCRs were conducted in a final 50 µl reaction mixture containing 1X reaction buffer (100 mM Tris/HCl (pH 8,3), 500 mM KCl, 25 mM Mg^{2+}; HotMaster™ *Taq* Buffer, Eppendorf), 200 µm of each dNTP (dAPT, dCTP, dGTP, dTTP), 0,3 µM of each primer, 1,5 U of *Taq* DNA polymerase (HotMaster™, Eppendorf), and 0,8 µg/µl of Bovine Serum Albumin (BSA) (Fermentas, Vilnius).

A negative control was performed without DNA to test any possible contamination of the reagents.

DNA amplification of the ITS regions was performed on a Mastercycler® ep gradient S (Eppendorf AG, Hamburg, Germany) thermocycler. Various thermocycling patterns were tested and then the best one was chosen, consisting of an initial denaturation at 95°C for 6 min, followed by 35 cycles of 94°C (1 min), 55°C (1 min), and 72°C (2 min), and a final extension step of 72°C for 10 min.

A sample of 10 µl of product was electrophoresed in a 1,2 % agarose gel and visualized by staining with ethidium bromide in an UVIpro Gold Gel Documentation System.

Images were elaborated by UVIpro Software (Image acquisition and analysis software – UVItec, Cambridge, UK).

The amplified products were first purified by *Exonuclease I* (USB Corporation, Cleveland, Ohio), in order to remove residual single-stranded primers and any extraneous single-stranded DNA produced by the PCR.

The *Shrimp Alcaline Phosphatase* (USB Corporation, Cleveland, Ohio) was then added to the PCR mixture to remove the remaining dNTPs which would interfere with the sequencing reaction.

The sequences were obtained by BMR Genomics (Padua) with Big Dye Terminator chemistry.

ITS sequences were checked, manually editing ambiguous readings, and compared to reference sequences in the GenBank database of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/BLAST/) (Benson et al. 1999) and the UNITE database (http://unite.ut.ee/) (Kõljalg et al. 2005) by using the BLASTn Search (Basic Local Aligment Search Total nucleotide) program (Altschul et al. 1997).

Database sequences yielding the greatest percent similarity to the ECM sequences were chosen as the best match for each of them.

Sequences taxon categories were assigned as follows (Landeweert et al. 2003; Mosca et al. 2007; Bidartondo & Read 2008): sequence similarity of ≥ 99%, identification to species level; sequence similarity of 95% to 99%, identification to genus level; sequence similarity of ≤ 95%, identification to family level.

All the anatomotypes were classified by an alphanumerical code (CAxx).

When possible, a sample of each of them was stored in water at -80°C and in FEA in the TeSAF Department Herbarium of the University of Padua.

Forty-five sequences were derived from ECM root tips.

Results: ECM community composition

Thanks to morphological, anatomical and molecular investigation 52 anatomotypes were revealed.

15 of them were assigned to family level (*Boletaceae*, *Clavulinaceae*, *Cortinariaceae*, *Helvellaceae*, *Pezizaceae*, *Sebacinaceae*, *Telephoraceae*), 25 to genus (*Chromelosporium* sp., *Clavulina* sp., *Geopora* sp., *Inocybe* sp., *Laccaria* sp., *Peziza* sp., *Pseudotomentella* sp., *Russula* sp., *Scleroderma* sp., *Sebacina* sp., *Tomentella* sp., *Trichophaea* sp.), 6 to species level [(*Cenococcum geophilum* Fr., *Geopora cervina* (Velen.) T. Schumach., *Scleroderma verrucosum* (Bull.) Pers., *Trichophaea woolhopeia* (Cooke & W. Phillips) Boud., *Tuber rapaeodorum* Tul. & C: Tul., *Tuber rufum* Pico], while 6 remained unidentified.

Ectomycorrhizal anatomotypes and their morphological or molecular identification are listed in Table 1.

Short descriptions and images of the ectomycorrhizal anatomotypes are reported in Annex 1.

Table 1: Ectomycorrhizal anatomotypes and their morphological or molecular identification. Additional references are available on the NCBI (www.ncbi.nih.gov/BLAST) or UNITE (www.unite.ut.ee) websites

Fungal taxa and Herbarium codex	**Best match sequence**	**Size (pair)**	**E value**	**Similarity**	**Accession number**	**Source**
Boletaceae (CA18)	*Boletus rubellus*	714	$2\ e^{-139}$	80%	EU819460	Gen Bank Database
CA34	-	-	-	-	-	-
CA43	-	-	-	-	-	-
CA88	-	-	-	-	-	-
CA89	-	-	-	-	-	-
CA94	-	-	-	-	-	-
CA96	-	-	-	-	-	-
Cenococcum geophilum (CA2)	-	-	-	-	-	-
Chromelosporium (CA92)	*Chromelosporium* sp. *JMP0018*	538	0	100%	EU819462	Gen Bank Database
Clavulina sp. (CA17)	*Clavulina cf. rugosa*	605	0	96%	DQ974712	Gen Bank Database
Clavulinaceae (CA19)	*Clavulina cf. rugosa src661*	605	$6\ e^{-118}$	84%	DQ974712	Gen Bank Database
Cortinariaceae (CA31)	*Inocybe cervicolor*	509	e^{-118}	85%	AM882937	Gen Bank Database
Cortinariaceae (CA68)	*Inocybe hirculus*	556	$2\ e^{-138}$	84%	FJ531872	Gen Bank Database
Cortinariaceae (CA73)	*Inocybe cf. friesii*	571	$3\ e^{-175}$	87%	FJ845413	Gen Bank Database
Cortinariaceae (CA20)	*Inocybe rimosa*	577	0	94%	AM882765	Gen Bank Database
Geopora sp. (CA30)	*Geopora cervina*	572	0	97%	FM206417	Gen Bank Database
Geopora cervina (CA11)	*Geopora cervina*	585	0	100%	FM206417	Gen Bank Database
Helvellaceae (CA6)	*U.E. Balsamia*	614	0	98%	EU668245	Gen Bank Database
Inocybe sp. (CA27)	*Inocybe malenconii*	685	0	96%	AM882862	Gen Bank Database
Inocybe sp. (CA83)	*Inocybe calida*	603	0	98%	AM882760	Gen Bank Database
Laccaria sp. (CA49)	*Laccaria amethystea*	610	0	98%	DQ499640	Gen Bank Database
Peziza sp. (CA60)	*Peziza succosa*	571	0	97%	DQ200840	Gen Bank Database
Peziza sp. (CA80)	*Peziza succosa*	566	0	96%	UDB000984	Unite Database

Peziza sp. (CA81)	*Peziza infossa*	571	0	96%	DQ974817	Gen Bank Database
Pezizaceae (CA28)	*Pachyphloeus virescens*	340	$2\ e^{-81}$	83%	EU543198	Gen Bank Database
Pseudotomentella sp. (CA67)	*U. Pseudotomentella*	589	0	100%	EU668196	Gen Bank Database
Russula sp. (CA39)	*Russula aff.delica*	233	$2\ e^{-103}$	96%	DQ422005	Gen Bank Database
Scleroderma sp. (CA91)	*Scleroderma areolatum*	628	0	99%	UDB001212	Unite Database
Scleroderma verrucosum (CA40)	*Scleroderma verrucosum*	607	0	100%	UDB000044	Unite Database
Sebacina sp. (CA66)	*Sebacina epigaea*	522	0	98%	AF490397	Gen Bank Database
Sebacina sp. (CA85)	*Sebacina incrustans*	553	0	98%	AF490395	Gen Bank Database
Sebacinaceae (CA14)	*U.E. (Sebacinaceae)*	457	0	98%	AJ879661	Gen Bank Database
Sebacinaceae (CA35)	*Sebacina incrustans*	552	0	94%	EU819442	Gen Bank Database
Sebacinaceae (CA71)	*Sebacina incrustans*	570	0	93%	EU819442	Gen Bank Database
Telephoraceae (CA3)	*Tomentella* sp. *J54*	589	0	93%	AJ534914	Gen Bank Database
Telephoraceae (CA32)	*Tomentella lapidum*	596	0	91%	AF272941	Gen Bank Database
Telephoraceae (CA33)	*Tomentella lapidum*	469	0	95%	AF272941	Gen Bank Database
Telephoraceae (CA95)	*Tomentella stuposa*	589	0	92%	EU819523	Gen Bank Database
Tomentella sp. (CA1)	*Tomentella ellisii*	602	0	96%	UDB000219	Unite Database
Tomentella sp. (CA21)	*U.E. (Tomentella)*	598	0	99%	AJ879644	Gen Bank Database
Tomentella sp. (CA42)	*Tomentella lapida*	587	0	97%	UDB001657	Unite Database
Tomentella sp. (CA54)	*Tomentella bryophila*	386	e^{-174}	96%	UDB000253	Unite Database
Tomentella sp. (CA55)	*Tomentella fuscocinerea*	614	0	95%	DQ974776	Gen Bank Database
Tomentella sp. (CA69)	*Tomentella ellisii*	557	0	96%	UDB000226	Unite Database
Tomentella sp. (CA70)	*Tomentella ellisii*	530	0	96%	UDB000219	Unite Database
Tomentella sp. (CA75)	*Tomentella ellisii*	612	0	96%	UDB000219	Unite Database
Tomentella sp. (CA79)	*Tomentella lateritia*	517	0	94%	UDB000267	Unite Database
Tomentella sp. (CA84)	*Tomentella* sp. *EDM19*	595	0	100%	EU444541	Gen Bank Database
Trichophaea sp. (CA82)	*Trichophaea woolhopeia*	483	0	97%	DQ200835	Gen Bank Database
Trichophaea woolhopeia (CA41)	*Trichophaea woolhopeia*	476	0	100%	DQ200835	Gen Bank Database
Tuber rapaeodorum (CA4)	*Tuber rapaeodorum*	476	0	99%	EU784430	Gen Bank Database
Tuber rufum (CA37)	*Tuber rufum*	584	0	100%	AY940646	Gen Bank Database

References

AGERER R (ed) (1987-2008) Colour atlas of ectomycorrhizae. 1-14th delivery, Einhorn, Schwäbisch Gmünd, D

AGERER R (1991) Characterization of ectomycorrhizae. In: NORRIS JR, READ DJ, VARMA AK, (eds) Techniques for the Study of Mycorrhiza. UK Academic Press., London, pp 25-73

AGERER R (2006) Fungal relationships and structural identity of their ectomycorrhizae. Mycol Progress 5: 67–107

AGERER R, RAMBOLD G (2004-2009) [update 2009-01-26] DEEMY – An Information System for Characterization and Determination of Ectomycorrhizae. www.deemy.de – München, D

ALTSCHUL S F, MADDEN T L, SCHÄFFER A A, ZHANG J, ZHANG Z, MILLER W, LIPMAN D (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research Vol 25, No. 17: 3389–3402

BENSON D A, BOGUSKI M S, LIPMAN D J, OSTELL J, OUELLETTE B F, RAPP B A, WHEELER D L (1999) GenBank. Nucleic Acids Research Vol 27, Issue 1: 12-17,

BIDARTONDO M I, READ D J (2008) Fungal specificity bottlenecks during orchid germination and development. Molecular Ecology 17:3707-3716

BRUNS T D, SHEFFERSON R P (2004) Evolutionary studies of ectomycorrhizal fungi: recent advances and future directions. Can J Bot 82:1122-1132

CABI BIOSCIENCE, CBS, LANDCARE RESEARCH (access from 2007-10 to 2009-10) Index Fungorum database www.indexfungorum.org

CAIRNEY JWG, CHAMBERS SM (eds) (1999) Ectomycorrhizal fungi: key genera in profile. Springer-Verlag, Berlin, D

DE ROMAN M, CLAVERIA V, DE MIGUEL AM (2005) A revision of the descriptions of ectomycorrhizas published since 1961. Mycol Res 109: 1063-1104

DIEDHIOU A.G., DUPOUEY JL, BUÉE M, DAMBRINE E, LAÜT L, GARBAYE J (2009) Response of ectomycorrhizal communities to past Roman occupation in an oak forest. Soil Biology & Biochemistry 41:2206-2213

DOUHAN G W, RIZZO D M (2005) Phylogenetic divergence in a local population of the ectomycorrhizal fungus *Cenococcum geophilum.* New Phytologist166:263-271

ERÖS-HONTI Z, JAKUCS E (2009) Characterization of beech ectomycorrhizae formed by species of the *Pachyphloeus – Amylascus* lineage. Mycorrhiza 19:337-345

GARDES M, BRUNS TD (1993) ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. Molecular Ecology 2:113-118

HORTON TR, BRUNS TD (2001) The molecular evolution in ectomycorrhizal ecology: peeking into the black box. Molecular Ecology 10:1855-1871

IOTTI M, ZAMBONELLI A (2006) A quick and precise technique for identifying ectomycorrhizas by PCR. Mycological Research 110:60-65

JAKUCS E, ERÖS-HONTI Z (2008) Morphological-anatomical characterization and identification of *Tomentella* ectomycorrhizas. Mycorrhiza 18:277-285

KOIDE RT, XU B, SHARDA J, LEKBERG Y, OSTIGUY N (2005a) Evidence of species interactions within an ectomycorrhizal fungal community. New Phytologist 165:305-316

KÕLJALG U, LARSSON K-H, ABARENKOV K, NILSSON RH, ALEXANDER IJ, EBERHARDT U, ERLAND S, HOILAND K, KJOLLER R, LARSSON E, PENNANEN T, SEN R, TAYLOR AFS, TEDERSOO L, VRALSTAD T, URSING BM (2005) UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. New Phytologist 166:1063-1068

LANDEWEERT R, LEEFLANG P, KUYPER TW, HOFFLAND E, ROSLING A, WERNARS K, SMIT E (2003) Molecular identification of ectomycorrhizal mycelium in soil horizons. Applied and Environmental Microbiology 69:327-333

LIU Y J, HALL B D (2004) Body plan evolution of ascomycetes, as inferred from an RNA polymerase II phylogeny. Proceedings of the National Academy of Sciences, USA 10:4507-4512

MOSCA E, MONTECCHIO L, SELLA L, GARBAYE J (2007) Short-term effect of removing tree competition on the ectomycorrhizal status of a declining peduncolate oak forest (*Quercus robur* L.). Forest Ecol Manage 244:129-140

NIELSEN J S, RASMUSSEN H N (1999) Mycorrhizal status and morphotype diversity in *Tilia cordata* – a pilot study of nurseries and urban habitats. Acta Horticulturae 496:451-459

PIGOTT CD (1982) Fine structure of mycorrhiza formed by *Cenococcum geophilum* Fr. on *Tilia cordata* Mill.. New Phytol 92: 501-512

TAYLOR F S, ALEXANDER I (2005) The ectomycorrhizal symbiosis. Life in the real world. Mycologist Volume 19, Part 3 August 2005, 102-112

TEDERSOO L, KÕLJALG U, HALLENBERG N, LARSSON K-H (2003) Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. New Phytologist 159:153-165

TEDERSOO L, HANSEN K, PERRY B.A., KJØLLER R (2005) Molecular and morphological diversity of pezizalean ectomycorrhiza. New Phytologist 170:581-596

TEDERSOO L, SUVI T, LARSSON E, KÕLJALG U (2006) Diversity and community structure of ectomycorrhizal fungi in a wooded meadow. Mycological Reasearch 110:734-748

TEDERSOO L, PARTEL K, JAIRUS T, GATES G, POLDMAA K, TAMM H (2009) Ascomycetes associated with ectomycorrhizas: molecular diversity and ecology with particular reference to the *Helotiales.* Environmental Microbiology, Early View, August 2009:1-13

TIMONEN S, KAUPPINEN P (2008) Mycorrhizal colonisation patterns of *Tilia* trees in street, nursery and forest habitats in southern Finland. Urban Forestry & Urban Greening 7:265-276

URBAN A, WEIB M, BAUER R (2003) Ectomycorrhizas involving sebacinoid mycobionts Mycol Res 107:3-14

Chapter 3

The ectomycorrhizal community structure in urban linden trees and its relationship with soil properties

Introduction

Urban trees experience a number of stresses related to the location where they are planted. Street trees are exposed to a relatively high stress level and the average lifespan is shorter than the one of park trees (Sæbø et al. 2003). In fact, under stress photosynthesis is reduced and carbon allocation is altered, resulting in the tree vitality decrease (Dobbertin 2005).

When decline begins, the mechanical strength of a tree decreases and consequently the risk of its collapse increases, becoming a potential threat for people and properties (Terho 2009). Therefore, the assessment of the vitality of mature urban trees is a major aspect of urban tree management.

The stresses that affect urban trees may be of biotic or abiotic nature and include polluting agents, mechanical damage, high and low temperature, de-icing road-salt, restricted space for crown and root development, drought (Pedersen et al. 2000; Sæbø et al. 2003; Percival 2006). In addition, construction works, utility trenching, and lack of tree care create unhealthy conditions for trees in towns (Pauleit et al. 2002).

Specifically soil may be affected by disturbance like incorporation of anthropic materials, contamination by pollutants, shortage of available water, soil compaction and subsequent root deoxygenation (Jim 1998; Pouyat et al. 2007).

Properties of surface soils can vary widely in urban landscapes, making it difficult to describe a typical “urban soil” (Pouyat et al. 2007). This goal is still harder for Italy where there has been little research on urban soils (Salvagio Manta et al. 2002; Imperato et al. 2003).

Among soil microorganisms, ectomycorrhizal (ECM) fungi represent a direct link between soil and tree roots (Leyval et al. 1997). They provide the tree with nutrients, protect its root system from microbial pathogens and enhance its drought tolerance (Smith & Read 1997; Alvarez et al. 2009). Therefore this symbiosis is a crucial factor in tree health (Peter et al, 2001).

Overall levels of mycorrhizal infection are known to be strongly influenced by the soil features. Soil nutrient status (Smith & Read 1997; Toljander et al. 2006), organic matter content and humus form (Rumberger et al. 2004; Scattolin et al. 2008a), soil horizons (Baier et al. 2006; Scattolin et al. 2008b), soil temperature and moisture (Kernaghan & Harper 2001; Toljander et al. 2006) are demonstrated to influence the vitality and ECM

degrees of the root tips as well as the species composition of the ECM community of natural stands. Moreover, the stability of the ECM community is a possible bioindicator of plant and forest health status (Trevisani et al. 1999; Montecchio et al. 2004; Montecchio et al. 2008; Mosca et al. 2007; Montecchio et al. 2009).

While the importance of ECM fungi in natural ecosystem is becoming increasingly appreciated and recent studies have described the ECM community composition and spatial distribution in forest natural stands (Kernaghan & Harper 2001; Tedersoo et al. 2003; Lilleskov et al. 2004; Izzo et al. 2005; Baier et al. 2006; Toljander et al. 2006; Scattolin et al. 2008a; Scattolin et al. 2008b) very few researches dealing with ECM fungi community in urban areas, especially as far as mature trees are concerned, have been done (Garbaye & Churin 1996; Garbaye et al. 1999; Nielsen & Rasmussen 1999; Appleton et al. 2003). Moreover no researches have been yet conducted on dynamics and ecological role of the ectomycorrhizal community in mature urban trees soils.

Tilia spp. is one of the most commonly used tree genera planted in streets and parks in central and north west European countries, and it is the most popular in Italy (Pauleit et al. 2002).

The city of Padova houses more than 3000 linden (*Tilia vulgaris* Hayne) trees along the streets, accounting for about the 30% of the total number of roadside trees of the town (Tree Inventory of Padova Municipality - Tree Management Department – December 2009 – personal communication).

The aim of this research was to verify if the tips vitality and the composition of the ECM consortium in mature urban linden trees growing in two different sites (roadside and park side sites) and at two decline levels (moderately and strongly declining) could be associated to main soil properties.

An increased knowledge of both the soil and the cause-effect relationship between ectomycorrhizal population dynamics and urban tree decline severity would in fact be able to increase the awareness of the importance of the belowground environment for urban trees and to enhance the cost-effectiveness of municipal tree programs.

Materials and methods

Study site and samples collection

The 16 trees used in the experiment are located in Padova (Italy), in two different sites. Padova is in the alluvial plain in the north east of Italy, at an average altitude of 12 m a.s.l.. The annual mean of daily maximum temperature is 18°C, the annual mean of daily minimum temperature is 7,5°C, the average annual rainfall ranges between 1,000 and 1,200 mm (ARPAV 2009).

The plantation sites were selected by means of the Tree Inventory of Padova Municipality - Tree Management Department.

The main queries used for the selection were the most representative trunk diameter (breast height, $d_{1,30}$ average diameter 44 cm), plant height (18-22 m), estimated age [50(±5)-year-old] and the presence of the two most characteristic decline classes, classified as "moderately declining" (CL1) and "strongly declining" (CL2).

Fig. 1 Examples of decline class 1 (left) and decline class 2 (right) linden trees.

The health situation of each tree was visually assessed considering a selected list of typical decline symptoms: defoliation degree, discoloured leaves, canopy transparency, presence of epicormic twigs and dead branches. In order to objectively record the presence of each symptom a modified version of the form usually adopted for Visual Tree Assessment by the Municipality of Padova – Tree Management Department was used (Fig. 6). A score was assigned to each symptom according to its degree of presence. Each tree thus obtained a final evaluation. The trees with a final score ranging from 30 to 60 were assigned to decline class 1 ("moderately declining", CL1), those with a final score inferior to 30 were assigned to decline class 2 ("severely declining", CL2) (Fig.1).

Final locations had to be searched among all the *Tilia vulgaris* Hayne plantations within which the two decline classes were present. From a first query the evidence turned out that only roadside plantations hosted both declining classes: the selection therefore proceeded among all the selected sites where the previously mentioned dendrometric characteristics were present but the query "CL2" was entered only for roadside lanes.

Among all the satisfactory results, 2 sites [Pio X (P) and Landucci (L)] were randomly selected.

Pio X (P) site houses 155 roadside linden trees standing in a single lane in a soil bed 150 cm wide.

In Landucci (L) site there are 31 linden trees standing in a single lane in a town garden.

In each site, 8 trees were selected with a distance of at least 8 m from the nearest tree and coded with a number (1÷16).

The 155 trees rooted in P site were previously classified according to the declining classes and among them four trees were chosen belonging to CL1 (no. 1-2-4-8) and four belonging to CL2 (no. 3-5-6-7).

In L site all 8 trees (coded 9÷16) belong to CL1.

Soil samples were collected for ECM evaluation once in each season, spring (05/2007), winter (03/2008), summer (08/2008) and autumn (09/2008).

Considering each tree as the central point of the main cardinal axes, and considering the four virtual quadrants obtained, 12 soil cores were collected for each plant at 3 distances from the stem base [100, 150, and 200 cm (Dist)], along two different directions: one

belonging to the first and/or fourth quadrant (N), the opposite one to the second and/or third quadrant (S).

Along each direction 6 cylindrical soil cores were taken with a soil corer (2,5 cm diameter, 30 cm depth) for each plant along two parallel directions having a distance of 20 cm one from each other. Each soil sample was thus coded with "dx" when situated on the right side if facing "N" direction, and with "sn" when situated on the left side.

The chosen sampling depth was the one that hosted the greatest density of root tips, according to previous investigations in the sampling sites, to better represent the root tips' status (vitality and ectomycorrhization).

The soil cores were stored in sealed plastic bags at +4°C in the dark until they reached the laboratory.

192 soil samples were collected at each sampling date, 768 in total.

During the autumn sampling, additional soil samples were collected to be analyzed for physical and chemical soil properties. For each tree two soil samples were collected on opposite directions (following the above-mentioned pattern) at 250 cm from the stem base.

The samples were collected to a depth of 30 cm by means of spades. The spades were washed with water and wiped dry with paper towels after each sampling. About 1 Kg of soil was taken at each sampling point and stored in a plastic bag.

Once in the laboratory, the soil samples were air-dried, gently crushed and sieved to < 2mm particle size with a plastic 2-mm sieve to avoid metal contamination (ISO, 2006). Subsamples were sieved to < 1mm particle size for total heavy metals analysis.

Laboratory observations and data analyses

Soil parameters

Using standard methods, basic physical and chemical soil parameters were determined: (1) pH; (2) organic carbon in %; (3) total organic matter in %; (4) total Nitrogen in ‰; (5) Carbon Nitrogen ratio ; (6) Cation Exchange Capability; (7) total concentration of selected heavy metals (Al, As, Cd, Cr, Cu, Fe, Na, Ni, Pb, Zn) in mg/Kg; (8) particle size distribution and texture by hydrometer and USDA textural classification (Gee & Bauder 1986); (9) extractable P in mg/Kg; (10) conductivity in µS/cm.

All the soil parameters were measured according to the national governmental regulations (G.U. 248/1999) .

The hydrological parameters (water content at Field Capacity, water content at permanent Wilting Point and Available Water capacity) were indirectly estimated using the following Pedotransfer Functions (Morari personal communication), which relate hydraulic properties to more easily measurable soil properties (Rawls et al. 1982; Pachepsky et al. 2005, Wösten 2006):

$$\text{Field Capacity} = 0.2576 - (0.002\ \text{sand}) + (0.0036\ \text{clay}) + 0.0299\ \text{organic matter})$$

$$\text{Wilting Point} = 0.026 + (0.005\ \text{clay}) + (0.0158\ \text{organic matter})$$

$$\text{Available Water} = \text{Field Capacity} - \text{Wilting Point}$$

Attributes from (1) to (7) were assessed at the Laboratories of the Department of Agricultural Biotechnology of the University of Padova.

Attributes from (8) to (10) were assessed at the CheLab srl – Chemical Laboratories (Resana – Italy).

Mann-Whitney *U*-Test was used to detect soil significant differences ($p \leq 0,05$) between the two sites P and L, and between the two decline classes, 1 and 2. Statistica 6, StatSoft, Tulsa, USA for Windows was used for data analysis.

Ectomycorrhizal (ECM) community

In the laboratory fine roots were sorted out from the soil and gently rinsed in water.

From each sample root fragments were randomly selected among those with diameter < 2 mm and observed. The first three fully developed and undamaged apexes of each root fragment were examined under stereomicroscope to determine their vitality, the presence or not of mycorrhization and, when present, the ectomycorrhizal anatomotype, until 20 living EM tips were found.

Totally 35,299 root tips were observed.

All observed tips were classified as “vital ectomycorrhized” (EM, well-developed ectomycorrhizae with a smooth, relatively thick mantle), “vital non ectomycorrhized” (NM,

well-developed, inflated and turgid tip, mantle lacking) and “non vital” (NV, scurfy surface and easily detachable cortex, with or without ectomycorrhizae) (Agerer, 1991; Baar & de Vries, 1995; Montecchio et al. 2004; Scattolin et al. 2008a).

The number of EM, NM and NV was calculated in each soil sample, for each decline class and each site.

After counting the total number of tips in each category (EM, NM and NV), the probability of finding each category (pEM, pNM, pNM) was determined as the ratio of total number of tips recorded.

The significance of the relationship between the different variables (pEM, pNM, pNM) was tested by the Mann-Whitney *U*-Test ($P<0.05$; Statistica 6, StatSoft, Tulsa, USA) in order to verify the effects of different sites or different decline classes on vitality and ectomycorrhization of root tips.

Microscope observations permitted then detailed morphological and structural descriptions of mycorrhizae: they were separated and coded according to colour, shape and type of ramification, features of mantle surface, type of outer and inner mantle, type of emanating hyphae, rhizomorphs and cystidia.

ECM anatomotypes were classified thanks to morphological, anatomical and molecular investigation, according to the available literature (Pigott 1982; Agerer 1991; Cairney & Chambers 1999; Urban et al. 2003; Liu & Hall 2004; Douhan & Rizzo 2005; De Roman et al. 2005; Tedersoo et al. 2005; Iotti et al. 2006; Agerer, 1987-2008; Bidartondo & Read 2008; Jakucs & Erös-Honti 2008; Agerer & Rambold 2004-2009; CABI Bioscience et al. 2007-2009; Erös-Honti & Jakucs 2009), as described in details in Chapter 2.

The relative abundance (R.a.) of each ECM species was then calculated, in each class and in each site per soil sample related to the total number of EM tips observed in that category (i.e. R.a. of ECMy in CL1= n° of tips with ECMy in CL1/ tot. N° of EM tips observed in CL1).

The statistical analyses were carried out on the whole data set consisting of the data belonging to the four sampling seasons.

It is known that ECM distribution is not homogeneous at distances between 0 and 17 m (Lilleskov et al., 2004). Since this autocorrelation among sampling points could influence

the community structure, the Mantel Test was performed to test the null hypothesis of no relationship among samples (total n° of ECM tips analysed in a soil core) from the same tree (Mc-Cune and Grace 2002).

The Mantel test ($P<0.01$, number of permutations =10000) compared species dissimilarity matrix and linear distance matrix between sampling points belonging to the same plant, using the XLSTAT-Pro Program (http://www.xlstat.com). If the Mantel test could not exclude a spatial correlation within a tree sampling area, this was excluded from the subsequent analyses.

The Sørensen similarity index was used to create the similarity matrix: $2a/(2a+b+c)$, where a = number of shared species, b = number of species unique to plot 1 and c = number of species unique to plot 2 (Izzo et al. 2005).

Relations among environmental variables and species abundance of ectomycorrhizae were analysed by means of multivariate ordination techniques (Jongman et al. 1995; Baier et al. 2006; Scattolin et al. 2008a) using CANOCO (software for Canonical Community Ordination, 4.5 Version).

The environmental parameters considered during the four sampling periods were the following: P and L sites, Decline Class 1 and Decline Class 2, chemical soil parameters [total concentration of selected heavy metals (Al, As, Cd, Cr, Cu, Fe, Na, Ni, Pb, Zn), pH, total organic matter, total nitrogen, carbon nitrogen ratio, Cation Exchange Capability, extractable P] and physical soil parameters (conductivity, water content at Field Capacity, water content at permanent Wilting Point and Available Water capacity).

A Detrended Correspondance Analysis (DCA; Hill and Gauch 1980) on the ECM relative abundances was performed to obtain estimates of gradient lengths in standard deviation units.

The detrending by segments method was applied with data not subjected to any transformation.

In order to choose the best ordination model, the lengths of gradient have been considered.

The use of weighted-averaging ordination models resulted to be appropriate for these data (ter Braak and Šmilauer, 2002) and unimodal (DCA and CCA; ter Braak 1986) analyses were performed.

DCA considered sampling points as cases, analyzing qualitative (sites P and L, class 1 and 2) and quantitative (chemical and physical soil parameters) variables.

In order to optimally display the differences in ECM species composition due to environmental variables, a constrained ordination was then calculated. A Canonical Correspondance Analysis (CCA) was therefore applied, scaling with a focus on inter-species distances and using a bi-plot scaling type, according to ter Braak and Šmilauer (2002).

A Monte Carlo permutation test was performed with 499 permutations ($p<0.05$).

By means of forward selection of environmental variables, the marginal effect [lambda-1 (λ_1)] (i.e. the variance singly expressed by each variable) and the conditional effect [lambda-A" (λ_A)] (whose value is strictly dependent on the inclusion sequence in the model), were investigated, according to ter Braak and Šmilauer (2002).

Results

Soil parameters

The results of the characterisation of the soil samples are given in Table 1 to Table 4.

According to USDA soil texture classification L soil was classified as "Loam", while P soil was classified as "Sandy Loam". Class 1 soil was classified as "Loam", while class 2 soil was classified as "Sandy Loam"**.** All soils were classified as alkaline (ARPAV 2009).

Average Potential Toxic Elements (PTE) concentrations were always below the national limit imposed for residential and recreational areas (G.U. 293/1999).

Descriptive statistics are reported in Table 5 to Table 8.

Significantly different means values (Mann-Whitney *U*-Test - $p\leq0.05$) of soil parameters between sites and between classes are reported in Tab. 9 and Tab. 10.

Attribute	P site	L site
Physical properties:		
Sand %	53,6	46,3
Clay %	7,1	11,1
field capacity m/m	0,186	0,220
wilt point m/m	0,067	0,089
conductivity µS/cm	299	324
Chemical properties:		
pH mg/Kg	7,9	7,8
OM %	0,36	0,49
N ‰	0,206	0,321
C/N ratio	10,187	8,824
CEC (cmol/Kg)	30,92	34,28
Cr mg/Kg	4,33	4,97
Cu mg/Kg	10,74	4,45
Na mg/Kg	293,30	244,84
Ni mg/Kg	3,17	3,82
Pb mg/Kg	52,24	28,32

Table 9 - Means values of significantly different ($p<0.05$) soil parameters in sites L and P

Attribute	Class 1	Class 2
Physical properties:		
conductivity µS/cm	310,83	313,75
Chemical properties:		
OM %	0,43	0,41
C/N ratio	9,02	10,96

Table 10 - Means values of significantly different ($p<0.05$) soil parameters in class 1 and 2

ECM community

Analyses of all the collected samples demonstrated that pNV, pNM and pEM among samples collected beneath the same tree (different directions and distances from the collar), among the trees of the same site and among the trees of the same decline class never differ significantly (Mann-Whitney *U*-Test, $p< 0.005$).

Decline classes as well do not differ in pNV, pNM and pEM distribution, and sites only differ significantly in pNM value (Table 11).

	pNV	pNM	pEM
decline Class			
CL1	0,579	0,0012	0,419
CL2	0,604	0,0020	0,393
site			
P	0,592	**0,0034**	0,406
L	0,541	**0,0002**	0,458

Table 11 – Mean values of pNV (Non Vital tips), pNM (vital but Not Mycorrhizal tips), pEM (EctoMycorrhizal tips) in different sites and different decline classes. Value in bolt characters are significantly different ($p<0.05$).

Site P had significantly more vital non ectomycorrhized tips than site L, and both values were very low (0.3 and 0.02% respectively).

From the characterization of the ECM tips during the four seasons 52 morphotypes were found. Among them, 15 were ascribed to a fungal family (*Boletaceae*, *Clavulinaceae*, *Cortinariaceae, Helvellaceae*, *Pezizaceae*, *Sebacinaceae*, *Telephoraceae*), 25 to a fungal genus (*Chromelosporium* sp., *Clavulina* sp., *Geopora* sp., *Inocybe* sp., *Laccaria* sp., *Peziza* sp., *Pseudotomentella* sp., *Russula* sp., *Scleroderma* sp., *Sebacina* sp., *Tomentella* sp., *Trichophaea* sp.), 6 to a fungal species [(*Cenococcum geophilum* Fr., *Geopora cervina* (Velen.) T. Schumach. , *Scleroderma verrucosum* (Bull.) Pers., *Trichophaea woolhopeia* (Cooke & W. Phillips) Boud. , *Tuber rapaeodorum* Tul. & C: Tul., *Tuber rufum* Pico], while 6 remained unidentified. All the anatomotypes were also classified by an alphanumerical code (CAxx).

The list of the ECM with their relative abundance in different sites and decline classes is reported in Table 12.

ECM n°	Morphotypes	Site L	Site P	class 1	class 2
18	*Boletaceae (CA18)*	0,422	0,052	0,105	0
34	*CA34*	4,842	0,315	0,183	0,447
43	*CA43*	1,187	0,459	0,236	0,683
88	*CA88*	3,008	0,551	0,786	0,315
89	*CA89*	0,303	0,747	0,157	1,340
94	*CA94*	0,976	0	0	0
96	*CA96*	0	0,039	0,079	0
2	*Cenococcum geophilum(CA2)*	0,528	58,904	54,491	63,304
92	*Chromelosporium(CA92)*	1,240	0	0	0
17	*Clavulina sp.(CA17)*	0,686	3,134	4,137	2,128
19	*Clavulinaceae(CA19)*	6,412	1,547	2,488	0,604
31	*Cortinariaceae (CA31)*	0,686	0,485	0,969	0
68	*Cortinariaceae (CA68)*	0,396	0,039	0	0,079
73	*Cortinariaceae (CA73)*	0,119	0,144	0,288	0
20	*Cortinariaceae (CA20)*	0,013	0,656	1,309	0
30	*Geopora sp.(CA30)*	2,230	0,879	0,759	0,998
11	*Geopora cervina (CA11)*	2,956	0,761	1,519	0
6	*Helvellaceae(CA6)*	0,501	0,944	0,995	0,893
27	*Inocybe sp.(CA27)*	0	0,092	0,183	0
83	*Inocybe sp.(CA83)*	0,092	0,800	0,550	1,051
49	*Laccaria sp.(CA49)*	0,910	0,839	1,126	0,552
60	*Peziza sp.(CA60)*	0,607	0,197	0	0,394
80	*Peziza sp.(CA80)*	1,016	0,538	0,655	0,420
81	*Peziza sp.(CA81)*	2,296	0,734	1,466	0
28	*Pezizaceae (CA28)*	6,531	2,111	4,059	0,158
67	*Pseudotomentella sp.(CA67)*	6,901	1,390	2,252	0,525
39	*Russula sp.(CA39)*	16,018	2,255	1,754	2,758
91	*Scleroderma sp.(CA91)*	0,185	0	0	0
40	*Scleroderma verrucosum (CA40)*	4,077	0,223	0,367	0,079
66	*Sebacina sp.(CA66)*	1,135	0	0	0
85	*Sebacina sp.(CA85)*	0	0,341	0,393	0,289
14	*Sebacinaceae (CA14)*	0,106	0,223	0,445	0
35	*Sebacinaceae (CA35)*	0,185	0,275	0,052	0,499
71	*Sebacinaceae (CA71)*	2,296	1,482	1,126	1,839
3	*Telephoraceae (CA3)*	4,671	0,826	0,340	1,313
32	*Telephoraceae (CA32)*	0,185	0,315	0,419	0,210
33	*Telephoraceae (CA33)*	7,956	4,931	4,032	5,831
95	*Telephoraceae (CA95)*	0,053	0,092	0,079	0,105
1	*Tomentella sp. (CA1)*	3,351	1,442	1,545	1,340
21	*Tomentella sp.(CA21)*	0,567	0,643	0,550	0,735
42	*Tomentella sp. (CA42)*	3,074	3,790	2,383	5,201
54	*Tomentella sp.(CA54)*	1,412	0,931	0,105	1,760
55	*Tomentella sp.(CA55)*	3,932	2,242	2,147	2,338
69	*Tomentella sp.(CA69)*	0	0,236	0,236	0,236
70	*Tomentella sp.(CA70)*	1,267	0,052	0,105	0
75	*Tomentella sp.(CA75)*	0	0,472	0,445	0,499
79	*Tomentella sp.(CA79)*	0,449	0,052	0,105	0
84	*Tomentella sp.(CA84)*	0,145	0,105	0	0,210
82	*Trichophaea sp.(CA82)*	0,106	0,367	0,419	0,315
41	*Trichophaea woolhopeia (CA41)*	1,359	0,210	0,105	0,315
4	*Tuber rapaeodorum (CA4)*	2,296	2,085	4,006	0,158
37	*Tuber rufum (CA37)*	0,317	0,052	0,052	0,053

Table 12 - Relative abundances (R.a.) of ECM in different sites (P – L) and different decline classes (CL1 – CL2)

The ECM species richness (number of species) is similar when comparing site L and site P, but the species consortium is rather dissimilar.

Forty-eight ECM types (12 with R.a. >1%) were found in site P while 47 ECM types (24 with R.a. >1%) in site L. Forty-one morphotypes were found in both sites, while 4 and 5 were found exclusively in site L and site P respectively. *Cenococcum geophilum* is the dominant species (58.9%) in site P, followed by *Telephoraceae* (CA33) (4.9%) , while it is rare in site L (0.52%).

The dominant species of site L is *Russula sp*. (CA39)(16%), less present in site P (2.25%)

In site L, ECM are distributed following a classic exponential trend, with relative abundance gradually decreasing from very few more frequent ECM species [(*Russula sp*. (CA39) with 16%, *Telephoraceae* (CA33) with 7,9%, *Pseudotomentella sp*. (CA67) with 6.9%)] to many others less frequent.

In site P and in both declining Classes 1 and 2 the ECM relative abundance follows as well a decreasing variation, but there is a sudden drop from the most frequent species (*Cenococcum geophilum* for all of them) and the others, since it is present in more than the 50% of the total number of ECM tips (58% in site P, 54,49% in class 1, 63,3% in class 2), whilst the second ECM species only covers the 5%, or less, of the total amount.

As far as declining classes are concerned, 45 ECM types (16 with R.a. >1%) were present in Class 1 and 37 ECM types (12 with R.a. >1%) in Class 2. Thirty-four morphotypes were found in both decline classes, while 10 and 3 were found exclusively in Class1 and Class 2 respectively.

Cenococcum geophilum is the dominant species in both classes and it is followed by *Clavulina* sp. (CA17) (4.13%) in class 1 and by *Telephoraceae* (CA33) (5.83%) in class 2.

Cenococcum geophilum was therefore the most frequent species in site P, followed by the family *Telephoraceae*.

The most frequent family in site L was *Telephoraceae*, followed by *Russulaceae*.

Altogether, 35 fungal genotypes belonging to Basidiomycota and 12 genotypes belonging to Ascomycota were found in site L; 37 Basidiomycota and 11 Ascomycota were found in site P; 35 Basidiomycota and 10 Ascomycota were found in Class 1; 29 Basidiomycota and 8 Ascomycota were found in Class 2. Nevertheless when comparing the two Phyla using relative abundances of each ECM (Fig. 2 and 3), Basidiomycota were the most represented

in site L (68%), while Ascomycota were the most represented in Site P (67%). The proportion between the two Phyla in the decline classes was comparable to that found in site P.

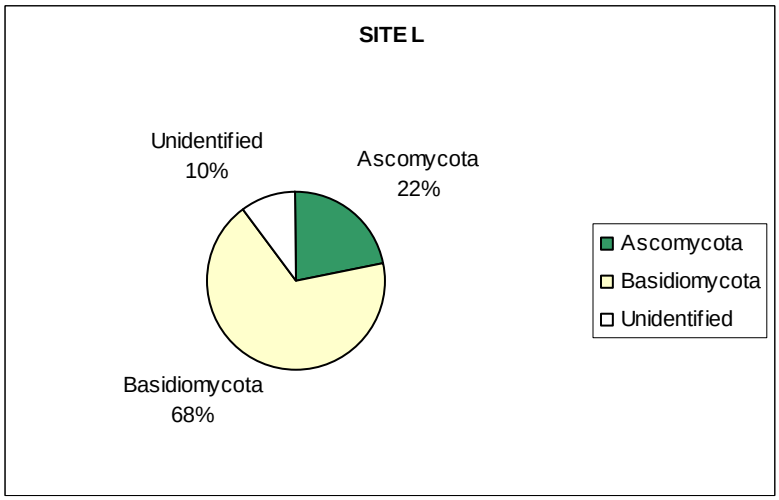


Fig. 2 Relative abundance of ECM Phyla in Site L

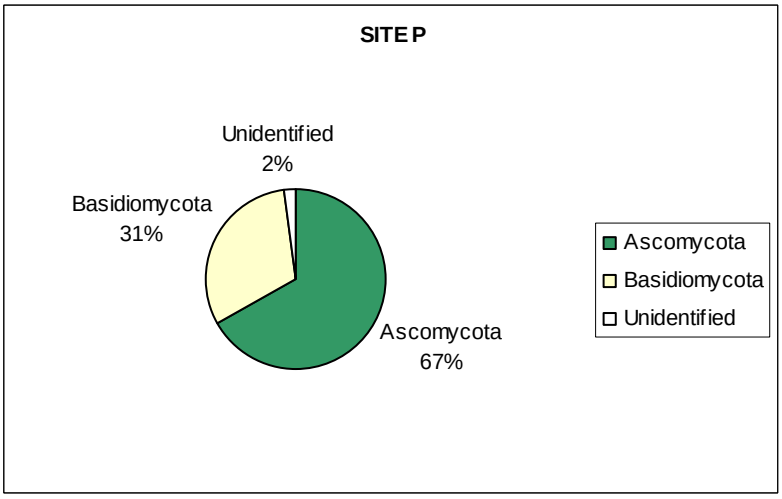


Fig 3 Relative abundance of ECM Phyla in Site L

The canonical analyses considered all the selected trees, as the Mantel Test excluded any spatial correlation within each one.

DCA, which considered 384 samplings, showed gradient lengths between 4 and 5 and demonstrated that the eigenvalues of axis 1 (horizontal) and 2 (vertical) are 0.702 and 0.510 respectively. Fig.4 is the DCA scatter plot of the ECM species in the plotted ordination plane.

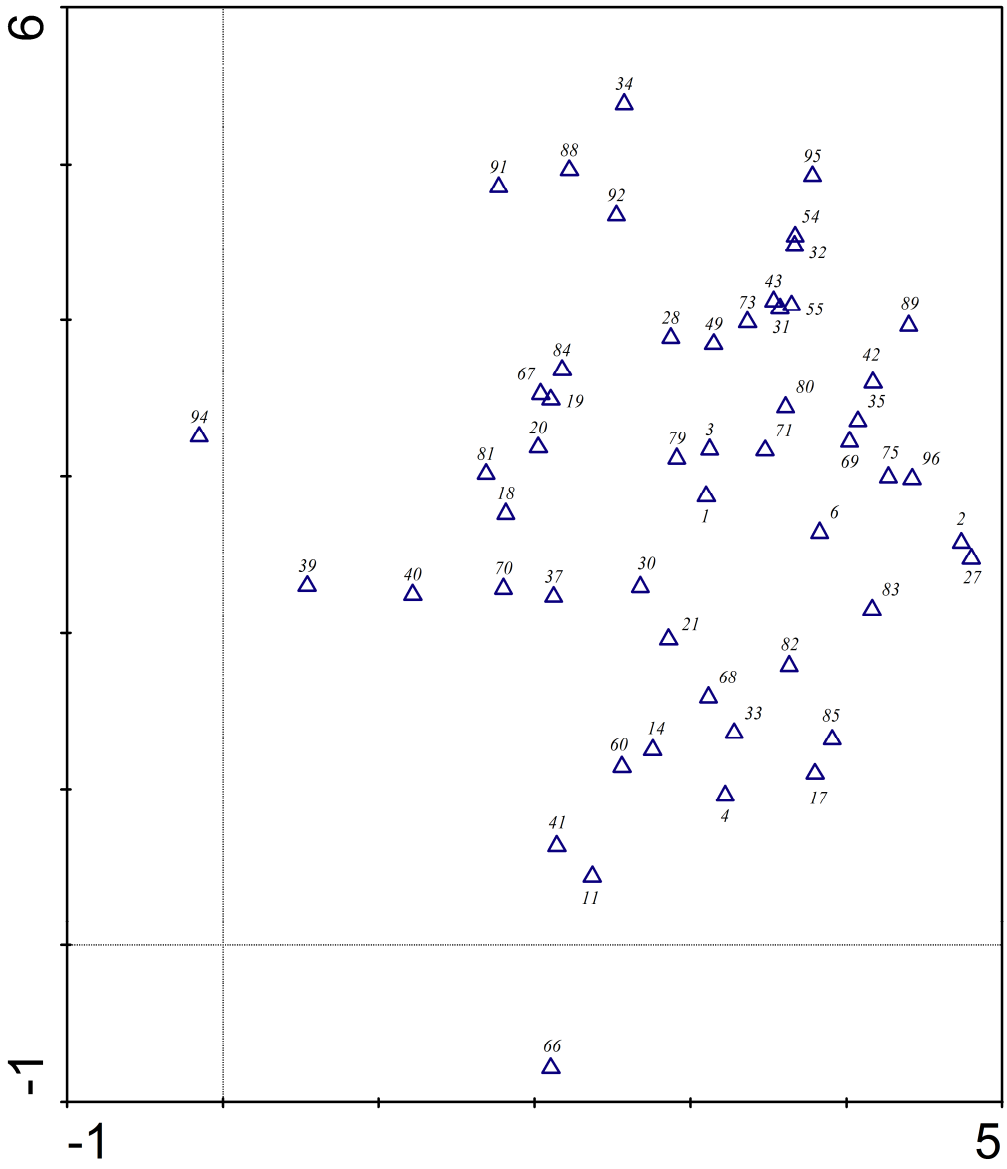


Fig. 4 Scatter plot of species from Detrended Correspondence Analysis. The ECM species corresponding to each number is reported in Table 12

It displays 9.5% of the inertia (i.e. the weighted variance) in species abundance, and 22.1 % of the variance in both the weighted average and class totals of species with respect to the environmental variables. Environmental correlation is 0.629 for axis 1 and 0.516 for axis 2.
As far as species distribution in the plot is concerned, the DCA scatter plot explained the rarity of a number of species (ter Braak & Prentice 1988), among which: *Telephoraceae* CA95, *Scleroderma sp.* CA91, CA96, *Inocybe* sp. CA83 and suggested that some species could be associated to well defined conditions (i.e. *Cenococcum geophilum* CA2 and *Russula sp.* CA39, the most frequent ECM species in site P and site L respectively; (CA34) most present in L, CA94, *Sebacina sp*. CA66, *Chromelosporium sp*.CA92 and *Tricophaea woolhopeia* (CA41) only present in L; *Sebacina* sp. (CA85), *Tomentella sp*. CA75 and *Inocybe sp*. (CA27) only present in P).
In CCA the eigenvalue of axis 1 and axis 2 are 0.204 and 0.153 respectively, indicating a high significance of the first axis and all the canonical axes were present when subjected to the Monte Carlo permutation test (P=0.0020).
Fig. 5 is the bi-plot of ECM species and environmental variables, displaying 2.8% of the inertia (i.e. the weighted variance) in abundances, and 27.8% of the variance in both the eighted average and species class totals with respect to the environmental variables.
By means of the correlation coefficents among variables and axes, we inferred that the first axis is defined by Pb, Zn, C/N and Cu. The second axis is defined by pH, Available Water, As and Field Capacity.
The intra-set correlations of axis 1 with Pb, Zn, C/N and Cu were -0.40, -0.35, -0.32 and -0.30 respectively, followed by class 1 and class 2 that displayed values of 0.28 and -0.28 respectively.
The ECM mainly associated with high values of Zn, Pb, Cu and C/N were in the left area of the diagram; those associated with basic pH and high values of As gathered in the upper part of the diagram, while those associated with acid pH and high values of Available Water and Field Capacity were in the lower part of the biplot.
The intra-set correlations of axis 2 with pH, Available Water, As and Field Capacity were 0.41, -0.39, 0.36 and -0.32 respectively.
The distance between species points in the bi-plot scaling (with a focus on species distances) approximated the chi-square distance between the species distributions.

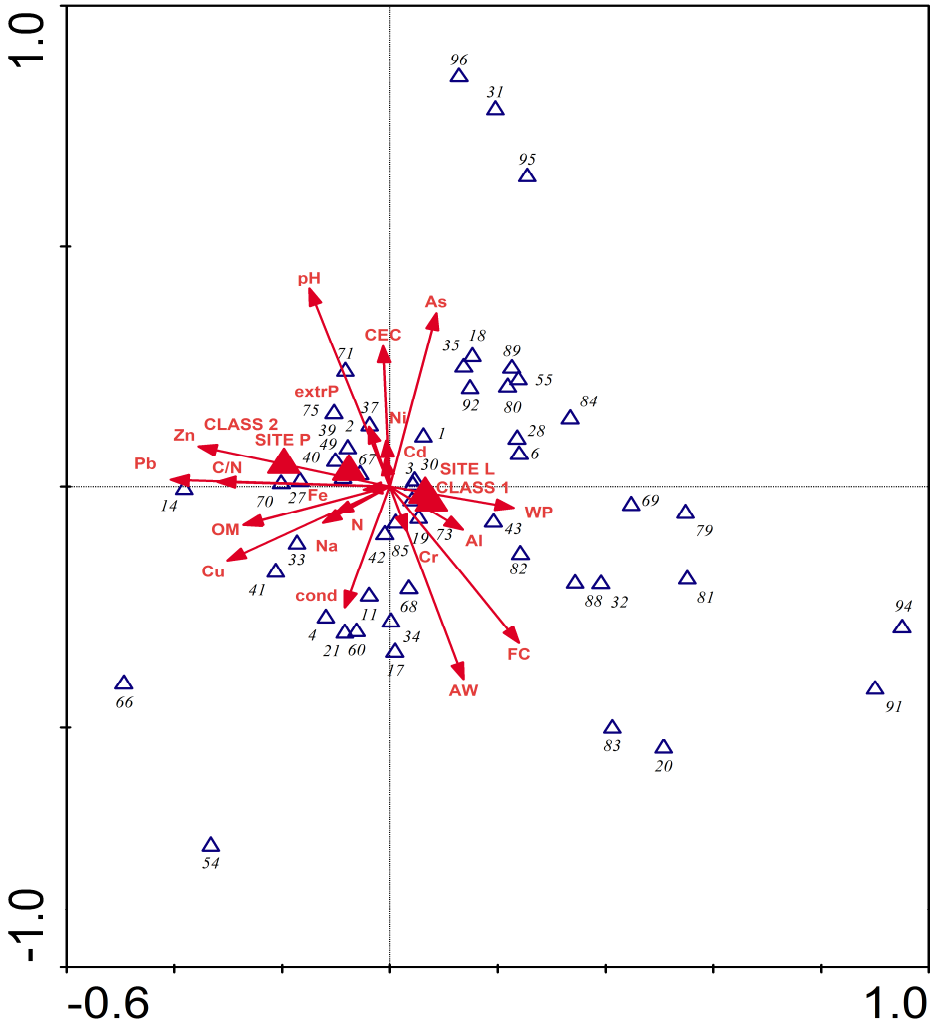


Fig 5 Canonical Correspondence Analysis ordination diagram of ECM species and environmental factors: sites (Site P and Site L), decline class (Class 1 and Class 2), heavy metals concentration (Al, As, Cd, Cr, Cu, Fe, Na, Ni, Pb, Zn), pH, total organic matter (OM), total Nitrogen (N), Carbon Nitrogen ratio (C/N), Cation Exchange Capability (CEC), extractable P (P), conductivity (cond), water content at Field Capacity (FC), water content at permanent Wilting Point (WP) and Available Water capacity (AW)

The ECM associated with Pb and C/N are *Sebacinaceae* (CA14*)* and *Tomentella sp*.(CA70); with Zn *Inocybe sp*.(CA27); with Cu *Trichophaea woolhopeia(CA41*) and *Telephoraceae* (CA33); with basic pH *Sebacinaceae* (CA71) and *Tomentella sp*.(CA75); with As CA96, *Cortinariaceae* (CA31) and *Telephoraceae* (CA95); with acid pH and

Available Water *Clavulina sp.*(CA17) and CA34; with Field Capacity *Cortinariaceae* (CA20) and *Inocybe sp.*(CA83).

The marginal effects in CCA demonstrated that the variables that are better suited to explain the model are Cu (λ_A =0.09), Pb (λ_A =0.09) and Organic Matter (λ_A =0.08).
The conditional effects, which show the environmental variables in order of their inclusion in the model, demonstrated that the most useful features to explain the model are Cu (λ_1 =0.09), Wilting Point (λ_1 =0.09) and pH (λ_1 =0.08); (P=0.002).

Discussion

The research was performed in two different sites of urban linden trees belonging to two different declining classes to verify if environmental variables influenced tips vitality and the ectomychorrhizal community.
The average values of the analyzed topsoil properties (Tab. 1 to 8) mirror an altered situation, regardless to different sites and decline classes: the shortage of organic matter, for instance, is reflected in the equally inadequate Nitrogen reserve, which is likely to act as a limiting factor on tree growth.
As expected, these results are consistent with those of other urban setting soils (Jim 1998; Manta et al. 2002; Imperato et al. 2003; Rodrigues et al. 2009) even if only a superficial comparison can be made due to the lack of an official common methodological approach.
It is well known that in contrast to natural ones, the properties and pedogenesis of urban soils may be dominated by their anthropogenic origin, and their biogeochemical cycles are strongly affected by urbanization. Processes in these soil often differ greatly from those in rural soils, contaminant loads are often much higher, parent material are diverse and often of extreme chemical composition (Rossiter 2007). The importance of urban and industrial soils has only recently been recognized and urban soils have been classified as Technosol by the WRB, World Reference Base for Soil Resources, only in 2006 (IUSS Working Group WRB, 2006). Recently studies on several cities around the world have highlighted similarities in their Technosols, despite their differences in geography, size, climate, etc. (Davidson et al. 2006; Biasioli et al. 2007; Lorenz & Lal 2009; Rodrigues et al. 2009). Anyway the use of WRB in urban soil mapping, characterized by the proportional

composition and spatial pattern of the constituents, has still to be developed and comparisons between urban soils can currently be only approximate.

Most of the tested soil qualities was significantly different between site P and site L, which showed average higher values of chemical-physical parameters (Table 9).

As far as declining classes are concerned, most of the tested soil properties was similar between the two declining classes (Table 10), except for the organic matter amount (significantly lower in class 2) and the conductivity (significantly higher in class 2).

Organic matter contents in urban soil may often be very low, reaching values of 1% (Jim, 1998; Scharenbroch et al. 2005), but Class 2 had an average value of 0.41%.

Organic matter is highly porous and when incorporated in the soil, it decreases soil bulk density. Soil compaction increases bulk density by reducing total pore volume and increasing the percentage of small pores, and this may happen more easily if organic matter is lacking (Scharenbroch et al. 2005).

Soil compaction reduces the availability of water and oxygen to plants, and probably due to this water stress situation P site, and especially Class 2, were dominated by *C. geophilum*. *C. geophilum* is one of the most frequently encountered ECM fungi in nature. This fungus is perhaps the least specialized of ECM fungi in respect to host species, forming ECM with many gymnosperms and angiosperms. It is a cosmopolitan ECM fungus well known for its extremely wide habitat range and for being competitive under adverse climates, due to active growth at low soil temperature, drought tolerance (Pigott, 1982), pioneering capabilities and persistence of sclerotia in the soil (Cairney & Chambers 1999). Most of the fine roots of urban trees may be found in the uppermost horizons of the soil where the nutrient cycling is most intense, even if altered (Jim 1998). Nevertheless the upper horizon suffers strongly from drought in summer, when water is scarce and the demand by the trees is high. This results in the periodical death of ectomycorrhizae which are formed again when drought ends (Courty et al. 2006). Consequently, since the regeneration of fine roots is carbon-costly, the presence of mycorrhizae that can survive and even express their maximal activity during drought periods (and *C. geophilum* is the most efficient drought-tolerant type) is an adaptive advantage. In the present study all trees were already 50 years old and the etomycorrhizal community associated to their roots was likely to be well-fitted

to the street habitat. The dominance of *C. geophilum* may be read as the trees response to the selective pressure exercised by environmental stresses.

In the investigated linden trees the degree of colonization was generally low regardless of their location and health condition (Table 11). One of the contributing factors could be the low inoculum potential in the urban soil, as hypothesized by Nielsen (1999) who compared young urban plantations to old forest trees of *Tilia cordata*.

Tips vitality and ectomycorrhization degree did not differ between sites and decline classes (Tab. 11).

Site P had significantly more vital non ectomycorrhized tips than site L, but since both values were very low this may mean that root tips had more chances to be vital when ectomycorrhized.

Other authors found that the fine roots of most declining trees in declining forests had a lower proportion of vital and ectomycorrhized tips (Montecchio et al. 2004; Mosca et al. 2007). Such a general trend did not occur in the present study whose results are anyway consistent with those of previous studies: Edwards (1992) did not found consistent effects of acidic precipitation and ozone on ECM formation in loblolly pine (*Pinus taeda* L.) seedlings; Swaty (2004) observed that the ectomycorrhizal colonization rate of pinyon pine (*Pinus edulis*) exposed to severe drought events did not differ significantly between trees experiencing differences in stress. These results corroborate the hypothesis that under severe conditions the ECM degree remains at an average low level because the plant invests into ECM mutualists until a threshold is reached at which further stress prevents plant resources from being allocated to ECM fungi, such stopping the increasing of ECM colonization (Swaty et al. 2004).

This might be due to the previously described altered situation of the urban soil, which turns out to be a generalized severe condition comparable to serious natural events (large-scale drought, fire, soil warming).

Timonen and Kauppinen (2008) have recently compared the mycorrhizal colonization patterns of *Tilia* spp. trees in street, nursery and forest habitats, also comparing healthy and unhealthy street trees. They found that healthy and unhealthy street trees didn't differ in mycorrhizal colonization intensity and that *Cenococcum* spp. were more dominating in the roots of the unhealthy street trees and that *Russula* spp. were common to healthy street

trees: the same dominant genera respectively found in site P and site L of the present study. Unfortunately the authors didn't analyze the influences of environmental variables on ECM community, and they couldn't explain whether the differences in the ECM community composition were cause, rather than effect, of the health state of the trees.

It may be hypothesized that also slight changes in Technosoil properties may indirectly affect the competitive ability of certain mycorrhizal fungi, resulting in species shifts without a subsequent change in total number. The application of the concepts of functional complementarity and functional groups has besides already been suggested for ECM communities in natural forests (Montecchio et al. 2004; Rumberger et al. 2004; Buée et al. 2005). For example Montecchio (2004) has hypothesized that the ECM community changes among forest trees at different stages of decline may be an indirect result of selective pressure exerted by environmental stresses on the trees: their rootlets, depending on individual susceptibility, loose their ability to select the most efficient fungal symbiont so that other less efficient fungi may take their place. In this hypothesis, while the ECM species common to both asymptomatic and declining trees may have had a wide adaptive range, the ones occurring exclusively on asyntomatic trees would be gradually replaced by ECM species growing exclusively on declining trees. Such ECM would be essential to maintain the stability of the modified ecosystem processes but would probably be less efficient than the previous ones. The results of the present study suggest that this hypothesis may be applied to urban environment as well.

In order to delve into this hypothesis the plant stress response of the present study has been therefore searched in the composition of the ECM consortia, and in its relationship with environmental variables.

From a qualitative point of view, the ECM richness was similar when comparing site L and site P, but decline class 2 had less species than class 1. (Table 12) and Ascomycete abundance was higher in site P (Fig. 2 and 3). These results are consistent with previous findings (Gehring et al., 1998; Swaty et al., 2004; Ruotsalainen et al. 2009; Sthultz et al. 2009) and with the hypothesis that ECM species richness declines and ascomycete fungi increase in response to stress.

As expected only a few taxa represented the majority of all ECM within each site, and within each decline class.

The abundance distribution of the number of species (Table 12) of all locations and decline classes, dominated in P by *Cenococcum geophilum* and in L by *Russula* sp.(CA39) and accompanied by a descending number of ECM of other taxa, showed a high similarity to those of other studies (Taylor 2002; Koide et al. 2005; Baier et al. 2006; Timonen & Kauppinen 2008). It is typical for mycorrhizal communities in natural stands that a few dominant species contrast with a high number of rare ECM. The present research confirms that this pattern is present in artificial environments as well.

Telephoraceae was the most abundant ECM taxa found in site L, and the second one in site P. It is widely known that species belonging to the *Telephoraceae*, generalist and important mycorrhizal partners of many deciduous trees and conifers, are among the most frequent and abundant ECM species in Europe and North America (Horton & Burns 2001; Kõljalg et al. 2000).

Multivariate techniques were chosen to study the influence of environmental variables on the ECM community composition. In particular an ordination method was applied because it permits to evaluate differences in species composition between sites and to identify the environmental variables responsible for these differences in a single analysis (Van den Brink et al. 2003).

The segregation in dependency on differences in soil properties was revealed by the ordination of the ECM community by DCA and CCA (Fig. 4 and 5).

Considering the variables taken into account (C/N, pH, Available Water, Field Capacity, Pb, Zn, Cu, As) *Clavulina sp*.(CA17),CA34, CA60 and CA21 were mainly present in sites with high Available Water value; the consortium *Sebacinaceae* (CA71) and *Tomentella sp*.(CA75) characterized basic sites; *Sebacinaceae* (CA14*)* and *Tomentella sp*.(CA70) were associated with high values of Pb and C/N , *Inocybe sp*.(CA27) with high values of Zn; *Trichophaea woolhopeia* (CA41) and *Telephoraceae* (CA33) were associated with Cu; CA96, *Cortinariaceae* (CA31) and *Telephoraceae* (CA95) with As; *Cortinariaceae* (CA20) and *Inocybe sp*.(CA83) with Field Capacity.

In conclusion the ordination of ECM species with CCA indicated that environmental variables, comprising the different soil properties and differences in sites and in class of decline, had the most pronounced effect on the ECM community structure. The results

suggest that the differences in ECM communities between sites and between decline classes may be related to the variation of the environmental variables as a whole, rather than to the influence of a single or few factors.

The influence of soil factors on ECM community was already known for natural stands. Peter et al. (2001) described changes in ECM species composition following nitrogen addition for two years to a subalpine *Picea abies* stand, while Toljander et al (2006) found strong correlations among ECM community structure and soil characteristics. Accordingly, Tedersoo et al. (2003) and Scattolin et al. (2008a) demonstrated the preference of ECM fungi for different substrate qualities, organic layers and mineral horizons.

The vertical niche differentiation of ECM in the soil horizons of a Norway spruce plantation was demonstrated by Baier et al. (2006) and more recently Scattolin et al. (2008b) confirmed that variability in the ECM community structure of Norway spruce stands could be explained by characteristics of the organic and mineral soil horizons.

Koide (2005) studied the ECM community of a red pine (*Pinus resinosa* Ait.) plantation and demonstrated the existence of species interactions in it. His study explained that significant associations among ECM species could occur because species with similar requirements towards soil resources would tend to occupy similar sites.

Few researches have dealt with the ECM community changes in declining trees. Montecchio et al. (2004) found that the severity of decline in natural *Quercus ilex* stands can be characterized determining the recovery frequency of the more frequent ECM independently of the least common ones. More recently Mosca et al. (2007) suggested that the stability of the ECM community of a natural stand of peduncolate oak could be a possible indicator of plant health status.

Unfortunately still less researches have been conducted in urban environment: Timonen & Kauppinen (2008) have described differences between the ECM community of *Tilia* spp. urban trees, but have not related them to environmental variables.

The results of the present work confirm that ECM groups arrange themselves according their preference for specific soil characteristics and favour the hypothesis that the observed change of ectomycorrhizal community is correlated with these changes in urban soil

properties. Further research efforts are needed to better understand the ECM community parameters that can be best used as indicators of plant health in urban environment.

Fig.6 Visual tree Assessment form used for the assignment of the decline class

VISUAL TREE ASSESSMENT

Date Assesser name Tree species Location Tree number

		SCORE	
Defolation degree	0-10%	20	
	10-30%	10	
	30-70%	5	
	70-100%	0	_____
Leaf decoloration	0-10%	10	
	10-30%	5	
	30-70%	3	
	70-100%	0	_____
Epicormic twigs	absent/rare	10	
	Frequent/abundant	5	_____
Dead branches	absent/rare	20	
	frequent	10	
	abundant	5	_____
Canopy transparency			
	0-20%	20	
	20-50%	10	
	50-90%	2	_____

TOTAL SCORE [ ] HEALTH CLASS [ ]

Calculate total score and assign each tree to the corresponding class as follows:

Total score	class
> 60	0- asymptomatic
30-60	1-moderately declining
<30	2-severly declining

Table 1 Main chemical characteristics of topsoil – site P

Tree number	1		2		3		4		5		6		7		8		
exposition	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	Max PTE conc*
Al mg/Kg	2033,57	1525,15	2325,32	2550,24	3209,61	3065,42	2969,41	2328,77	1964,80	2492,11	1470,07	1839,52	2046,18	1968,25	2445,73	3034,16	
As mg/Kg	7,51	7,29	7,26	37,30	7,73	8,62	6,96	6,80	8,29	6,66	8,83	3,93	5,65	5,43	4,32	6,17	20
Cd mg/Kg	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	2
Cr mg/Kg	4,63	3,17	4,14	5,25	5,02	5,29	5,24	4,33	4,02	5,09	3,53	3,48	3,78	3,67	4,02	4,65	150
Cu mg/Kg	7,81	6,93	5,40	4,04	8,99	6,55	8,37	8,16	25,27	8,52	6,92	4,13	29,67	23,95	8,55	8,65	120
Fe mg/Kg	2996,79	2803,35	3352,88	2663,55	3309,10	3236,69	3091,80	3002,40	3301,65	2703,68	3214,67	1574,00	2999,44	2415,62	2452,52	3104,64	
Na mg/Kg	230,67	213,49	265,65	352,90	244,27	310,42	236,87	292,52	362,62	304,06	229,24	295,41	447,59	345,99	262,32	298,81	
Ni mg/Kg	2,77	2,61	3,28	3,63	3,36	3,58	3,28	3,27	3,06	3,13	3,58	2,32	3,43	3,22	2,97	3,19	120
Pb mg/Kg	57,68	62,85	51,18	34,37	51,46	63,53	48,66	57,47	55,35	44,38	52,50	20,41	57,42	56,50	69,09	52,97	100
Zn mg/Kg	30,22	28,14	31,01	26,40	31,32	30,76	34,85	38,83	43,05	41,30	34,78	22,78	54,85	49,41	66,72	32,13	150
pH mg/Kg	7,9	8,1	8,0	8,1	8,0	7,8	7,9	7,7	7,9	7,7	8,0	7,9	8,1	8,0	8,1	7,9	
C org %	0,14	0,15	0,14	0,14	0,18	0,24	0,17	0,23	0,20	0,33	0,22	0,31	0,19	0,24	0,21	0,24	
OM %	0,24	0,27	0,25	0,24	0,31	0,41	0,29	0,39	0,34	0,57	0,38	0,53	0,33	0,41	0,35	0,42	
N ‰	0,156	0,193	0,219	0,168	0,178	0,220	0,181	0,248	0,196	0,316	0,230	0,237	0,136	0,250	0,153	0,210	
C/N ratio	8,834	8,040	6,581	8,362	10,106	10,723	9,287	9,213	10,023	10,525	9,685	13,125	13,992	9,499	13,518	11,483	
CEC (cmol/Kg)	24,62	23,56	64,11	23,19	25,85	28,29	24,37	39,81	26,73	38,18	28,74	27,08	28,91	31,00	29,41	30,93	
Extractable P(mg/Kg)	23	23	34	34	26	26	36	36	34	34	34	34	26	26	32	32	

*Maximum acceptable Potentially Toxic Elements concentrations for residential and recreational areas in Italy

Table 2 Main chemical characteristics of topsoil – site L

Tree number	9		10		11		12		13		14		15		16		
esposition	E	W	E	W	E	W	E	W	E	W	E	W	E	W	E	W	Max PTE conc*
Al mg/Kg	2034,23	2085,12	2374,94	1784,68	2285,17	2386,69	1913,29	2209,95	2460,98	2869,92	2042,52	2971,93	2089,17	2486,52	2347,14	3048,45	
As mg/Kg	8,37	6,03	5,29	8,84	6,94	11,73	5,55	9,16	5,31	4,10	5,09	4,18	5,02	4,20	4,64	3,83	20
Cd mg/Kg	0,00	0,05	0,00	0,05	0,00	0,00	0,05	0,15	0,00	0,00	0,00	0,00	0,00	0,05	0,00	0,00	2
Cr mg/Kg	4,97	5,67	4,99	4,30	4,86	5,03	3,53	4,98	4,40	6,12	5,04	5,95	4,51	5,16	4,84	5,14	150
Cu mg/Kg	5,17	5,47	5,90	4,65	4,10	4,16	3,48	5,84	3,44	3,94	2,77	4,64	4,21	4,91	3,73	4,84	120
Fe mg/Kg	2919,99	2898,97	3084,15	3052,02	3548,94	3393,63	2554,63	3817,41	3271,52	3028,97	2816,60	3335,61	3135,07	2809,85	2762,98	3009,06	
Na mg/Kg	182,98	338,91	167,68	207,60	247,89	231,40	192,47	298,73	161,84	338,98	225,96	299,70	276,65	216,47	251,78	278,42	
Ni mg/Kg	3,50	3,85	3,92	3,59	5,01	3,45	2,88	3,83	3,69	4,50	3,48	4,49	3,65	4,05	3,58	3,63	120
Pb mg/Kg	41,38	52,82	48,53	31,58	28,11	29,10	24,62	38,41	23,61	17,49	14,97	20,37	20,06	25,86	19,98	16,29	100
Zn mg/Kg	34,44	42,49	48,89	47,60	34,99	27,22	25,48	48,08	27,25	25,08	20,06	25,46	25,89	29,60	23,26	21,44	150
pH mg/Kg	7,8	7,8	7,7	7,8	7,9	7,9	7,9	7,9	7,8	7,8	7,8	7,9	7,9	7,8	7,8	7,8	
C org %	0,32	0,50	0,39	0,33	0,29	0,25	0,30	0,26	0,31	0,25	0,17	0,21	0,23	0,27	0,26	0,20	
OM %	0,56	0,87	0,68	0,56	0,51	0,42	0,51	0,45	0,53	0,43	0,30	0,37	0,40	0,47	0,45	0,35	
N ‰	0,385	0,378	0,440	0,389	0,380	0,311	0,299	0,291	0,370	0,294	0,219	0,256	0,303	0,284	0,280	0,255	
C/N ratio	8,444	13,301	8,926	8,361	7,750	7,903	9,975	8,949	8,388	8,515	7,836	8,365	7,628	9,598	9,367	7,874	
CEC (cmol/Kg)	37,55	38,78	40,86	35,04	38,89	37,62	32,97	31,44	35,44	29,32	26,70	32,86	34,61	33,27	33,05	30,09	
Extractable P (mg/Kg)	30	30	27	27	27	27	46	46	29	29	27	27	36	36	24	24	

*Maximum acceptable Potentially Toxic Elements concentrations for residential and recreational areas in Italy

Table 3 Main physical and hydrological characteristics of topsoil - site P

Tree number	1		2		3		4		5		6		7		8	
esposition	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S
Sand %	47,8		50,6		47		52		47		60,1		70,3		54,2	
Silt %	48		40,3		48,7		41,2		41,4		33,1		23		38,8	
Clay %	4,2		9,1		4,3		6,8		11,6		6,8		6,7		7	
textural classes	Sandy Loam		Loam		Sandy Loam		Sandy Loam		Loam		Sandy Loam		Sandy Loam		Sandy Loam	
conductivity µS/cm	240	240	275	275	280	280	355	355	310	310	280	280	385	385	270	270
field capacity m/m	0,184	0,185	0,197	0,196	0,188	0,191	0,187	0,190	0,215	0,222	0,173	0,178	0,151	0,153	0,185	0,187
wilting point m/m	0,051	0,051	0,075	0,075	0,052	0,054	0,065	0,066	0,089	0,093	0,066	0,068	0,065	0,066	0,067	0,068
available water m/m	0,133	0,134	0,121	0,121	0,136	0,137	0,122	0,124	0,126	0,129	0,107	0,109	0,086	0,087	0,118	0,119

Table 4 Main physical and hydrological characteristics of topsoil – site L

Tree number	9		10		11		12		13		14		15		16	
esposition	E	W	E	W	E	W	E	W	E	W	E	W	E	W	E	W
Sand %	52,4		57,5		46,9		50,9		36,6		42,5		43,3		40,4	
Silt %	35,8		33		43,6		35,7		54,2		45,7		44,9		47,8	
Clay %	11,8		9,5		9,5		13,4		9,2		11,8		11,8		11,8	
textural classes	Sandy Loam		Sandy Loam		Loam		Loam		Silt Loam		Loam		Loam		Loam	
conductivity µS/cm	310	310	370	370	310	310	300	300	390	390	295	295	310	310	305	305
field capacity m/m	0,212	0,221	0,197	0,194	0,213	0,211	0,219	0,217	0,233	0,230	0,224	0,226	0,225	0,228	0,233	0,230
wilting point m/m	0,094	0,099	0,084	0,082	0,082	0,080	0,101	0,100	0,080	0,079	0,090	0,091	0,091	0,092	0,092	0,090
available water m/m	0,118	0,122	0,113	0,111	0,132	0,130	0,118	0,117	0,153	0,152	0,134	0,135	0,134	0,135	0,141	0,139

Attribute	Mean	S.D.	Variance	Minimum	Maximum
Physical properties:					
Sand %	53,6	7,8	60,5	47,0	70,3
Silt %	39,3	8,0	63,7	23,0	48,7
Clay %	7,1	2,3	5,4	4,2	11,6
field capacity m/m	0,186	0,018	0,000	0,151	0,222
wilting point m/m	0,067	0,012	0,000	0,051	0,093
available water m/m	0,120	0,015	0,000	0,086	0,137
conductivity µS/cm	299	47	2170	240	385
Chemical properties:					
pH mg/Kg	7,9	0,1	0,0	7,7	8,1
OM%	0,36	0,10	0,01	0,24	0,57
N ‰	0,206	0,046	0,002	0,136	0,316
C/N ratio	10,187	2,026	4,105	6,581	13,992
CEC (cmol/Kg)	30,92	10,02	100,39	23,19	64,11
Extractable P (mg/Kg)	31	5	22	23	36
Al mg/Kg	2329,27	539,62	291194,29	1470,07	3209,61
As mg/Kg	8,67	7,76	60,24	3,93	37,30
Cd mg/Kg	0,00	0,00	0,00	0,00	0,00
Cr mg/Kg	4,33	0,71	0,50	3,17	5,29
Cu mg/Kg	10,74	7,94	63,04	4,04	29,67
Fe mg/Kg	2888,92	458,22	209969,81	1574,00	3352,88
Na mg/Kg	293,30	61,69	3806,18	213,49	447,59
Ni mg/Kg	3,17	0,36	0,13	2,32	3,63
Pb mg/Kg	52,24	11,68	136,32	20,41	69,09
Zn mg/Kg	37,28	11,53	132,83	22,78	66,72

Table 5 - Statistical summary of soil properties of 16 soil samples collected at site P

Attribute	Mean	S.D.	Variance	Minimum	Maximum
Physical properties:					
Sand %	46,3	6,70	44,87	36,60	57,50
Silt %	42,6	6,96	48,47	33,00	54,20
Clay %	11,1	1,46	2,13	9,20	13,40
field capacity m/m	0,220	0,01	0,00	0,19	0,23
wilting point m/m	0,089	0,01	0,00	0,08	0,10
available water m/m	0,130	0,01	0,00	0,11	0,15
conductivity µS/cm	324	34,33	1178,33	295,00	390,00
Chemical properties:					
pH mg/Kg	7,8	0,05	0,00	7,71	7,91
OM%	0,49	0,14	0,02	0,30	0,87
N ‰	0,321	0,06	0,00	0,22	0,44
C/N ratio	8,824	1,38	1,90	7,63	13,30
CEC (cmol/Kg)	34,28	3,85	14,84	26,70	40,86
Extractable P (mg/Kg)	31	6,85	46,87	24,00	46,00
Al mg/Kg	2336,92	368,94	136113,95	1784,68	3048,45
As mg/Kg	6,14	2,26	5,12	3,83	11,73
Cd mg/Kg	0,02	0,04	0,00	0,00	0,15
Cr mg/Kg	4,97	0,63	0,40	3,53	6,12
Cu mg/Kg	4,45	0,89	0,79	2,77	5,90
Fe mg/Kg	3089,96	321,58	103413,41	2554,63	3817,41
Na mg/Kg	244,84	56,55	3197,50	161,84	338,98
Ni mg/Kg	3,82	0,51	0,26	2,88	5,01
Pb mg/Kg	28,32	11,48	131,72	14,97	52,82
Zn mg/Kg	31,70	9,88	97,54	20,06	48,89

Table 6 - Statistical summary of soil properties of 16 soil samples collected at site L

Attribute	Mean	S.D.	Variance	Minimum	Maximum
Physical properties:					
Sand %	47,93	6,05	36,56	36,60	57,50
Silt %	42,42	6,00	36,00	33,00	54,20
Clay %	9,66	2,60	6,78	4,20	13,40
field capacity m/m	0,21	0,02	0,00	0,18	0,23
wilting point m/m	0,08	0,01	0,00	0,05	0,10
available water m/m	0,13	0,01	0,00	0,11	0,15
conductivity µS/cm	310,83	41,80	1747,10	240,00	390,00
Chemical properties:					
pH mg/Kg	7,87	0,11	0,01	7,71	8,11
OM%	0,43	0,15	0,02	0,24	0,87
N ‰	0,28	0,08	0,01	0,15	0,44
C/N ratio	9,02	1,65	2,73	6,58	13,52
CEC (cmol/Kg)	33,69	8,34	69,52	23,19	64,11
Extractable P (mg/Kg)	30,92	6,26	39,21	23,00	46,00
Al mg/Kg	2358,46	402,45	161969,49	1525,15	3048,45
As mg/Kg	7,58	6,62	43,77	3,83	37,30
Cd mg/Kg	0,01	0,03	0,00	0,00	0,15
Cr mg/Kg	4,79	0,68	0,47	3,17	6,12
Cu mg/Kg	5,38	1,78	3,18	2,77	8,65
Fe mg/Kg	3037,80	312,21	97474,73	2452,52	3817,41
Na mg/Kg	252,95	53,26	2836,29	161,84	352,90
Ni mg/Kg	3,59	0,56	0,31	2,61	5,01
Pb mg/Kg	36,98	16,58	274,95	14,97	69,09
Zn mg/Kg	33,15	10,92	119,30	20,06	66,72

Table 7 - Statistical summary of soil properties of 16 soil samples collected - class 1

Attribute	Mean	S.D.	Variance	Minimum	Maximum
Physical properties:					
Sand %	56,10	10,46	109,50	47,00	70,30
Silt %	36,55	10,24	104,76	23,00	48,70
Clay %	7,35	2,83	8,03	4,30	11,60
field capacity m/m	0,18	0,03	0,00	0,15	0,22
wilting point m/m	0,07	0,01	0,00	0,05	0,09
available water m/m	0,11	0,02	0,00	0,09	0,14
conductivity µS/cm	313,75	45,88	2105,36	280,00	385,00
Chemical properties:					
pH mg/Kg	7,92	0,14	0,02	7,66	8,11
OM%	0,41	0,10	0,01	0,31	0,57
N ‰	0,22	0,05	0,00	0,14	0,32
C/N ratio	10,96	1,67	2,78	9,50	13,99
CEC (cmol/Kg)	29,35	3,90	15,24	25,85	38,18
Extractable P (mg/Kg)	30,00	4,28	18,29	26,00	34,00
Al mg/Kg	2257,00	612,14	374717,63	1470,07	3209,61
As mg/Kg	6,89	1,77	3,13	3,93	8,83
Cd mg/Kg	0,00	0,00	0,00	0,00	0,00
Cr mg/Kg	4,24	0,77	0,59	3,48	5,29
Cu mg/Kg	14,25	10,21	104,14	4,13	29,67
Fe mg/Kg	2844,36	604,46	365375,67	1574,00	3309,10
Na mg/Kg	317,45	69,32	4805,87	229,24	447,59
Ni mg/Kg	3,21	0,41	0,17	2,32	3,58
Pb mg/Kg	50,19	13,22	174,88	20,41	63,53
Zn mg/Kg	38,53	10,60	112,44	22,78	54,85

Table 8 - Statistical summary of soil properties of 16 soil samples collected - class 2

References

AGERER R (ed) (1987-2008) Colour atlas of ectomycorrhizae. 1-14th delivery, Einhorn, Schwäbisch Gmünd, D

AGERER R (1991) Characterization of ectomycorrhizae. In: NORRIS JR, READ DJ, VARMA AK, (eds) Techniques for the Study of Mycorrhiza. UK Academic Press., London, pp 25-73

ALVAREZ M, HUYGENS D, FERNANDEZ C, GACITÚA Y, OLIVARES E, SAAVEDRA I, ALBERDI M, VALENZUELA E (2009) Effect of ectomycorrhizal colonization and drought on reactive oxygen species metabolism of *Nothofagus dombeyi* roots. Tree physiology 29:1047-1057

APPLETON B, KOCI J, FRENCH S, LEYSTAN M, HARRIS R (2003) Mycorrhizal fungal inoculation of established street trees. Journal of Arboriculture 29:107-111

ARPAV (2009) – Agenzia Regionale per la Prevenzione e Protezione Ambientale del Veneto – provider: http://www.arpa.veneto.it

BAAR J, DE VRIES FW (1995) Effects of manipulation of litter and humus layers on ectomycorrhizal colonization potential in Scots pine stands of different age. Mycorrhiza 5:267-272

BAIER R, INGENHAAG J, BLASCHKE H, GÖTTLEIN A, AGERER R (2006) Vertical distribution of an ectomycorrhizal community in upper soil horizons of a young Norway spruce (*Picea abies* [L.] Karst.) stand of the Bavarian Limestone Alps. Mycorrhiza 16(3):197-206

BIASIOLI M, GRČMAN H, KRALJ T, MADRID F, DÍAZ-BARRIENTOS E, AJMONE-MARSAN F (2007) Potentially toxic elements contamination in urban soils: a comparison of three european cities. J Environ Qual 36:70-79

BIDARTONDO MI, READ DJ (2008) Fungal specificity bottlenecks during orchid germination and development. Molecular Ecology 17:3707–3716

BRAND F (1992) Mixed associations of fungi in ectomycorrhizal roots. In: Read DJ, Lewis DH, Fitter AH, Alexander IJ, eds. Mycorrhizas in ecosystems. Wallingford, UK: CAB International, 142-17.

BUÉE M, VAIRELLES D, GARBAYE J (2005) Year-round monitoring of diversity and potential metabolic activity of the ectomycorrhizal community in a beech (*Fagus silvatica*) forest subjected to two thinning regimes. Mycorrhiza 15:235-245

CABI BIOSCIENCE, CBS, LANDCARE RESEARCH (access from 2007-10 to 2009-10) Index Fungorum database www.indexfungorum.org

CAIRNEY JWG, CHAMBERS SM (eds) (1999) Ectomicorrhizal fungi: key genera in profile. Springer, Berlin Heidelberg New York

COURTY PE, POUYSEGUR R, BUÉE M, GARBAYE J (2006) Laccase and phosphatase activities of the dominant ectomycorrhizal types in a lowland oak forest. Soil Biology & Biochemistry 38:1219-1222

DAVIDSON CM, URQUHART GJ, AJMONE-MARSAN F, BIASIOLI M, DA COSTA DUARTE A, DÍAZ-BARRIENTOS E, GRČMAN H, HOSSACK I, HURSTHOUSE AS, MADRID L, RODRIGUES S, ZUPAN M (2006) Fractionation of potentially toxic elements in urban soils from five European cities by means of a harmonized sequential extraction procedure. Analytica Chimica Acta 565:63-72

DE ROMAN M, CLAVERIA V, DE MIGUEL AM (2005) A revision of the descriptions of ectomycorrhizas published since 1961. Mycol Res 109: 1063-1104

DIEDHIOU AG, DUPOUEY JL, BUEE M, DAMBRINE E, LAUT L, GARBAYE J (2009) Response of ectomycorrhizal communities to past Roman occupation. Soil Biology & Biochemistry 41:2206-2213

DOBBERTIN M (2005) Tree growth as indicator of tree vitality and of tree reaction to environmental stress: a review. Eur J Forest Res 124:319-333

DOUHAN GW, RIZZO DM (2005) Phylogenetic divergence in a local population of the ectomycorrhizal fungus Cenococcum geophilum. New Phytologist 166:263-271

EDWARDS GS & KELLY JM (1992) Ectomycorrhizal colonization of loblolly pine seedlings during three growing seasons in response to ozone, acidic precipitation, and soil Mg status. Environ Pollut 76:71-77

ERÖS-HONTI Z, JAKUCS E (2009) Characterization of beech ectomycorrhizae formed by species of the *Pachyphloeus – Amylascus* lineage. Mycorrhiza 19:337-345

GARBAYE J & CHURIN JL (1996) Effect of ectomycorrhizal inoculation at planting on growth and foliage quality of *Tilia tomentosa*. Journal of Arboriculture 22:29-34

GARBAYE J, LOHOU C, LAURENT P, CHURIN JL (1999) Ectomycorrhizal inoculation of avenue trees in Paris. Acta Hort. 496:445-449

GARDES M, BRUNS T D (1996) Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest. Above- and below-ground views. Can J Bot 74:1572-1583

GAZZETTA UFFICIALE (1999) Decreto Ministeriale del 13/09/1999 Approvazione dei "Metodi ufficiali di analisi chimica del suolo". G.U. Suppl. Ord. N° 248 , 21/10/1999

GAZZETTA UFFICIALE (1999) Decreto Ministeriale del 25/10/1999 Regolamento recante criteri, procedure e modalità per la messa in sicurezza, la bonifica e il ripristino

ambientale dei siti inquinati, ai sensi dell'articolo 17 del decreto legislativo 5 febbraio 1997, n. 22, e successive modificazioni e integrazioni. G.U. n.293 Suppl. Ord. N° 218 , 15/12/1999

GEE G W, BAUDER J W (1986) Particle-size analysis. In Methods of Soil Analysis, Part1: Physical and Mineralogical Methods, 2nd ed. (A. Klute ed.) pp 383-411. American Society of Agronomy, Madison, WI.

GEHRING CA, THEIMER TC, WHITHAM TG, KEIM P (1998) Ectomycorrhizal fungal community structure of pinyon pines growing in two environmental extremes. Ecology 79:1562–1572

HILL MO, GAUCH HG JR (1980) Detrended correspondence analysis, an improved ordination technique. Vegetatio 42:47-58

HORTON T R, BRUNS T D (2001) The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. Molecular Ecology 10:1855-1871

IMPERATO M, ADAMO P, NAIMO D, ARIENZO M, STANZIONE D, VIOLANTE P (2003) Spatial distribution of heavy metals in urban soils of Naples city (Italy). Environmental Pollution 124:247-256

ISO (2006) Soil quality-pretreatment of samples for physic-chemical analysis. ISO 11464. International Standards Organization, Geneva, Switzerland

IOTTI M, ZAMBONELLI A (2006) A quick and precise technique for identifying ectomycorrhizas by PCR. Mycological Research 110:60-65

IUSS WORKING GROUP WRB (2006) World reference base for soil resources. World Soil Resources Reports No.103. Rome, Italy; FAO

IZZO A, AGBOWO J, BRUNS TD (2005) Detection of plot-level changes in ectomycorrhizal communities across years in an old-growth mixed-conifer forest. New Phytologist 166:619-630

JAKUCS E, ERÖS-HONTI Z (2008) Morphological-anatomical characterization and identification of *Tomentella* ectomycorrhizas. Mycorrhiza 18:277-285

JIM CY (1998) Physical and chemical properties of a Hong Kong roadside soil in relation to urban tree growth. Urban Ecosystem 2:171-181

JONGMAN RHG, TER BRAAK CIF, VAN TONGEREN OFR (eds) (1995) Data analysis in community and landscape ecology. 2nd ed., Cambridge Univ. Press, Cambridge, UK

KERNAGHAN G & HARPER KA (2001) Community structure of ectomycorrhizal fungi across an alpine/subalpine ecotone. Ecography 24:181-188

KOIDE RT, XU B, SHARDA J, LEKBERG Y, OSTIGUY N (2005) Evidence of species interactions within an ectomycorrhizal fungal community. New Phytologist 165:305-316

KÕLJALG U, DAHLBERG A, TAYLOR AFS, LARSSON E, HALLENBERG N, STENLID J, LARSSON KH, FRANSSON PM, KÅRÉN O, JONSSON L (2000) Diversity and abundance of resupinate thelephoroid fungi as ectomycorrhizal symbionts in Swedish boreal forests. Molecular Ecology 9:1985-1996

KRAIGHER H, AL SAYEGH PETKOVSEK S, GREBENC T, SIMONCIC P (2007) Types of ectomycorrhiza as pollution stress indicators: case studies in Slovenia. Environ Monit Assess 128:31-45

LANDEWEERT R, LEEFLANG P, KUYPER TW, HOFFLAND E, ROSLING, WERNARS K, SMITH E (2003) Molecular identification of ectomycorrhizal mycelium in soil horizons. Appl Environ Microbiol 69:327-333

LEYVAL C, TURNAU K, HASELWANDTER K (1997) Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. Mycorrhiza 7.139-153

LILLESKOV EA, BRUNS TD, HORTON TR, TAYLOR DK, GROGAN P (2004) Detection of forest stand-level spatial structure in ectomycorrhizal fungal communities. FEMS Microbiol Ecol 49:319-332

LIU Y J, HALL B D (2004) Body plan evolution of ascomycetes, as inferred from an RNA polymerase II phylogeny. Proceedings of the National Academy of Sciences, USA 10:4507-4512

LORENZ K, LAL R (2009) Biogeochemical C and N cycles in urban soils. Environment International 35:1–8

MAMOUNM, OLIVIER JM (1993) Competition between *Tuber melanosporum* and other ectomycorrhizal fungi under two irrigation regimes. Competition with *Tuber brumale*. Plant and Soil 149:211-218

MANTA DS, ANGELONE M, BELLANCA A, NERI R, SPROVIERI M (2002) Heavy metals in urban soils: a case study from the city of Palermo (Sicily), Italy.Sci Total Environ 300:229-243

MC-CUNE B, GRACE JB (eds) (2002) Analysis of ecological communities. MjM Software Design, Gleneden Beach, Oregon, USA

MONTECCHIO L, CAUSIN R, ROSSI S, MUTTO ACCORDI S (2004) Changes in ectomycorrhizal diversity in a declining *Quercus ilex* coastal forest. Phytopathologia Mediterranea 43:26-34

MONTECCHIO L, MOTTA E, MUTTO ACCORDI S (2008) Le ectomicorrize come indicatori di salute delle piante forestali (Ectomycorrhizae as forest plant's health indicators) [in Italian] Atti del III Congresso Nazionale di Selvicoltura per il miglioramento e la conservazione dei boschi italiani. Taormina Ottobre 2008

MONTECCHIO L, DI MARINO E, SCATTOLIN L, ABS C, AGERER R (2009) The ectomycorrhizal community structure in European beech forests differing in coppice shoot age and stand features. Journal of Forestry 107:250-259

MOSCA E, MONTECCHIO L, SELLA L, GARBAYE J (2007) Short-term effect of removing tree competition on the ectomycorrhizal status of a declining peduncolate oak forest (*Quercus robur* L.). Forest Ecology and Management 244:129-140

NIELSEN JS & RASMUSSEN HN(1999) Mycorrhizal status and morphotype diversity in *Tilia cordata* – a pilot study of nurseries and urban habitats. Acta Hort. 496:451-459

PACHEPSKY Y A, RAWLS W J, LIN H S (2006) Hydropedology and pedotransfer functions Geoderma 131:308– 316

PAULEIT S, JONES N, GARCIA-MARTIN G, GARCIA-VALDECANTOS JL, RIVIÈRE LM, VIDAL-BEAUDET L, BODSON M, RANDRUP TB (2002) Tree establishment practice in towns and cities – Results from a European survey. Urban Forestry & Urban Greening 1:83-96

PEDERSEN LB, RANDRUP TB, INGERSLEV M (2000) Effects of road distance and protective measures in deicing NaCl deposition and soil solution chemistry in planted median strips. Journal of Arboriculture 26(5): 238-245

PERCIVAL GC, KEARY IP, AL-HABSI S (2006) An assessment of the drought tolerance of *Fraxinus* genotypes for urban landscape plantings. Urban Forestry & Urban Greening 5:17-27

PETER M, AYER F, EGLI S (2001) Nitrogen addition in a Norway spruce stand altered macromycete sporocarp production and below-ground ectomycorrhizal species composition. New Phytologist 149:311-325

PIGOTT CD (1982) Fine structure of mycorrhiza formed by *Cenococcum geophilum* Fr. on *Tilia cordata* Mill.. New Phytol 92: 501-512

POUYAT RV, RUSSELL-ANELLI J, NEERCHAL NK (2007) Soil chemical and physical properties that differentiate urban land-use and cover types. Soil Sci Soc Am J 71:1010-1019

RAWLS WI, BRAKENSIEK DL, SAXTON KE (1982) Estimation of soil water properties. American Society of Association Executives, paper n 81:2510

RODRIGUES S, URQUHART G, HOSSACK I, PEREIRA ME, DUARTE AC, DAVIDSON C, HURSTHOUSE A, TUCKER P, ROBERSTON D (2009) The influence of anthropogenic and natural geochemical factors on urban soil quality variability: a comparison between Glasgow, UK and Aveiro, Portugal. Environ Chem Lett 7:141-148

ROSSITER DG (2007) Classification of urban and industrial soils in the World Reference Base for Soil Resources. J Soils Sediments 7(2):96-100

RUMBERGER MD, MÜNZENBERGER B, BENS O, EHRIG F, LENTZSCH P, HÜTTL RF (2004) Changes in diversity and storage function of ectomycorrhiza and soil organoprofile dynamics after introduction of beech into Scots pine forests. Plant and Soil 264:111-126

RUOTSALAINEN AL, MARKKOLA AM, KOZLOV MV (2009) Mycorrhizal colonisation of mountain birch (*Betula pubescens* ssp. *Czerepanovii*) along three environmental gradients: does life in harsh environments alter plant-fungal relationship? Environ Monit Assess 148:215-232

SALVAGIO MANTA D, ANGELONE M, BELLANCA A, NERI R, SPROVIERI M (2002) Heavy metals in urban soils: a case study from the city of Palermo (Sicily), Italy. Sci Total Environ 300:229-243

SCATTOLIN L, MONTECCHIO L, AGERER R (2008a) The ectomycorrhizal community structure in high mountain Norway spruce stands. Trees 22:13-22

SCATTOLIN L, MONTECCHIO L, MOSCA E, AGERER R (2008b) Vertical distribution of the ectomycorrhizal community in the top soil of Norway spruce stands. Eur J Forest Res 127:347-357

SCHARENBROCH BC, LLOYD JE, JOHNSON-MAYNARD JL (2005) Distinguishing urban soils with physical, chemical, and biological properties. Pedobiologia 49:283—296

SMITH SE, READ DJ (eds) (1997) Mycorrhizal symbiosis. London, UK; Academic Press.

STHULTZ CM, WHITHAM TG, KENNEDY K, DECKERT R, GEHRING CA (2009) Genetically based susceptibility to herbivory influences the ectomycorrhizal fungal communities of a foundation tree species. New Phytologist 184:657-667

SWATY RL, DECKERT R, WHITHAM TG, GEHRING CA (2004) Ectomycorrhizal abundance and community composition shifts with drought: predictions from tree rings. Ecology 85(4):1072-1084

SÆBØ A, BENEDIKZT, RANDRUP TB (2003) Selection of trees for urban forestry in the Nordic countries. Urban Forestry & Urban Greening 2:101-114

TAYLOR AFS (2002) Fungal diversity in ectomycorrhizal communities: sampling effort and species detection. Plant Soil 244:19-28

TEDERSOO L, KÕLJALG U, HALLENBERG N, LARSSON K-H (2003) Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. New Phytologist 159:153-165

TEDERSOO L, HANSEN K, PERRY B.A., KJØLLER R (2005) Molecular and morphological diversity of pezizalean ectomycorrhiza. New Phytologist 170:581-596

ter BRAAK CJF (1986) Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. Ecology 67:1167-1179

ter BRAAK CJF, PRENTICE C (1988) A theory of gradient analysis. Adv in Ecol Res 18:271-317

ter BRAAK CJF, ŠMILAUER P (eds) (2002) CANOCO reference annual and CanoDraw for windows user's guide. Software for canonical community ordination (version 4.5). Microcomputer Power, Ithaca, NY, USA

TERHO M (2009) An assessment of decay among urban *Tilia*, *Betula* and *Acer* trees felled as hazardous. Urban Forestry & Urban Greening 8:77-85

TOLJANDER JF, EBERHARDT U, TOLJANDER YK, PAUL LR, TAYLOR AFS (2006) Species composition of an ectomycorrhizal fungal community along a local nutrient gradient in a boreal forest. New Phytologist 170:873-884

TREE MANAGEMENT DEPARTMENT OF PADOVA (2009) – Tree Inventory of Padova Municipality

TREVISANI M, CAUSIN R, MONTECCHIO L, KASTNER C, HEUMANN C (1999) Analysing the relationship between ectomycorrhizal infection and forest decline using marginal models. Sonderforschungsbereich 386:1-20

URBAN A, WEIB M, BAUER R (2003) Ectomycorrhizas involving sebacinoid mycobionts Mycol Res 107:3-14

VAN DEN BRINK PJ, VAN DEN BRINK N, TER BRAAK CJF (2003) Multivariate analysis of ecotoxicological data using ordination: demonstrations of utility on the basis of various examples. Australasian Journal of Ecotoxicology 9:141-156

WÖSTEN J H M (2006) Pedotransfer Functions. In Encyclopedia of Soil Science, Vol.2, 2nd ed. (R. Lal ed.) pp 1257-1261 The Ohio State University, Columbus, Ohio, USA

WURZBURGER N, HARTSHORN A S, HENDRICK R L (2004) Ectomycorrhizal fungal community structure across a bog-forest ecotone in southeastern Alaska. Mycorrhiza 14:383-389 (Wurburger-Hartshorn 2004)

Chapter 4

SPAD Chlorophyll meter readings as a diagnostic tool for linden trees stress

Introduction

Urban trees management requires continuous monitoring of health condition in order to quickly detect signs of decline and to discover and remove the source of the stress.

Stress detection in woody plants usually relies on visual assessment of symptoms (Mattheck & Breloer 1994; Dobbertin 2005).

Unfortunately, visual assessment of tree vitality is very difficult due to the fact that most inventories are based on parameters such as percentage of foliage reduction and leaf color that need individual interpretation (Percival 2004; Dobbertin 2005). Furthermore, in many cases leaves size and number are subjected to considerable annual fluctuations as a result of drought and other physiological and biological damage. As a consequence, tree health visual evaluations may be untrustworthy.

On the contrary, the foliar chlorophyll content and other related variables, such as foliar nitrogen content, can be useful and objective diagnostic indicators of the health and physiological performance of a plant (Nielsen et al. 1995; Matile et. al 1996; Richardson et al. 2002; Percival 2004; Cartelat et al. 2005; Percival et al. 2008).

First of all, since the foliar concentration of photosynthetic pigments affects the absorption of solar radiation, a low chlorophyll content may be a limit to photosynthetic potential (Filella et al. 1995). Secondly, the breakdown of leaf chlorophyll and the resulting depigmentation may be associated with a variety of environmental stress, since leaf chlorophyll concentration decreases under stress and during senescence (Matile et al., 1996; Peñuelas & Filella, 1998; Matile 2000; Neufeld et al. 2006; Mansouri-Far et al. 2010). Moreover, much of leaf nitrogen is carried by chlorophyll, so quantifying chlorophyll content provides a rapid estimation of nitrogen and, consequently, nutrient plant status (Filella et al. 1995; Percival et al. 2008).

The leaf chlorophyll content may be assayed directly by *in vitro* chemical techniques (e.g., Lichtenthaler et al. 2007) or, thanks to its absorption features in the visible and in the UV part of the spectrum, it can be measured by rapid and non-destructive in vivo optical methods as, for instance, reflectance – based indices and hand-held Chl absorbance meters (Richardson et al, 2002; Cartelat et al. 2005; Percival et al. 2008). The latter may be of great interest when the investigation of the physiological status of trees vegetation is needed, since they are fast, easy to use, and cheaper than other optical methods.

A potentially useful device to quickly and non-destructively measure foliar N and Chl concentration is the Minolta Chlorophyll Meter SPAD-502 (Konica Minolta Sensing).

The SPAD meter utilizes two LEDs that emit light onto the upper surface of a leaf; a red LED with a peak wavelength of 650 nm and an infrared LED with a peak wavelength of 940 nm. The light enters the leaf where a portion of the light is absorbed by chlorophyll and the remainder is transmitted through the leaf where it contacts a silicon photodiode detector and is converted into an electrical signal. The amount of light reaching the photodiode detector is inversely proportional to the amount of chlorophyll in the light path. Using these two transmittances the meter calculates a numerical value from 0 to 199 which is proportional to the amount of Chl present in the leaf.

Once the relation between SPAD index and Chl content has been established by standard chemical techniques, SPAD can provide a fast and non-destructive assay of various photosynthetic pigments.

Chl handheld meters are used extensively in agriculture for estimation of foliar Chl and the assessment of the crop nitrogen (N) nutrition status during the growth cycle for numerous crop species as rice (Ramesh et al. 2002), wheat (Cartelat et al. 2005), maize (Muthuri et al. 2009), potato (Uddling et al. 2007), cotton (Chen & Ruberson 2008), and they have also been successfully tested for monitoring physiological variables of threatened and endangered plants (Hawkins et al. 2009).

As far as trees are concerned, there are several publications about the application of the SPAD meter to measure Chl content in fruit trees: pear trees (Peryea & Kammereck 1997), apple trees (Nielsen et al. 1995), papaya trees (Torres Netto et al. 2002), coffee trees (Torres Netto et al. 2005), all of them related to the assessment of the state of the photochemical process of the plant and to the application of the tool in nutrition programs.

On the contrary few studies are available on forest or ornamental trees, and all of them concern juvenile trees. Richardson (et al. 2001) compared the performance of different non invasive methods to estimate foliar Chl content in young trees of *Betula papyrifera* Marsh. cultivated in greenhouse while Chang & Robison (2003) evaluated the utility of SPAD for assessing foliar N in young trees of hardwood species (*Liquidambar styraciflua* L., *Platanus occidentalis* L., *Populus heterophylla* L., *Fraxinus pennsylvanica* Marsh.). Percival (et al. 2006) used SPAD to measure chlorophyll concentration of 6-year-old

containerised trees of a range of *Fraxinus* spp.genotypes, in order to test their different drought tolerance. In a more recent research he studied the correlation between the leaf photosynthetic pigment content and total leaf nitrogen content with the SPAD readings in *Acer pseudoplatanus, Fagus sylvatica* and *Quercus robur* (Percival et al 2008). A similar study was also accomplished by Uddling (et al. 2007) on juvenile trees of *Betula pendula*.

As foliar concentration of pigments, most notably the chlorophylls and carotenoids, are affected by a variety of stress factors (Ögren 1990; Maki & Colombo 2001; Neufeld et al. 2006), SPAD index can also provide a useful tool to assess the plant stress degree (Peñuelas & Filella 1998; Percival et al.2006).

It is known that while short-term stress events (minutes to hours) disturb the photosynthetic performance, long-term stress events (days to weeks) and mineral deficiencies result in a decline in the foliar chlorophyll content (Lichtenthaler & Miehé 1997), which can easily be monitored by chlorophyll meter readings.

In this study the possibility of using SPAD as a quick and handheld system for the evaluation of urban linden tree health was tested, as no similar application was previously reported.

Hölscher (2004) compared morphological and chemical leaf traits and photosynthetic parameters of four broad-leaved tree species, which showed different values between leaves collected in the lower and in the upper canopy level. He also confirmed the difference of photosynthetic capacities between juvenile and adult trees. Large trees in fact support leaves acclimated to high light intensities in the upper canopy and may produce leaves acclimated to low light densities in lower canopy layers, while leaves of seedling experience essentially the same light environment.

Since the present research concerned mature trees, that display differences in leaf structure among crown positions (Hölscher 2004; Lichtenthaler et al. 2007), a screening study was necessary to previously locate the best sampling point position in the tree crown.

Specifically, the aims of this study were: (1) to evaluate the impact of the sampling point position in the crown on the SPAD-foliar Chl relation; (2) to determine the mathematical relationship between SPAD-502 readings and the foliar chlorophyll content and total foliar nitrogen content in linden trees (*Tilia vulgaris* Hayne) in urban environment, displaying

different stages of visual symptoms of decline; (3) to evaluate if ECM community variability can be associated to variations in chlorophyll content and to different classes of decline; (4) to verify the possibility of using chlorophyll meter readings as a diagnostic tool for linden trees health.

Materials and methods

Samples collection

The trees used in this experiment were the same previously described in Cap.3.

In a screening study the effect of sampling position within a tree on the SPAD meter index, foliar Chl and N content was studied. A test tree was selected among the 16 aforementioned linden trees. Thirty-six sampling points were chosen around the crown: their position varied according to the tree height (lower, mid and upper crown), to the distance from the trunk (inner, mid and external crown) and to the cardinal point (north, east, south and west) (Fig.1).

Once a week on July, on five sampling dates, three fully expanded, sunlit leaves were chosen in each sampling point. The sampling points (at 6 – 14 and 22 m height) were reached by a swing-platform. Three SPAD meter readings were taken on each leaf in the inter-venal area and the average reading was recorded to represent the SPAD value of that leaf. Immediately after the readings the leaves were collected and placed in a plastic bag which was closed and kept on ice in an insulated box. The samples were then brought into the laboratory and processed within two hours of collection.

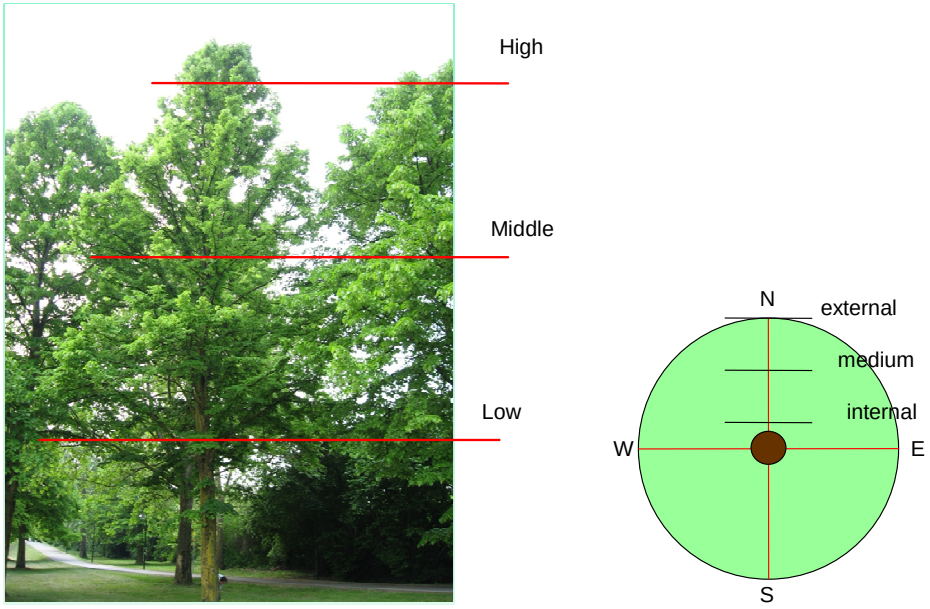


Fig. 1 Distribution of the 36 sampling points in the test tree

A second experiment, based on the result of the screening study, was designed to investigate the effectiveness of SPAD-502 readings in assessing Chl status and to evaluate if ECM community variability could be explained by the variation of foliar Chl content.

Once a week from August to the end of September on seven dates three fully expanded, sunlit leaves were chosen on a south exposed branch in the high-crown and external region of each of the 16 chosen trees. SPAD readings and leaves collections were done according to the aforementioned procedures. In addition, further foliar samples collected near the sampling point were dried to constant weight, ground and analyzed for N concentration.

The SPAD readings were always taken in the morning, and approximately at the same hour for each tree, in order to restrain errors due to the irradiance effect. It is in fact known that there may be high variability in light transmission caused by irradiance induced chloroplast movement which can affect results in SPAD measurements (Hoel & Solhaug 1998).

Two of the SPAD readings overlapped with the summer (08/2009) and autumn (09/2009) soil sample collection carried out for the ECM community analysis which is described in details in Chapter 3.

Laboratory observations

Chlorophyll determination

Leaves collected both in the screening study and in the second experiment were processed as follows.

A variable number of leaf discs with an area of 201 mm^2 were removed from each leaf, in order to obtain 250 mg of fresh foliar tissue which was ground in an aqueous ethanol solution (98% v/v) by Ultra -Turrax. The samples were kept in the dark for 24 hours at 4°C, then the extracts were filtered and subsequently analysed by means of a spectrophotometer at 665 nm and 649 nm. Totally 112 samples were processed (16 trees, 7 repetitions).

The chlorophyll a (Chla) and chlorophyll b (Chlb) concentrations were calculated using the Welburn and Lichtenthaler (1984) formula, and expressed in mg pigment per g of leaf f.w.

$$Chla = (13.95*A665 – 6.88*A649)*V/f.w.$$

$$Chlb = (24.96*A649-7.32*A665)*V/f.w.$$

Where A665 = chlorophyll absorbance at 665 nm
A649 = chlorophyll absorbance at 649 nm
V = final ethanol volume in ml
f.w. = foliar fresh weight in mg

The total chlorophyll concentration ($\mu gChlg^{-1}$)and the total chlorophyll content ($\mu gChlcm^{-2}$) were then calculated.

Nitrogen determination

Leaves deriving from further foliar sampling of the second experiment were put in paper bags and placed in a forced air chamber at 65 °C. After 48 hours the material was ground in

a mill, weighted (400 mg on average) and stored in paper bags for later analysis. The Kjeldahl method was used to determine the total nitrogen content. Totally 112 samples were processed (16 trees, 7 repetitions).

Ectomycorrhizal (ECM) community

The processing of fine roots in order to analyze the ECM community is described in chapter 3.

Data analyses

Screening study – Test Tree

To identify the spatial structure of the variables sampled (SPAD index and chl content), semivariogram analysis was carried out by means of the Geostatistical Analyst Extension (Environmental System Research Institute, ESRI Inc., 2006 Redlands, CA) of the ArcGIS software (vers. 9.1).

This analysis creates statistically interpolated continuous surfaces from measured samples, representing a prediction of where a given phenomenon may occur (ESRI 2001).

The spherical model (showing a progressive decrease in spatial autocorrelation and an increase in semivariance until given distances, beyond which autocorrelation is zero) was used according to Liao et al. (2006).

Kriging, built on the principle that points that are close to one another are more alike than those farther away (spatial autocorrelation), uses the empirical semivariogram as a means to explore this relationship (Johnston et al. 2001).

Semivariance (γ (h)) is defined as one-half the variance between two sample values (Johnston et al. 2001). The semivariance for all pair of samples within each distance class is calculated and plotted against the distance between samples to produce a semivariogram. (Fig. 2).

According to Avendaño et al. (2003), the shape of the experimental semivarogram may take many forms, depending on the data and sampling interval used. Generally, the semivariance increases when the distance between sample points increases. Then the variogram tends to level off at a SILL equal to the variance of the variable. The distance at which this occur (i.e. the distance where the $\gamma(h)$ stops increasing) is referred to as the RANGE of the variable. The range is the distance over which the sampling units are not spatially correlated any longer and gives the measure of the spatial influence of the structuring process. Samples spatially related are separated by distances closer than the range; samples no longer spatially autocorrelated are separated by distances greater than the range. The discontinuity at the origin (non-zero intercept) is called the NUGGET effect. It corresponds to the local variation occurring at scales finer than the minimum sampling interval and includes methodological errors (Johnston et al. 2001; Fig. 2).

The Range, Nugget, Partial Sill (= Sill-Nugget) model parameters and the Spatial Dependence (= Partial Sill/Sill, in percentage) were calculated for each semivariogram of SPAD index and total Chl content for each height, distance, cardinal point, date.

The goodness of fit of each semivariogram was then assessed using five cross-validation parameters: the mean prediction error (ME); the root mean square prediction error (RMS); the average of standardized prediction error (ASE); the mean standardized prediction error (MS); the root mean square standardized (RMSS), according to Johnston et al. (2001). The expectations for a good-fitting semivariogram and kriging model are: an average ME and MS close to 0, an RMSS close to 1, a small RMS and an ASE close to RMS. If RMSS<1 or ASE>RMS, there is a tendency toward overestimation of the variance. If RMSS>1 or ASE<RMS, there is a tendency toward underestimation, according to Johnston et al. (2001).

All the above parameters were calculated using SPAD indexes taken at each sampling point and Chl contents determinated for each sampling point.

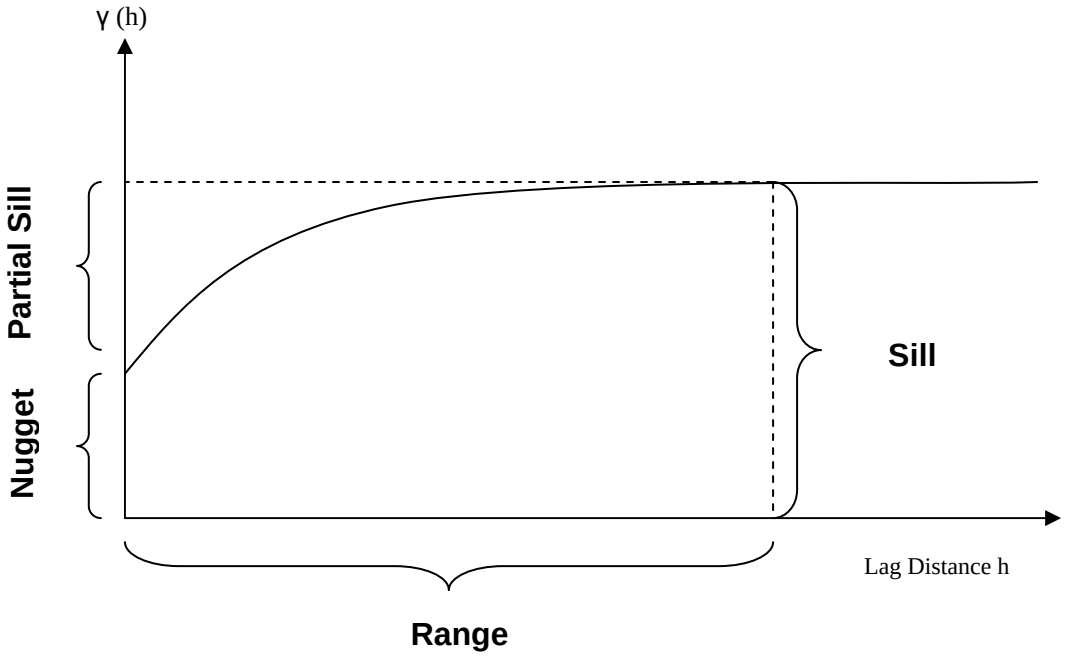


Fig. 2. Schematic diagram of a semivariogram showing the proportion of semivariance (γ, axis Y) found at increasing distances of paired samples (distance h, axis X)

In addition, for each sampling date and each crown level, regressions were fitted for foliar Chl content on SPAD readings. Regression analysis was performed using Statistica 6, StatSoft, Tulsa, USA for Windows.

Evaluation of the relationship between leaf Chl and N content and SPAD readings

Regressions were fitted for foliar Chl content and foliar N concentration on SPAD readings, both considering all 16 trees together and according to the corresponding decline class.

Mean Chl content and N content between decline classes (Class 1 and Class 2) were compared by a one-way analysis of variance.

Regression analysis and analysis of variance were performed using Statistica 6, StatSoft, Tulsa, USA for Windows.

Analysis of the association among ECM community, total foliar chlorophyll content and decline classes

Multivariate techniques were chosen to study the differences in ECM species composition associated to the leaves total chlorophyll content and to the different decline classes.

The ECM data set consisted of the relative abundances of ECM anatomotypes counted in the soil samples collected on summer (08/2008) and autumn (09/2008); the Chlorophyll data set consisted of total chlorophyll content measured in leaves collected from the same trees considered for the soil samples, and in the same day of the soil sampling.

It is known that ECM distribution is not homogeneous at distances between 0 and 17 m (Lilleskov et al., 2004). Since this autocorrelation among sampling points could influence the community structure, the Mantel Test was performed to test the null hypothesis of no relationship among samples (total n° of ECM tips analysed in a soil core) from the same tree (Mc-Cune & Grace 2002).

The Mantel test ($P<0.01$, number of permutations =10000) compared species dissimilarity matrix and linear distance matrix between sampling points belonging to the same plant, using the XLSTAT-Pro Program (http://www.xlstat.com). If the Mantel test could not exclude a spatial correlation within a tree sampling area, this was excluded from the subsequent analyses.

The Sørensen similarity index was used to create the similarity matrix: $2a/(2a+b+c)$, where a = number of shared species, b = number of species unique to plot 1 and c = number of species unique to plot 2 (Izzo et al. 2005).

Relations among the considered variable and species abundance of ectomycorrhizae were analysed by means of multivariate ordination techniques (Jongman et al. 1995) using CANOCO (software for Canonical Community Ordination, 4.5 Version).

The environmental parameters considered during the two sampling periods were Decline Class 1 and Decline Class 2 (qualitative variables) and total chlorophyll content (quantitative variable).

A Detrended Correspondance Analysis (DCA; Hill & Gauch 1980) on the ECM relative abundances was performed to obtain estimates of gradient lengths in standard deviation units.

The detrending by segments method was applied with data not subjected to any transformation.

In order to choose the best ordination model, the lengths of gradient have been considered.

The use of weighted-averaging ordination models resulted to be appropriate for these data (ter Braak & Šmilauer, 2002) and unimodal (DCA and CCA; ter Braak 1986) analyses were performed

DCA considered sampling points as cases, analyzing qualitative (class 1 and 2) and quantitative (total chlorophyll content) variables.

In order to detect ECM species-environment relations, decline classes and total chlorophyll content were then used as environmental variables to constrain the ordination axes (ter Braak 2004; Van den Brink et al. 2003)

A Canonical Correspondance Analysis (CCA) was therefore applied, scaling with a focus on inter-species distances and using a bi-plot scaling type, according to ter Braak and Šmilauer (2002).

A Monte Carlo permutation test was performed with 499 permutations ($p<0.05$).

By means of forward selection of environmental variables, the marginal effect [lambda-1 (λ_1)] (i.e. the variance singly expressed by each variable) and the conditional effect [lambda-A" (λ_A)] (whose value is strictly dependent on the inclusion sequence in the model), were investigated, according to ter Braak and Šmilauer (2002).

Results

Screening study – Test Tree

Considering each sampling date, a semivariogram and a kriging map were drawn for each variable (SPAD index and chlorophyll content) and for each crown level (high, medium, low). Each sampling date was therefore represented by a group of six plots and semivariograms.

The goodness of fit of each group of semivariograms was then assessed analysing the cross validation parameters.

All groups displayed a similar performance and the sampling date August 19th is described hereafter as an example.

At this sampling date, the models of SPAD index and chlorophyll content obtained by the semivariograms, described by their main parameters (Range, Nugget, Partial Sill) in Table 1 fitted the data very well, according to the cross validation results (Table 2). The Root Mean Square (RMS) and Average Standard Error (ASE) values, in fact, were close, demonstrating that the model accurately represented the variability of the data. The Mean Standardized (MS) value showed a good fit and was close to zero, indicating a rather small error in the estimation of our predicted values. The Root Mean Square Standardized (RMSS) value, close to the optimum value of 1, showed a good fit of the model's predicted values with the data collected.

The cross validation results for SPAD index at all levels and Chl content at Low and Medium level indicated that the model slightly overestimated the data variability. The overestimation was expressed by the ASE values higher than RMS values. The RMSS value of Chl at high level was 1.104, showing a good fit to the data and that the model's predicted values tended to slightly underestimate the actual data.

The mean errors (ME) and the MS values were close to zero, indicating a good fit of the two models to the data.

The Spatial Dependence (Table 1, last column) was high for SPAD high level (100%) indicating a strongly spatially structured system.

Kriged (interpolated) plots, with the tree stem located in the centre, showed spatial patterning of SPAD index and of chlorophyll content at three crown levels (Fig 3, 4, 5) with

gradients associated to the north-south direction. Values increased from north to south, and from inner to more external positions.

	Range	Nugget	Partial Sill	Spatial dependence %
SPAD l	1155.92	3.068	3.512	53%
SPAD m	479.93	11.27	4.13	26%
SPAD h	191.85	0	35.30	100%
Chl l	1155.92	9.6781	6.06	62%
Chl m	361.827	5.1193	7.908	60%
Chl h	191.85	14.967	14.98	50%

Table 1 Model parameters and spatial dependence for each semivariogram (sampling date August 19th). Letters l, m and h correspond to the sampling level: low, medium and high.

	Mean (ME)	Root mean sq. (RMS)	Av.St. err. (ASE)	Mean St. (MS)	Root Mean Sq. St. (RMSS)
SPAD l	-0.07971	2.114	2.256	-0.011	0.9261
SPAD m	-0.5982	3.894	4.127	-0.1242	0.9575
SPAD h	0.1629	3.634	3.769	0.01463	0.9757
Chl l	0.1798	3.44	3.753	0.05534	0.9285
Chl m	-0.2151	3.472	3.712	-0.03176	0.9312
Chl h	-0.061	5.631	4.98	-0.0077	1.104

Table 2 Cross validation results for each semivariogram (sampling date August 19th). Letters l, m and h correspond to the sampling level: low, medium and high.

Regression analysis was performed to describe the relationship between Chl and SPAD for each level. Several models were tested and the best fit was a linear regression. The strongest relationship (best r^2 value) between Chl and SPAD was found in the high level (Fig.6).

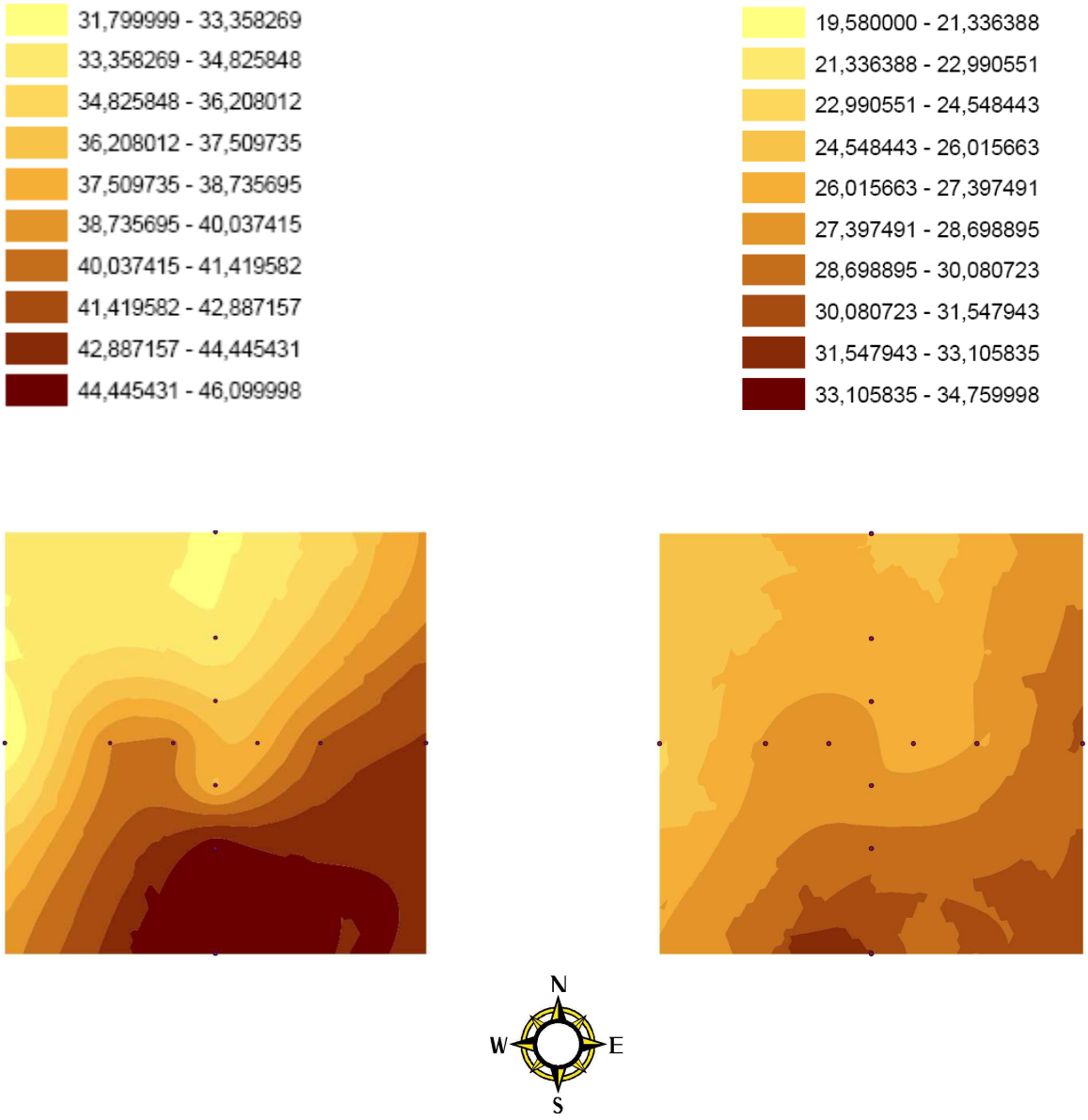


Fig. 3 Kriged (interpolated) plots for SPAD values (SPAD units, left) and foliar Chl content (μgChlcm^{-2}, right) with the stem located in the centre – high crown level. Dots indicate sampling points, whose position is summarized in Table 3.

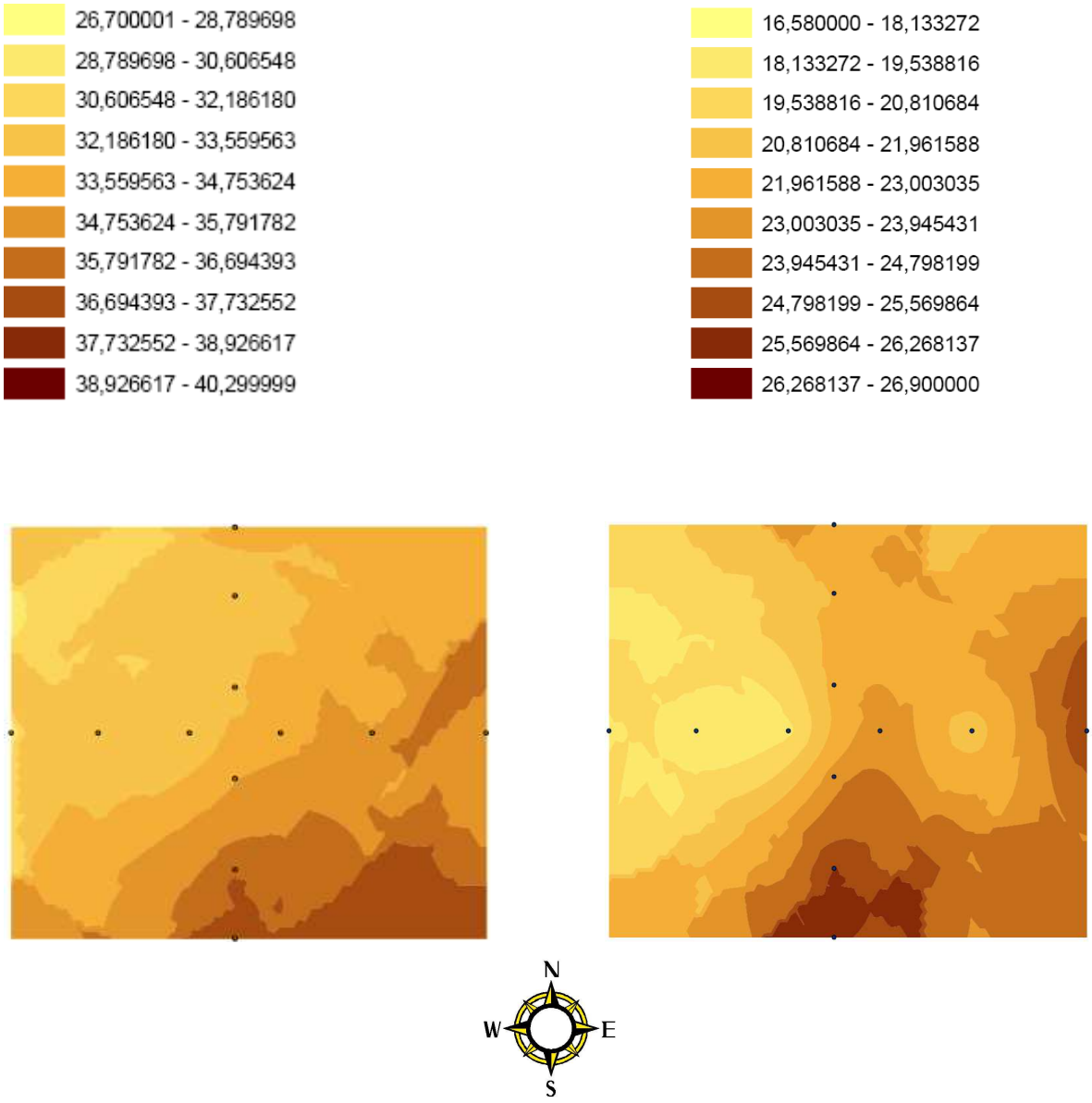


Fig. 4 Kriged (interpolated) plots for SPAD values (SPAD units, left) and foliar Chl content (μgChlcm^{-2}, right) with the stem located in the centre – middle crown level. Dots indicate sampling points, whose position is summarized in Table 3.

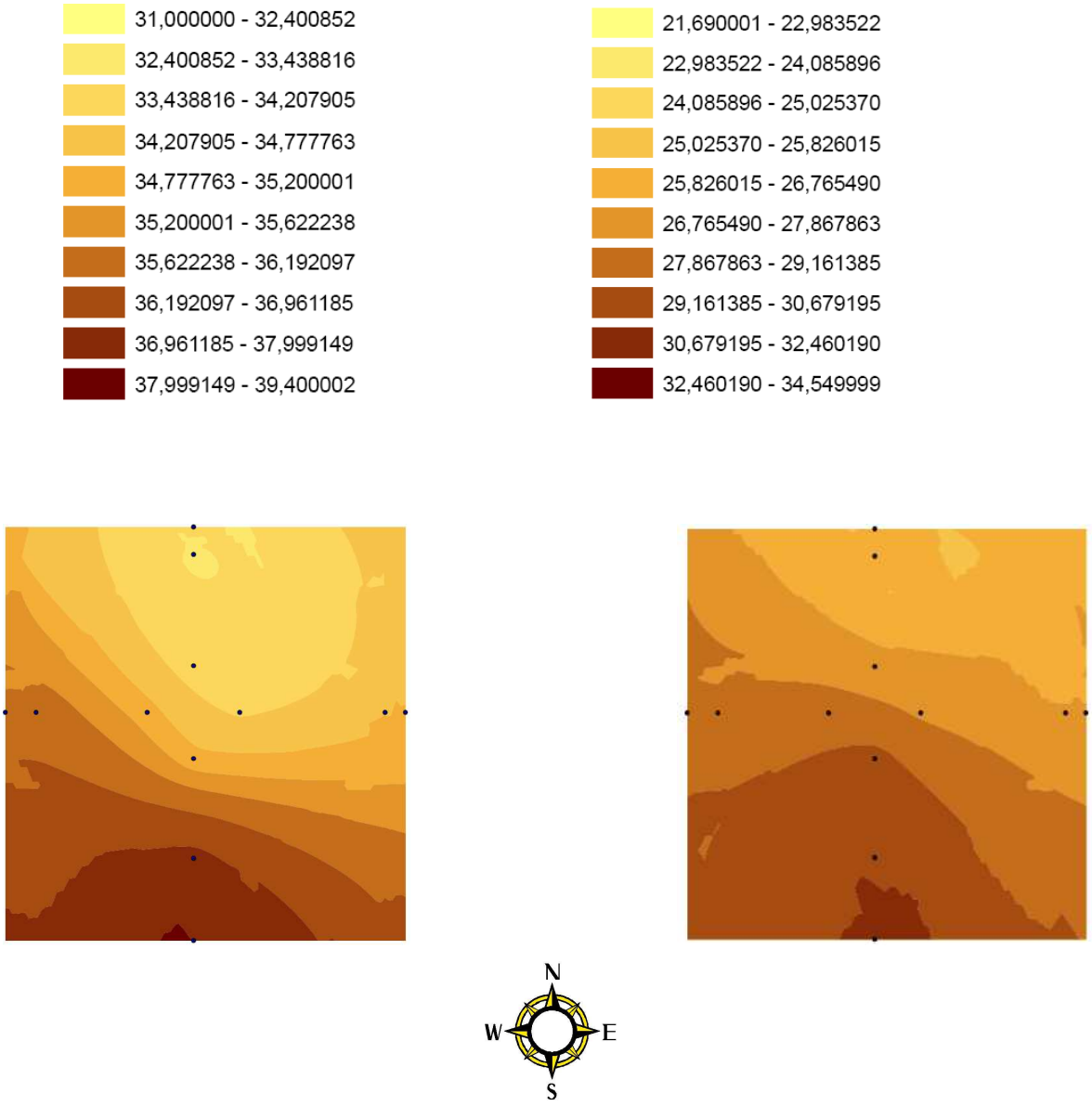


Fig. 5 Kriged (interpolated) plots for SPAD values (SPAD units, left) and foliar Chl content (μgChlcm^{-2}, right) with the stem located in the centre – low crown level. Dots indicate sampling points, whose position is summarized in Table 3.

Sampling point	Distance from the tree stem (cm)	Sampling point	Distance from the tree stem (cm)	Sampling point	Distance from the tree stem (cm)
HN1	20	MN1	100	LN1	135
HN2	50	MN2	300	LN2	460
HN3	100	MN3	450	LN3	540
HE1	20	ME1	100	LE1	135
HE2	50	ME2	300	LE2	560
HE3	100	ME3	550	LE3	620
HS1	20	MS1	100	LS1	135
HS2	50	MS2	300	LS2	425
HS3	100	MS3	480	LS3	660
HW1	20	MW1	100	LW1	135
HW2	50	MW2	300	LW2	460
HW3	100	MW3	490	LW3	550

Table 3. Sample points' average distances from the tree stem. H: high level; M: middle level; L: low level; N: north; E: east; S: south; W: west; 1: inner point; 2: central point; 3: external point.

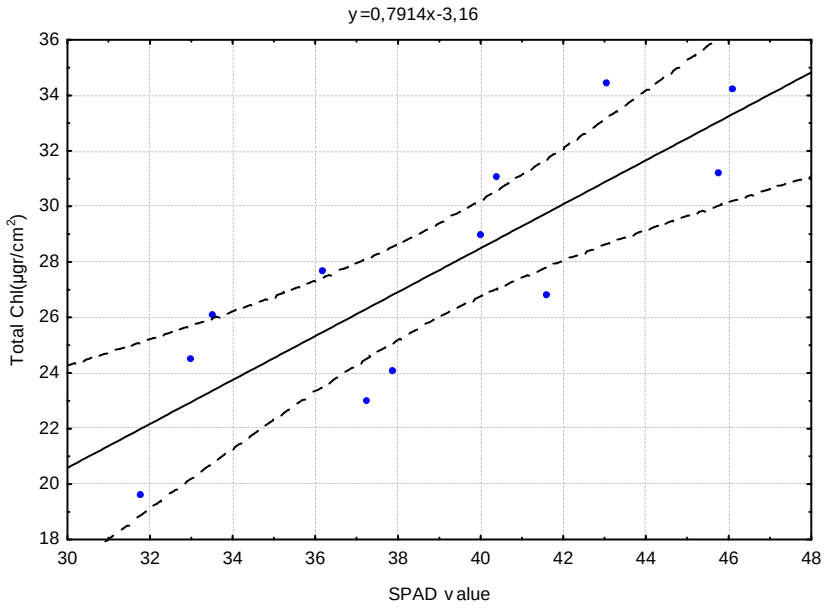


Fig 6. Leaf chlorophyll content in relation to SPAD values – Sample date August 19th, high sampling level. The regression parameters are given in Table 5.

Evaluation of the relationship between leaf Chl and N content and SPAD readings

The relationships between SPAD readings and total leaf Chl content (Fig.7) and between SPAD readings and total leaf N content (Fig. 8) were established.

A linear regression model best fit both relationships between the parameters (Table 5).

Parameter	Decline class	
	Class 1	Class 2
Total Chl (mg/cm2)	90.56±2.80	64.36±7.49
Total Foliar N %	1.73±0.024	1.30±0.046

Table 4. Total Chlorophyll Content and Total Foliar Nitrogen means values as a function of decline class. Means are different at $P<0.01$

Tree type	Model	Slope	R^2	P value
Total Chl, all trees	Y=2.9085x-13,70	0.86	0.74	<0.001
Total Chl, Class 1 Trees	Y=3.2254x-26.14	0.81	0.66	<0.001
Total Chl, Class 2 Trees	Y=2.7941x-8.35	0.77	0.603	<0.001
N content, all trees	Y=0.0314x+0.56	0.86	0.73	<0.001
Total Chl level H 19.08	Y=0.7914x-3.16	0.834	0.69	<0.001

Table 5. Equations to predict total chlorophyll content and N content from SPAD meter readings

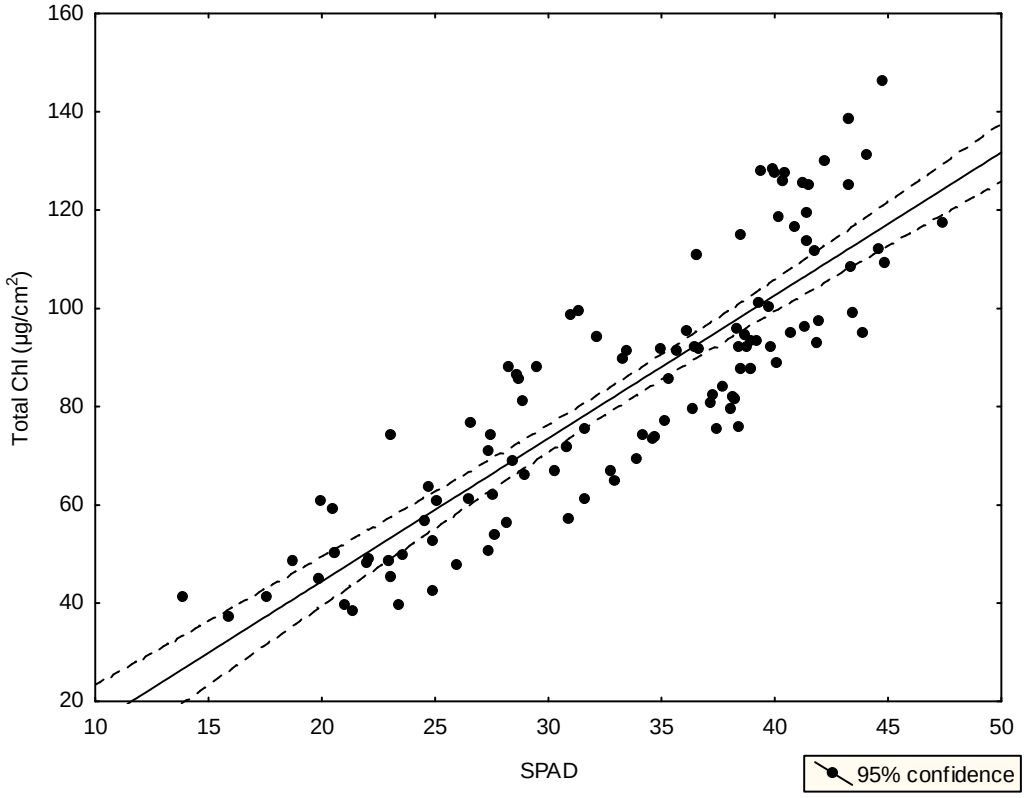


Fig. 7. Scatterplot indicating the relationship between SPAD values and total chlorophyll content in *Tilia vulgaris* Hayne leaves. The values refer to leaves belonging to 16 analyzed trees. See Table 5 for equation.

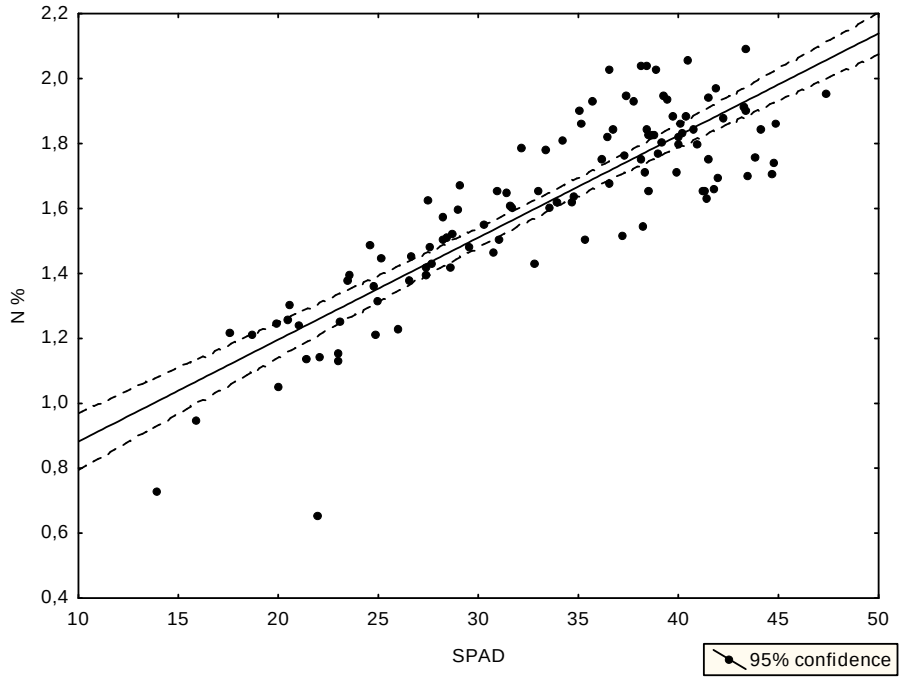


Fig.8. Scatterplot indicating the relationship between SPAD values and total foliar nitrogen content (%) in *Tilia vulgaris* Hayne leaves. The values refer to leaves belonging to 16 analyzed trees. See Table 5 for equation.

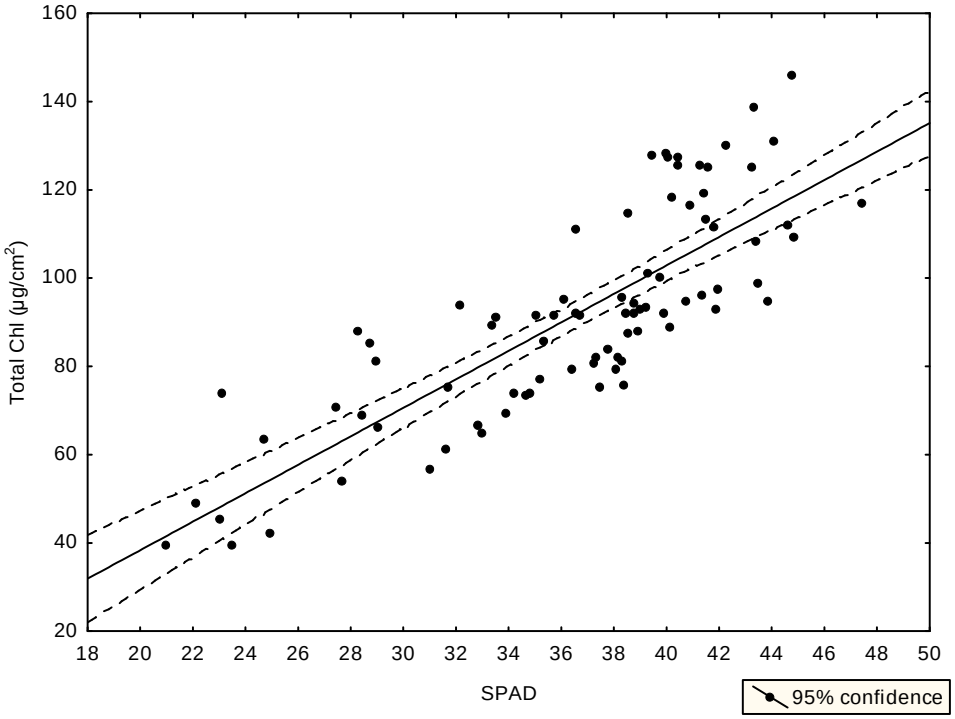


Fig. 9 Scatterplot indicating the relationship between SPAD values and total chlorophyll content in *Tilia vulgaris* Hayne leaves. The values refer to leaves collected from 12 trees belonging to Decline Class 1. See Table 5 for equation.

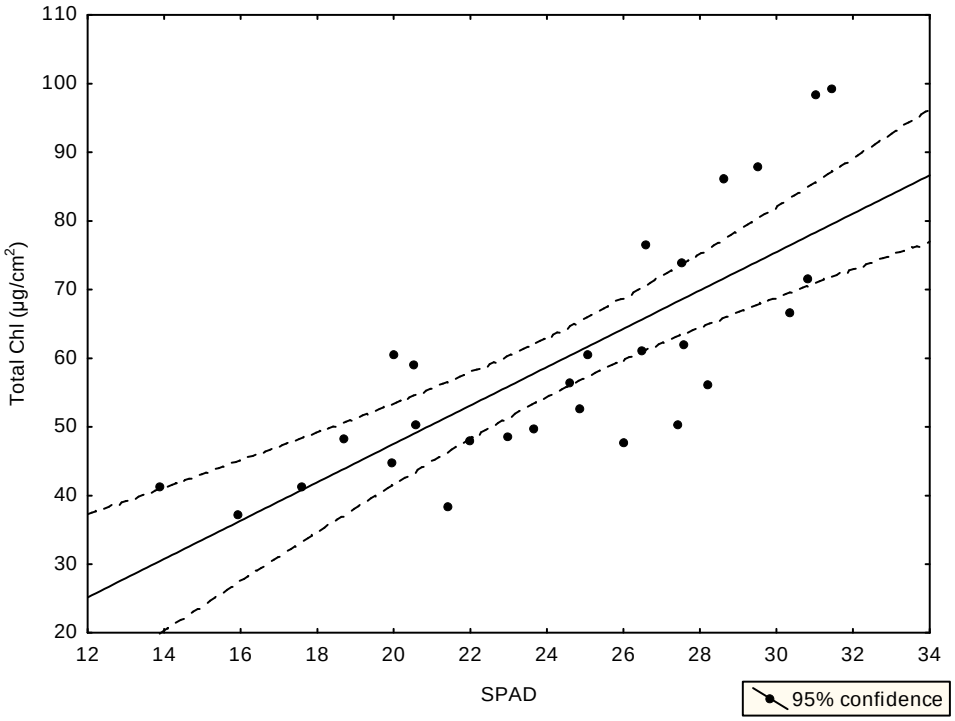


Fig. 10. Scatterplot indicating the relationship between SPAD values and total chlorophyll content in *Tilia vulgaris* Hayne leaves. The values refer to leaves collected from 4 trees belonging to Decline Class 2. See Table 5 for equation.

Total Chl content ranged from 39.7 μg/cm^2 to 146.16 μg/cm^2 in Class 1 and from 37.2 μg/cm^2 to 99.3 μg/cm^2 in Class 2. SPAD values ranged from 21 to 47.4 in decline class1 and from 13.8 to 31.5 in decline class 2.

Both mean foliar Chl content and N content of decline Class 1 were highly significantly greater than those of Class 2 (Table 4).

Analysing the data set of decline class 1 and decline class 2 separately, a close linear correlation between Chl content and SPAD values was found as well (Fig. 9 and 10). The corresponding calibration equations are given in Table 5.

Data from Kopinga & van den Burg (1995) indicate that the percentage leaf N associated with *Tilia vulgaris* Hayne ranges between 1.7% and 2.8% with values less than 1.7% generally associated with a low foliar N content.

Results of the present study indicate that the SPAD threshold value indicating low foliar N (less than 1.7%) in the detected trees is 36. Consequently, results of this investigation indicate that all trees belonging to decline class 2 (severely declining) show a SPAD value inferior to the critical value of 36 (Fig.10).

The scatterplot referring to decline class 1 (Fig. 9) shows SPAD values ranging from 21 to 47.7, including the critical value among them.

Analysis of the association among ECM community, total foliar chlorophyll content and decline classes

From the characterization of the ECM tips collected during summer and autumn sampling 45 morphotypes were found. The list of the ECM morphotypes is reported in Table 6.

Table 6 - ECM morphotypes found in summer and autumn sampling

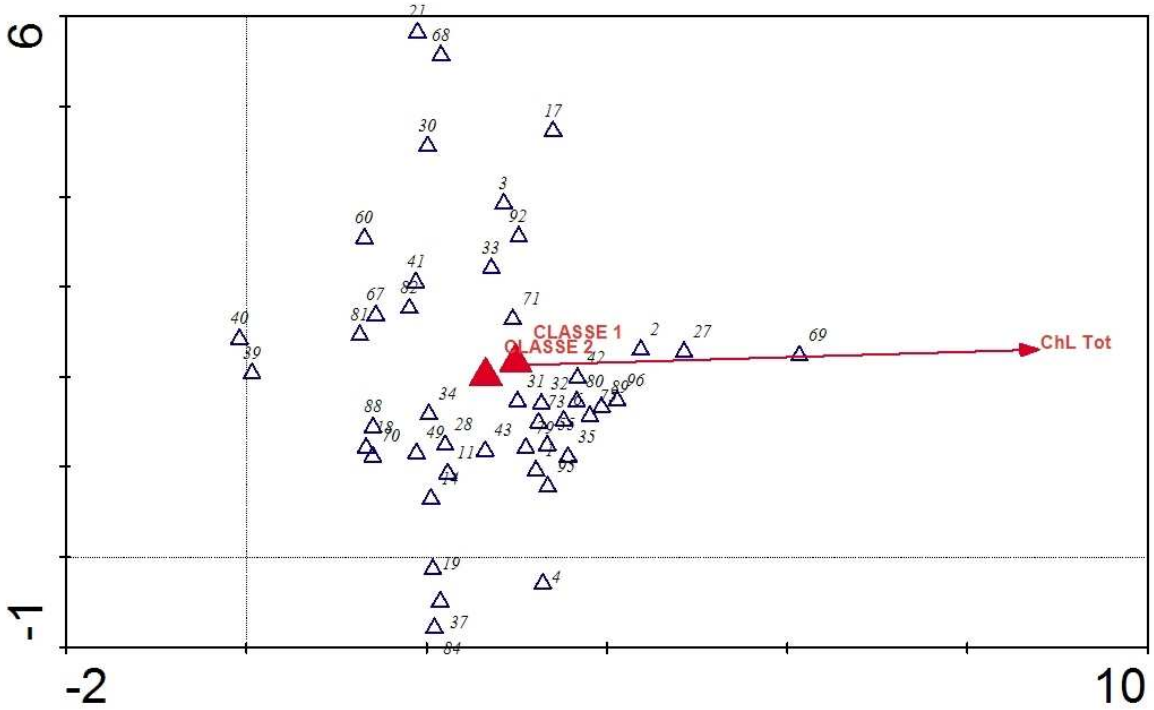
ECM n°	Morphotypes
18	*Boletaceae (CA18)*
34	*CA34*
43	*CA43*
88	*CA88*
89	*CA89*
96	*CA96*
2	*Cenococcum geophilum(CA2)*
92	*Chromelosporium(CA92)*
17	*Clavulina sp.(CA17)*
19	*Clavulinaceae(CA19)*
31	*Cortinariaceae (CA31)*
68	*Cortinariaceae (CA68)*
73	*Cortinariaceae (CA73)*
30	*Geopora sp.(CA30)*
11	*Geopora cervina (CA11)*
6	*Helvellaceae(CA6)*
27	*Inocybe sp.(CA27)*
49	*Laccaria sp.(CA49)*
60	*Peziza sp.(CA60)*
80	*Peziza sp.(CA80)*
81	*Peziza sp.(CA81)*
28	*Pezizaceae (CA28)*
67	*Pseudotomentella sp.(CA67)*
39	*Russula sp.(CA39)*
40	*Scleroderma verrucosum (CA40)*
14	*Sebacinaceae (CA14)*
35	*Sebacinaceae (CA35)*
71	*Sebacinaceae (CA71)*
3	*Telephoraceae (CA3)*
32	*Telephoraceae (CA32)*
33	*Telephoraceae (CA33)*
95	*Telephoraceae (CA95)*
1	*Tomentella sp. (CA1)*
21	*Tomentella sp.(CA21)*
42	*Tomentella sp. (CA42)*
55	*Tomentella sp.(CA55)*
69	*Tomentella sp.(CA69)*
70	*Tomentella sp.(CA70)*
75	*Tomentella sp.(CA75)*
79	*Tomentella sp.(CA79)*
84	*Tomentella sp.(CA84)*
82	*Trichophaea sp.(CA82)*
41	*Trichophaea woolhopeia (CA41)*
4	*Tuber rapaeodorum (CA4)*
37	*Tuber rufum (CA37)*

.

The canonical analyses considered all the selected trees, as the Mantel Test excluded any spatial correlation within each one.

DCA, which considered 192 samplings, showed long gradient lengths (>4) and demonstrated that the eigenvalues of axis 1 (horizontal) and 2 (vertical) are 0,742 and 0,479 respectively.

Fig.11 is the DCA scatter plot of the ECM species in the plotted ordination plane. The ECM species corresponding to each number is reported in Table 6.



It displays 13.2% of the inertia (i.e. the weighted variance) in species abundance, and 33.2 % of the variance in both the weighted average and class totals of species with respect to the environmental variables.

Environmental correlation is 0.548 for axis 1 and 0.346 for axis 2.

As far as species distribution in the plot is concerned, the DCA scatter plot (Fig.11) explained the rarity of the following anatomotypes: *Tomentella sp.(CA21, Cortinariaceae (CA68) Tuber rufum (CA37), Tomentella sp.(CA84), Tomentella sp.(CA69)* (ter Braak & Prentice 1988).

In CCA the eigenvalue of axis 1 and axis 2 are 0.199 and 0.122 respectively, indicating a high significance of the first axis and all the canonical axes were present when subjected to the Monte Carlo permutation test (P=0.0020).

Fig.12 is the bi-plot of ECM species and environmental variables, displaying 3.5% of the inertia (i.e. the weighted variance) in abundances, and 100% of the variance in both the weighted average and species class totals with respect to the environmental variables.

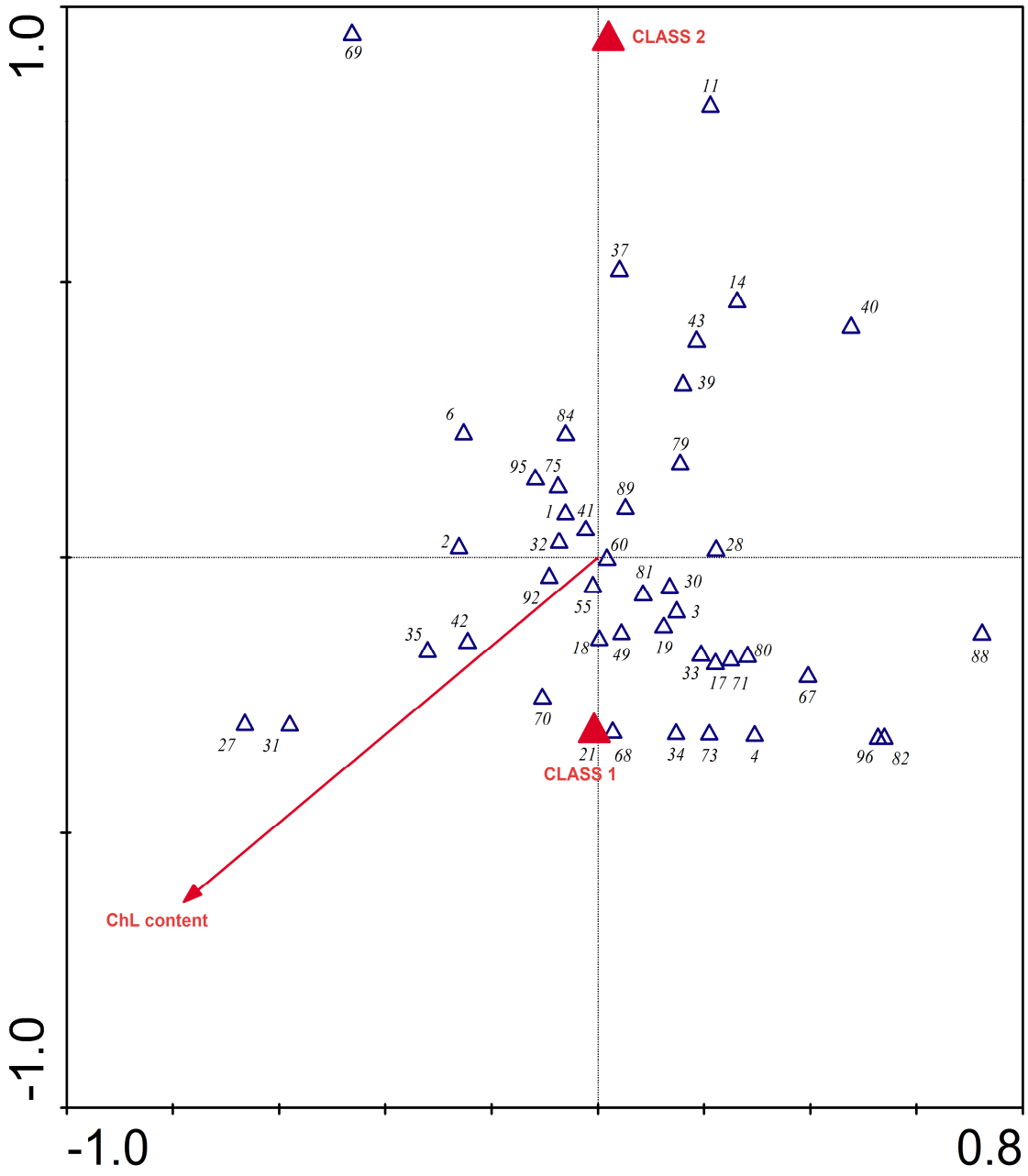


Fig 12 Canonical Correspondence Analysis ordination diagram of ECM species and environmental factors: decline class (Class 1 and Class 2) and foliar Chl content (ChL content). The ECM species corresponding to each number is reported in Table 6.

By means of the correlation coefficents among variables and axes, we inferred that the first axis is defined by chlorophyll content, the second axis is defined by decline classes.

The intra-set correlation of axis 1 with chlorophyll content was -0.78; the intra-set correlations of axis 2 with class 1 and class 2 were -0.99 and 0.99 respectively.

The ECM mainly associated with class 2 were in the upper part of the diagram, those associated with class 1 were in the lower part of the diagram. The ECM mainly associated with high foliar chlorophyll content were in the lower left area of the diagram, while those associated with low foliar chlorophyll content were in the upper right area of the biplot.

The distance between species points in the bi-plot scaling (with a focus on species distances) approximated the chi-square distance between the species distributions.

The ECM associated with high values of foliar chlorophyll content were *Inocybe sp*.(CA27*)*, *Cortinariaceae* (CA31); with class 2, *Geopora cervina* (CA11), *Tomentella sp*.(CA69); with class 1, *Tomentella sp*. (CA21), *Cortinariaceae* (CA68), *Tomentella sp*.(CA70).

The marginal effects in CCA demonstrated that the variable that is better suited to explain the model is chlorophyll content (λ_1 = 0.17) followed by the decline classes (λ_1 = 0.12 for both).

The conditional effects, which show the environmental variables in order of their inclusion in the model, demonstrated that the most useful features to explain the model are chlorophyll content (λ_A =0.17) and decline class 1(λ_A =0.15).

Discussion

The quantification of foliar chlorophyll concentration may provide a robust and accurate estimation of tree vitality (Percival et al. 2008), and it may be achieved by hand held Chl meters (Richardson et al. 2002; Chang & Robinson 2003; Uddling et al. 2007).

It is ascertained that it is essential to derive species-specific calibration equations for SPAD index when estimating Chl content (Richardson et al. 2002), due to the differences in leaf structure among plant species. On the contrary, equally important differences shown by leaf structure among crown positions in the tree itself have not yet been considered by studies concerning the use of SPAD meter in trees.

Hölscher (2004) found differences in Chl and N concentrations between leaves of lower and upper canopy of adult *Tilia platyphyllos* trees, with higher levels in the higher canopy.

It has been moreover established that sun leaves, growing on end-branches and in the higher part of the tree crown, compared to shade leaves are generally thicker, have smaller stomata but higher stomata density, have higher Chl content per leaf area unit and possess sun-type chloroplast with different ultrastructure and biochemical organization (Lichtenthaler et al. 2007). These are essential prerequisite for their higher photosynthetic rates.

It is therefore important to take in regard differences shown by leaves at different canopy levels when Chl measurement are dealing with mature trees.

The first aim of this study was the detection of the best sampling point position in a mature tree crown for the analysis of the SPAD values – foliar Chl content relation. The best point had therefore to be located in a crown level with strong spatial correlation and in a position with high Chl-SPAD relationship.

The concerted results of geostatistical and regression analyses carried out in the screening study permitted to detect it.

Similar studies on the subject were not found in the scientific literature.

Only a small amount of the published studies on SPAD in the scientific literature quantifies the relationship between the in vitro determined leaf Chl and the SPAD readings (Uddling et al. 2007), and many of the studies that do perform such calibrations parameterise linear relationships (Chang & Robison 2003; Torres Netto et al. 2005; Neufeld et al. 2006;

Percival et al. 2008). This is consistent with the results of the present study which found a significant linear correlation both between SPAD units and chlorophyll content and SPAD units and N concentration in foliar tissue of mature urban trees of *Tilia vulgaris* Hayne.
When comparing leaves belonging to trees of different decline classes, the mean Chl and N values were statistically different and the relationship between Chl and SPAD had to be represented with separate slopes and intercepts.
This showed that SPAD meter was reliable for estimating leaf chlorophyll amounts and N contents both in severely and slightly declining trees.
Results of this study moreover indicate that it is possible to determine a critical foliar N content below which a reduction in photosynthetic efficiency occurs. Investigation recorded a SPAD value less than 36 as a level when foliar N-deficiency was detected, and below which linden tree declining might be considered severe (decline class 2).
Anyway when SPAD values were comprised between 21 and 31, there was an overlap area where it was no more possible to distinguish between the two decline classes. That could mean that an ulterior decline class is needed between the two to better describe the photosynthetic efficiency and the health status of the considered linden trees. It furthermore suggests that SPAD meter permits an accurate decline classification in urban environment, more objective than the one performed by visual assessment.

Finally, the ordination of the ectomycorrhizal community by multivariate techniques revealed differences in species composition between decline classes and identified Chl content as a useful variable to detect these differences.
The segregation in dependency on differences in Chl content was revealed by the ordination of the ECM community by CCA (Fig. 12).
The ordination of ECM species with CCA indicated that Chl content, primarily, and decline Classes, secondly, had influence on the ECM community structure.
Few researches have dealt with the ECM community changes in declining trees (Montecchio et al. 2004; Mosca et al. 2007), and equally few have investigated the relationship between ECM variations and perturbations of the photochemical processes (Alberdi et al. 2007; Ruotosalinen et al. 2009), reaching the conclusion that

ectomycorrhizal fungi favour the maintenance of a higher functionality of the photosynthetic apparatus under stress conditions.

No information about this topic exists for mature urban trees.

Results presented here are one of the first to deal with the subject and can contribute to inquire into the associations between ECM community structure and photosynthetic efficiency in urban environment trees.

References

ALBERDI M, ÁLVAREZ M, VALENZUELA E, GODOY R, OLIVARES E, BARRIENTOS M (2007) Response to water deficit of *Nothofagus dombeyi* plants inoculated with a specific (*Descolea antarctica* Sing) and non-specific (*Pisolithus tinctorious* (Pers.) Coker & Couch) ectomycorrhizal fungi Revista Chilena de Historia Natural 80:479-491

AVENDAÑO F, SCHABENBERGER O, PIERCE FJ, MELAKABERHAN H (2003) Geostatistical analysis of field spatial distribution patterns of soybean cyst nematode. Agron J 95:936-948

CARTELAT A, CEROVIC ZG, GOULAS Y, MEYER S, LELARGE C, PRIOUL JL, BARBOTTINA A, JEUFFROY MH, GATE P, AGATI G, MOYA I (2005) Optically assessed contents of leaf polyphenolics and chlorophyll as indicators of nitrogen deficiency in wheat (*Triticum aestivum* L.). Field Crops Research 91:35-49

CHANG SX & ROBINSON DJ (2003) Nondestructive and rapid estimation of hardwood foliar nitrogen status using the SPAD-502 chlorophyll meter. Forest Ecology & Management 181:331-338

CHEN Y, RUBERSON JR (2008) Impact of variable nitrogen fertilisation on arthropods in cotton in Georgia, USA. Agric Ecosys & Environ 126:281-288

DOBBERTIN M (2005) Tree growth as indicator of tree vitality and of tree reaction to environmental stress: a review. Eur J Forest Res 124:319-333

ESRI Inc. 2001. ArcGIS Geostatistical Analyst: Statistical Tools for Data Exploration, Modeling and Advanced Surface Generation. An ESRI White Paper. 19 p.

ESRI Inc. 2006. ArcGIS Vers 9.0 with Geostatistical Extension. Environmental System Reasearch Institute, Redlands, CA.

FILELLA I, SERRANO L, SERRA J, PEÑUELAS J (1995) Evaluating Wheat Nitrogen Status with Canopy Reflectance Indices and Discriminant Analysis. Crop. Sci. 35:1400-1405

HAWKINS TS, GARDINER ES, COMER GS (2009) Modeling the relationship between extractable chlorophyll and SPAD-502 readings for endangered plant species research. J for Nat Conserv 17:123-127

HILL MO, GAUCH HG JR (1980) Detrended correspondence analysis, an improved ordination technique. Vegetatio 42:47-58

HÖLSCHER D (2004) Leaf traits and photosynthetic parameters of saplings and adult trees of co-existing species in a temperate broad-leaved forest. Basic Appl Ecol 5:163-172

HOEL BO & SOLHAUG KA (1998) Effect of irradiance on chlorophyll estimation with the Minolta SPAD-502 leaf chlorophyll meter. Annals of Botany 82:389-392

IZZO A, AGBOWO J, BRUNS TD (2005) Detection of plot-level changes in ectomycorrhizal communities across years in an old-growth mixed-conifer forest. New Phytologist 166:619-630

JONGMAN RHG, TER BRAAK CIF, VAN TONGEREN OFR (eds) (1995) Data analysis in community and landscape ecology. 2nd ed., Cambridge Univ. Press, Cambridge, UK

JOHNSTON K, VER HOEF JM, KRIVORUCHKO K, LUCAS N (2001) Using ArcGIS Geostatistical Analyst. Redlands, CA: ESRI Inc. 300 p.

KOPINGA J & VAN DEN BURG J (1995) Using soil and foliar analysis to diagnose the nutritional status of urban trees. Journal of Arboriculture 21:17-23

LIAO D, PEUQUET DJ, DUAN Y, WHISTEL EA, DOU J, SMITH R, LIN HM, CHEN JC, HEISS G (2006) GIS approaches for the estimation of residential-level ambient PM concentrations. Envir Hth Persp 114:1374-1380

LICHTENTHALER H K, MIEHÉ JA (1997) Fluorescence imaging as a diagnostic tool for plant stress. Trends in Plant Science 2-8:316-320

LICHTENTHALER H K, AC A, MAREK M V, KALINAJ, URBAN O (2007) Differences in pigment composition, photosynthetic rates and chlorophyll fluorescence images of sun and shade leaves of four tree species. Plant Physiol Biochem 45:577-588

LILLESKOV EA, BRUNS TD, HORTON TR, TAYLOR DK, GROGAN P (2004) Detection of forest stand-level spatial structure in ectomycorrhizal fungal communities. FEMS Microbiol Ecol 49:319-332

MAKI DS, COLOMBO SJ (2001) Early detection of the effects of warm storage on conifer seedlings using physiological tests. Forest Ecology & Management 154:237-249

MANSOURI-FAR C, MODARRES SANAVY SAM, SABERALI SF (2010) Maize yield response to deficit irrigation during low-sensitive growth stages and nitrogen rate under semi-arid climatic conditions. Agric Water Manag 97:12-22

MATILE P, HOTENSTEINER S, THOMAS H, KRAUTLER B (1996) Chlorophyll Breakdown in Senescent Leaves. Plant Physiol. 112:1403-1409

MATILE P (2000) Biochemistry of Indian summer: physiology of autumnal leaf coloration. Exper Gerontology 35:145-158

MATTHECK C & BRELOER H (1994) Field guide for visual tree assessment (VTA). Arboric J 18:1-23

MC-CUNE B, GRACE JB (eds) (2002) Analysis of ecological communities. MjM Software Design, Gleneden Beach, Oregon, USA

MONTECCHIO L, CAUSIN R, ROSSI S, MUTTO ACCORDI S (2004) Changes in ectomycorrhizal diversity in a declining *Quercus ilex* coastal forest. Phytopathologia Mediterranea 43:26-34

MOSCA E, MONTECCHIO L, SELLA L, GARBAYE J (2007) Short-term effect of removing tree competition on the ectomycorrhizal status of a declining peduncolate oak forest (*Quercus robur* L.). Forest Ecology and Management 244:129-140

MUTHURI CW, ONG CK, CRAIGON J, MATI BM, NGUMI VW, BLACK CR (2009) Gas exchange and water use efficiency of trees and maize in agroforestry systems in semi-arid Kenya. Agric Ecosyst & Environ 129:497-507

NEUFELD HS, CHAPPELKA AH, SOMERS GL, BURKEY KO, DAVISON AW, FINKELSTEIN PL (2006) Visible foliar injury caused by ozone alters the relationship between SPAD meter readings and chlorophyll concentrations in cutleaf coneflower. Photosynthesis Research 87:281-286

NIELSEN D, HOGUE E J, NEILSEN G H, PARCHOMCHUK P (1995) Using SPAD-502 values to assess the nitrogen status of apple trees. HortScience 30:426-444

ÖGREN E (1990) Evaluation of chlorophyll fluorescence as a probe for drought stress in willow leaves. Plant Physiol 93:1280-1285

PEÑUELAS J, FILELLA I (1998) Visible and near-infrared reflectance techniques for diagnosing plant physiological status. Trends in Plant Science 3:151-156

PERCIVAL G C (2004) Evaluation of physiological tests as predictors of young tree establishment and growth. J. Arboric. 30 (2): 80-86

PERCIVAL G C, KEARY I P, AL-HABSI S (2006) An assessment of the drought tolerance of *Fraxinus* genotypes for urban landscape plantings. Urban Forestry & Urban Greening 5:17-27

PERCIVAL G C, KEARY I P, NOVISS K (2008) The potential of a Chlorophyll Content SPAD Meter to Quantify Nutrient Stress in Foliar Tissue of Sycamore (*Acer pseudolatanus*), English Oak (*Quercus robur*), and European Beech (*Fagus sylvatica*). Arboricolture & Urban Forestry 34(2):89-99

PERYEA FJ, KAMMERECK R (1997) Use of Minolta SPAD-502 chlorophyll meter to quantify the effectiveness of mid-summer trunk injection of iron on chlorotic pear trees. J of Plant Nutrition 20:1457-1463

RAMESH K, CHANDRASEKARAN, BALASUBRAMANIAN N, BANGARUSAMY U, SIVASAMY R, SANKARAN N (2002) Chlorophyll dynamics in Rice (*Oryza sativa*)

before and after flowering based on SPAD (Chlorophyll) meter monitoring and its relation with grain yeld. J Agronomy & Crop Science 188:102-105

RICHARDSON AD, DUIGAN SP, BERLYN GP (2002) An evaluation of noninvasive methods to estimate foliar chlorophyll content. New Phytologist 153:185-194

RUOTSALAINEN AL, MARKKOLA AM, KOZLOV MV (2009) Mycorrhizal colonisation of mountain birch (*Betula pubescens* ssp. *Czerepanovii*) along three environmental gradients: does life in harsh environments alter plant-fungal relationship? Environ Monit Assess 148:215-232

ter BRAAK CJF (1986) Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. Ecology 67:1167-1179

ter BRAAK CJF, PRENTICE C (1988) A theory of gradient analysis. Adv in Ecol Res 18:271-317

ter BRAAK CJF, ŠMILAUER P (eds) (2002) CANOCO reference annual and CanoDraw for windows user's guide. Software for canonical community ordination (version 4.5). Microcomputer Power, Ithaca, NY, USA

ter BRAAK CJF (2004) The analysis of vegetation-environment relationship by canonical correspondence analysis. Plant Ecology 69:69-77

TORRES NETTO A, CAMPOSTRINI E, GONÇALVES DE OLIVEIRA J, KIYOSHI YAMANISHI O (2002) Portable chlorophyll meter for the quantification of photosynthetic pigments, nitrose and the possible use for assessment of the photochemical process in *Carica papaya* L.Braz J Plant Physiol 14(3):203-210

TORRES NETTO A, CAMPOSTRINI E, GONÇALVES DE OLIVEIRA J, BRESSAN-SMITH RE (2005) Photosynthetic pigments, nitrose, chlorophyll α fluorescence and SPAD-502 readings in coffee leale. Scientia Horticulturae 104:199-209

UDDLING J, GELANG-ALFREDSSON J, PIIKKI K, PLEIJEL H (2007) Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. Phtosynth Res 91:37-46

VAN DEN BRINK PJ, VAN DEN BRINK N, TER BRAAK CJF (2003) Multivariate analysis of ecotoxicological data using ordination: demonstrations of utility on the basis of various examples. Australasian Journal of Ecotoxicology 9:141-156

WELBURN AR, LICHTENTHALER H K (1984) Formulae and program to determine carotenoids and chlorophyll a and b of leaf extracts in different solvents.Advances in Photosyntesis Research. Vol. II. Martinus Njhorff/Dr. W. Junk Publisher, The Hague, The Netherlands.

General conclusions

Ectomycorrhizal symbiosis with fungi is obligatory for most trees in temperate and boreal regions. Many ectomycorrhizal tree species are commonly planted in cities as ornamental but their mycorrhizal status in these environments is poorly documented.

Ectomycorrhizal fungal communities can be species-rich, with more than 50 species observed in monospecific natural stands of trees (Koide et al. 2005) but studies of mycorrhizal status and fungal diversity of urban trees are few.

A first goal of this research was to describe the ECM community of mature urban linden trees (*Tilia vulgaris* Hayne) growing in two different sites (roadside and parkside sites) and at two decline levels (moderately and strongly declining) and to verify if its characteristics (root tips vitality and composition) can be associated to main soil properties.

This study demonstrated first of all that urban soils as well, even if altered and nutrient depleted, consent to urban linden trees to host a rich ECM community.

Furthermore the ordination of ECM species indicated that environmental features, comprising the different soil properties and differences in sites and in class of decline, are highly correlated to ECM species.

That may be explained by the consideration that the adaptive potential of tree species might be too limited to quickly evolve tolerance to soil modification or depletion and therefore symbiotic microorganisms, especially ectomycorrhizae that can potentially adapt more quickly, come on the forefront (Krznaric et al. 2009).

ECM groups arranged themselves according to their preference for specific soil characteristics and the observed changes of ectomycorrhizal community were correlated with variations in urban soil properties. Moreover, differences in species composition between decline classes were revealed and foliar chlorophyll content turned out to be a useful variable to detect these differences.

Since the quantification of foliar chlorophyll content may provide an accurate estimation of tree vitality, part of the research was committed to verifying the reliability of a hand held Chl meter, SPAD-205, for estimating leaf chlorophyll amounts and nitrogen contents both in severely and slightly declining trees.

The results highlighted that SPAD meter, conceived for the estimation of the nutrition status of crop species, may be a useful tool for field diagnosis in urban environment as well even for adult trees.
Once assessed the best sampling point position in the tree crown, it was possible to find a significant correlation between SPAD units and both chlorophyll content and nitrogen concentration in foliar tissue of mature urban trees of *Tilia vulgaris* Hayne.
Thanks to the SPAD analysis it was emphasized that the two visually determined declining classes were not able to properly describe the health status of the investigated linden trees, and that one intermediate decline class was necessary.
The inaccuracy of a subjective tree health visual assessment might therefore be improved by the objective evaluation of the foliar chlorophyll content.

Further researches could be addressed to better understand the ECM community parameters that can be best used as indicators of plant health in urban environment. A deepened knowledge of the soil and of the cause-effect relationship between ectomycorrhizal population dynamics and urban tree decline severity would in fact be able to increase the awareness of the importance of the belowground environment for urban trees and to enhance the cost-effectiveness of municipal tree programs

References

KOIDE R, XU B, SHARDA J (2005) Contrasting below-ground views of an ectomycorrhizal fungal community. New Phytologist 166:251-262

KRZNARIC E, WEVERS JHL, CLOQUET C, VANGRONSVELD J, VANHAECKE F, COLPAERT J (2009) Zn pollution counteracts Cd toxicity in metal-tolerant ectomycorrhizal fungi and their host plant, *Pinus sylvestris*. Environ Microb Published Online**:** 14 Oct 2009

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Thanks to all the staff of the department and to the new young friends of mine who have shared with me these years regardless of my age.

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Thanks to Linda Scattolin, who has been a true friend besides a helpful tutor.

I above all thank my young sons who have coped with a crazy mum who has gone back to school, with unconditioned love and juvenile strength.

Annex 1

Images and short descriptions of the ectomycorrhizal anatomotypes

Codex	Fungal taxa	Macroscopic description	Fig.	Outer mantle	Fig.	Inner mantle	Fig.	Emanating hyphae	Fig.	Cystidia	Fig.	Rhyzomorphs
CA18	*Boletaceae*	yellow-gold; dichotomous; grainy	1	Plectenchymatous, hyphae arranged net-like, repeteadly and squarrosely branched	2	Plectenchymatous	3	Yellowish; abundant; without clamps		Lacking	4	Undifferentiated (A); yellow-gold
CA34		Brown; dichotomous; smooth	5 6	Pseudoparenchymatous, mantle with epidermoid cells	7	Pseudoparenchymatous	8	Not observed		Lacking		Not observed
CA43		yellowish brown; dichotomous; smooth	9	Pseudoparenchymatous, mantle with epidermoid cells	10	Pseudoparenchymatous	11	Colourless, ramified, without clamps, non specific distribution	12	Lacking		Not observed
CA88		Orange to yellowish light brown; dichotomous; smooth, locally woolly	13 14	Pseudoparenchymatous, mantle with epidermoid cells	15	Pseudoparenchymatous		Scarce; short; white		Awl-shaped, bristle-like	16	Not observed
CA89		Dark brown; Monopodial-pinnate; woolly	17	Pseudoparenchymatous, mantle with angular cells	18	Plectenchymatous, star-like	19	Abundant, brownish, septa with clamp, ramified	20	Lacking		Not observed
CA94		Orange to yellowish light brown; monopodial-pinnate; smooth	21 22	Pseudoparenchymatous, mantle with angular cells	23	Transitional type between plectenchymatous and pseudoparenchymatous, with oily droplets	24	Scarce; short; white		Lacking		Not observed
CA96		Brown to light brown; Monopodial-pinnate; densely short spiny	25	Plectenchymatous, hyphae arranged net-like with prominent cystidia	26	Transitional type between plectenchymatous and pseudoparenchymatous	27	Not observed		Awl-shaped, bristle-like with clamp	28	Not observed
CA92	*Chromelosporium* sp.	Yellow to orange; Monopodial-pinnate; smooth	29 30	Pseudoparenchymatous, mantle with angular cells	31	Pseudoparenchymatous	32	Abundant; short; colourless		Lacking		Not observed

Codex	Fungal taxa	Macroscopic description	Fig.	Outer mantle	Fig.	Inner mantle	Fig.	Emanating hyphae	Fig.	Cystidia	Fig.	Rhyzomorphs
CA17	*Clavulina* sp.	Yellowish grey to yellowish brown	33	Transitional type between plectenchymatous and pseudoparenchymatous, irregularly shaped hyphae form a coarse net	34	Transitional type between plectenchymatous and pseudoparenchymatous with oily droplets	35	Colourless, ramified, with septa and clamp	36	Lacking		Not observed
CA19	*Clavulinaceae*	Greyish white; monopodial pinnate; loosely grainy loosely woolly	37	Plectenchymatous, with gelatinous matrix between the hyphae	38	Plectenchymatous, with oily droplets	39	Abundant, tortuous, with clamps; warty with soil particles	40	Lacking		Not observed
CA31	*Cortinariaceae*	Brownish orange; dichotomous, grainy long spiny	41	Pseudoparenchymatous, mantle with angular cells	42	Pseudoparenchymatous	43	Not observed		Capitate or thin-walled slightly acuminated, with clamp, basal cell yellow	44	Slightly differentiated (C)
CA68	*Cortinariaceae*	light yellow; monopodial pinnate; smooth	45 46	Plectenchymatous, hyphae arranged net-like, repeteadly and squarrosely branched	47	Transitional type between plectenchymatous and pseudoparenchymatous	48	Colourless, infrequent, with clamp		Lacking		Not observed
CA73	*Cortinariaceae*	Light yellow yo light brown; unramified; not smooth	49	Pseudoparenchymatous, mantle with epidermoid cells	50	Transitional type between plectenchymatous and pseudoparenchymatous	51	Abundant; whitish; Y shaped ramifications	52	Lacking		Not observed
CA20	*Cortinariaceae*	Brown; simple; grainy	53	Transition type, a net of coarse and irregularly shaped hyphae	54 55	Plectenchymatous, star-like	56	Not observed		Lacking		Not observed

Codex	Fungal taxa	Macroscopic description	Fig.	Outer mantle	Fig.	Inner mantle	Fig.	Emanating hyphae	Fig.	Cystidia	Fig.	Rhyzomorphs
CA30	*Geopora* sp.	Brown to light brown; monopodial pyramidal; grainy	57	Pseudoparenchymatous, mantle with angular cells	58	Pseudoparenchymatous mantle with angular to spherical cells	59	Not observed	60	Lacking		Not observed
CA6	*Helvellaceae*	Orange to brown; monopodial pyramidal; grainy	61 62	Pseudoparenchymatous, mantle with angular cells	63	Pseudoparenchymatous	64	Infrequent; colourless, tortuous, with septa		Lacking		Not observed
CA27	*Inocybe* sp.	yellow orange; dichotomous; cottony	65 66	Plectenchymatous, hyphae rather irregularly arranged, no special pattern discernable, but hyphae often growing in longitudinal directions regarding root orientation	67	Transitional type between plectenchymatous and pseudoparenchymatous		Abundant; whitish; Y shaped ramifications; clamps with a hole	68	Lacking		Not observed
CA83	*Inocybe* sp.	Black, withish on the very tip; monopodial-pinnate; loosely cottony	69 70	Plectenchymatous, hyphae rather irregularly arranged, no special pattern discernable, but hyphae often growing in longitudinal directions regarding root orientation	71	Plectenchymatous		Abundant; whitish; wavy; with clamp	72	Lacking		Not observed
CA49	*Laccaria* sp.	yellow orange; monopodial pinnate; cottony	73 74	Plectenchymatous, hyphae rather irregularly arranged, no special pattern discernable	75	Plectenchymatous, with broad streaks of parallel hyphae		Abundant; whitish; concentrated distally ; clamps with a hole	76	Lacking		Not observed
CA60	*Peziza* sp.	Orange to light brown; monopodial pinnate; grainy	77	Pseudoparenchymatous, mantle with angular cells	78	Pseudoparenchymatous	79	Abundant, concentrated distally	80	Lacking		Not observed

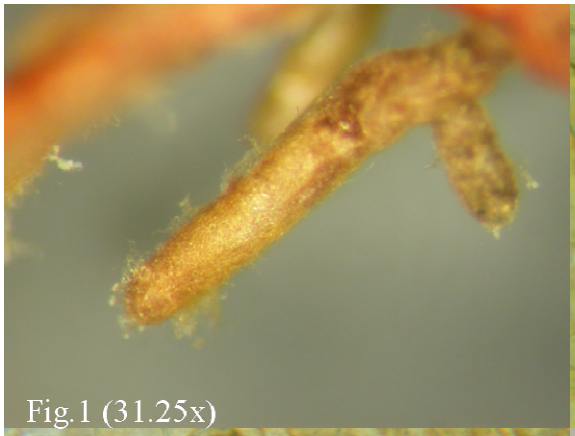
Codex	Fungal taxa	Macroscopic description	Fig.	Outer mantle	Fig.	Inner mantle	Fig.	Emanating hyphae	Fig.	Cystidia	Fig.	Rhyzomorphs
CA80	*Peziza* sp.	Yellowish whitish; unramified cottony	81 82	Pseudoparenchymatous, mantle with angular cells	83	Pseudoparenchymatous		Infrequent, colourless; with septa	84	Lacking		Not observed
CA81	*Peziza* sp.	Light yellow to brown; unramified; smooth	85	Pseudoparenchymatous, mantle with angular cells	86	Pseudoparenchymatous	87	Colourless, ramified, with septa and H-shape open anastomosis	88	Lacking		Not observed
CA28	*Pezizaceae*	Dark brown; irregularly pinnate; densely grainy/short spiny	89	Pseudoparenchymatous, mantle with angular cells	90	Pseudoparenchymatous	91	Browinish, septa without clamps		Awl-shaped, bristle-like, thick-walled	92	Not observed
CA67	*Pseudotomentella* sp.	Dark brown to black; monopodial pyramidal; densely warty	93	Plectenchymatous, hyphae rather irregularly arranged, no special pattern discernable, bearing a network of angular-triangular, horn-shaped cells	94	Plectenchymatous, with star-like pattern	95	Browinish, septa without clamps		Lacking	96	Undifferentiated (A); brownish
CA39	*Russula* sp.	Brownish orange; monopodial pinnate; densely short spiny	97	Plectenchymatous, hyphae arranged net-like with prominent cystidia	98	Plectenchymatous	99	Not observed		Bottle_shaped with a straight neck and flask-shaped with an apical knob	100	Not observed
CA91	*Scleroderma* sp.	Orange; irregularly pinnate; densely woolly	101 102	Plectenchymatous, hyphae arranged net-like, repeteadly and squarrosely branched	103	Plectenchymatous, ring-like		Abundant; whitish; Y shaped ramifications	104	Lacking		Not observed
CA66	*Sebacina* sp.	Yellow to orange; unramified; loosely cottony	105 106	Pseudoparenchymatous, mantle with angular cells	107	Pseudoparenchymatous	108	Infrequent, concentrated distally		Lacking		Not observed

Codex	Fungal taxa	Macroscopic description	Fig.	Outer mantle	Fig.	Inner mantle	Fig.	Emanating hyphae	Fig.	Cystidia	Fig.	Rhyzomorphs
CA85	*Sebacina* sp.	Brownish; irregularly pinnate; loosely woolly	109 110	Plectenchymatous, hyphae arranged net-like, repeteadly and squarrosely branched	111	Plectenchymatous, with broad streaks of parallel hyphae	112	Abundant, tortuous, whitish		Lacking		Not observed
CA14	*Sebacinaceae*	Yellowish to greenish light brown; monopodial pinnate; densely cottony	113 114	Plectenchymatous, hyphae arranged net-like, repeteadly and squarrosely branched	115	Plectenchymatous		Abundant, not striking, whitish	116	Lacking		Not observed
CA35	*Sebacinaceae*	Yellow to orange; unramified; loosely cottony	117 118	Transitional type between plectenchymatous and pseudoparenchymatous, irregularly shaped hyphae form a coarse net	119	Plectenchymatous		Abundant, tortuous, short, with soil particles		Lacking	120	Not observed
CA71	*Sebacinaceae*	Orange to light brown; irregularly pinnate; grainy	121 122	Pseudoparenchymatous, with epidermoid cells bearing a delicate hyphal net	123	Pseudoparenchymatous	124	Scarce, tortuous		Lacking		Not observed
CA3	*Telephoraceae*	Dark brown; irregularly pinnate; densely woolly	125	Plectenchymatous, hyphae rather irregularly arranged, no special pattern discernable, but hyphae often growing in longitudinal directions regarding root orientation	126	Plectenchymatous, star-like	127	Brownish, ramified, with septa and H-shape open anastomosis	128	Lacking		Differentiated (E)

Codex	Fungal taxa	Macroscopic description	Fig.	Outer mantle	Fig.	Inner mantle	Fig.	Emanating hyphae	Fig.	Cystidia	Fig.	Rhyzomorphs
CA32	*Telephoraceae*	Dark brown; irregularly pinnate; long spiny	129	Plectenchymatous, hyphae rather irregularly arranged, no special pattern discernable, bearing a network of angular-triangular, horn-shaped cells	130	Transitional type between plectenchymatous and pseudoparenchymatous	131	Not observed		Awl-shaped, bristle-like; foot cell present	132	Not observed
CA33	*Telephoraceae*	Black; irregularly pinnate; grainy	133	Pseudoparenchymatous, mantle with angular cells	134	Transitional type between plectenchymatous and pseudoparenchymatous	135	Abundant, hyaline, wavy, long	136	Lacking		Not observed
CA95	*Telephoraceae*	Dark brown; irregularly pinnate; loosely grainy	137 138	Pseudoparenchymatous, mantle with angular cells, bearing mounds of roundish cells (type K)	139	Plectenchymatous, star-like	140	Not observed		Lacking		Not observed
CA1	*Tomentella* sp.	Yellow to light brown; monopodial-pinnate; grainy	141	Pseudoparenchymatous, with epidermoid cells bearing a delicate hyphal net	142	Plectenchymatous	143	Infrequent, concentrated distally, colourless, with clamps	144	Lacking		Not observed
CA21	*Tomentella* sp.	Brown; monopodial pinnate; grainy	145	Pseudoparenchymatous, with epidermoid cells bearing a delicate hyphal net	146	Transitional type between plectenchymatous and pseudoparenchymatous	147	Infrequent, whitish, tortuous, with clamps	148	Clamped “fibulocystidium-type”		Undifferentiated (B); brownish
CA42	*Tomentella* sp.	Dark brown; irregularly pinnate; short spiny	149	Pseudoparenchymatous, mantle with angular cells	150	Plectenchymatous, with star-like pattern	151	Scarce		Awl-shaped, bristle-like with broad basal part	152	Not observed
CA54	*Tomentella* sp.	Dark brown; irregularly pinnate; short spiny	153	Pseudoparenchymatous, mantle with angular cells	154	Plectenchymatous, with star-like patter	155	Scarce, colourless, warty, with septa		Ramified (verticillate)	156	Not observed

Codex	Fungal taxa	Macroscopic description	Fig.	Outer mantle	Fig.	Inner mantle	Fig.	Emanating hyphae	Fig.	Cystidia	Fig.	Rhyzomorphs
CA55	*Tomentella* sp.	Brown; irregulary pinnate; grainy	157	Pseudoparenchymatous, mantle composed of angular-triangular cells	158	Plectenchymatous	159	Infrequent, concentrated distally, colourless, fringed tips	160	Lacking		Not observed
CA69	*Tomentella* sp.	Greyish orange to brown; irregularly pinnate; smooth	161	Pseudoparenchymatous, mantle composed of angular-triangular cells	162	Plectenchymatous, with broad streaks of parallel hyphae	163	Abundant, concentrated distally, colourless, with clamps	164	Lacking		Not observed
CA70	*Tomentella* sp.	Orange to brown; monopodial-pinnate; short spiny	165	Pseudoparenchymatous, mantle composed of angular-triangular cells	166	Transitional type between plectenchymatous and pseudoparenchymatous		Not observed		Awl-shaped with clamp "fibulocystidium" type; basal cell yellow	167 168	Not observed
CA75	*Tomentella* sp.	Yellowish to orange; irregularly pinnate; woolly	169	Pseudoparenchymatous, mantle composed of angular-triangular cells	170	Plectenchymatous, with broad streaks of parallel hyphae	171	Abundant, colourless, with clamps		Lacking	172	Undifferentiated
CA79	*Tomentella* sp.	Dark brown; simple; grainy	173	Pseudoparenchymatous, mantles with angular cells	174	Plectenchymatous, with star-like pattern	175	Infrequent, brownish, with clamps, warty	176	Lacking		Not observed
CA84	*Tomentella* sp.	Brown to orange; irregularly pinnate; loosely grainy	177	Pseudoparenchymatous, mantle with angular cells bearing a delicate hyphal net	178	Transitional type between plectenchymatous and pseudoparenchymatous	179	Infrequent		Cpitate with clamps	180	Undifferentiated, hyphae compacted arranged and of uniform diameter
CA82	*Trichophaea* sp.	Brown to yellow in the apical part; monopodial pinnate; loosely grainy	181 182	Pseudoparenchymatous, mantle with angular cells bearing a delicate hyphal net	183	Pseudoparenchymatous		Abundant; whitish; Y shaped ramifications	184	Lacking		Not observed

Boletaceae (CA18)



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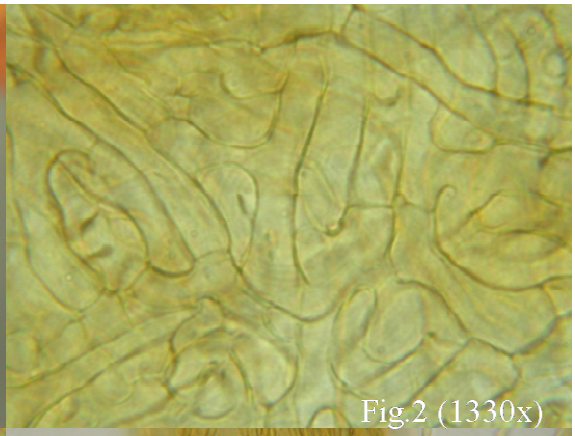


Fig.2 (1330x)

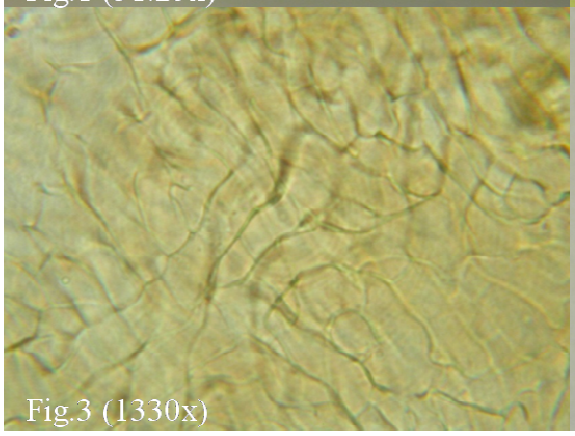


Fig.3 (1330x)

Fig.4 (520x)

CA34

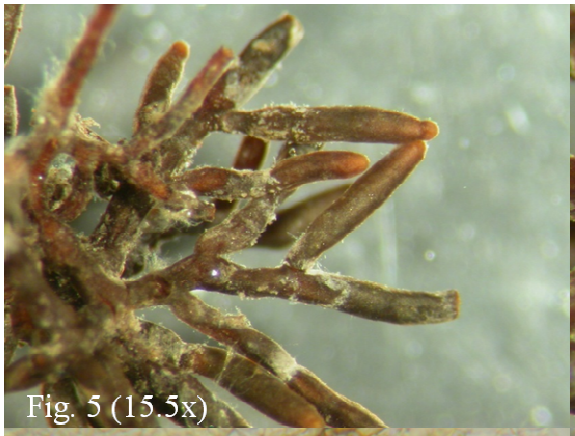


Fig. 5 (15.5x)

Fig. 6 (31.25x)

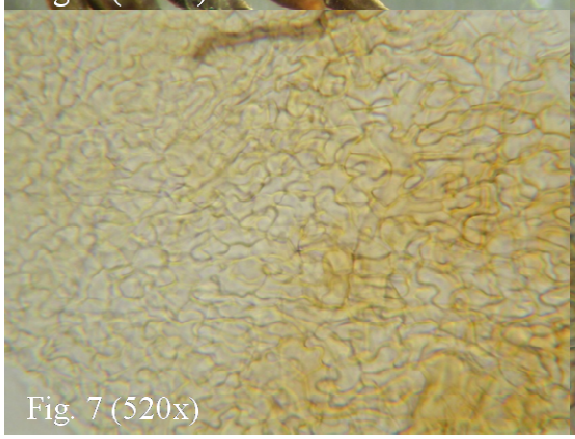


Fig. 7 (520x)

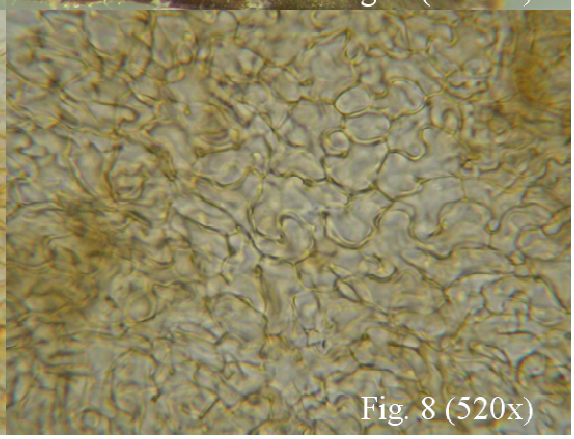


Fig. 8 (520x)

CA43

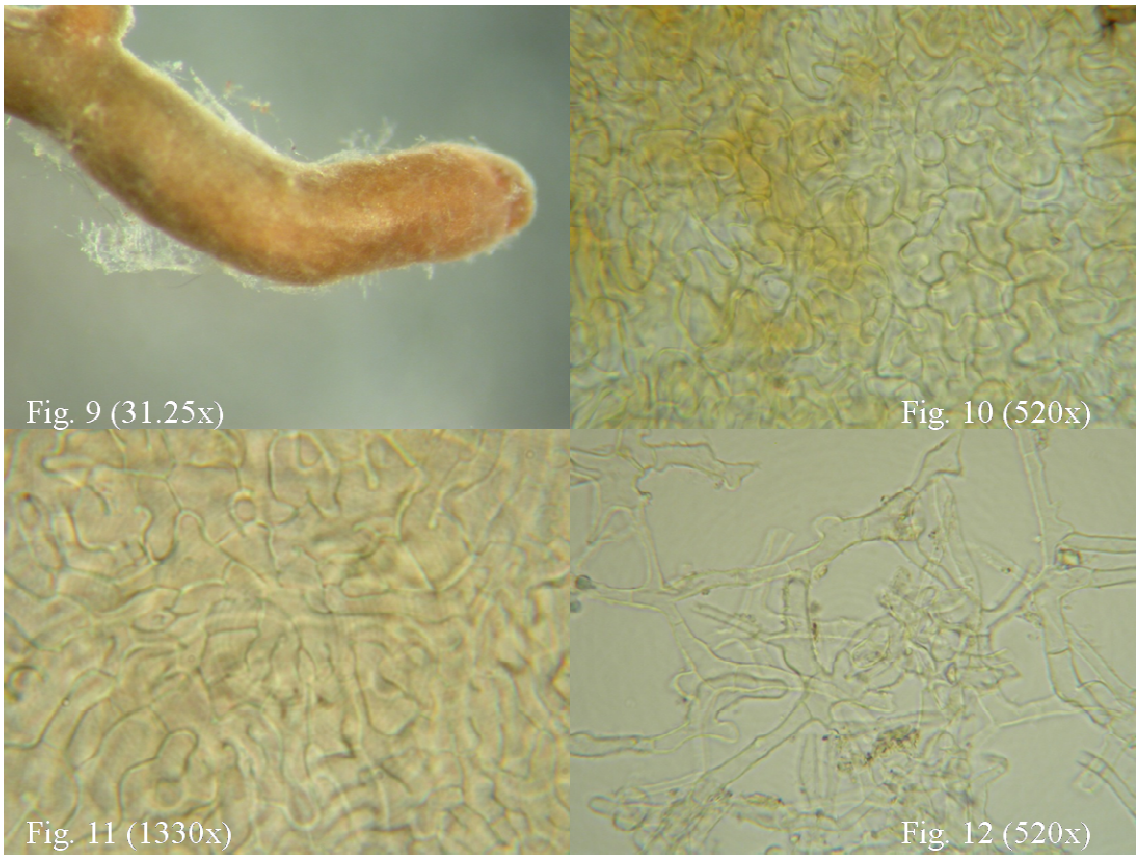


Fig. 9 (31.25x)

Fig. 10 (520x)

Fig. 11 (1330x)

Fig. 12 (520x)

CA88

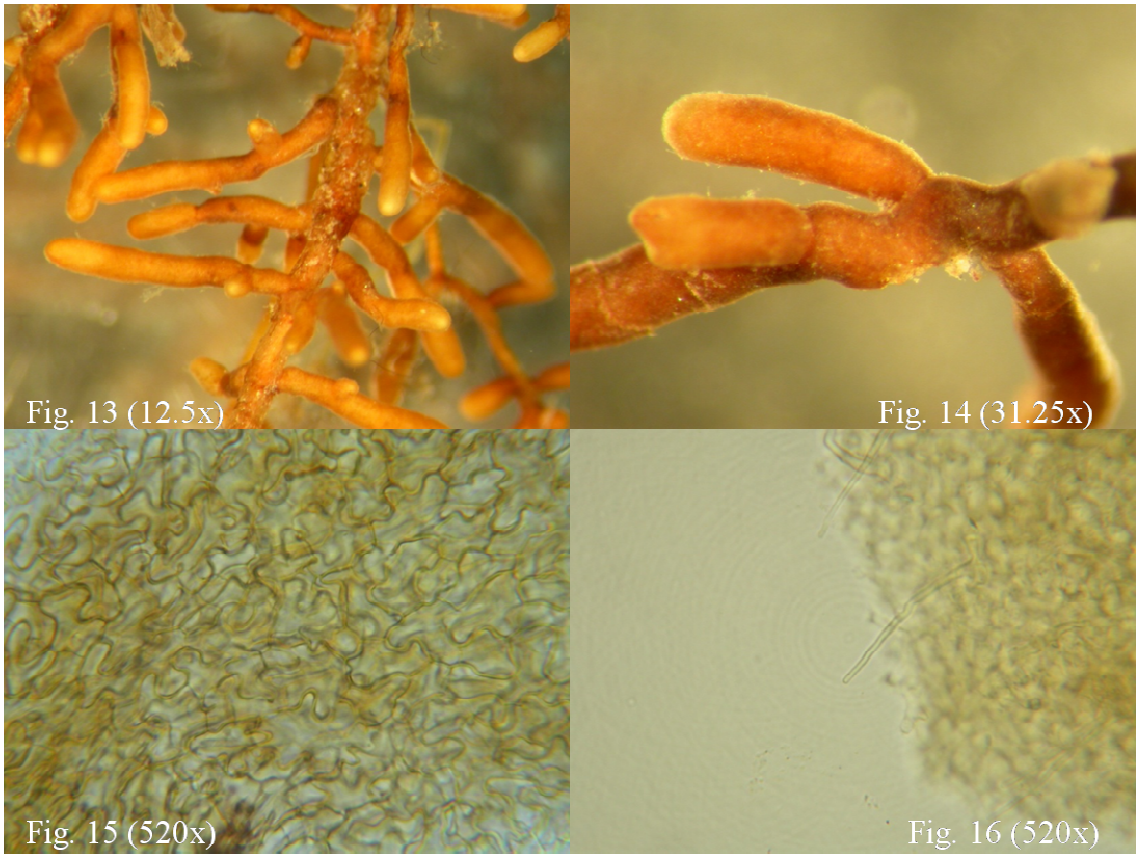


Fig. 13 (12.5x)

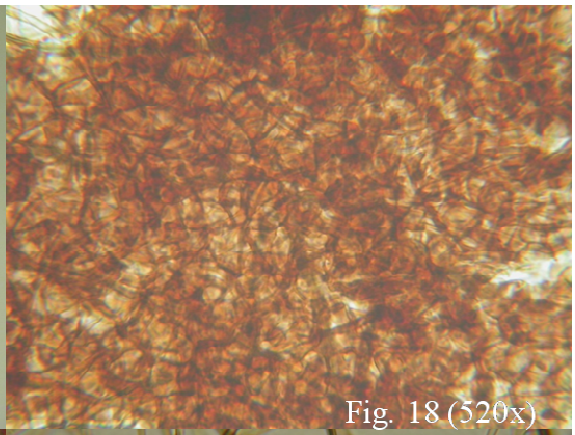
Fig. 14 (31.25x)

Fig. 15 (520x)

Fig. 16 (520x)

CA89

Fig. 17 (60x)



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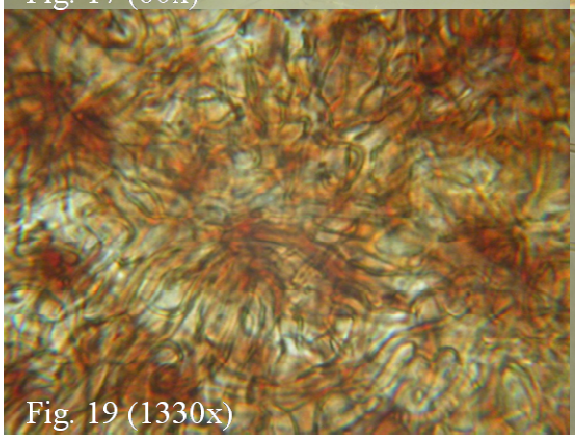

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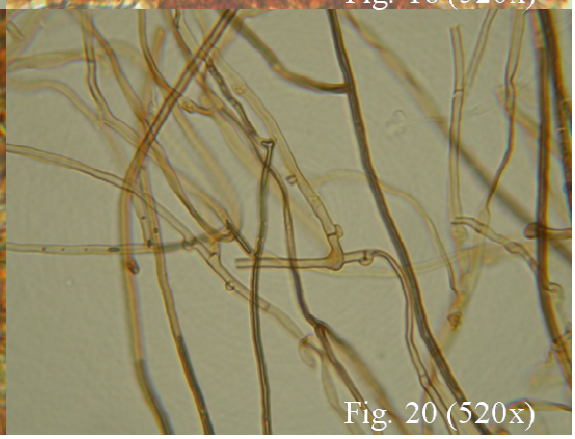

Fig. 20 (520x)

CA94

Fig. 21 (12.5x)

Fig. 22 (31.25x)

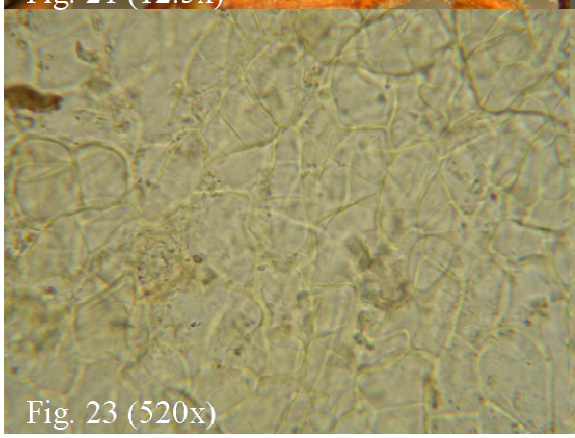

Fig. 23 (520x)

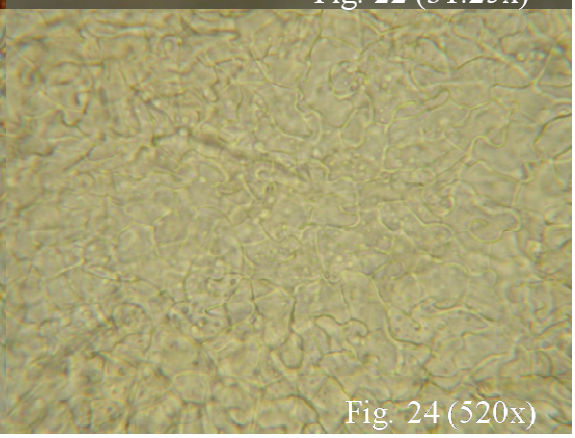

Fig. 24 (520x)

CA96

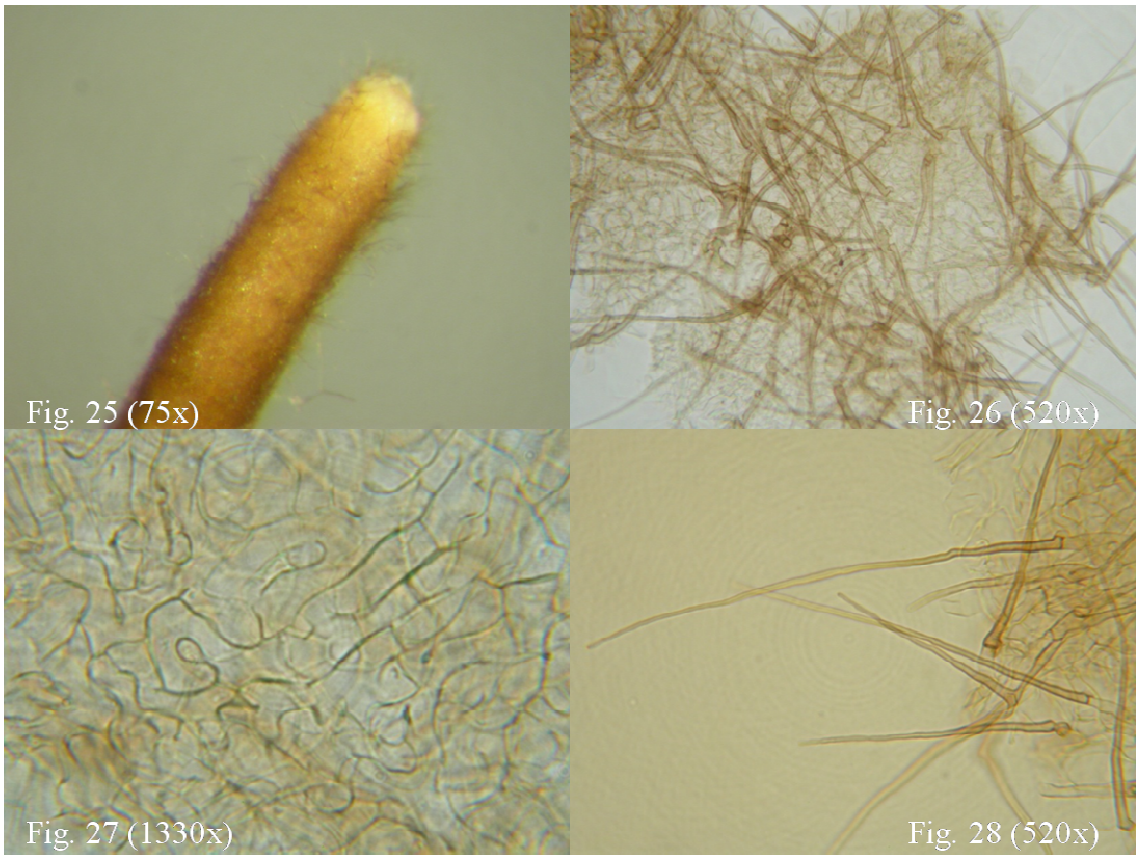

Fig. 25 (75x)

Fig. 26 (520x)

Fig. 27 (1330x)

Fig. 28 (520x)

Chromelosporium (CA92)

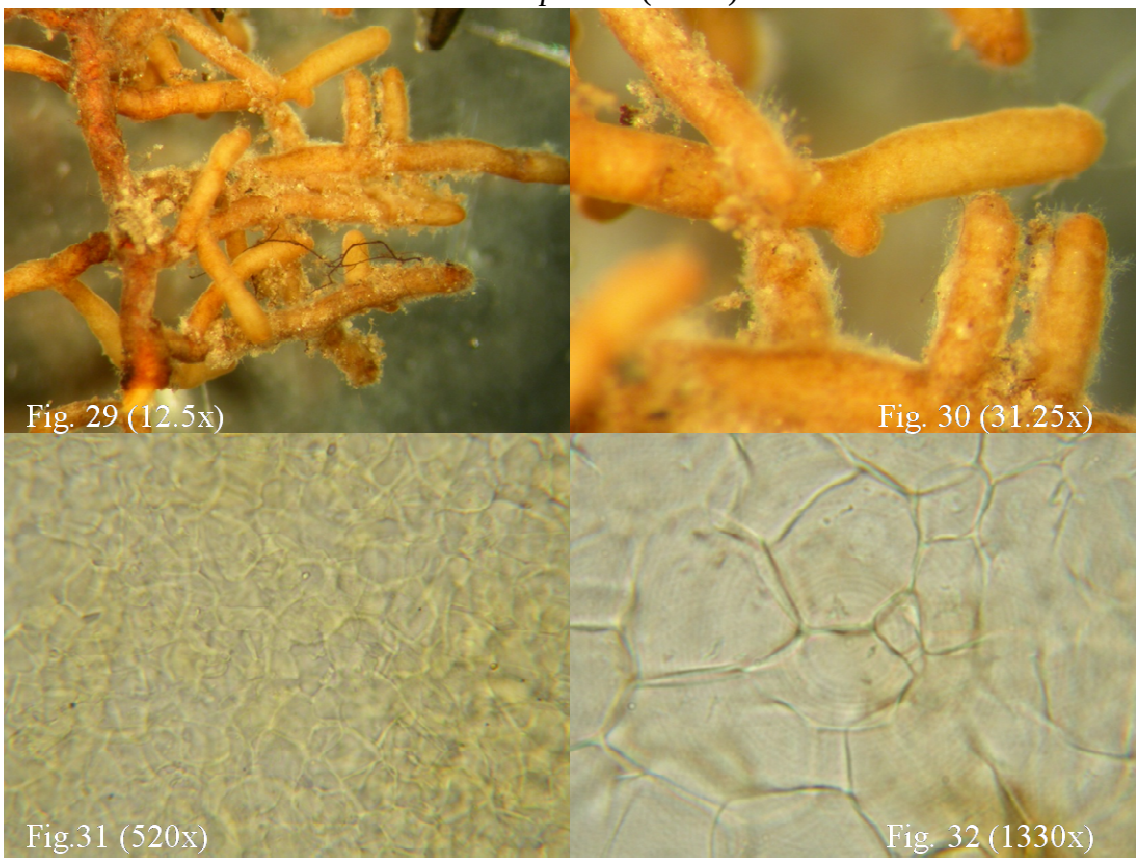

Fig. 29 (12.5x)

Fig. 30 (31.25x)

Fig.31 (520x)

Fig. 32 (1330x)

Clavulina sp.(CA17)

Fig. 33 (75x)

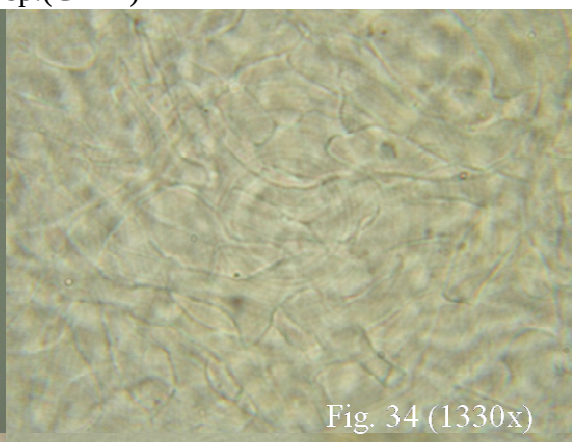

Fig. 34 (1330x)

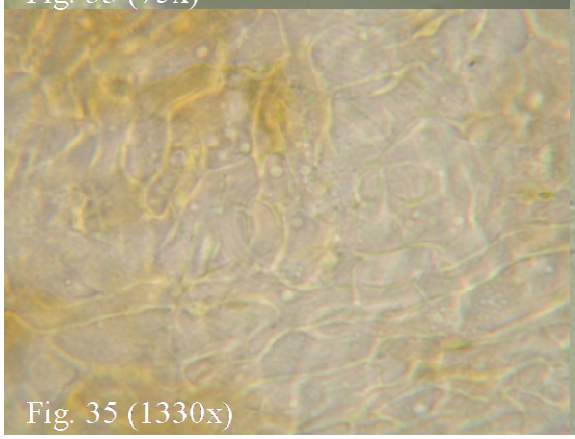

Fig. 35 (1330x)

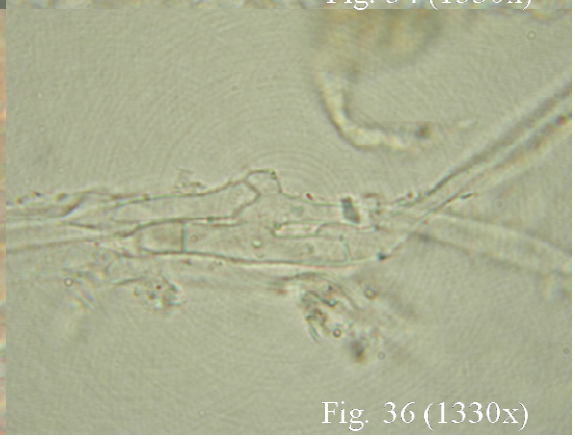

Fig. 36 (1330x)

Clavulinaceae (CA19)

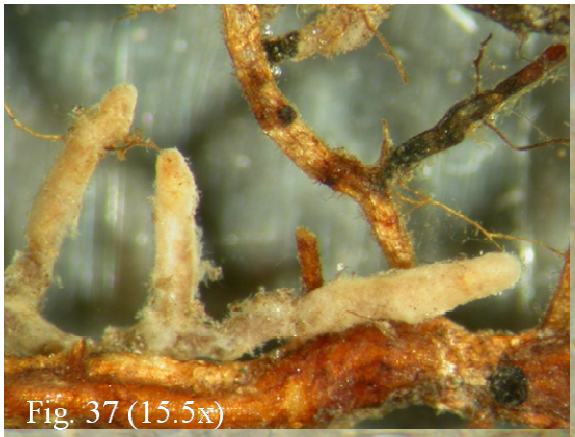

Fig. 37 (15.5x)

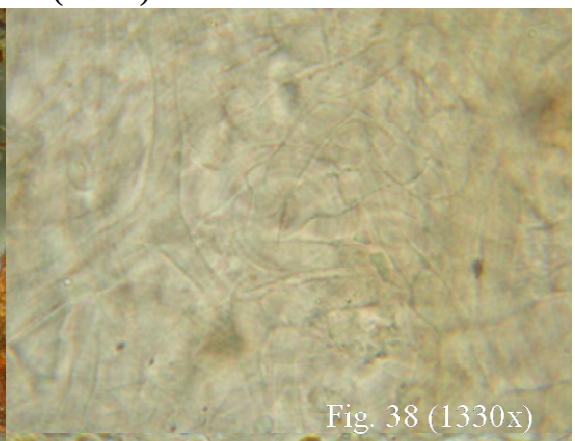

Fig. 38 (1330x)

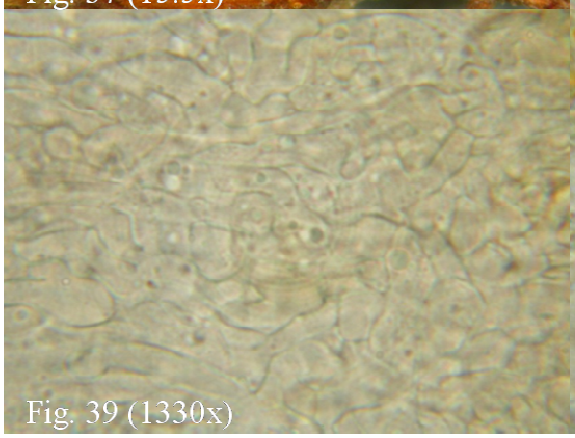

Fig. 39 (1330x)

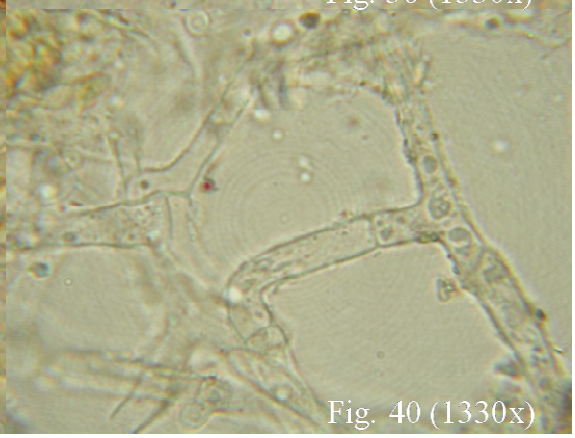

Fig. 40 (1330x)

Cortinariaceae (CA31)

Fig. 41 (75x)

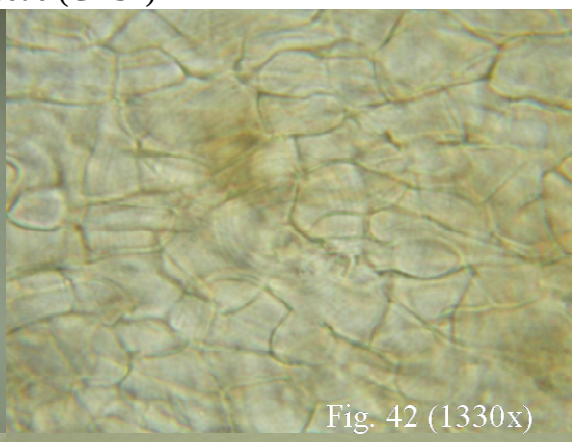

Fig. 42 (1330x)

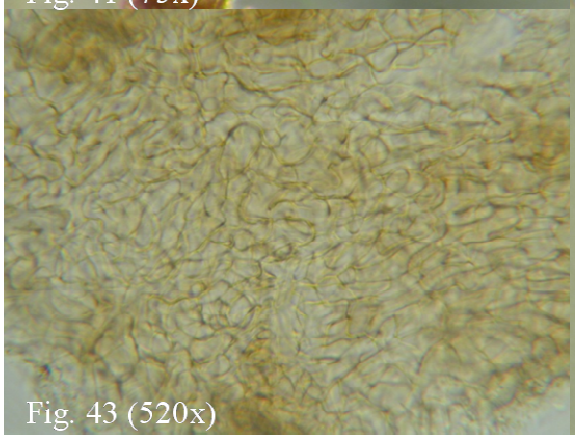

Fig. 43 (520x)

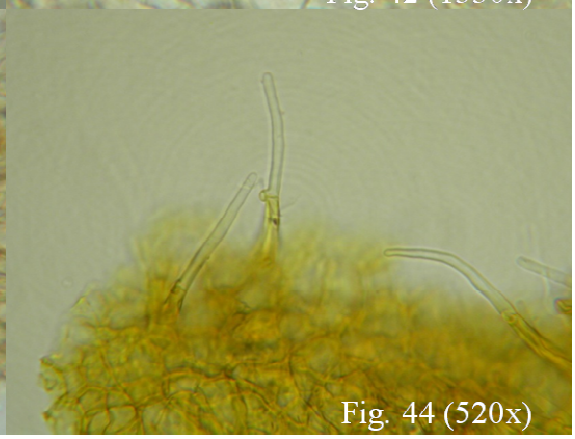

Fig. 44 (520x)

Cortinariaceae (CA68)

Fig. 45 (15.5x)

Fig. 46 (75x)

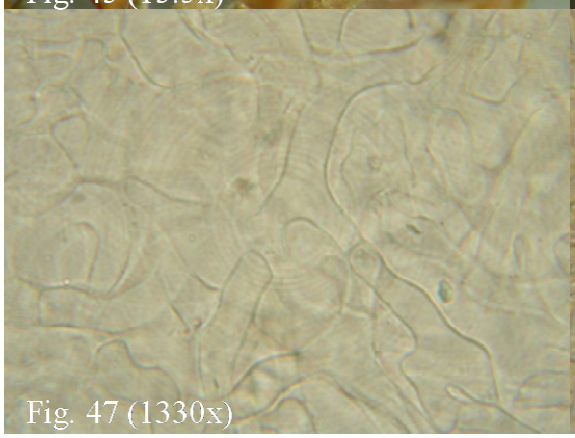

Fig. 47 (1330x)

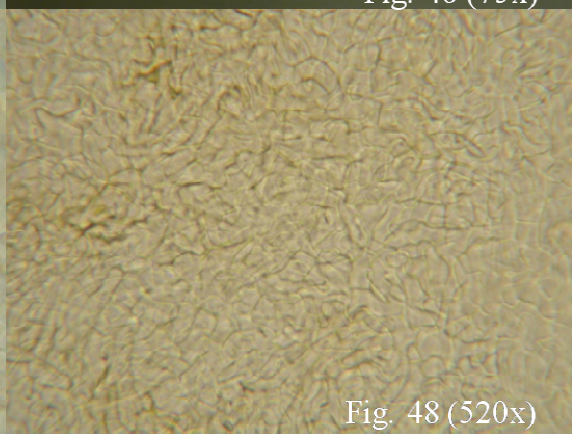

Fig. 48 (520x)

Cortinariaceae (CA73)

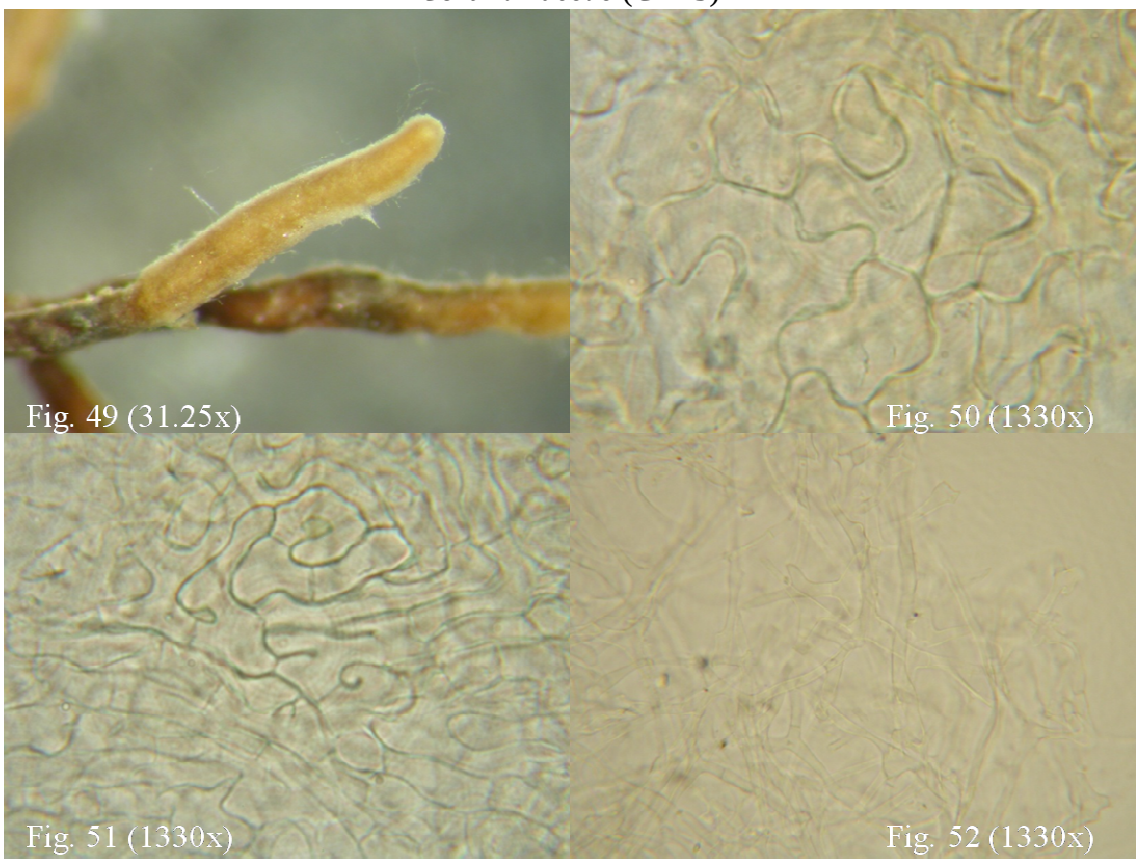

Fig. 49 (31.25x)

Fig. 50 (1330x)

Fig. 51 (1330x)

Fig. 52 (1330x)

Cortinariaceae (CA20)

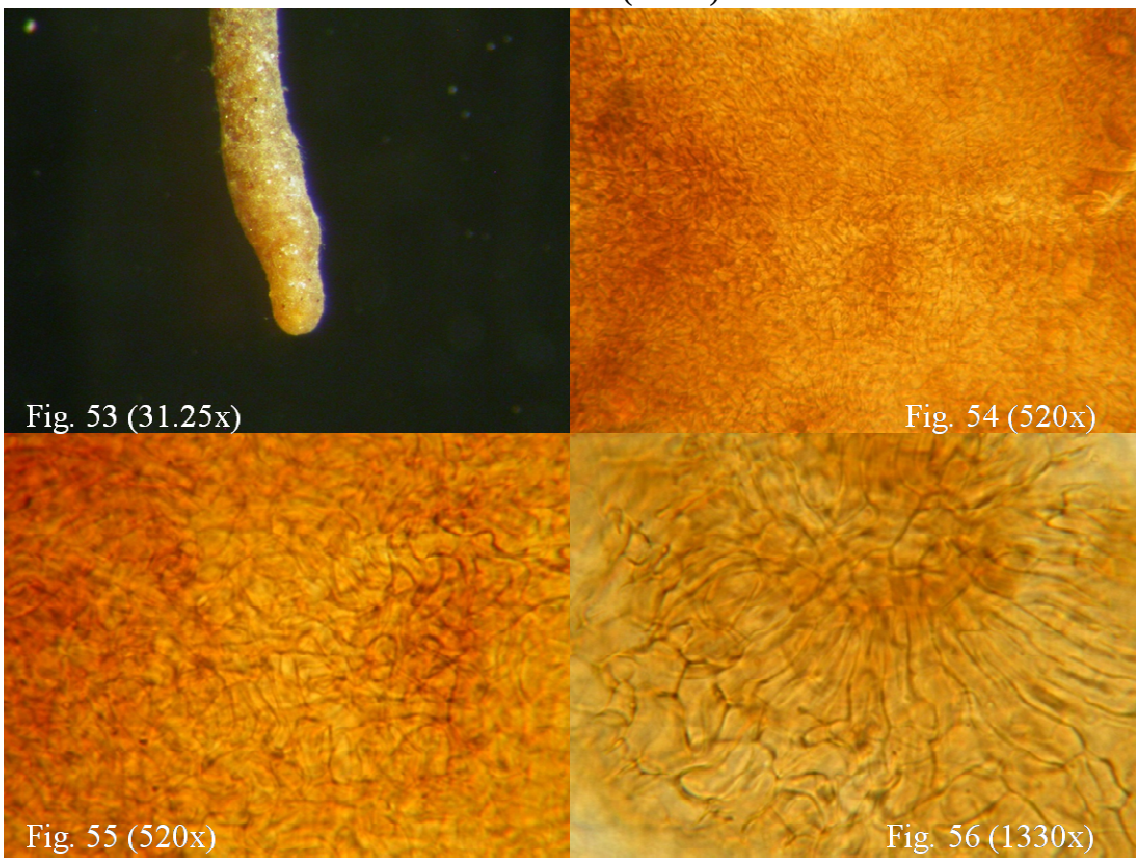

Fig. 53 (31.25x)

Fig. 54 (520x)

Fig. 55 (520x)

Fig. 56 (1330x)

Geopora sp.(CA30)

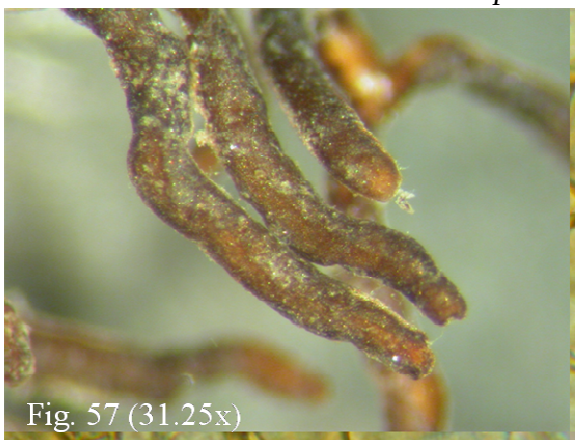

Fig. 57 (31.25x)

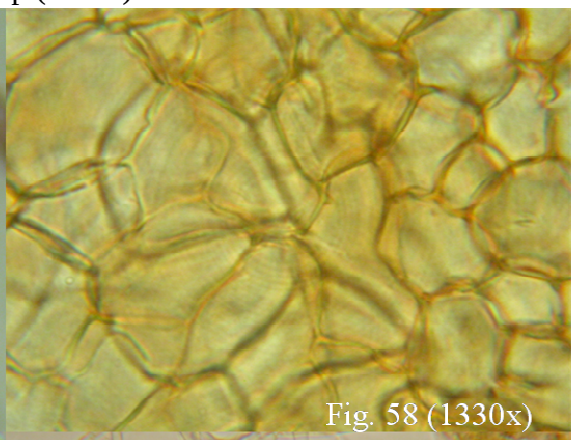

Fig. 58 (1330x)

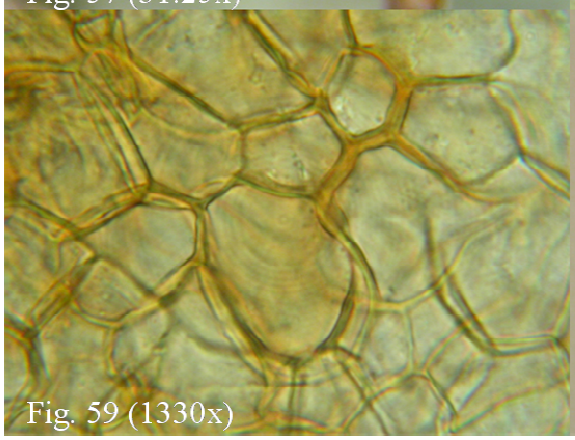

Fig. 59 (1330x)

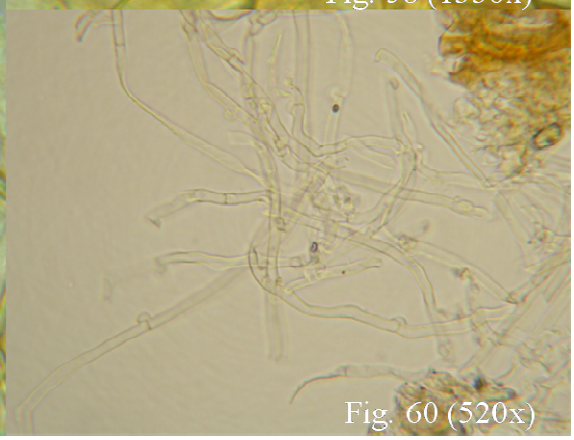

Fig. 60 (520x)

Helvellaceae (CA6)

Fig. 61 (31.25x)

Fig. 62 (75x)

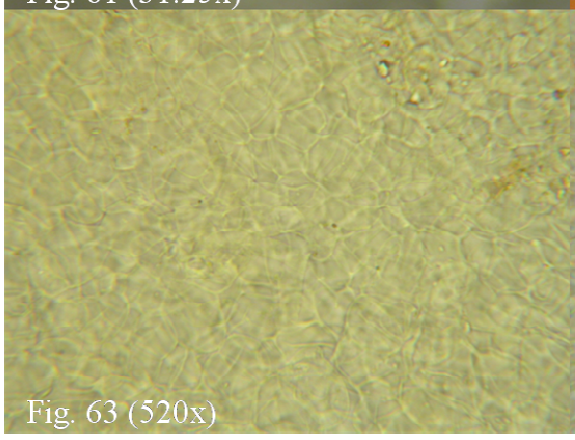

Fig. 63 (520x)

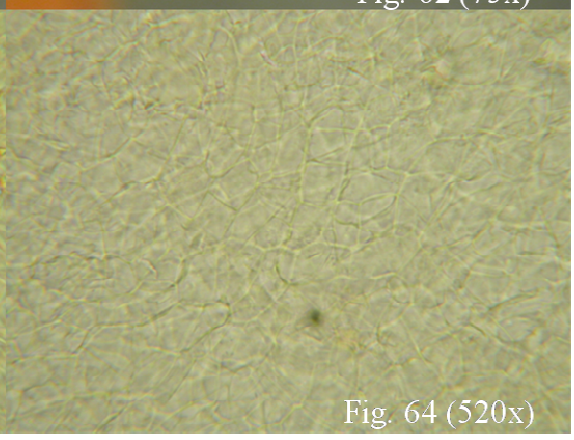

Fig. 64 (520x)

Inocybe sp.(CA27)

Fig. 65 (15.5x)

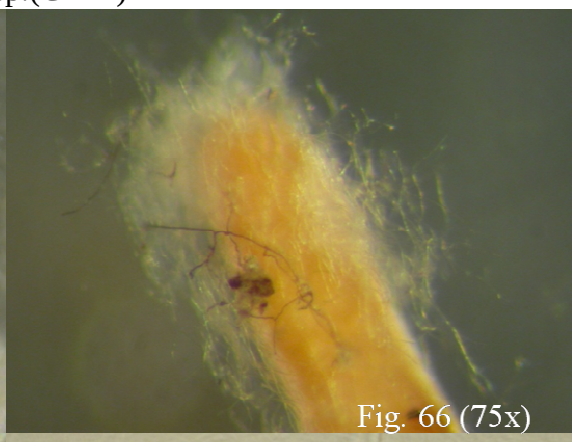

Fig. 66 (75x)

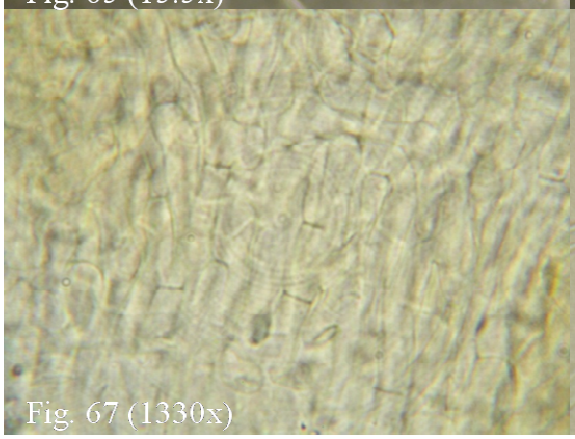

Fig. 67 (1330x)

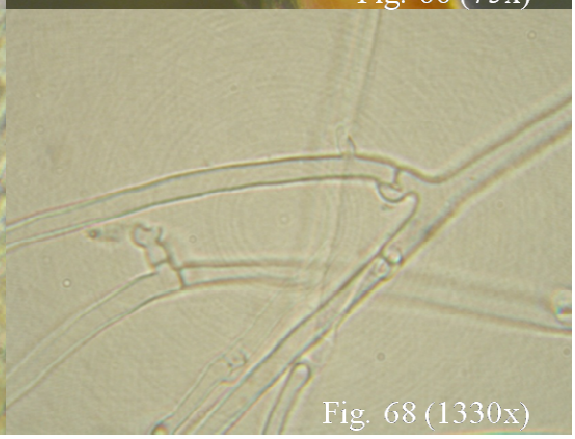

Fig. 68 (1330x)

Inocybe sp.(CA83)

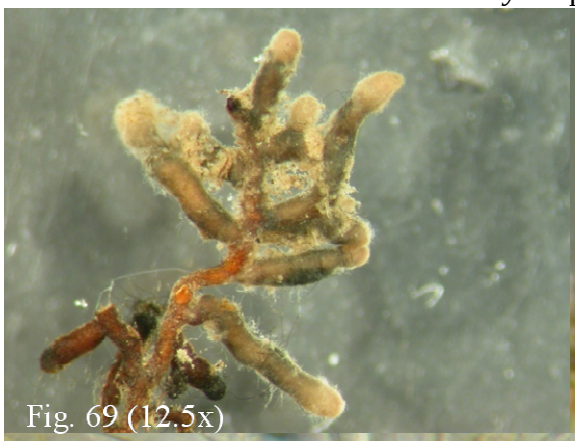

Fig. 69 (12.5x)

Fig. 70 (31.25x)

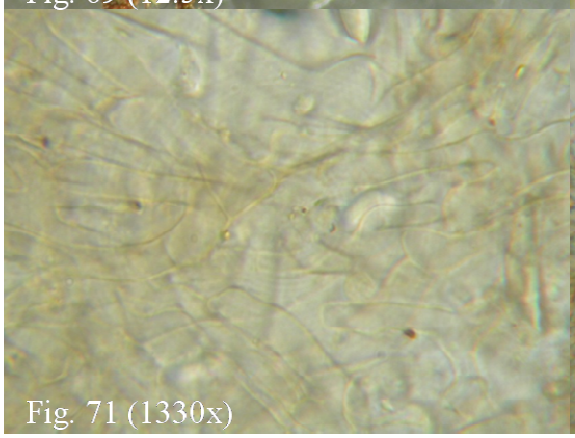

Fig. 71 (1330x)

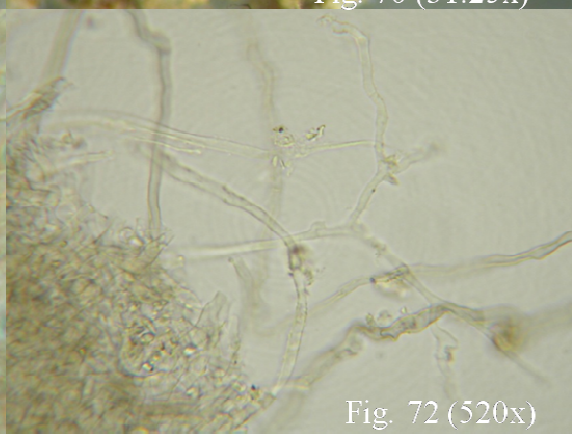

Fig. 72 (520x)

Laccaria sp.(CA49)

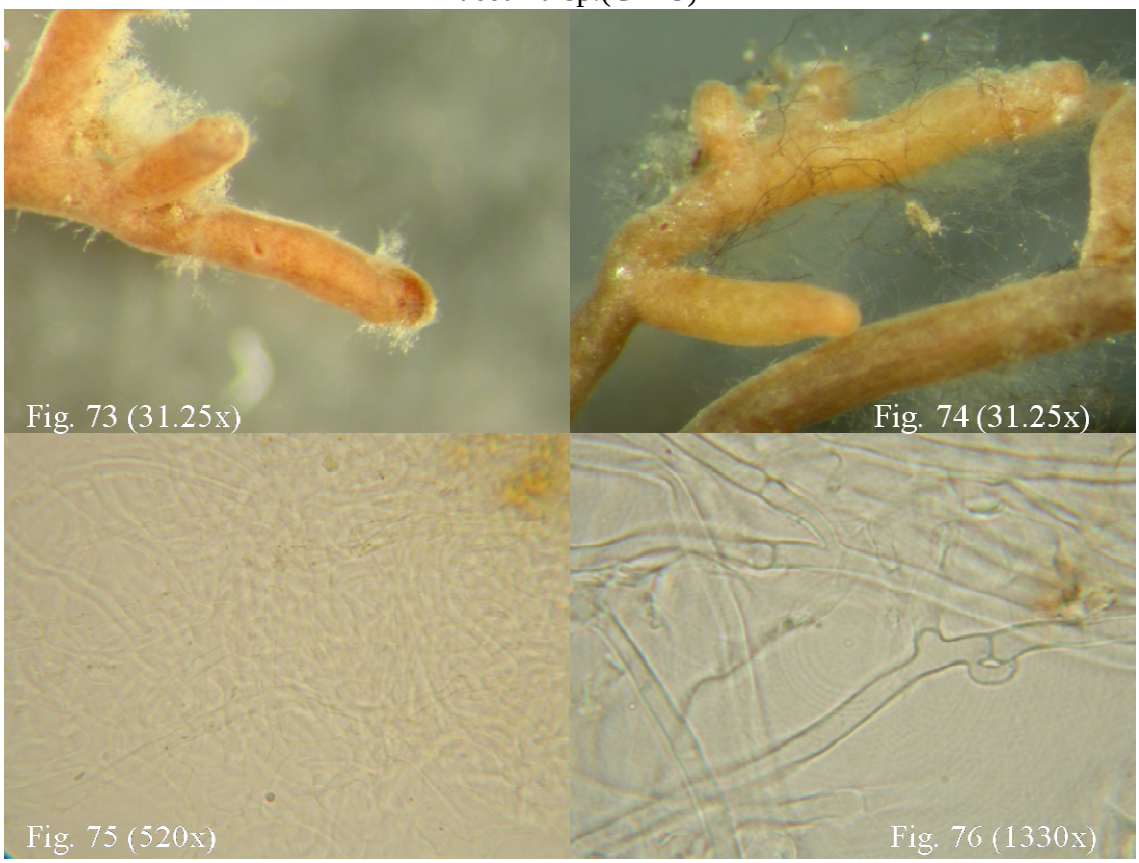

Fig. 73 (31.25x)

Fig. 74 (31.25x)

Fig. 75 (520x)

Fig. 76 (1330x)

Peziza sp.(CA60)

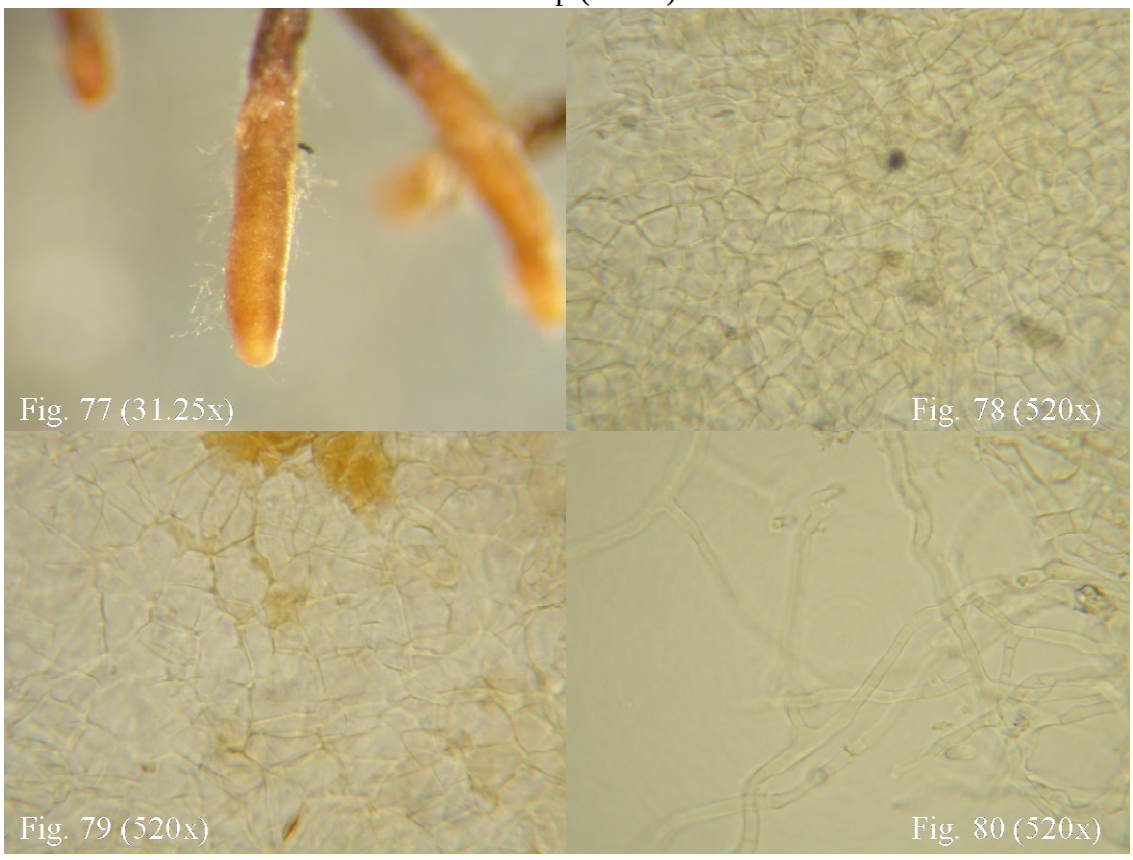

Fig. 77 (31.25x)

Fig. 78 (520x)

Fig. 79 (520x)

Fig. 80 (520x)

Peziza sp.(CA80)

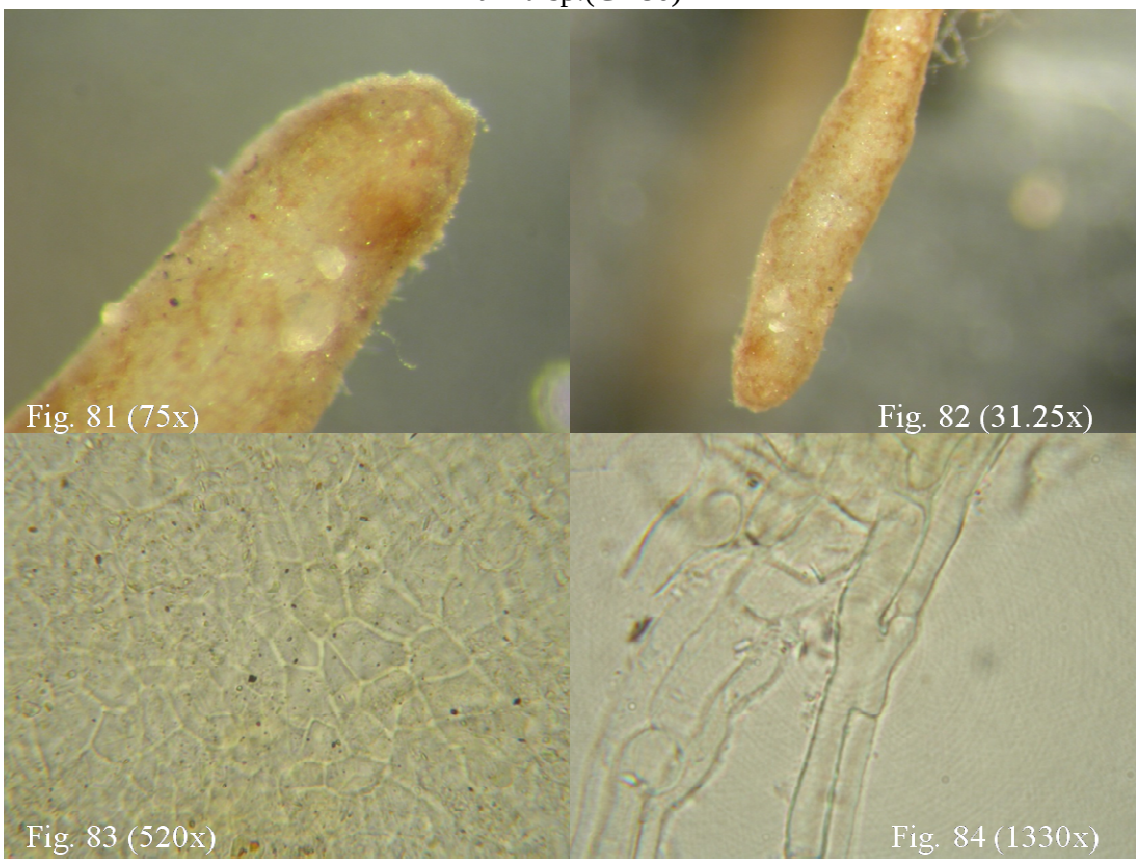

Fig. 81 (75x)

Fig. 82 (31.25x)

Fig. 83 (520x)

Fig. 84 (1330x)

Peziza sp.(CA81)

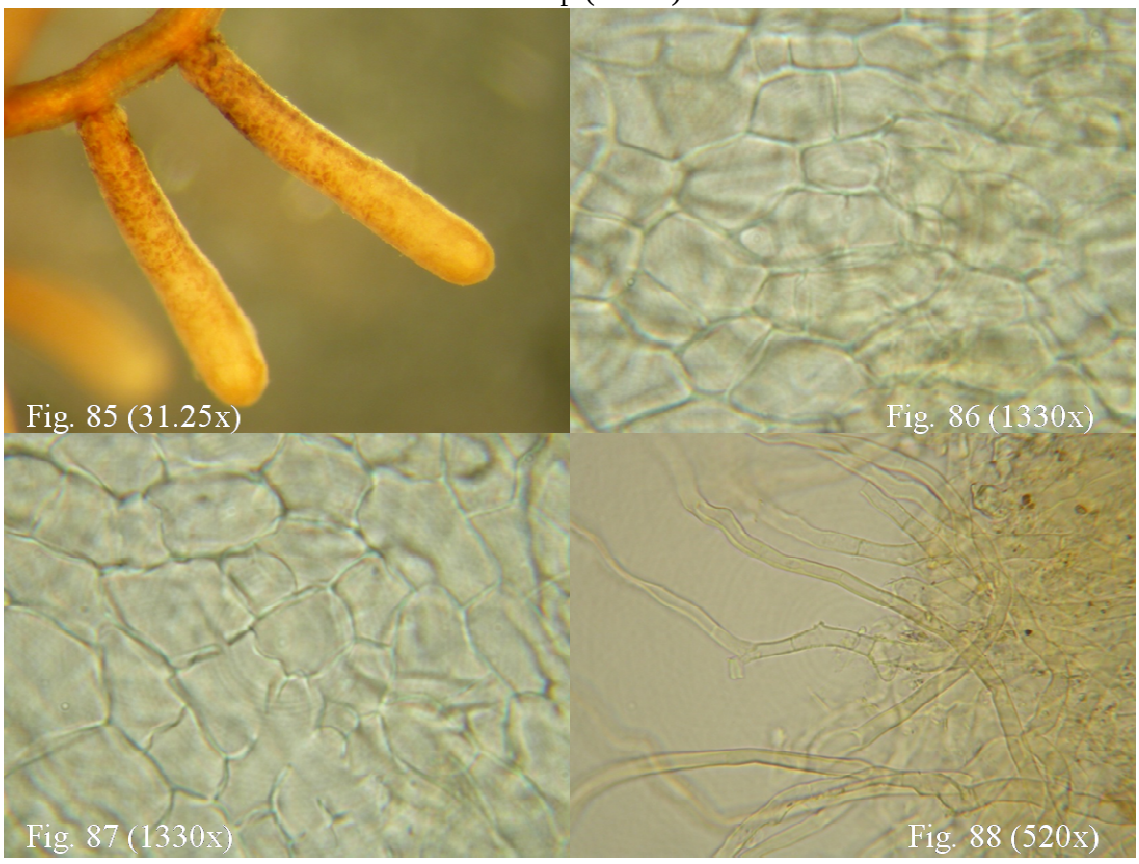

Fig. 85 (31.25x)

Fig. 86 (1330x)

Fig. 87 (1330x)

Fig. 88 (520x)

Pezizaceae (CA28)

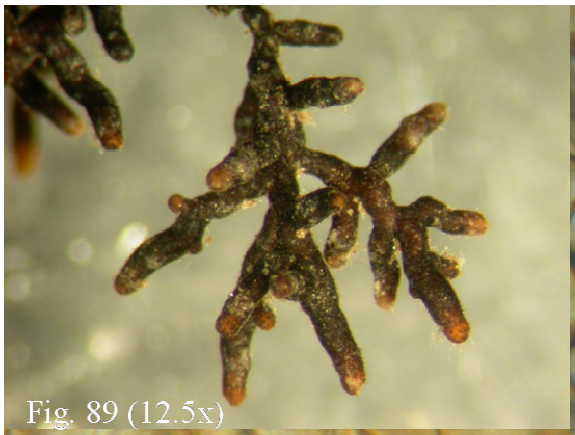

Fig. 89 (12.5x)

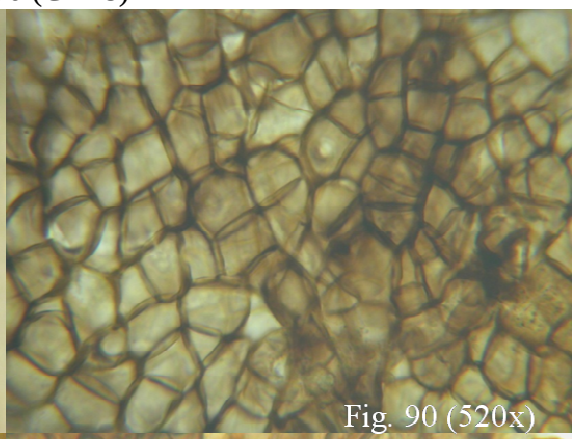

Fig. 90 (520x)

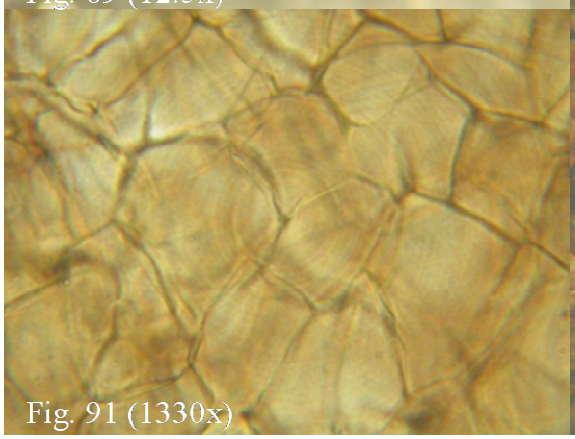

Fig. 91 (1330x)

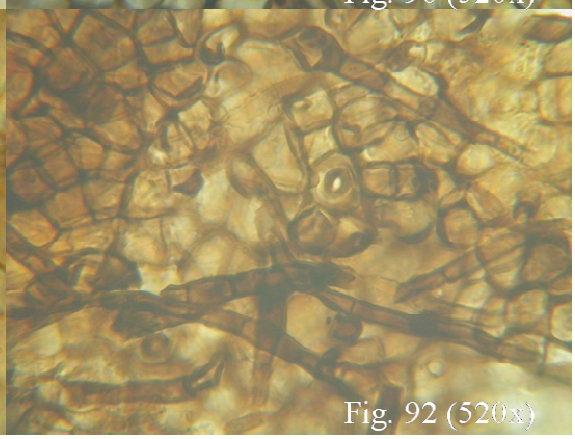

Fig. 92 (520x)

Pseudotomentella sp. (CA67)

Fig. 93 (40x)

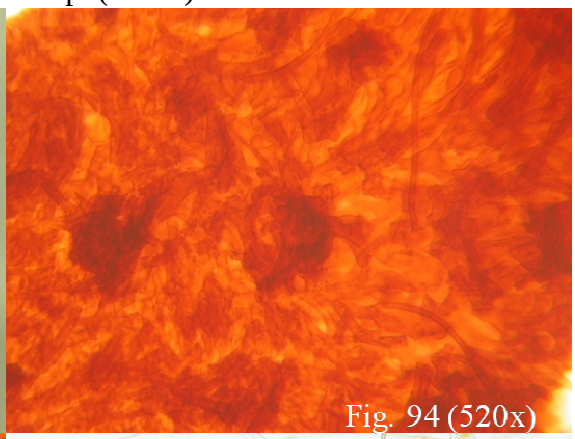

Fig. 94 (520x)

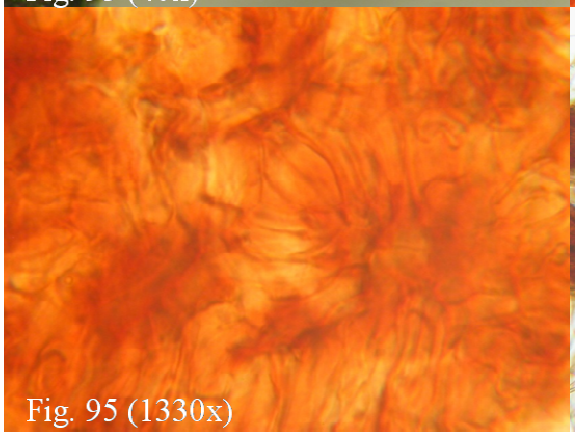

Fig. 95 (1330x)

Fig. 96 (520x)

Russula sp. (CA39)

Fig. 97 (75x)

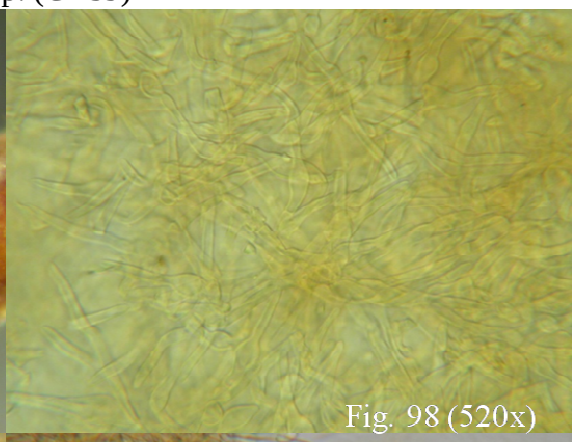

Fig. 98 (520x)

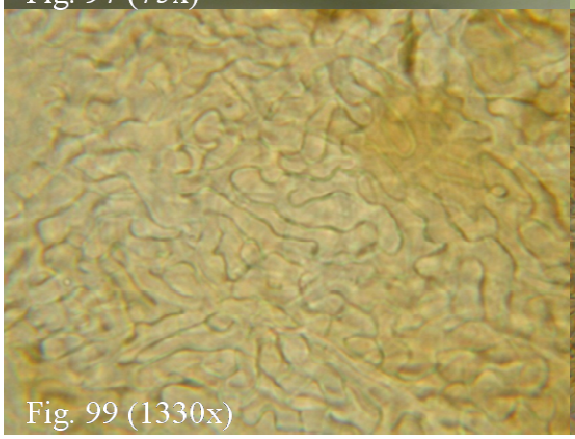

Fig. 99 (1330x)

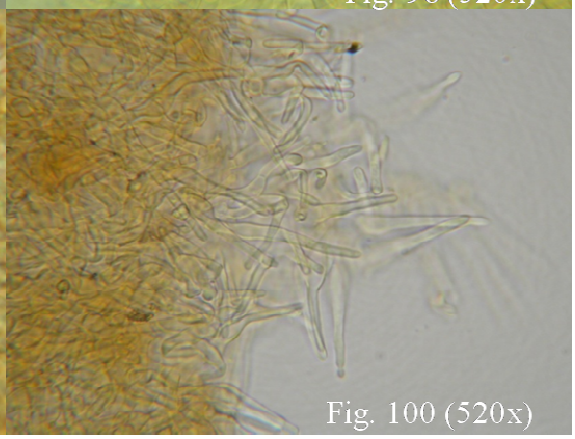

Fig. 100 (520x)

Scleroderma sp.(CA91)

Fig. 101 (15.5x)

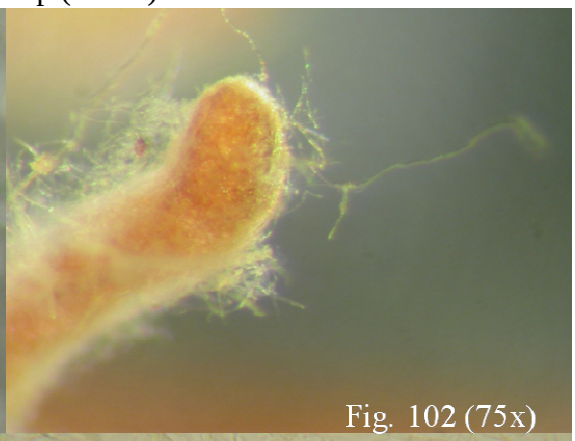

Fig. 102 (75x)

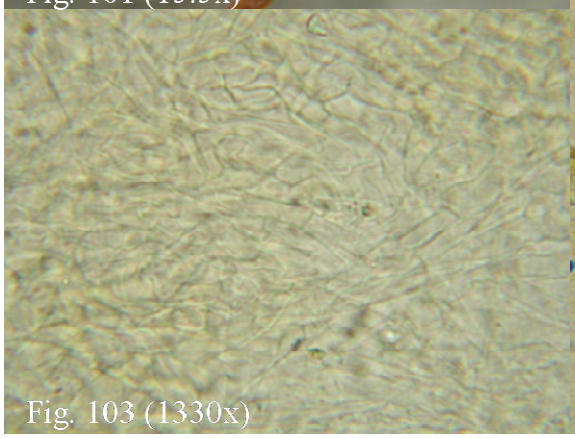

Fig. 103 (1330x)

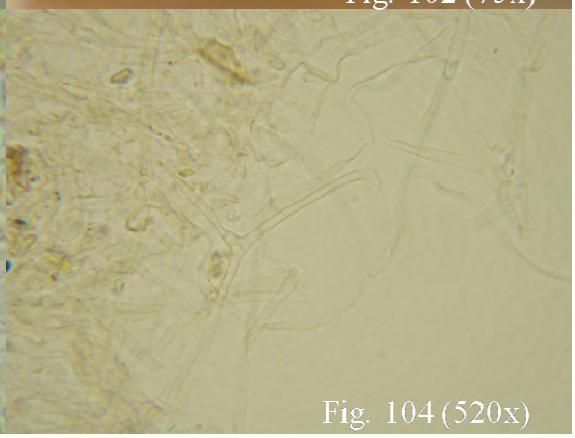

Fig. 104 (520x)

Sebacina sp. (CA66)

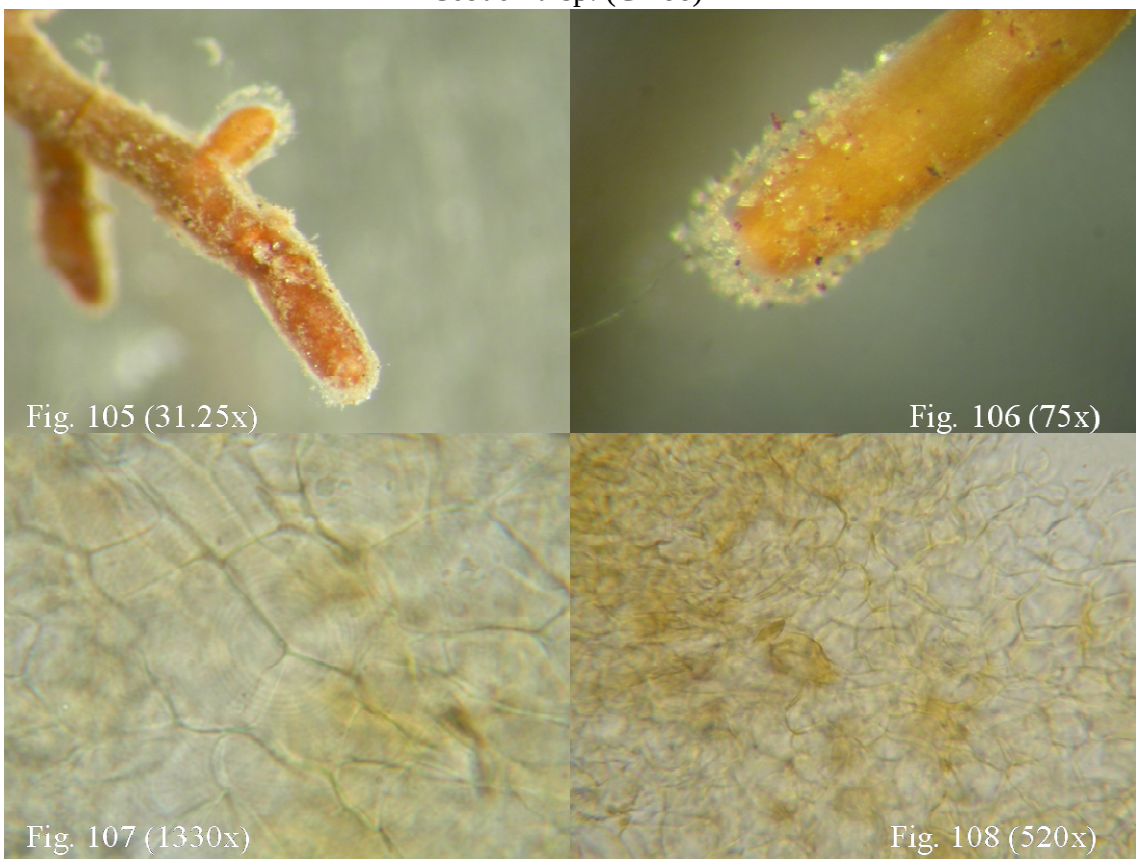

Fig. 105 (31.25x)

Fig. 106 (75x)

Fig. 107 (1330x)

Fig. 108 (520x)

Sebacina sp. (CA85)

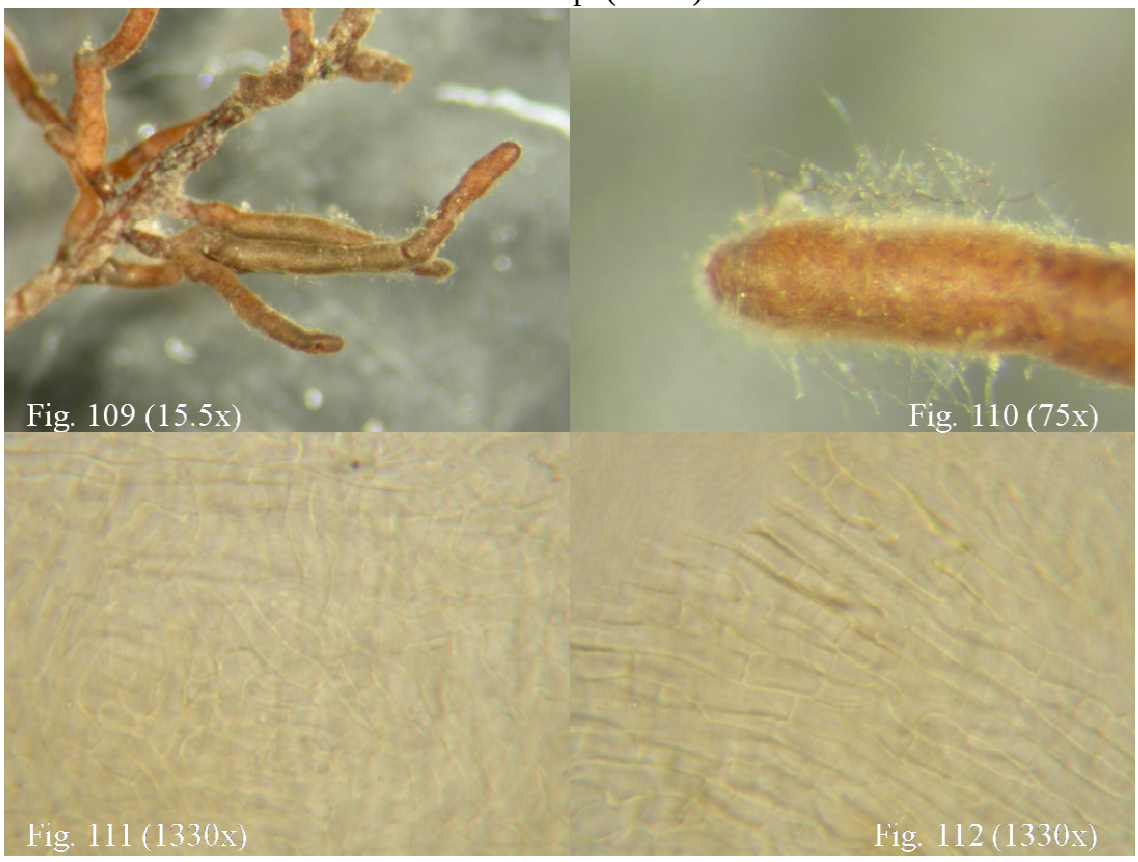

Fig. 109 (15.5x)

Fig. 110 (75x)

Fig. 111 (1330x)

Fig. 112 (1330x)

Sebacinaceae (CA14)

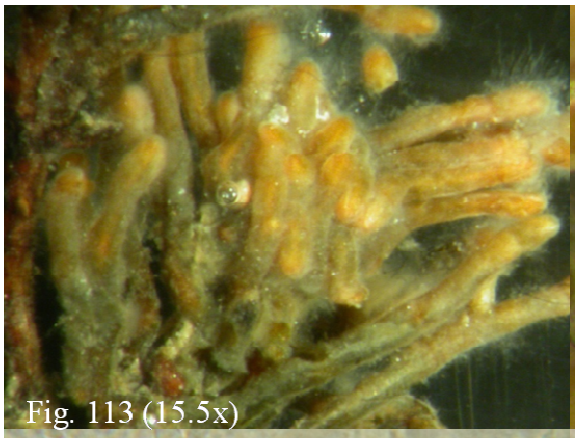

Fig. 113 (15.5x)

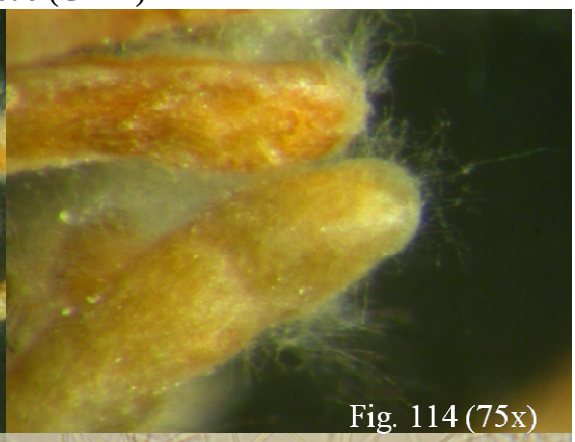

Fig. 114 (75x)

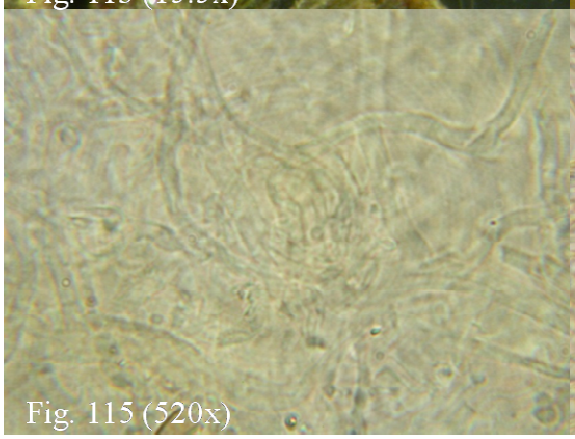

Fig. 115 (520x)

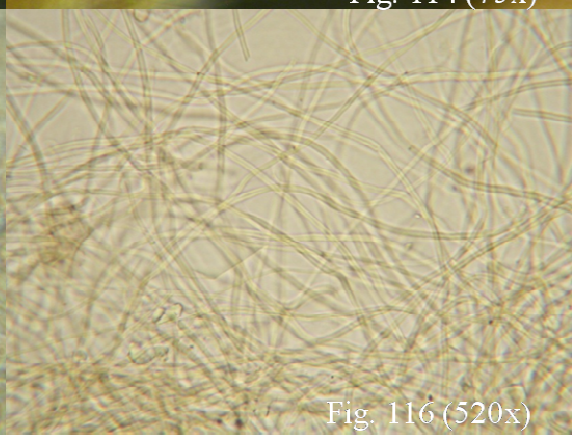

Fig. 116 (520x)

Sebacinaceae (CA35)

Fig. 117 (31.25x)

Fig. 118 (75x)

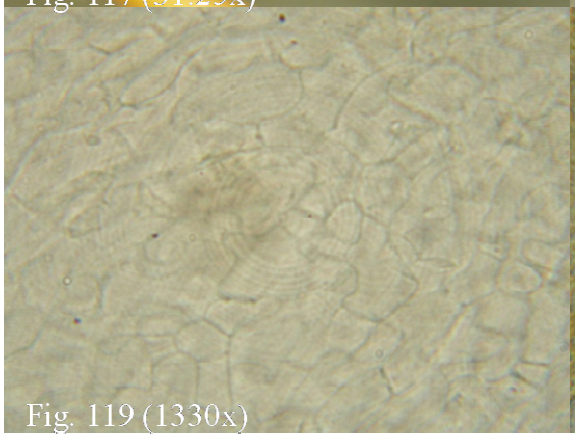

Fig. 119 (1330x)

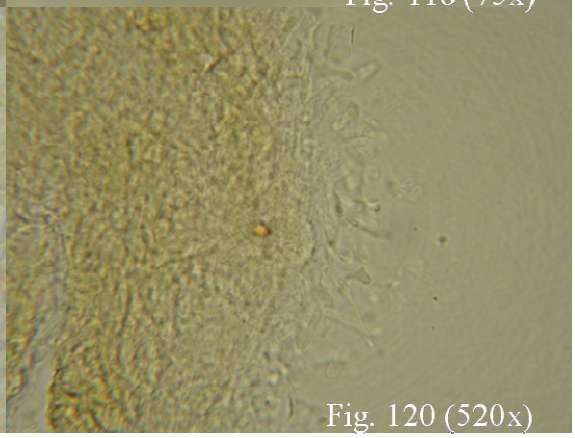

Fig. 120 (520x)

Sebacinaceae (CA71)

Fig. 121 (31.25x)

Fig. 122 (75x)

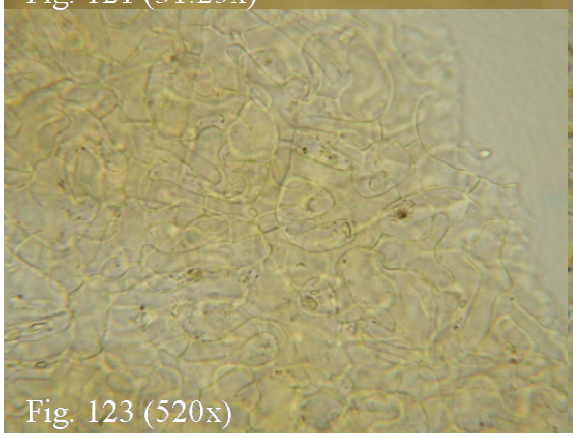

Fig. 123 (520x)

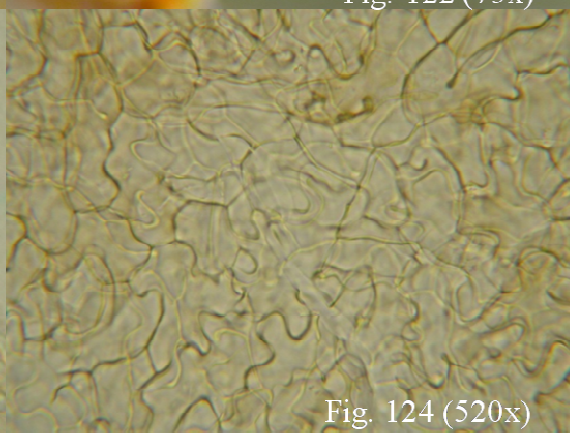

Fig. 124 (520x)

Telephoraceae (CA3)

Fig. 125 (31.25x)

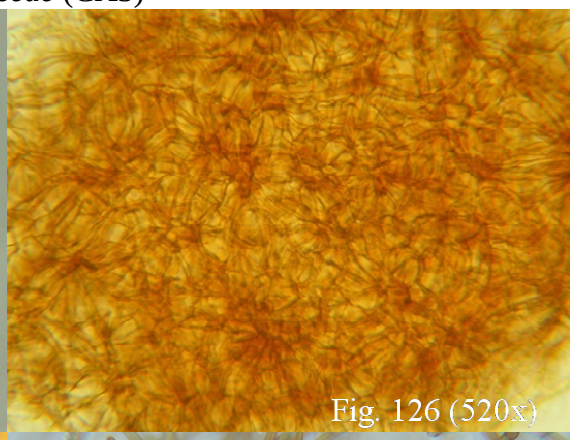

Fig. 126 (520x)

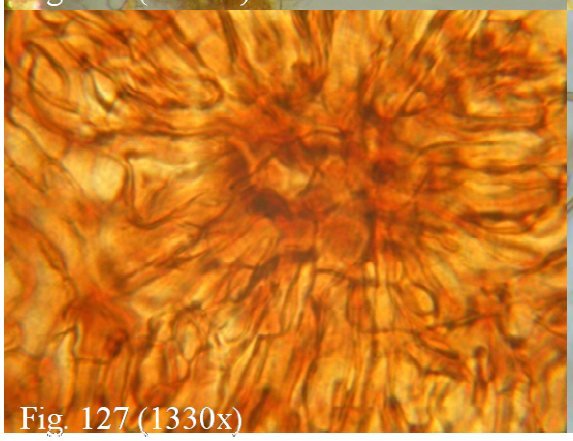

Fig. 127 (1330x)

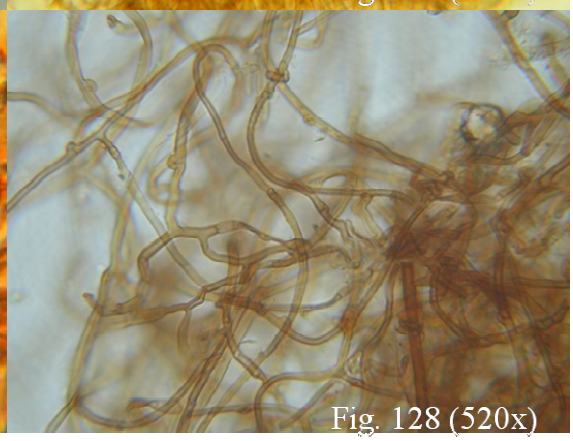

Fig. 128 (520x)

Telephoraceae (CA32)

Fig. 129 (31.25x)

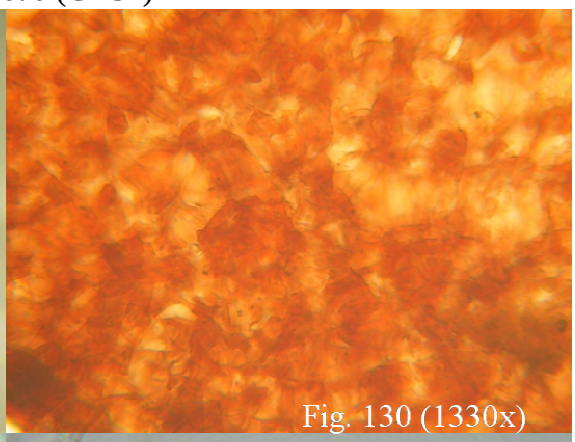

Fig. 130 (1330x)

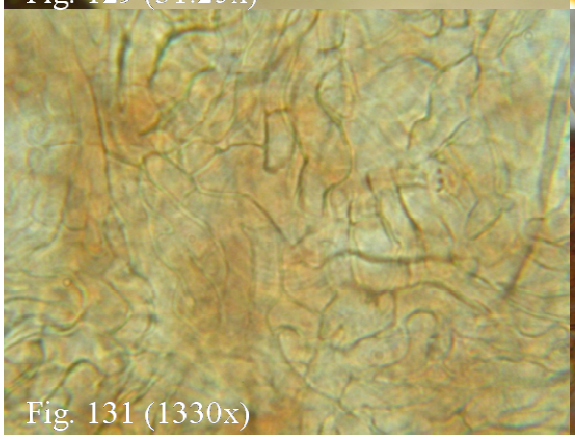

Fig. 131 (1330x)

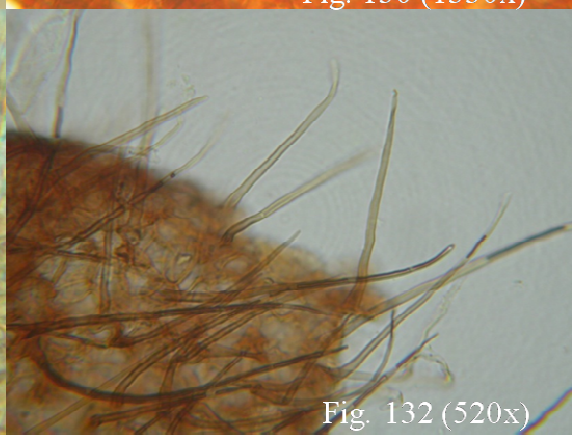

Fig. 132 (520x)

Telephoraceae (CA33)

Fig. 133 (31.25x)

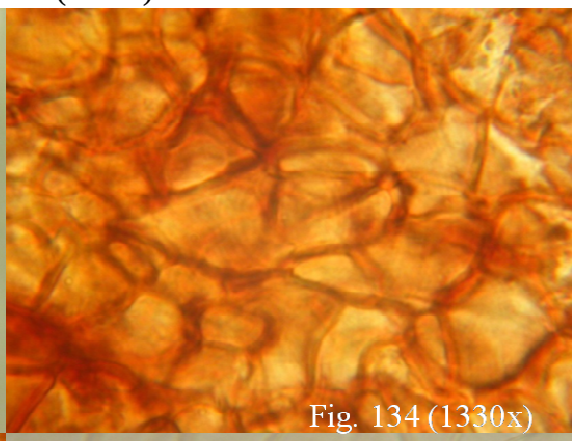

Fig. 134 (1330x)

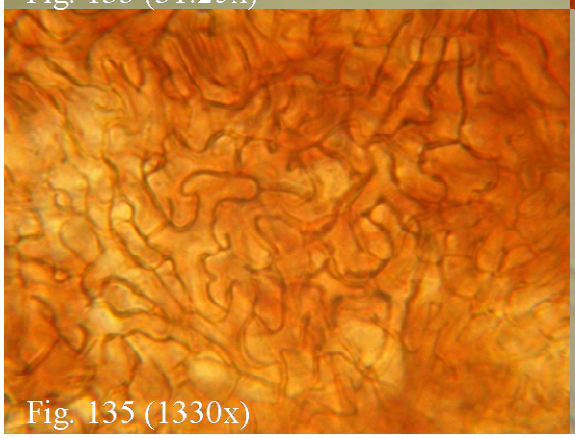

Fig. 135 (1330x)

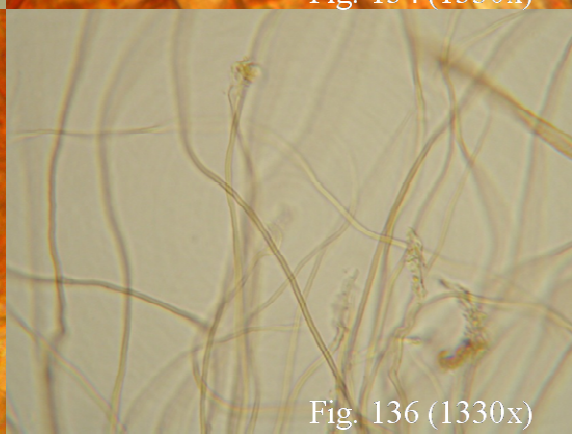

Fig. 136 (1330x)

Telephoraceae (CA95)

Fig. 137 (15.5x)

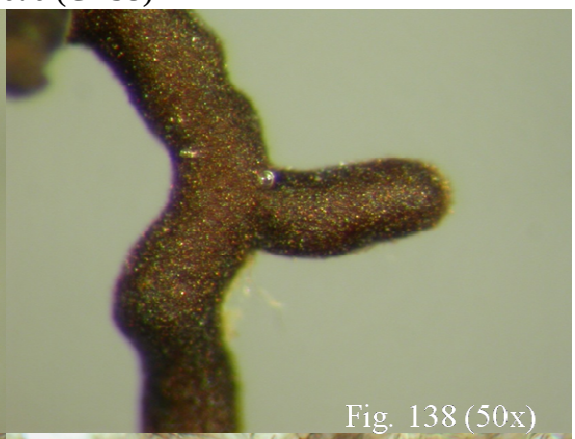

Fig. 138 (50x)

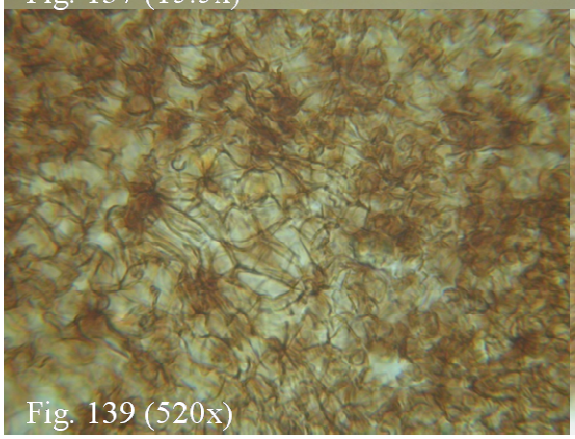

Fig. 139 (520x)

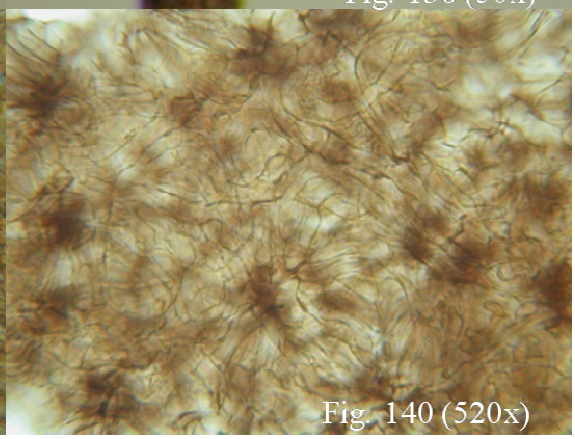

Fig. 140 (520x)

Tomentella sp.(CA1)

Fig. 141 (31.25x)

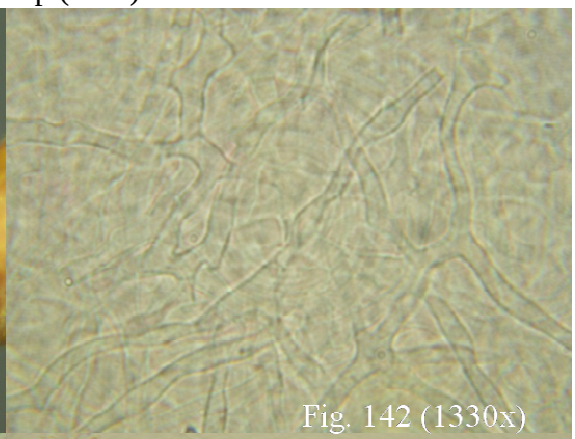

Fig. 142 (1330x)

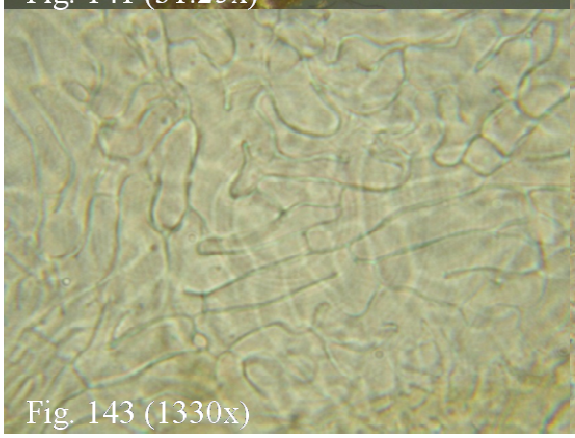

Fig. 143 (1330x)

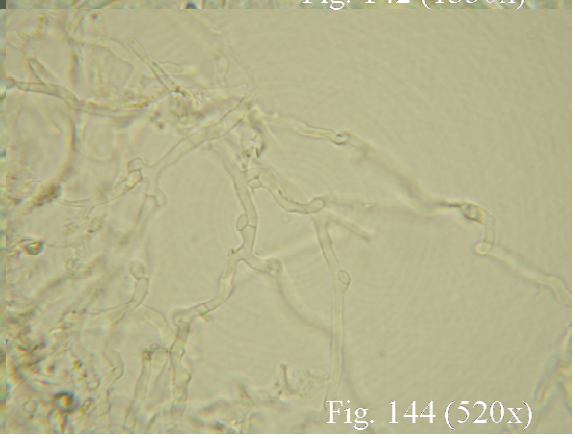

Fig. 144 (520x)

Tomentella sp.(CA21)

Fig. 145 (12.5x)

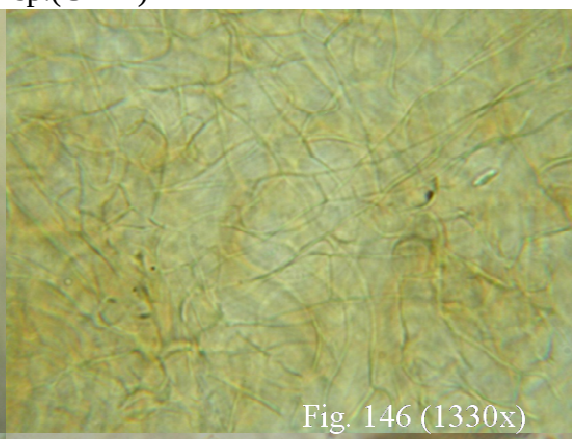

Fig. 146 (1330x)

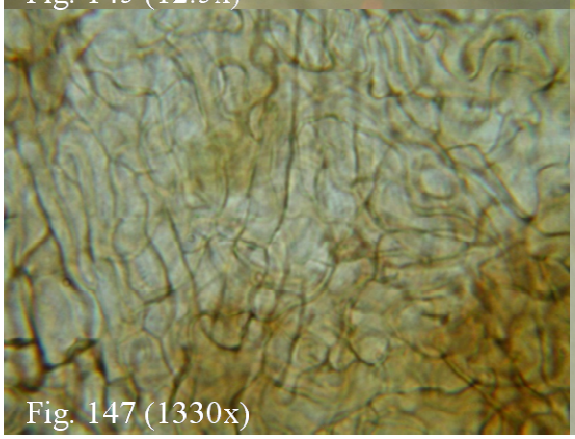

Fig. 147 (1330x)

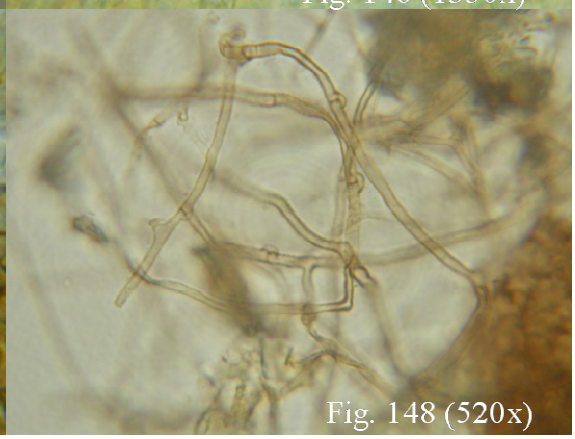

Fig. 148 (520x)

Tomentella sp.(CA42)

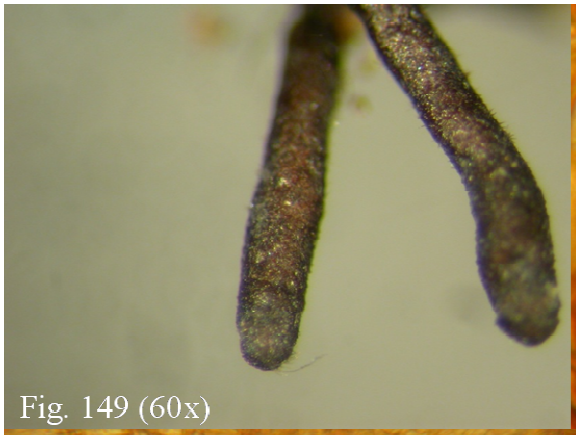

Fig. 149 (60x)

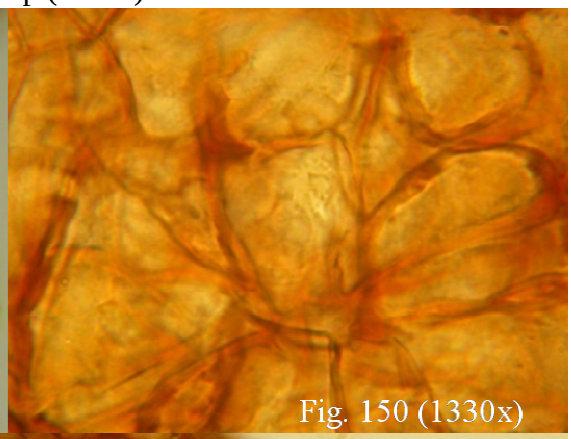

Fig. 150 (1330x)

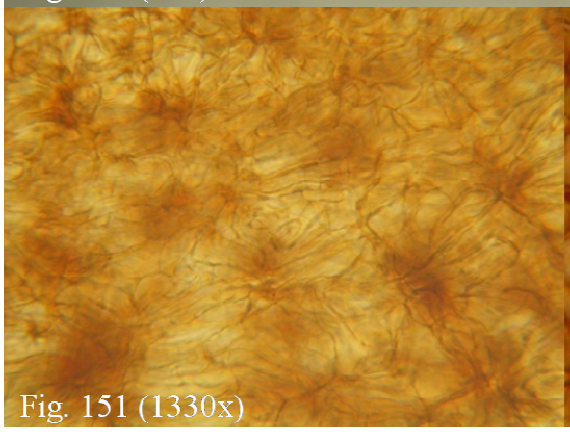

Fig. 151 (1330x)

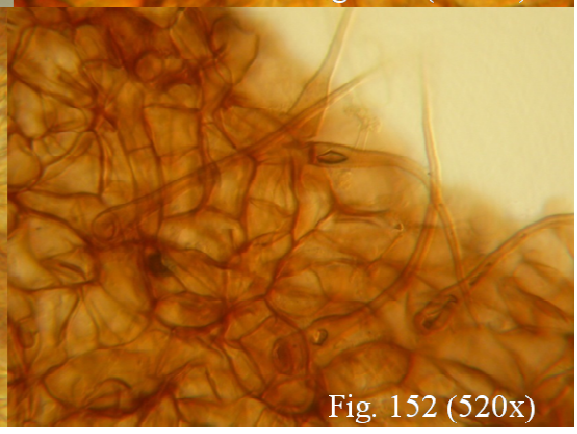

Fig. 152 (520x)

Tomentella sp.(CA54)

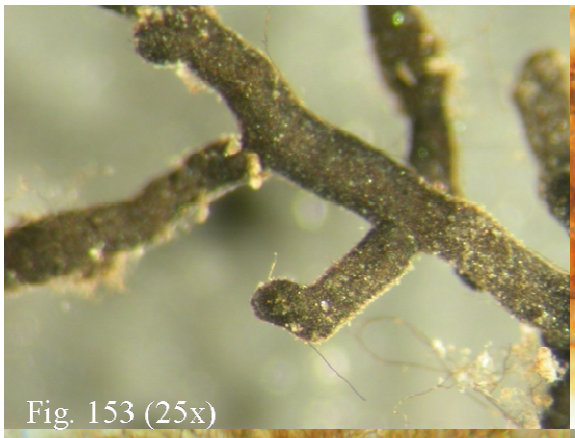

Fig. 153 (25x)

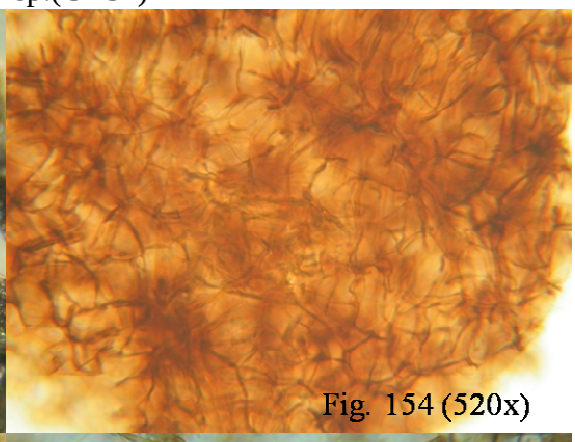

Fig. 154 (520x)

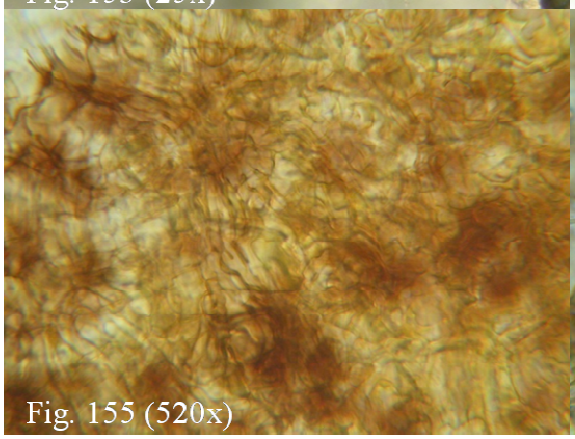

Fig. 155 (520x)

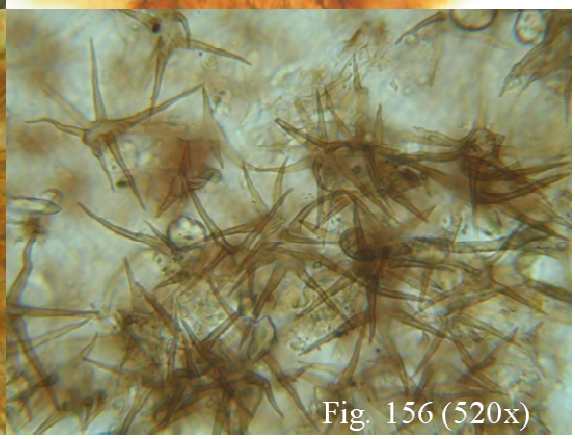

Fig. 156 (520x)

Tomentella sp.(CA55)

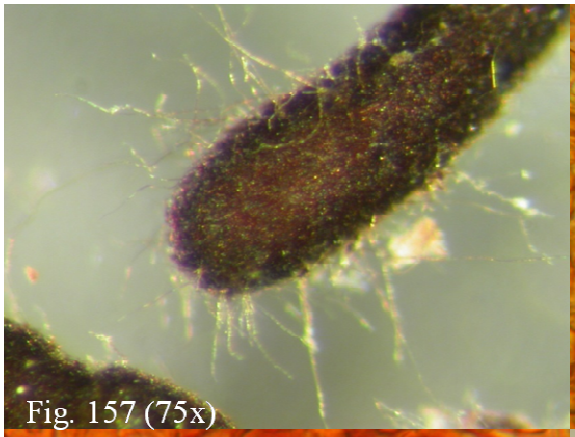

Fig. 157 (75x)

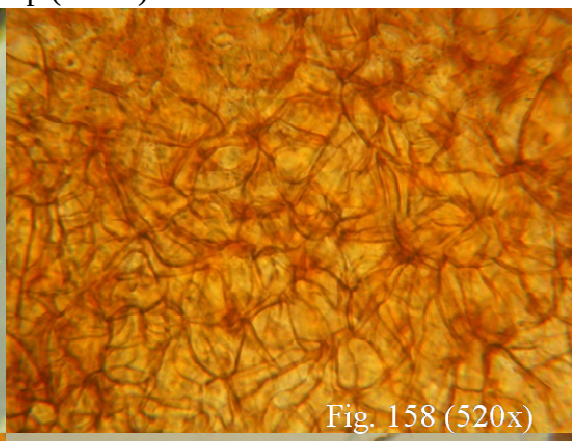

Fig. 158 (520x)

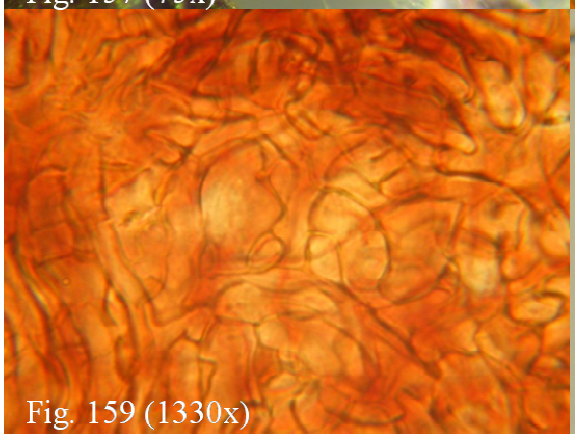

Fig. 159 (1330x)

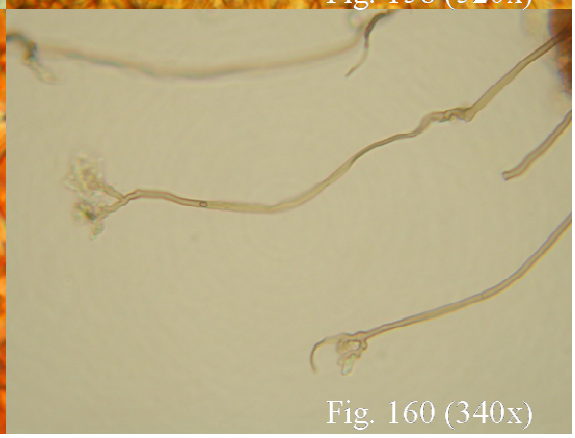

Fig. 160 (340x)

Tomentella sp.(CA69)

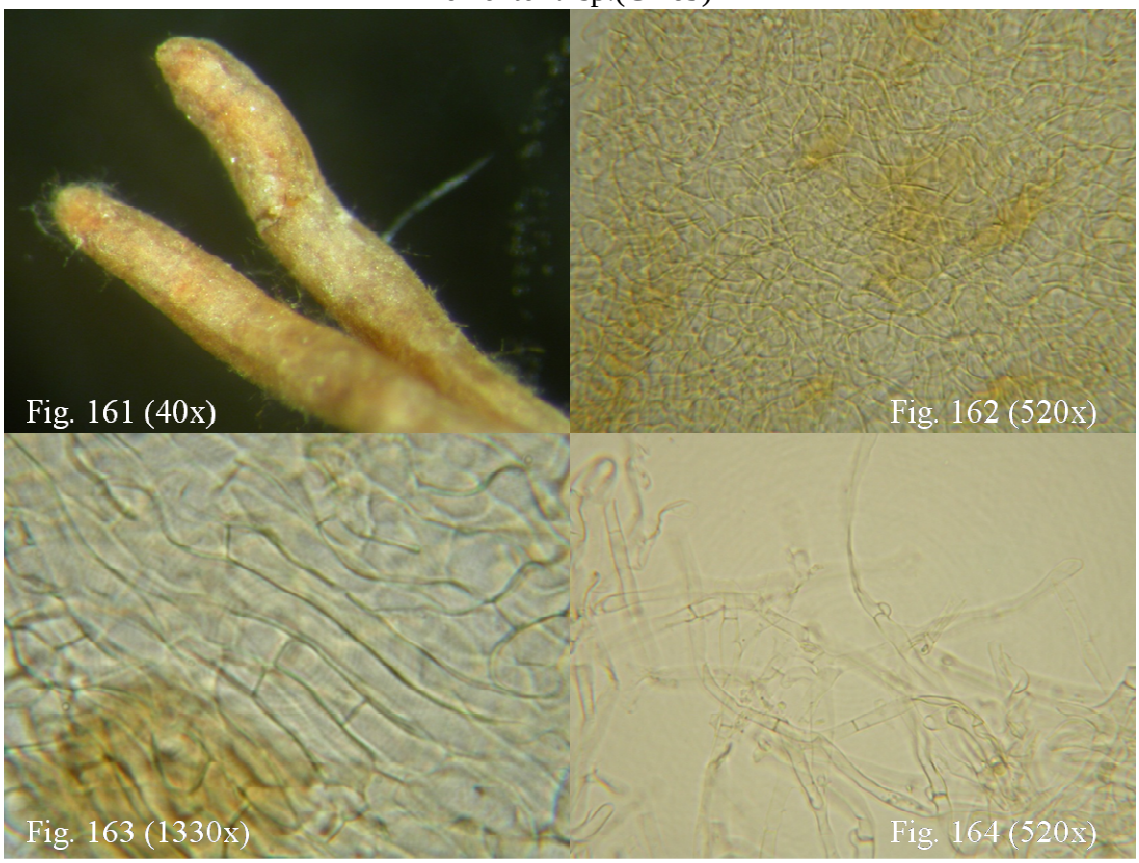

Fig. 161 (40x)

Fig. 162 (520x)

Fig. 163 (1330x)

Fig. 164 (520x)

Tomentella sp.(CA70)

Fig. 165 (31.25x)

Fig. 166 (520x)

Fig. 167 (520x)

Fig. 168 (1330x)

Tomentella sp.(CA75)

Fig. 169 (31.25x)

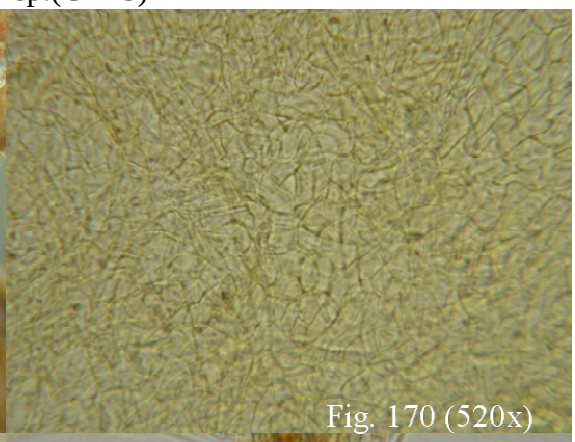

Fig. 170 (520x)

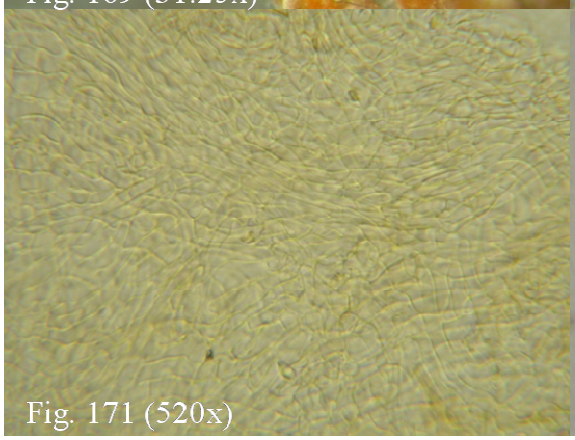

Fig. 171 (520x)

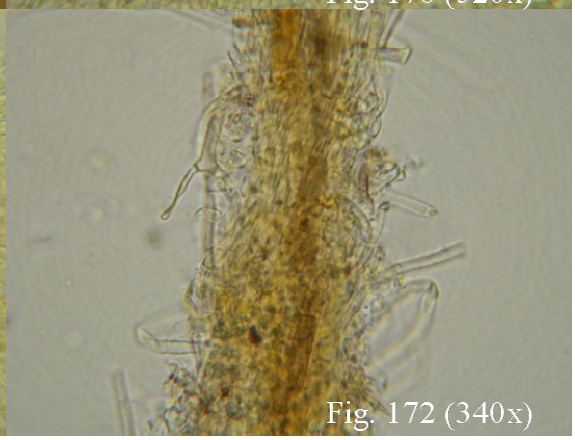

Fig. 172 (340x)

Tomentella sp.(CA79)

Fig. 173 (31.25x)

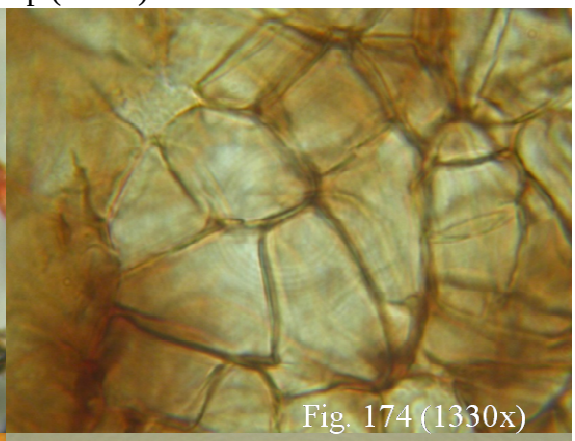

Fig. 174 (1330x)

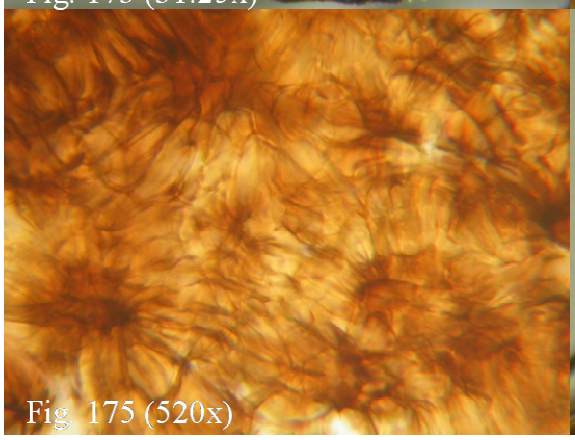

Fig. 175 (520x)

Fig. 176 (520x)

Tomentella sp.(CA84)

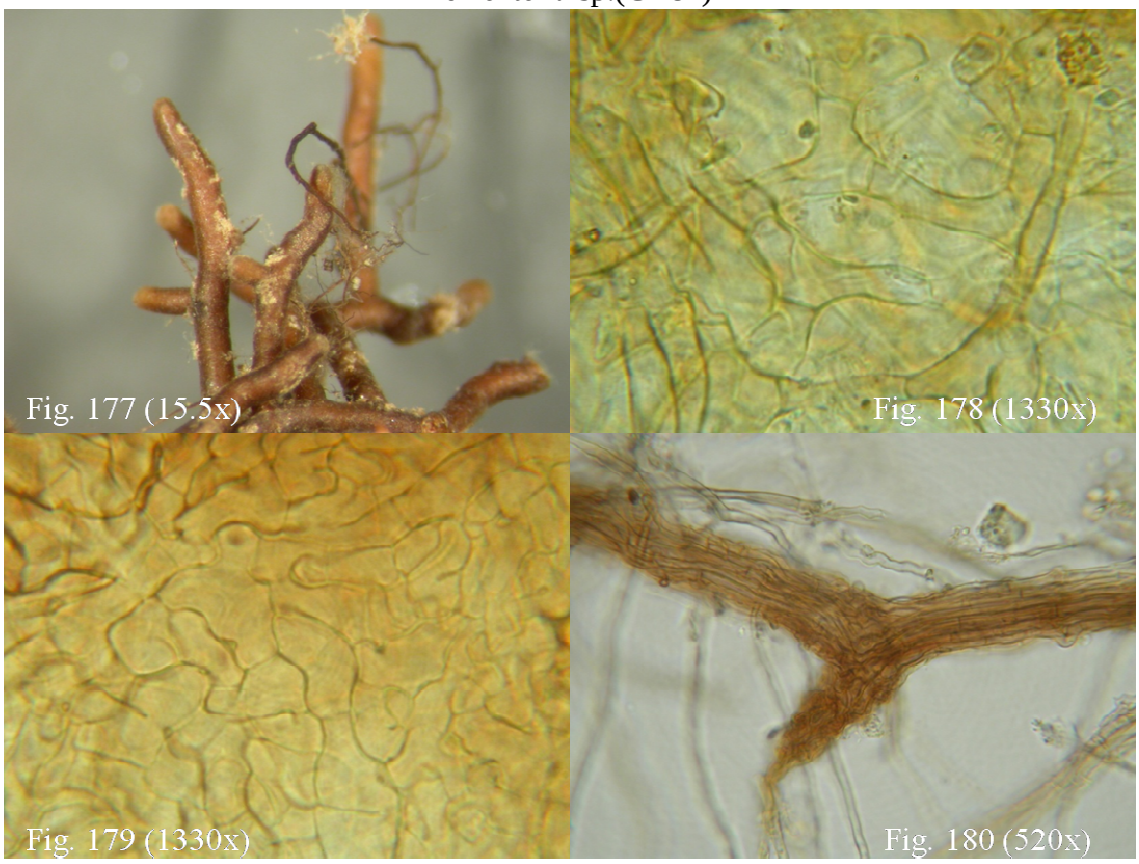

Fig. 177 (15.5x)

Fig. 178 (1330x)

Fig. 179 (1330x)

Fig. 180 (520x)

Trichophaea sp. (CA82)

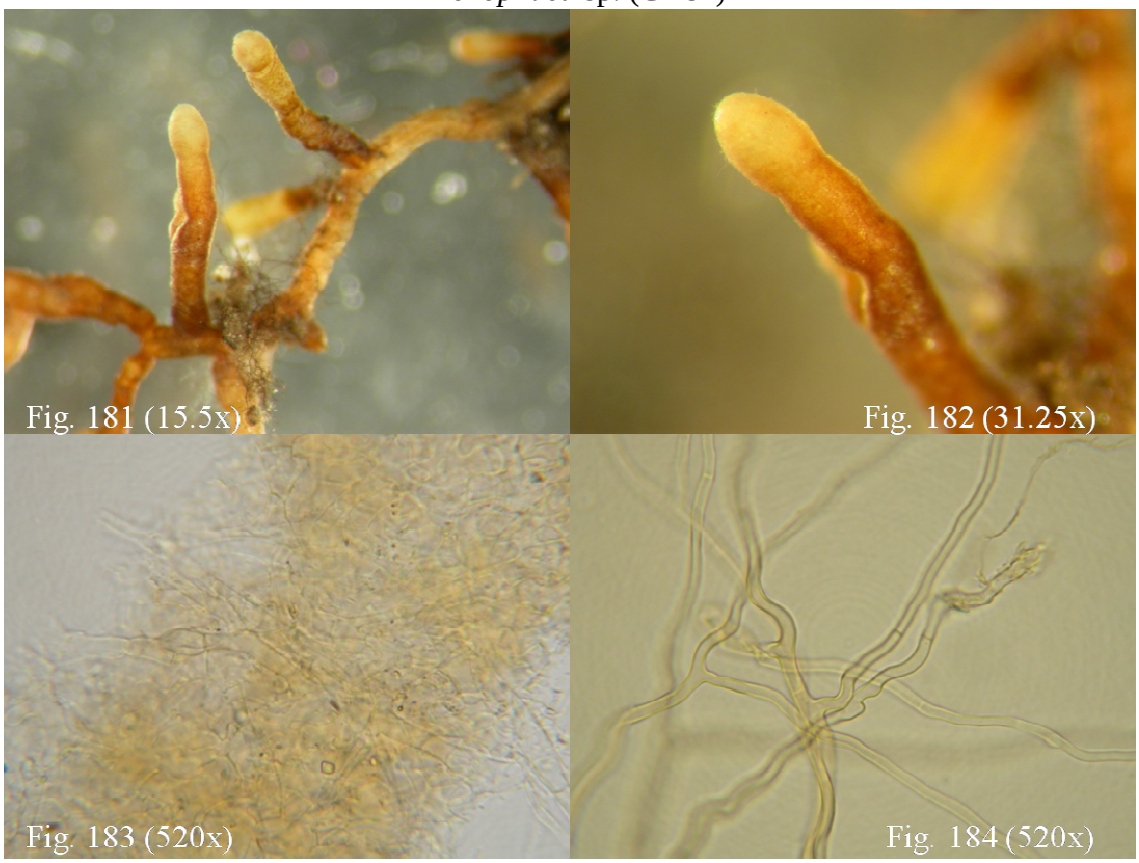

Fig. 181 (15.5x)

Fig. 182 (31.25x)

Fig. 183 (520x)

Fig. 184 (520x)