PDF

MONGEFITOFOR HANDBOOKS

# MONITORING AND MANAGEMENT OF ASH DIEBACK IN TRANSBOUNDARY ALPINE AREAS

GUGLIELMO LIONE, LUCA DOVIGO, CHIARA FERRACINI, ROBERTO MARTINIS, SILVIA ONGARO, MARTINA PELLICCIARO, SIMONA PRENCIPE, SIMONE PROSPERO, ANDREA RETTORI, IVAN ROLLET E PAOLO GONTHIER

**MONITORING AND MANAGEMENT OF ASH DIEBACK IN TRANSBOUNDARY ALPINE AREAS**
Series: “MONGEFITOFOR HANDBOOKS”


**Authors**
Guglielmo Lione [1], Luca Dovigo [2], Chiara Ferracini [1], Roberto Martinis [3], Silvia Ongaro [1], Martina Pellicciaro [1], Simona Prencipe [1], Simone Prospero [3], Andrea Rettori [3], Ivan Rollet [2], Paolo Gonthier [1]

**Scientific co-ordination**
Paolo Gonthier [1]
Chiara Ferracini [1]
Simone Prospero [4]
Guglielmo Lione [1]

[1] University of Torino - Department of Agricultural, Forest and Food Sciences (DISAFA), Largo P. Braccini 2, I-10095 Grugliasco (TO), Italy.
[2] Regione Autonoma Valle d’Aosta, Assessorato Agricoltura e Risorse Naturali, Corpo Forestale della Valle d’Aosta, Loc. La Grande Charrière 14, 11020 Saint-Christophe (AO), Italy.
[3] Studio Associato Planta, Via Chiesa 19, 10090 Rosta (TO), Italy.
[4] Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), Zürcherstrasse 111, 8903 Birmensdorf, Switzerland.



**Recommended form of citation**
Lione G., Dovigo L., Ferracini C., Martinis R., Ongaro S., Pellicciaro M., Prencipe S., Prospero S., Rettori A., Rollet I., Gonthier P. (2023). Monitoring and management of ash dieback in transboundary alpine areas. Series: “MONGEFITOFOR handbooks”. Regione Autonoma Valle d’Aosta. ISBN 9791280561237

# INDICE

# PREFACE

Forests are an important component of the landscape of the Autonomous Region of Valle d'Aosta and of the Italian-Swiss cross-border areas, performing many essential functions for the conservation, preservation and protection of the territory and of the communities that inhabit it. In order to guarantee in time and space the fulfilment of these functions, forest health must be constantly monitored and preserved. The need to pool experience, acquired knowledge and projects in relation to the monitoring and management of the main threats to forests has therefore led the Autonomous Region of Valle d'Aosta, the two Swiss cantons of Grisons and Ticino, the Department of Agricultural, Forest and Food Sciences (DISAFA) of the University of Torino and the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL) in Birmensdorf - Zurich to cooperate in an ambitious project named MONGEFITOFOR (Linee Guida per il MONitoraggio e la Gestione delle Emergenze FITOsanitarie nelle FOReste delle Alpi centro-occidentali - Emerging Threats to Tree Health in Forests of the Central and Western Alps: Guidelines for Monitoring and Management), implemented within the framework of the European Territorial Cooperation Programme INTERREG V-A Italy-Switzerland 2014/2020. Several threats to forest health were addressed within the project, including the decline of European ash. This handbook presents the results of monitoring activities aimed at investigating the causes of the decline of the European ash, quantifying its impact and proposing operational strategies for its management in forest ecosystems of cross-border alpine areas. In fact, this handbook was created thanks to the knowledge gained from the synergy among the project partners who shared experience, knowledge and skills during the various activities conducted in Italy and Switzerland.

This handbook is an operational tool for the forestry sector but, at the same time, contributes to disseminating to technicians, administrators, stakeholders and to the general public knowledge and good practices to protect the health of the European ash in forest ecosystems.

*The Councillor for Agriculture and Natural Resources*
*Marco Carrel*

# THE MONGEFITOFOR PROJECT

***MONGEFITOFOR*** (Linee Guida per il **MON**itoraggio e la **G**estione delle **E**mergenze **FITO**sanitarie nelle **FOR**este delle Alpi centro-occidentali - Emerging Threats to Tree Health in Forests of the Central and Western Alps: Guidelines for Monitoring and Management) is a project funded by the European Union through the Territorial Cooperation Programme ***INTERREG*** V-A Italy-Switzerland 2014/2020 in which Italian and Swiss local institutions and research bodies cooperate to monitor the ***forest health*** of ***cross-border areas*** and propose sustainable strategies for their management and protection, thus promoting the ***resilience of forest ecosystems***. The project is coordinated by the Forestry Corps of the Autonomous Region of Valle d'Aosta (Struttura Corpo Forestale della Valle d'Aosta) (IT) and has as partners the University of Torino - Department of Agricultural, Forest and Food Sciences (DISAFA) (IT), the Swiss Federal Institute for Forest, Snow and Landscape Research WSL (Birmensdorf) (CH), Canton Grisons - Office for Forests and Natural Hazards (CH) and Canton Ticino - Forestry Section (CH).

The project relies on the consideration that forests are an essential element of the ***landscape*** of the Alpine valleys and have a multifunctional value related not only to the ***production*** of timber, but also to the promotion of ***biodiversity***, to the ***hydrogeological protection*** of slopes and to the provision of ***recreation*** and leisure for tourists, visitors and citizens. However, to ensure these fundamental functions, forests must be adequately protected. The MONGEFITOFOR project aims to address, on a cross-border level, some of the most important threats to plant health that have affected the forests of the foothill and submontane zones of the central-western Alps in recent years (**Box 1**). These include the ***decline of European ash trees*** caused by the ***fungal pathogen Hymenoscyphus fraxineus*** to which this ***technical-scientific field handbook*** is dedicated. This handbook aims to support not only owners, managers and administrators of forest resources, but also technicians and operators who wish to deepen their knowledge and improve their skills.

# BOX 1

## Insights into the MONGEFITOFOR project

The MONGEFITOFOR project, which started in 2019 and will end in 2023, focuses on the monitoring of emerging threats to forest health affecting tree species that play a key role in forest ecosystems of the foothill and submontane zones of the cross-border areas between Italy and Switzerland: **chestnut** (*Castanea sativa*), **European ash** (*Fraxinus excelsior*) and **Scots pine** (*Pinus sylvestris*), to which specific technical-scientific handbooks of the series "**MONGEFITOFOR HANDBOOKS**" are dedicated. Moreover, the MONGEFITOFOR project also released multimedia content providing technical information and additional insights, accessible on the following platforms:

**WEB SITE**
https://fitosanitario.regione.vda.it/progetto-mongefitofor

**FACEBOOK**
https://www.facebook.com/Mongefitofor-103015101617192/

**INSTAGRAM**
https://instagram.com/mongefitofor?igshid=1f0k8nykdbkwl

**YOUTUBE**
https://www.youtube.com/channel/UCeafnk1hcccn8Vlm4wqFvSg

# EUROPEAN ASH: GENERAL OVERVIEW OF THE SPECIES

European or common ash (*Fraxinus excelsior* L.) (**Figure 1**), hereafter simply referred to as ash, is a forest tree species of particular interest for the cross-border areas between Italy and Switzerland, which is also highly appreciated in arboriculture for wood production, or as an ornamental species in urban parks or other anthropic landscapes.
Ash is a long-living tree that can reach 40 m in height, exceeds one meter in diameter and has a good ***suckering ca-***

***FIGURA 1***
*EUROPEAN OR COMMON ASH (FRAXINUS EXCELSIOR). AN ADULT AND VIGOROUS ASH TREE GROWN IN A GRASSLAND FORMATION IN THE ALPS NEAR THE MUNICIPALITY OF COURMAYEUR (AO). THE PRESENCE OF SEVERAL CO-DOMINANT STEMS INDICATES PREVIOUS COPPICING.*

1

***pacity at the stem and root levels***. These characteristics allow the management of ash in coppice or ***high forest stands***. In the foothill and submontane zones of the Alps, ash is generally a sporadic or ***companion species***, participating in the constitution of various tree forest communities with mixed composition. However, locally and under particularly favourable conditions, ash can attain a canopy cover of 50-75% in mixed forests, where it can also be present in monospecific groups. Ash seeds are characterised by a long ***dormancy*** period, in fact they can take up to two years after maturity to germinate under natural conditions.

At the ecological level, ash does not tolerate prolonged periods of ***drought***, to which it is more susceptible than other broadleaves growing in the same forest stands. The search for soils with a high water content makes ash a forest species suitable for colonising riparian or floodplain habitats. In terms of soil conditions, ash adapts to slope and plain areas, as the species is widespread in widely different geomorphological districts (e.g. Alps, Po Valley). However, ash prefers ***fertile soils*** and tends to shy away from soils with an acid reaction, to which it shows reduced adaptability. The species adapts well not only to soils typical of the forest environments, but also to those more common in agricultural areas. Because of this adaptability, ash is frequently reported as a sort of pioneer species, namely a tree that can most easily colonise former cultivated or marginal areas, forming so-called "***invasion ash forests***". The requirement of ash towards light input is mesophilic, although some distinctions are reported depending on the age of the tree. Indeed, ***shade tolerance*** is markedly more pronounced for ash seedlings and for young trees, whereas adult ashes tend to be characterised by a more pronounced heliophilicity and a lower ability to tolerate competition from other dominant or co-dominant trees. In relation to climate, ash can withstand low temperatures, especially during the vegetative rest period, but it does not react well to ***late frosts***, which can cause the death of young sprouts or ***seedlings***, especially if temperatures below -3°C continue for more than 18 consecutive hours. The ***ecological*** needs of ash trees can be summarised by means of a series of quantitative indices (**Table 1**).

## SYNECOLOGY AND CHARACTERISATION OF FOREST STANDS WITH ASH

Given the sporadic nature of this companion species and its ecological needs, ash can participate to different plant communities and shows a rather articulated synecology, characterised by a ***high*** geographical

### TABLE 1

Summary description of European ash ecology. For each environmental factor, the corresponding Landolt index is reported (1 to 5, values extrapolated from Lauber and Wagner, 2001) with its description. The asterisk next to the numerical score indicates the tolerance of the species to changes in the factor considered.

| Environmental factor | Index | Description |
|---|---|---|
| **Water needs** | **3*** | Needs medium-humid soils but tolerates large variations of even more than 2 points on the Landolt scale |
| **Soil reaction (pH)** | **4** | Prefers soils with neutral to sub-alkaline or basic pH (5.5-8.5). |
| **Nutrient requirements** | **3** | It grows on soils with an intermediate nutrient supply between the extremes represented by pioneer and nitrophilic species. |
| **Light** | **3** | It is neither a shade-tolerant nor a distinctly heliophilous species and is therefore adapted to intermediate light conditions . |
| **Temperature** | **3*** | It preferably grows in stands with temperatures typical of the upper-foothill and submontane zones, but it also adapts to some temperature variations. |
| **Continentality** | **3** | Adapted to moderately subcontinental to sub-Atlantic climates. |

***variability***. In terms of forest type, ash mainly grows in ***multi-specific forests*** classifiable as ***maple-linden-ash or maple-ash forests***, more rarely in monospecific ***ash forests***. However, ash is also present in many other forest types where it colonises different habitats associating mainly with deciduous trees (e.g. maples, linden, alder, hornbeam, chestnut, elm, cherry and hazelnut), but also with conifers (e.g. larch and pine). In the cross-border areas between Italy and Switzerland, some of the forest types that most frequently host ash trees are:

- "Invasion" maple-linden forests;
- Black alder or white alder forests;
- Linden forests;
- "Invasion" hazelnuts stands;
- Chestnut stands;
- Scots pine forests;
- Larch forests.

This high synecological variability also has repercussions on the dynamics of the forests, which may differ depending on the environmental and silvicultural conditions. In some cases, for the above-mentioned forest types, variants "with" or "at" ash, have been identified: the canopy cover of ash varies between 25 and 50% in the first case, and exceeds 75% in the second.

## FUNCTIONS AND ECOSYSTEM SERVICES OF FORESTS HOSTING ASH

The large variability of the forests in which ash occurs implies that, virtually, the species is able to exert a wide range of ***functions*** and provide various ***ecosystem services***, including:

- Production of ***timber and firewood***;
- Production of secondary products for niche markets;
- ***Hydrogeological protection*** and slope consolidation;
- Provision of services related to ecological-environmental functions (e.g. support for ***biodiversity***, sequestration of atmospheric carbon dioxide and generation of ***carbon stocks***, contribution to biogeochemical cycles);
- Participation in forests that play an important role as ***landscape*** elements, providing suitable spaces for ***tourism*** and recreation.

The extent to which ash fulfils the various functions and provides the related ecosystem services depends on the forest type where it grows, its management (***coppice*** or ***high forest***) and the environment. Ash is included among the so-called ***noble broadleaves*** in view of the valuable aesthetic and physical-mechanical characteristics of its wood. Ash provides woods for ***furniture*** production and ***veneer logs***, or also for special assortments for niche markets. For example, ash wood is used in the production of tool handles, oars, boat masts, hockey sticks and is also valued in ***cabinetmaking***. ***Ash*** firewood obtained from coppices is also of good quality. Non-wood production includes the harvesting of leaves, bark and fruits for the preparation of ***phytotherapeutic products*** or other herbal preparations, while the traditional use of ash for livestock feed is now minor. The morphology of the root system makes the ash tree a valuable species for ***slope consolidation***, due to the presence of a taproot and abundant vertical lateral roots that explore the soil in depth (**Figure 2**). Furthermore, the crown and stem, by intercepting rainfall, contribute to the regular flow of water and help prevent erosive phenomena.

Ash is also a key species of ***newly-formed forests*** (**Figure 3**) which settle and colonise former crops, terraces and meadow or pasture areas in which active management is no longer practised, mainly as a result of land abandonment, depopulation of the foothills and mountains and the contraction of traditional agricultural and zootechnical activities. Newly-formed forests contribute to the spontaneous reforestation of the territory, amplifying the capacity of forest systems to provide ecosystem services. Ash not only contributes to structure ***ecological networks*** and ***habitats*** that promote animal, plant and microbial biodiversity, but also creates a favourable substrate for colonisation by various organisms and microorganisms, including ***fungi*** and ***lichens*** (**Box 2**).

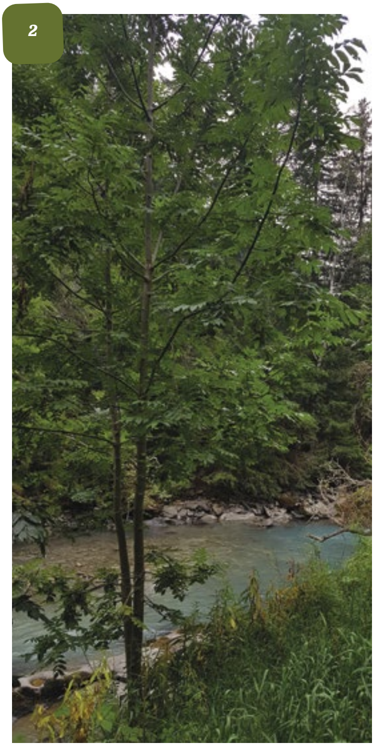


***FIGURE 2***

*ASH TREES ON A SLOPE NEAR A STREAM. IN THIS ECOSYSTEM, ASH PLAYS AN IMPORTANT ROLE IN CONSOLIDATING THE SLOPE AND PROTECTING THE BANK.*

***FIGURE 3***

*ASH IS ALSO A KEY SPECIES OF NEWLY-FORMED FORESTS (FIGURE 3) WHICH SETTLE AND COLONISE FORMER CROPS, TERRACES AND MEADOW OR PASTURE AREAS IN WHICH ACTIVE MANAGEMENT IS NO LONGER PRACTISED, MAINLY AS A RESULT OF LAND ABANDONMENT, DEPOPULATION OF THE FOOTHILLS AND MOUNTAINS AND THE CONTRACTION OF TRADITIONAL AGRICULTURAL AND ZOOTECHNICAL ACTIVITIES. NEWLY-FORMED FORESTS CONTRIBUTE TO THE SPONTANEOUS REFORESTATION OF THE TERRITORY, AMPLIFYING THE CAPACITY OF FOREST SYSTEMS TO PROVIDE ECOSYSTEM SERVICES.*

With the exception of specialised wood plantations which, in the cross-border areas between Italy and Switzerland

# BOX 2

## Ash trees as reservoirs of biodiversity

Ash trees in the cross-border areas between Italy and Switzerland are a resource whose preservation is crucial to maintain and improve the biodiversity levels of forest ecosystems. Promoting biodiversity is a priority in forest management and its importance is recognised at the international level. The resilience of ecosystems against threats, whether natural or anthropogenic, can be increased by preserving their biodiversity. Forest stands in which ash is present are habitats that host different organisms and microorganisms, which establish a complex network of ecological interactions. Recent studies have shown that, precisely in the areas covered by the MONGEFITOFOR project, ash trees represent an important reservoir of biodiversity. For example, the bark of the ash tree is able to host a wide range of lichen species, organisms in which a mutualistic symbiosis involving fungi and algae is established (**Figure 4**). Some of the lichen species found on ash in the north-western Alps are rare and included in special protection lists. Safeguarding ash is therefore an imperative in order to protect other fairly rare species and to maintain the structural and functional efficiency of forest ecosystems

4

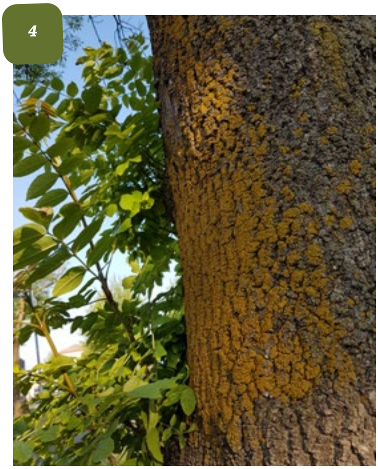


***FIGURE 4***
*ASH TREES SUPPORT THE BIODIVERSITY OF FOREST ECOSYSTEMS. THE BARK OF THE ASH TREE SHOWN IN THE PICTURE IS COLONISED BY LICHENS, PARTICULAR ORGANISMS IN WHICH ALGAE AND FUNGI COEXIST, ESTABLISHING A MUTUALISTIC SYMBIOSIS.*

are either absent, or not significant, ash silviculture is highly influenced by the synecological and structural ***variability*** of the forests in which the species is present. Given its ecological and environmental values, there is a general interest in the ***preservation of ash*** as a noble broadleaf tree. In particular, when possible, ash is favoured by guiding the dynamic processes of succession towards a ***multi-specific composition*** in balance with the environmental characteristics of the stand, mainly following the principles of ***naturalistic*** or ***systemic silviculture***. For ash trees, ***high forest*** management is preferred over coppice, although the use of compound coppice with cuttings foreseen every 80- or 100-year and the maintaining of standard trees for one, two or even three rotations cannot be ruled out. Under conditions of adequate fertility, ash is a suitable species to enrich coppice forests dominated by other broadleaves. In forests where ash is already present, ***thinnings*** are recommended, at intervals of 5-10 years, in order to favour those trees that seem most promising in terms of bearing, growth capacity and vegetative vigor, adapting the number, intensity and frequency of thinnings to the situation in situ. When there is a productive interest, woody assortments should be obtained from ash trees of approximately 70 years, as senescent trees may display sub-optimal wood technological characteristics (e.g. appearance of defects such as ”black heart”). ***Selective thinnings*** and ***regeneration cuts***, applied to mixed forests (e.g. maple-ash forest type) have provided promising results in contexts not too dissimilar to those of the cross-border areas between Italy and Switzerland, promoting the natural regeneration of ash. In practice, however, coppicing and free evolution of ash in the absence of silvicultural planning represent established realities in marginal contexts, where traditional management prevails, consolidated by local uses, also as a consequence of high land property fragmentation.

The silvicultural indications provided in this chapter are valid for forests in which ash trees do not show evident symptoms of decline. Otherwise, it is indeed advisable to supplement good silvicultural practices with appropriate operations that take into account the presence and impact of diseases caused by fungi or other biotic or abiotic agents, as well as damages caused by phytophagous insects. The MONGEFITOFOR project, by monitoring and investigating the emerging threats to the health of ash trees in forest stands through a solid scientific basis, therefore aims to outline possible ***forest management guidelines***. ***Such guidelines*** should be applied to situations in which diseases or insect infestations may require a silvicultural approach driven by the principles of ***plant pathology***.

## ASH DIEBACK CAUSED BY *HYMENOSCYPHUS FRAXINEUS*

The ***decline*** of a forest tree species is a complex phenomenon, which determines an overall loss of vitality, often accompanied by a variety of ***symptoms***, which may result in high ***mortality*** rates. Forest decline may depend on different factors, abiotic and biotic, which mainly include pathogens, but also natural disturbances (e.g. fire, hail, windstorms, climate change, infestations of phytophagous insects). The role of ***infectious diseases*** in forest decline can be of the outmost importance. In the case of forest tree species, the main causal agents of diseases (***pathogens***) are ***fungi***, chromistans, bacteria, phytoplasmas, viruses and parasitic plants. Pathogens can infect healthy trees causing epidemics in a variety of ways. Infections may occur locally, affecting individuals of susceptible tree species present in a given forest stand, or they may affect larger areas, even at the regional, national, continental or intercontinental scale.

Among the infectious diseases that affect ash, the most prominent one is the ***ash dieback*** caused by the ascomycete ***fungus Hymenoscyphus fraxineus*** (T. Kowalski) Baral, Queloz, Hosoya, comb. nov. (formerly known as *Chalara fraxinea* or *H. pseudoalbidus*). In Europe, the first reports of this disease date back to the early 1990s, when extensive mortality of both mature and young ash trees occurred in several forests in Poland. The pathogen had been likely introduced to Europe from East Asia, where it does not, however, cause any particular disease symptom to the local ash species (*Fraxinus mandshurica* and *F. chinensis*). Since the early 2000s, the pathogen has spread widely and rapidly, colonizing a large part of the ash tree's distribution area across the European continent. For example, between 1992 and 2012, *H. fraxineus* was reported in at least 26 different countries. The introduction into new areas and the rapid spread of the pathogen may have been due to different factors, such as the movement of ***infected*** seedlings or other ***propagation material*** and wood, as well as to the pathogen's natural diffusion via infectious spores. At the European level, the ***ecological impact*** and ***economic losses*** caused by *H. fraxineus* have been extremely significant. For instance, in certain areas, due to the high impact of the ash dieback, a local extinction of the species was foreseen. It is estimated that in Lithuania, the forest area occupied by ash trees dropped from 53,000 to 38,000 ha between 2001 and 2009, while in Denmark, as a result of the effects of the disease, the establishment of new ash plantations has been strongly discouraged. Some studies have reported mortality peaks of up to 70% in forest stands and up to 85% in plantations, which is why the ***conservation of the species*** has become a priority in ***forest planning and management policies*** at an international level.

In Italy, the dieback of ash trees associated with *H. fraxineus* was observed for the first time in 2009, in Friuli-Venezia-Giulia. Subsequently, the disease rapidly spread to most regions in northern and central Italy. In particular, *H. fraxineus* was reported in 2016 in Piedmont, at La Mandria park (Turin), and it was therefore possible to estimate that the pathogen's spread rate in Italy could be around 70 km/year. A similar situation was observed in Switzerland. In fact, since the first report in 2008 in the Basel region, by 2015 the pathogen spread and virtually colonised the entire distribution area of ash trees in Switzerland. Before the start of the MONGEFITOFOR project, however, it was not known whether H. fraxineus was present in the cross-border areas of the Western and Central Alps, especially on the Italian side. In fact, in the Aosta Valley, a systematic phytosanitary monitoring of ash trees had not been undertaken before the beginning of the project MONGEFITOFOR.

The life cycle of *H. fraxineus* mainly takes place on the leaves and leaf rachises of the host. Depending on the climatic conditions, the pathogen differentiates reproductive structures called ***apothecia***, which are typical of certain ascomycetes (**Box 3**).

# BOX 03

## The apothecia of *Hymenoscyphus fraxineus*: so small, so important!

Apothecia are some of the structures produced by ascomycete fungi in which the spores are differentiated and mature, and from which they are subsequently released. In the case of Hymenoscyphus fraxineus, the apothecia appear as tiny whitish cups with a roughly circular receptacle supported by a peduncle that stands outside the growth substrate. The size of the apothecia is very small and, although visible to the naked eye, a magnifying glass is often required to observe their morphological characteristics. In fact, the diameter of the receptacle ranges from 1 to 5 mm, but can exceed 8 mm under certain conditions. However, especially if the apothecium is not newly formed or is dehydrated, its size may shrink well below one millimeter in diameter and the colouration may also appear darker. The thickness of the receptacle ranges from about 0.3 to 0.7 mm and is thinner near the margins. The peduncle is up to 2 mm long and submillimetric in thickness, increasing near the receptacle. The apothecia of H. fraxineus are produced at the level of the litter, on the rachis and leaf petioles that have fallen to the ground at the end of the previous year's growing season (**Figure 6**). Apothecia are mainly differentiated during the summer months, more rarely as early as late spring, and generally persist until the end of September. Several dozen or hundreds of apothecia may form on a single rachis, depending on the case. The apothecia release the mature spores of the pathogen, which are essential for the infection processes and for the onset of the disease

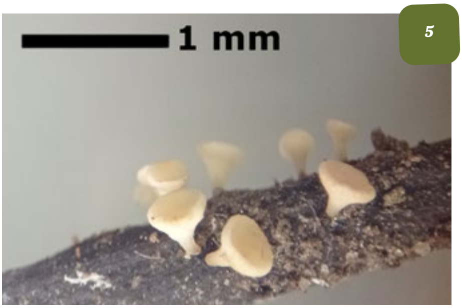


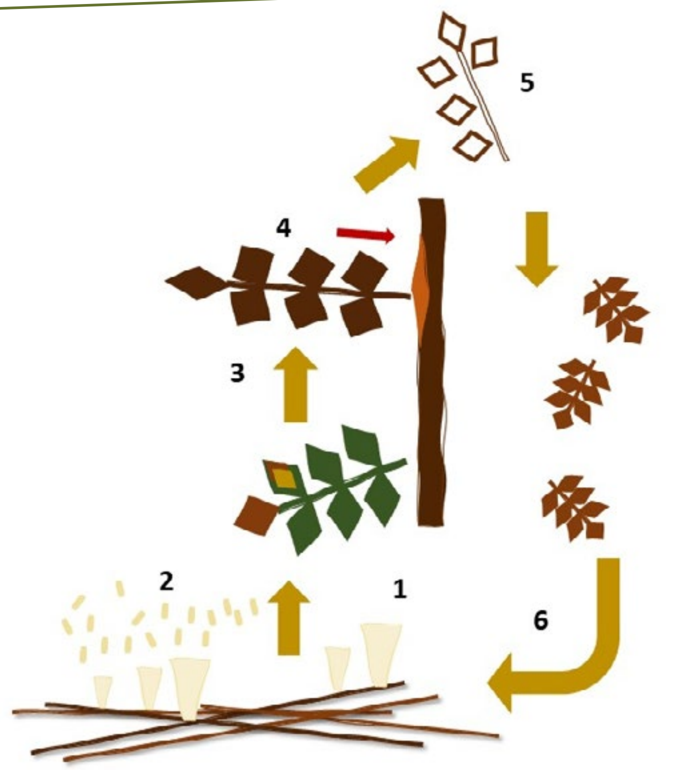

***FIGURE 5***
*APOTHECIA DIFFERENTIATED BY HYMENOSCYPHUS FRAXINEUS ON LEAF RACHIS PRESENT AT LITTER LEVEL. THE RACHIS DATES BACK TO THE PREVIOUS GROWING SEASON, AS CAN BE SEEN FROM THE ALTERED STATE OF THE TISSUE. THE SCALE OF THE IMAGE HIGHLIGHTS THE SMALL SIZE OF THE APOTHECIA*

The ***life cycle*** of the pathogen can be exemplified from the differentiation of apothecia. The apothecia allow the formation, maturation and release of the ***spores*** of the pathogen, which are airborne and play an extremely important ***infectious*** and ***epidemiological role***. The spores are the main means by which healthy trees are infected and through which the pathogen can spread. Spore production and release are generally concentrated during the summer/autumn period, with peaks of sporulation occurring between mid-July and the first half of August. The ***spores*** of *H. fraxineus* ***are not visible*** to the naked eye, as they are unicellular and hyaline, with an average size of a few thousandths of a millimeter (16-21 x 4-4.5 μm). The spores are ***airborne*** and are therefore carried by the ***wind***, which allows them to land on the host tissues. Once the pathogen reaches the leaf surface, it penetrates the tissues through the stomata.

The development of the hyphae in the leaf tissues allows the fungus to ***absorb the nutrients*** it needs and this leads to a progressive ***death*** of the leaf ***cells and tissues***, technically defined as ***necrosis***. The infection can also affect the petioles and leaf rachises, again leading to the development of necrotic areas. As the colonisation of the host tissues by the fungus progresses, the leaves wilt and drop prematurely. The mycelium of the fungus can also colonise living tissues present subcortically in stems and branches, particularly the cambium. When this occurs, the colonised tissues also tend to display necrosis followed by the onset of longitudinal lesions of the bark (***cankers***), associated with branch desiccation. Colour alterations in the stems may subsequently be observed too. Necrotic lesions that may occur at the base of the stem can be subsequently colonised by fungal pathogens causing wood decay or root rot (e.g. *Armillaria* spp.), foreshadowing possible future risks to the mechanical stability of the tree. Progressive crown wilting proceeds from the distal portions inwards, a phenomenon known as ***dieback***. After the leaves have fallen, on these, *H. fraxineus* produces resistance structures (pseudosclerotial plates) that allow the fungus to remain viable, protecting it from dehydration. On top of these structures, the following year, the biological cycle of the pathogen is completed with the differentiation of new apothecia and the production of spores that will allow new infections to occur. The simplified life cycle of *H. fraxineus* is shown in (**Figure 5**).

***FIGURE 6***
*LIFE CYCLE OF HYMENOSCYPHUS FRAXINEUS: DIFFERENTIATION OF THE APOTHECIA ON THE LEAF RACHIS OF THE PREVIOUS YEAR (1) AND SPORE RELEASE (2). THE SPORES SPREAD BY THE WIND, ONCE LANDED ON THE LEAF SURFACE, GERMINATE AND START THE INFECTION PROCESS IN THE LEAF TISSUE (3). GRADUALLY THE CROWN WILTS (DIEBACK) (4) AND THE PATHOGEN CAN ALSO COLONISE THE BRANCHES, ON WHICH IT CAUSES CAMBIAL NECROSIS AND CANKERS (4). FOLLOWING THE LEAF FALL (5), ON THE LEAF RACHIS ACCUMULATED IN THE LITTER (6), H. FRAXINEUS PRODUCES PSEUDOSCLEROTIAL PLATES THAT ALLOW THE FUNGUS TO REMAIN VIABLE AND, IN THE FOLLOWING YEAR, TO COMPLETE THE BIOLOGICAL CYCLE WITH THE DIFFERENTIATION OF NEW APOTHECIA.*

6



## MONITORING ASH DIEBACK

During the MONGEFITOFOR project, a systematic ***phytosanitary*** monitoring of ash was conducted through a series of ***forest surveys***, accompanied by the collection of ***biological samples*** and their subsequent laboratory analysis. With reference to ash decline, the following operations were carried out:

• Qualitative and quantitative assessment of the ***dieback symptoms*** revealed by ash trees in stands representative of the forests present in the cross-border areas between Italy and Switzerland;

• ***Diagnosis*** of the presence and assessment of the incidence, spatial distribution and sporulation capacity of H. fraxineus;

• Monitoring of other phytosanitary problems.

In order to select the sites for the survey, a preliminary characterisation of forest stands with ash was performed based on forest type data held by the Forestry Corps of Valle d'Aosta. Monitoring was conducted in Valle d'Aosta because in Switzerland the presence and distribution of H. fraxineus were already known. Further selection criteria to identify suitable forest stands relied on technical and scientific requirements, yet also logistical and administrative aspects were accounted for in order to select sites:

• spread throughout the natural distribution area of ash in Aosta Valley;

• located at elevations representative of the altitudinal zones in which ash trees grow in the region;

• balanced in relation to aspect;

• located at different slopes;

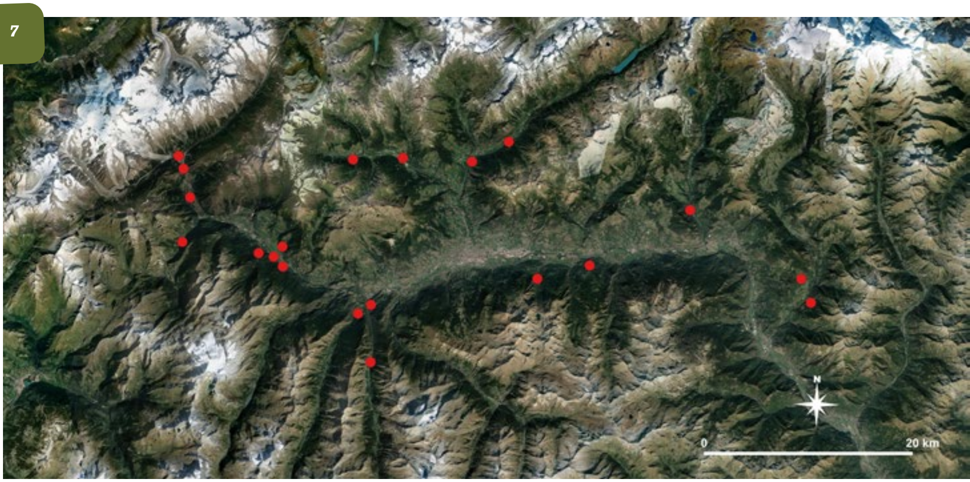
• accessible as they were served by roads;

• preferably of public or consortium property.

The phytosanitary monitoring of ash dieback and decline in Valle d'Aosta was conducted over two years (2020-2021), performing field surveys during the summer months, from the end of July to the beginning of September. In the 20 sites selected (**Figure 7**), relevant dendrometric and pathological variables were acquired (**Figure 8**). In particular, a visual analysis of the symptoms potentially associated with the dieback caused by H. fraxineus was performed.

During the surveys, biological samples were taken from leaf and/or wood tissues of ash trees in vegetation, or from the litter, in the latter case verifying the presence of apothecia of H. fraxineus on the leaf rachises of the previous year (**Figure 9**). An assessment of the presence and abundance of airborne spores of the pathogen was also performed by using special ***volumetric samplers*** located in some specif-

7



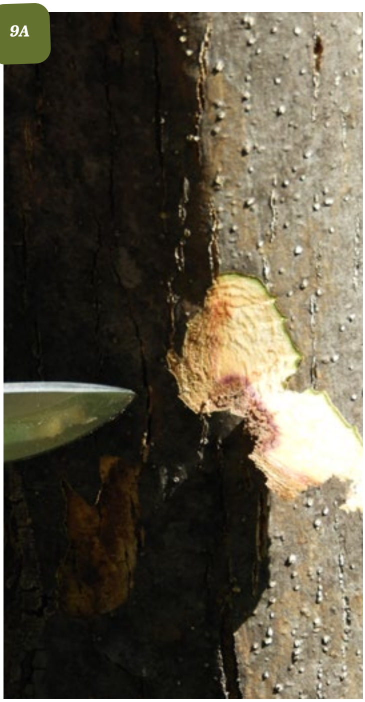

8

***FIGURE 7***
*XXXXX.*

***FIGURE 8***
*ACQUISITION OF VARIABLES USEFUL FOR THE DENDROMETRIC CHARACTERISATION OF ASH AND FOR THE ASSESSMENT OF SYMPTOMS POTENTIALLY RELATED TO DIEBACK OR OTHER DISEASES. THE PICTURE SHOWS THE OPERATOR MEASURING THE HEIGHT OF THE TREE AND QUANTIFYING THE SEVERITY OF DISEASE SYMPTOMS POTENTIALLY ASSOCIATED WITH INFECTIONS OF HYMENOSCYPHUS FRAXINEUS.*

***FIGURE 9***
*COLLECTION OF SAMPLES IN THE FOREST. SAMPLING WAS CONDUCTED BY REMOVING LEAF AND/OR WOOD TISSUES FROM ASH TREES IN VEGETATION (A) OR BY INSPECTING THE LITTER IN SEARCH OF LEAF RACHISES SHOWING THE PRESENCE OF APOTHECIA MORPHOLOGICALLY REFERABLE TO HYMENOSCYPHUS FRAXINEUS (B).*

ic sites previously identified (**Figure 10**). The samples were then transferred to the Laboratory of Forest Pathology and the Laboratory of Phytopathological Biotechnologies of Forest Trees of the Department of Agricultural, Forest and Food Sciences (DISAFA) of the University of Torino, where they were analysed using ***microbiological and molecular diagnostic techniques***. The analyses allowed to verify the presence of H. fraxineus, to quantify its incidence and to test its sporulation capacity (**Box 4**)


9A


9B

***FIGURE 10***

*SET-UP OF VOLUMETRIC SAMPLERS FOR THE MONITORING OF AIRBORNE SPORES OF HYMENOSCYPHUS FRAXINEUS (A) AND DETAILED IMAGE OF THE SAMPLER (B). POWERED BY SOLAR PANELS AND COUPLED TO A THERMOPLUVIOMETRIC AND ANEMOMETRIC STATION, THE DEVICE SAMPLES EQUAL VOLUMES OF AIR AT REGULAR INTERVALS, CAPTURING THE SPORES OF THE PATHOGEN THAT MAY BE PRESENT. SPORES ADHERE TO A ROTATING TAPE THAT IS PERIODICALLY REPLACED AND ANALYSED IN THE LABORATORY*

**10A**

**10B**

## BOX 4

### Laboratory analyses for the diagnosis of *Hymenoscyphus fraxineus*

*Hymenoscyphus fraxineus is an ascomycete fungal pathogen and as such is a micro-organism whose diagnosis cannot be conducted simply in the forest using expeditious methods, but requires laboratory analyses. While the observation of symptoms in the field allows to verify the presence of a disease and to formulate some hypotheses on its possible causes, laboratory analyses can diagnose the presence of the pathogen and confirm its identity. During the MONGEFITOFOR project, various diagnostic tests were conducted, some of which were based on classical microbiology and microscopy techniques, including microbial isolation assays. Following the collection of samples of symptomatic plant tissues, or apothecia, the work is conducted in a sterile environment by placing a number of fragments of the sample in culture on dedicated substrates, with the aim of obtaining in vitro cultures of the pathogenic micro-organism. The colonies obtained can be examined through microscopic or molecular approaches to identify the micro-organism.*

*Microbial isolation may sometimes prove ineffective for some micro-organisms and, in this case, more sophisticated diagnostic tools based on DNA extraction and analysis are needed. DNA extraction is a technique that can be performed on various types of sample (host tissues or elements of the pathogen, such as mycelium or apothecia, spores captured by the volumetric sampler tape, etc.). Through subsequent analyses, such as real-time PCR, it is*

## SYMPTOMS ASSOCIATED WITH ASH DIEBACK

The phytosanitary monitoring of the ash trees conducted during the MONGEFITOFOR project allowed to assess the overall health conditions of this species, verifying the presence of symptoms of dieback caused by H. fraxineus. The symptoms observed in Valle d'Aosta were consistent not only with those reported in the scientific literature, but also with those observed in forest stands with ash tree in Piedmont, at least based on some recent surveys conducted in that region (see Ebone et al., 2023). Disease symp-

*possible to assess the presence of H. fraxineus DNA and to quantify it. During the MONGEFITOFOR project, real-time PCR analyses (Figure 11) were essential to assess the presence, incidence, spatial distribution and sporulation capacity of H. fraxineus.*

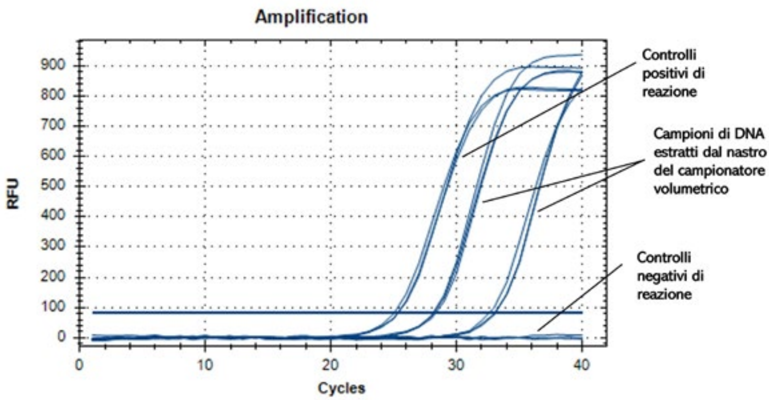



***FIGURE 11***
*EXAMPLES OF AMPLIFICATION CURVES OBTAINED FROM REAL-TIME PCR ANALYSIS OF POSITIVE REACTION CONTROLS, OF DNA EXTRACTED FROM ENVIRONMENTAL SAMPLES AND OF NEGATIVE REACTION CONTROLS. THE SELECTED SAMPLES WERE POSITIVE FOR THE PRESENCE OF HYMENOSCYPHUS FRAXINEUS DNA*

toms were detected on leaves, branches and stems of ash, both on young and adult trees, as described below.

It is likely that at an early stage of the disease, an incipient symptom is the ***withering*** of the leaves, especially those in the distal portions of the branches, followed by their progressive ***wilting***. However, withering and wilting are non-specific symptoms, as they can be caused by a variety of biotic or abiotic factors unrelated to *H. fraxineus* infections. Most commonly, ***spots*** are observed on the leaves as a result of the death of cells and entire portions of the leaf tissues, a phenomenon technically referred to as ***necrosis*** (**Figure 12**). The necrotic areas, fairly irregular in shape and of different size, are generally characterised by a ***dark colour***. At the edge of the necrosis, or in portions of the foliar surface that are infected but not yet necrotic, a localised or diffuse ***yellowing*** may occur, which pro-

12

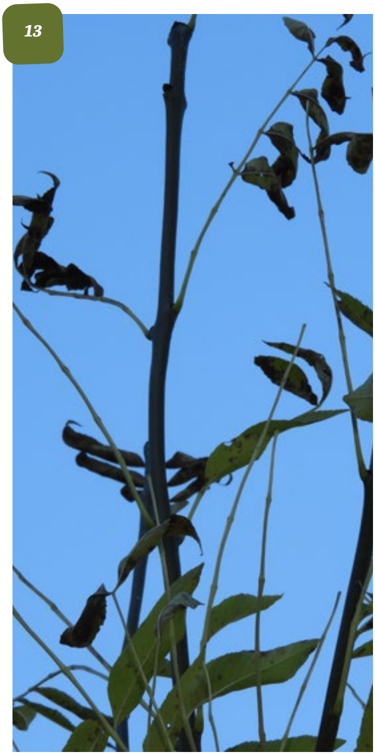
duces a symptom called ***chlorosis*** (Figure 12). When the necrosis is particularly extensive, ***deformations of the leaves*** can be observed (**Figure 13**). The deformed leaves appear as crumpled due to irregular growth. Necrosis may also extend to the petioles and leaf rachises,

***FIGURE 12***
*SYMPTOMATIC ASH LEAVES, SHOWING NECROTIC SPOTS AND CHLOROTIC YELLOWING FOLLOWING THE INFECTION OF HYMENOSCYPHUS FRAXINEUS.*

***FIGURE 13***
*ASH LEAVES DEFORMED DUE TO EXTENSIVE FOLIAR NECROSES INDUCED BY HYMENOSCYPHUS FRAXINEUS.*

***FIGURE 14***
*PREMATURE FALL OF THE LEAVES OF ASH TREES INFECTED BY HYMENOSCYPHUS FRAXINEUS. IN THE SUMMER, PREMATURELY FALLEN LEAVES ARE ALREADY VISIBLE IN THE LITTER.*

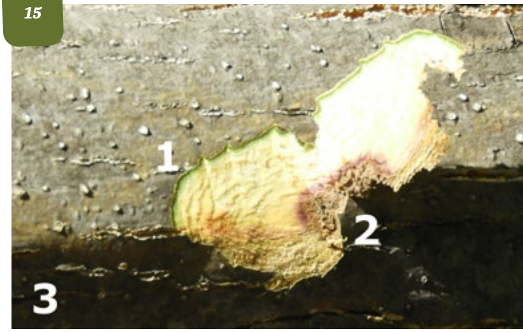
13



14

which turn brown. Not infrequently, the wilting leaves of the ash remain on the branches for some time before they prematurely fall (**Figure 14**). Branches may also exhibit ***subcortical necrosis*** affecting the ***cambium***, a vital tissue whose main function is to allow the diametrical growth of woody organs (**Figure 15**). Localised death of the cambium lead to the onset of depressions appearing on the surface of the affected branches, as their diametrical growth is inhibited (**Figure 15**). Subsequently, the same phenomenon leads to the appearance of ***cankers***, i.e. cracks and lesions in the bark and underlying tissues, which develop parallel to the direction of branch distension (**Figures 15** and **16**). As a general rule, the distal portion of the branch affected by canker dies. The main stem or mature suckers may show a discolouration or ***colour alteration*** of the bark, which under normal conditions appears olive-green in young trees, or grey-brown in mature trees, while symptomatic ash trees show browned areas with reddish-brown shades (**Figure 17**). The main stem may also show the occurrence of ***cankers*** following the establishment of the pathogen in the cambial tissues. Leaves fall and cankers contribute to a symptom of decline known as crown transparency (**Figure 18**). Crown transparency means a progressive thinning of the foliage density, which in the case of ash trees infected by *H. fraxineus* has a characteristic progression (**Figure 18**). In fact, *H. fraxineus* causes a disease known as dieback, essentially indicating a progressive wilting of the foliage and death (die-) of the twigs, which moves backwards (-back), starting from the outer portions of the crown and progressively advancing inwards (**Figure 18**). Declining ash trees may attempt to react by emitting ***shoots***, the proliferation of which over time causes a significant alteration in the architecture of the crown (**Figure 18**). The decline process often ends with the ***death of the tree***, which not infrequently remains standing for longer or shorter periods (**Figure 18**), until its failure occurs. The ***failure*** of the tree is not a direct effect due to the action of *H. fraxineus*, but occurs as a result of the intervention of other factors such as, for example, wood decay fungi and mechanical loads due to wind, precipitations or gravity. The scientific literature suggests that not all ash trees, although belonging to the same species, are equally susceptible to the disease. The phytosanitary monitoring carried out in the course of the MONGEFITOFOR project seems to corroborate this observation. In fact, the coexistence of symptomatic and completely asymptomatic trees was not rarely observed. This marked difference in the expression of symptoms, when observed among trees of comparable age, grown a few meters apart, in a site that is homogeneous in terms of environmental and silvicultural conditions, seems likely related to genotypic differences, rather than to epidemiological dynamics (e.g. infections occurring at different times, different exposure to environmental stress factors), at least in the majority of cases. This phenomenon is known as ***genetic resistance*** or ***tolerance*** to the pathogen and indicates the possibility that some individuals are either immune to the pathogen or display markedly attenuated disease symptoms (**Figure 19**).

15



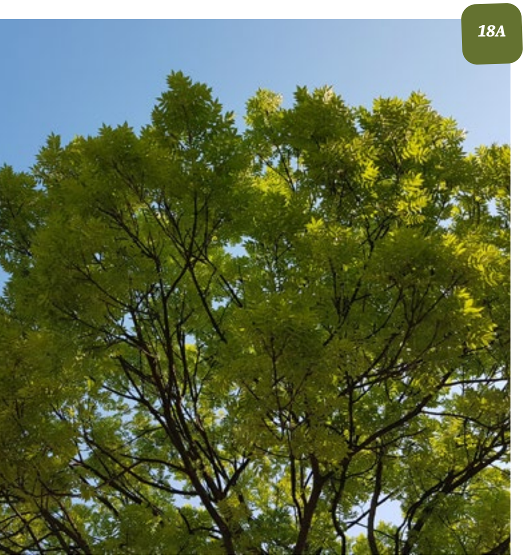

16

17

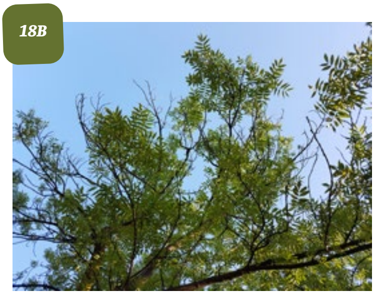
***FIGURE 15***
*ASH TREE BRANCH FROM WHICH THE BARK WAS REMOVED WITH A SCALPEL TO SHOW THE OUTERMOST PORTION OF THE UNDERLYING WOODY TISSUES. IN THE UPPER PART OF THE IMAGE, THE CAMBIUM IS VISIBLE IMMEDIATELY BELOW THE BARK AS A THIN GREEN LAYER (1). IN THE CENTER OF THE IMAGE, A DEPRESSED AREA CAN BE OBSERVED, BELOW WHICH THE CAMBIUM NO LONGER APPEARS GREEN, BUT DARKENED AS A RESULT OF THE ALTERATION CAUSED BY HYMENOSCYPHUS FRAXINEUS (2). BELOW, IT IS POSSIBLE TO SEE THE PRESENCE OF CANKER LESIONS RUNNING LONGITUDINALLY ALONG THE BRANCH (3).*

***FIGURE 16***
*SYMPTOMS OF CANKER ON A YOUNG BRANCH OF ASH DUE TO HYMENOSCYPHUS FRAXINEUS INFECTION.*

***FIGURE 17***
*COLOUR CHANGES OF THE STEM OF A DECLINING ASH TREE INFECTED BY HYMENOSCYPHUS FRAXINEUS.*







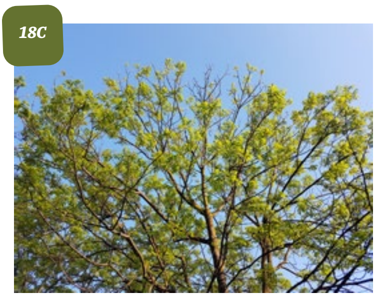




***FIGURE 18***

*RECONSTRUCTION OF THE TYPICAL PROGRESSION OF THE ASH DIEBACK CAUSED BY HYMENOSCYPHUS FRAXINEUS. A NON-INFECTED ASH TREE DISPLAYS AN ASYMPTOMATIC, DENSE AND VIGOROUS CROWN (A). FOLLOWING INFECTION, THE FIRST SYMPTOMS ARE VISIBLE AS A PROGRESSIVE THINNING OF THE CROWN, FIRST AFFECTING THE DISTAL PORTIONS OF THE BRANCHES AND THE OUTER SECTORS OF THE CROWN (B). THE LATTER SHOWS GRADUALLY INCREASING LEVELS OF TRANSPARENCY FOLLOWING AN EXTENSIVE PREMATURE FALL OF THE LEAVES (C). THE COURSE OF THE DISEASE LEADS TO A NOTICEABLE CONTRACTION OF THE CROWN, AFFECTED BY WIDESPREAD WILTING OF THE BRANCHES (D). THE ARCHITECTURE OF THE CROWN APPEARS ALTERED, WITH THE REDUCTION OF THE SECONDARY BRANCHES AND CONCOMITANT PRODUCTION OF EPICORMIC SPROUTS, OR SHOOTS, CLOSE TO THE MAIN BRANCHES (E). AT AN ADVANCED STAGE OF THE DISEASE, THE CROWN SHOWS AN EXTREMELY REDUCED VOLUME AND APPEARS CONCENTRATED IN THE LOWER AND INNER PORTIONS: THIS IS THE MAIN DIEBACK SYMPTOM (F). THE OUTCOME OF THE DISEASE IS NOT INFREQUENTLY LETHAL (G).*

***FIGURE 19***

*ASH TREES GROWN IN HOMOGENEOUS CONDITIONS. THE COEXISTENCE OF ASH TREES SHOWING SEVERE DIEBACK SYMPTOMS (FOREGROUND) AND OTHERS ASYMPTOMATIC (BACKGROUND) CAN BE OBSERVED. THIS PHENOMENON COULD BE LINKED TO THE RESISTANCE OR GENETIC TOLERANCE OF THE ASYMPTOMATIC ASH TREES TO HYMENOSCYPHUS FRAXINEUS.*

18E

18G

18F

19

As described above, the apothecia of H. fraxineus are differentiated at the level of the litter, but it should be pointed out that there are several other ascomycetes that may form reproductive structures that are morphologically similar to those of *H. fraxineus*, fuelling the risk of ***confusion among species***. In addition, if apothecia are dehydrated, not fully developed, or too old, they may not display their distinctive morphological characteristics. For example, in the course of the MONGEFITOFOR project, apothecia visually associated to *H. fraxineus* turned out to belong to other non-pathogenic species (e.g. *Cyathicula fraxinicola*, *Hymenoscyphus scutula*) once identified through molecular techniques. It is worth noting that the majority of fungal species are not pathogenic, but play a fundamental ***ecological role*** in the functioning of forest ecosystems, contributing to the cycle of the organic matter (e.g. saprotrophic fungi), establishing synergic relationships with certain plant species (e.g. symbiotic fungi), or coexisting neutrally with the latter without causing damage (e.g. endophytic fungi).

## INCIDENCE, SEVERITY AND DISTRIBUTION OF ASH DIEBACK

The assessment of the ash dieback symptoms described in the previous chapter allowed appraising quantitatively the incidence and severity levels of the disease within the surveyed area. In detail, the widespread presence of ***foliar necrosis detected*** in different sites looked compatible with the infection of *H. fraxineus*. The incidence of the above symptom ranged between 3% and 92%, with an average value of around ***34%***. In other words, on average around 1/3 of the sampled leaves were affected by visible symptoms of necrosis. The ***crown transparency***, one of the most relevant symptoms for estimating the impact the disease, averaged around ***34%***, ranging from a minimum of 12% to a maximum of 68%. Crown transparency is quantitative, assessing the ***percentage of canopy lost*** due to the premature fall of the leaves and to the death of the branches (**Figure 20**).

Discolouration at the base of the stem was observed in only 5 sites, while ***cankers*** were observed in all the forests monitored, albeit with low averages of up to 2 lesions per tree.

On the whole, ***symptoms*** of ash ***dieback*** were detected ***in all the***

***monitored sites***, despite the fact that the latter were very different in terms of environmental and silvicultural characteristics. In fact, the forest monitored were located at elevations ranging from 785 m a.s.l. to 1480 m a.s.l. The majority of these (55%) were located in the upper valley, while the remainder in the middle (30%) and lower valley (15%), with slopes varying from approximately 0 to 100%. The dendrometric measurements showed that the ash trees present in the project areas were young, although sites with mature trees were abundant. The average diameters at breast height ranged from 5.99 (± 2.04 cm) to 22.68 cm (± 12.43 cm). The data seem to suggest that in the cross-border areas between Italy and Switzerland ash dieback is widespread on ash trees of different age classes and growing in sites characterised by different environmental and silvicultural conditions.


***FIGURE 20***
*ASH TREE WITH DIEBACK SYMPTOMS (A) ON WHICH THE OUTLINE OF THE LOST PORTION OF THE CROWN IS SCHEMATICALLY INDICATED (B). THE CONTINUOUS RED LINE INDICATES VOIDS CAUSED BY PREMATURE FALL OF THE LEAVES AND WILTING IN THE OUTER PORTION OF THE CROWN, WHILE THE ISOLATED DOTS IDENTIFY INCIPIENT THINNING, PROGRESSING DOWNWARDS AND TOWARDS THE INNERMOST PORTIONS OF THE CROWN. QUANTITATIVELY, THE PERCENTAGE OF CROWN TRANSPARENCY OF THE ASH TREE SHOWN IN THE FIGURE IS 45%.*

The phytosanitary survey of ash carried out during the MONGEFITOFOR project highlighted that in Valle d'Aosta ash tree ***dieback*** is present and ***widespread***, affecting a large part of the ash tree's distribution area in the foothill and submontane zones of the region. The sites investigated are in fact located over a vast area, extending approximately 60 km in longitude and 20 km in latitude, virtually covering a topographical area of 1200 km2 over a 700 m range of altitude.

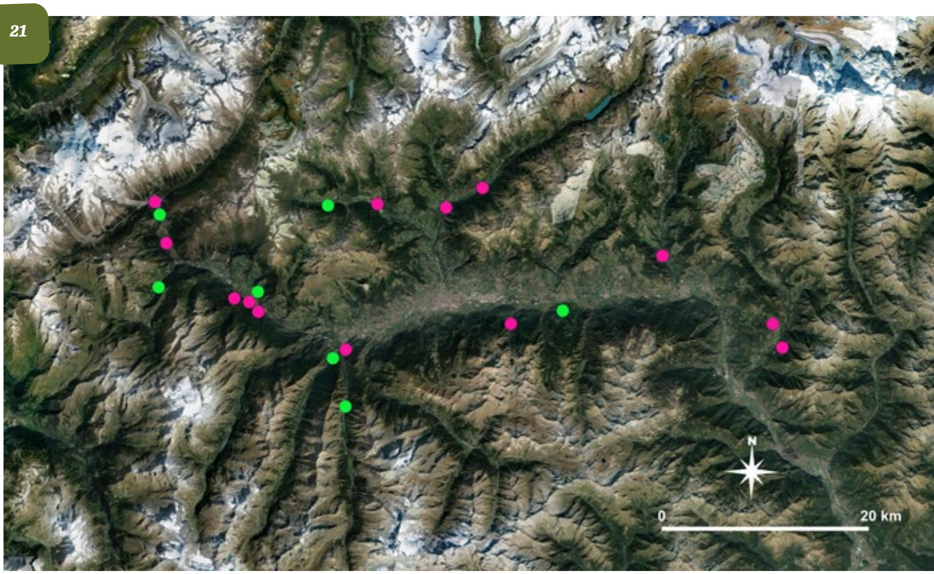
## PRESENCE, SPORULATION CAPACITY AND SPATIAL DISTRIBUTION OF *HYMENOSCYPHUS FRAXINEUS*

While the assessment of the ash dieback symptoms revealed the impact of the disease in terms of incidence and severity, the MONGEFITOFOR project activities also allowed to diagnose the presence of H. fraxineus, assess its sporulation capacity and determine its spatial distribution. From host tissue samples and apothecia, colonies of the fungal pathogen could not be isolated in the laboratory. The failure of microbial isolation does not imply, however, that H. fraxineus was absent. In fact, some pathogens establish such a specific relationship with the host that it is difficult to separate them from the tissues they colonise. Hence, their growth in vitro on artificial substrates can be rather difficult, despite the fact that the most up-to-date international standards were used to perform the isolation trials in the laboratory (e.g. the use of a specific protocol for H. fraxineus included in a PM7 standard of the European and Mediterranean Plant Protection Organisation - EPPO). However, the ***diagnosis*** conducted by using the ***molecular techniques*** described above revealed the ***widespread presence*** of ***H. fraxineus***. The pathogen was detected in ***65%*** of the ***sites monitored*** (**Figure 21**). At the ***site level***, where the pathogen was present, its ***incidence*** (percentage of infected trees) ranged between ***11%*** and ***80%***. It is interesting to note that an increasing incidence of *H. fraxineus* was detected, lower in the upper (16%), higher in the middle (35%) and highest (40%) in the lower Valle d'Aosta. This phenomenon could be related to epidemiological dynamics (e.g. the pathogen's invasion front follows an east-west direction coming from Piedmont) or could depend

on environmental and local conditions, which in the lower valley could be more favourable to the pathogen. *H. fraxineus* was detected both in leaf samples and in woody tissues, confirming the association between the symptoms observed in the forests and the presence of the pathogen.
Volumetric samplers for the monitoring of airborne spores of *H. fraxineus* showed that the pathogen was able to sporulate in the areas covered by phytosanitary survey, confirming the adaptation of the fungus to the environmental conditions typical of the north-western Alpine sectors. Apparently, the autumn period was particularly favourable for the sporulation of the pathogen, which was higher where ash trees were more abundant.

***FIGURE 21***
*MAP OF THE SITES WHERE HYMENOSCYPHUS FRAXINEUS WAS DETECTED AS A RESULT OF THE PHYTOSANITARY SURVEY CONDUCTED DURING THE MONGEFITOFOR PROJECT. THE SITES WHERE THE PATHOGEN WAS PRESENT ARE SHOWN IN FUCHSIA, THE OTHERS IN GREEN. IN THE BACKGROUND, SATELLITE IMAGE OF THE CROSS-BORDER AREAS BETWEEN ITALY AND SWITZERLAND (SOURCE: GOOGLE MAPS, 2023).*

21



The results obtained have a practical methodological relevance, in relation to ***phytosanitary monitoring options***, for which the MONGEFITOFOR project stands as a pilot study for cross-border areas and beyond. In fact, in terms of diagnostic efficiency, real-time PCR analyses could detect the pathogen even when microbial isolation assays failed. For future monitoring programmes, it might therefore be advantageous to use molecular diagnostic approaches in order to avoid significant underestimates of pathogen incidence.

Other pathogens affecting ash

In addition to ash dieback caused by *H. fraxineus*, the phytosanitary surveys carried out during the project MONGEFITOFOR sought for the presence of any symptoms potentially related to other disease agents or damage caused by phytophagous insects.

In particular, wood decays and root rots are diseases that can result in the onset of symptoms at least partly overlapping with those caused by *H. fraxineus*. However, it should be considered that *H. fraxineus* generally results in the onset of characteristic symptoms leading to a peculiar progress of the dieback. Furthermore, ***wood decay fungi*** causing the above-mentioned diseases often infect senescent trees or trees already debilitated due to the action of other disease agents, such as *H. fraxineus*. In order to detect the possible presence of wood decay fungi, wood samples positive to *H. fraxineus* were further analyzed by using molecular techniques (multiplex-PCR).

The four pathogens identified following multiplex-PCR analysis were: ***Laetiporus sulphureus*** in 89% of the samples, *Stereum* spp. in 68%, ***Armillaria spp.*** in 26%, and *Pleurotus* spp. in 5%. *L. sulphureus* is a basidiomycete fungus causing brown rot in broadleaves and conifers. Different white-rot fungi infecting both broad-leaved trees and conifers belong to the genus *Stereum*. The genus *Armillaria* includes species that are well known for their ability to colonize dead wood causing white rot, but also for their ability to act as primary pathogens affecting living trees. Indeed, it is well known that infections by *Armillaria* spp. are particularly frequent on living hosts already debilitated as a result of abiotic or biotic stresses. Finally, *Pleurotus* spp. is a saprotrophic basidiomycete, sometimes agent of white rot on living hosts. However, no typical signs (e.g. fruiting bodies, *Armillaria* rhizomorphs) of the presence of the above-mentioned pathogens were observed in the forest.

The results suggest that *H. fraxineus* may be associated with ***other pathogens*** and that therefore the ascomycete-triggered disease may evolve into a more articulated decline syndrome, in which wood decay and root rot agents are also involved.

## SILVICULTURAL AND PHYTOSANITARY STRATEGIES FOR THE MANAGEMENT OF FORESTS WITH DECLINING ASH INFECTED BY *HYMENOSCYPHUS FRAXINEUS*

The presence of symptomatic ash trees infected by *H. fraxineus* suggests that appropriate strategies should be implemented to supplement traditional management practices. Given the large variability that characterises forests with ash trees, even apart from the presence of biotic or abiotic threats, it is not possible to outline an exhaustive protocol that covers all the different cases. The knowledge gained from an in-depth review of the international scientific literature and the experience acquired following the MONGEFITOFOR project, also deriving from the ***exchange*** of expertise among the project partners, led to define a series of ***management guidelines*** to support ***silvicultural*** and ***phytosanitary operations*** in order to enhance the functions and ecosystem services provided by forests hosting ash trees. In particular, the ***conservation of ash*** is a core objective to promote the perpetuation of the species and of the vegetal communities where it is present. However, the achievement of such an objective needs a prior assessment of the health conditions of the tree and of the forest stand as a whole.
In relation to the cross-border areas involved in the MONGEFITOFOR project, a general set of operative guidelines has been defined, nonetheless each case needs to be assessed singularly based on overall characteristics of the forest stand. The guidelines are a sort of ***toolbox*** that can be applied to different situations, while maintaining the flexibility needed to manage a species that is often not overly abundant in the forests of the foothill and submontane zones of the Alps. It should be emphasised that some of the proposed guidelines derive from empirical observations and inferences drawn from other model systems, as their experimental assessment on ash would take decades. The guidelines are set out as a numbered list of options: starting from point 1, the user assesses the congruence of the scenarios described with the

situation he can observe in the forest and then goes on by selecting at each step the most appropriate option. Each chosen option leads to the next point to be considered () until the final option describing the operational guidelines to implement in the forest is reached.

**1.** Assess whether ash trees are in ***good vegetative conditions*** (→**2**) or whether, and to what extent, the species shows a state of decline displaying symptoms consistent with the ***dieback*** caused by *H. fraxineus* (→**3**);

**2.** The operations suggested if ash trees are in good vegetative condition depend on the environmental and silvicultural characteristics of the forest stand, as indicated in the scenarios below.
If the forest stand shows environmental and silvicultural characteristics suitable to ash and putatively adequate for its long-term conservation based on its ecological needs, it may be convenient selecting one the following options:

**a.** A ***non-intervention*** strategy is recommended because active management would be hardly justifiable. In fact, either the current situation is in line with the expectations, or the technical and operational conditions to carry out rational silvicultural operations are lacking. Instead, if the aim is to ***increase the abundance of ash trees*** and the technical and operational conditions are adequate to conduct rational silvicultural operations, go to (→**2b**);
However, if climatic, edaphic, ecological, synecological or structural characteristics of the forest stand are not fully consistent with the needs of ash, three options could be considered:

**b.** The conditions of the forest stand can be likely modified to promote ash trees through ***standard silvicultural operations***. The site is adequate to host such operations, for instance because roads are present within, or in the close proximity of the forest stand. The silvicultural operations (e.g. thinnings, successive shelterwood cuttings, establishment of artificial regeneration) aim at ***regulating*** the ***specific composition***, modifying the horizontal and vertical ***structure of the forest stand*** and mitigating intra- and interspecific ***competition***. Such operations act directly or indirectly on the availability of essential ecological factors (e.g. light, water and nutrients) and must be implemented to promote the ***conservation of ash trees*** and increase their abundance, if possible, while maintaining a balanced and mixed composition of the forest stand;

**c.** Ash trees look apparently not well-adapted to the characteristics of the forest stand. Although some of the conditions characterizing the forest stand can be modified to promote ash trees through standard silvicultural operations, in the long run the conservation of ash looks unlikely (e.g. the station is too arid, the soil physical and chemical properties are unfavorable). If the technical and operational conditions are adequate to conduct rational silvicultural operations in the stand, such operations should be designed and implemented to drive the ***succession of the forest*** by promoting the establishment and spread of the native tree species more adapted to the local environmental conditions.

**d.** The forest stand displays characteristics compatible with the scenarios described in points 2b or 2c, technical and operational conditions are inadequate to conduct rational silvicultural operations. In this case, a ***non-intervention*** strategy is recommended.

**3.** In case declining ash trees are present, the options suggested are differentiated based on the abundance of the species, on its spatial distribution within the forest stand and on the severity of the symptoms, as indicated below.

**a.** ***Ash trees are present sporadically***, not reaching 15% of the canopy cover, and there are no significant monospecific groups of the species. Ash trees show incipient, overt or severe symptoms of decline. Given these premises, even if the technical and operational conditions are adequate to conduct rational silvicultural operations in the stand, the limited abundance of the species and the uncertain outcome of the ongoing decline suggest to avoid any active silvicultural or phytosanitary operation for the conservation of ash. Depending on the characteristics of the forest stand, options (→**2c**) or (→**2d**) may apply.

**b.** ***Ash abundance*** attains at least ***15%*** or ash trees are locally present in ***large groups*** of 5-10 individuals or stumps. Ash trees show ***early symptoms*** of decline, crown transparency is not excessive attaining an average around 20%. In terms of disease severity, differences among trees do not appear too evident and the impact of the disease looks fairly uniform. Dead ash trees are absent, rare, or sporadic. In this case,

***waiting*** 1-3 years and re-evaluating the situation may help to undertake better decisions based on the evolution of decline symptoms. At the end of this "waiting time", if the severity of the decline symptoms of ash do not show a substantial increase, go to option (→**2**), otherwise (→**3c**).

**c.** ***Ash abundance*** attains at least ***15%*** or ash trees are locally present in ***large groups*** of 5-10 individuals or stumps. However, in this case, the ***symptoms*** of ***decline*** are ***severe***, the impact of the dieback is evident, the crown transparency attains on average more than 20%. ***Declining*** ash trees are systematically present, some of which are severely affected by dieback or are dead. However, the distribution of the symptoms is heterogeneous and ***substantial differences*** of disease severity can be observed among the ash trees present in the forest stand. In fact, ash trees can be scored either as ***ash trees+*** or ***ash trees-***. Ash ***trees+*** are dominant trees, or trees that are likely to become dominant, whose symptoms of decline are significantly less severe than those displayed by the surrounding ash trees (ash trees-). Hence, ash ***trees+*** are either asymptomatic, or affected by minor symptoms, with a crown transparency not exceeding 25%. The management strategy will therefore aim at ***preserving*** ash trees+, mainly based on the scenario discussed in point 2a above. Indeed, ash trees+ might be ***resistant*** or ***tolerant*** to *H. fraxineus* due to their genetic profile. The release and conservation of ash trees+ is aimed not only at preserving ash as a key species of the forest stand, but also at promoting its natural regeneration. In fact, the progeny of the ash trees+ may inherit favourable genetic traits related to the resistance or tolerance to the pathogen. In the long term, management practices aimed at preserving ash trees+ and promote their natural regeneration should lead to the establishment of ash populations that are less and less susceptible to *H. fraxineus*, thus reducing the ***impact*** of the dieback on ash. If a low abundance of seedlings from ash trees+ is observed, or if seedlings display an overall state of stress, thinnings providing additional light input at the ground level may help to promote the establishment of vigorous seedlings from ash trees+. However, if the above operation was unfeasible or impractical, or if it failed, ***artificial regeneration might be an option***. The artificial regeneration of ash is obtained by planting groups of seedlings

belonging to ash trees+ growing in other sites. It is worth noting that seedlings for planting should be carefully inspected and must not show any disease symptoms. The introduction of native genotypes of *F. excelsior*, although not present in the planting site, should increase the genetic ***variability*** of ash trees in the forest stand, potentially increasing its ***resilience***.
However, silvicultural operations to promote ash trees+ should be combined with the removal of ash trees-, namely ash trees severely affected by dieback and displaying a crown transparency exceeding 25%. Their removal is aimed at ***preventing further infections*** by progressively lowering of the amount of substrate available for the production of apothecia of *H. fraxineus*, mainly consisting of leaf rachises. In fact, the removal of ash trees- prevents the accumulation of further leaf rachises in the coming years, resulting in a predictable reduction of inoculum pressure at the site level. In the long term, a reduced production of infectious spores should lead to a progressive improvement of the ash tree's health. Furthermore, the removed ash trees- no longer contribute to the transmission of potentially unfavourable genetic traits to the progeny, including traits potentially correlated with a pronounced susceptibility to *H. fraxineus.* In addition, the removal of ash trees- also contributes to improve the stability of the forest stand.
If ash trees+ are absent in the forest stand, go to option (→**3d**).
**d.** If ash trees+ are not present or insufficiently abundant to allow the long-term conservation of the species and if ash dieback is particularly severe, the option of ***non-intervention*** should be considered. Alternatively, cuttings should be carried out as described in point **3c**, subsequently proceeding with the planting of ***artificial regeneration*** using seedlings obtained from native ash tree+ growing in other sites. The seedlings should be planted a few years after the removal of ash trees- to allow for a gradual degradation of the litter. Such degradation should reduce the amount of substrate available for the production of *H. fraxineus* apothecia, thus reducing the risk of new infections.

The guidelines described are summarised in the diagram of **Figure 22**.

22

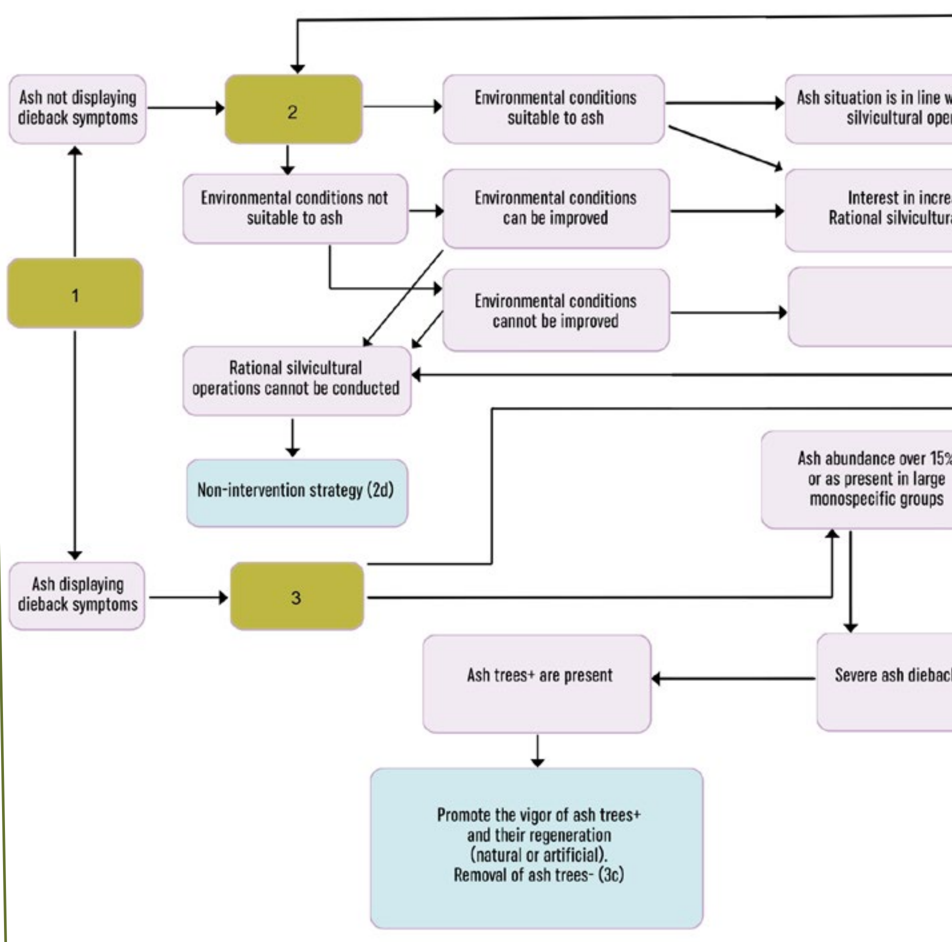
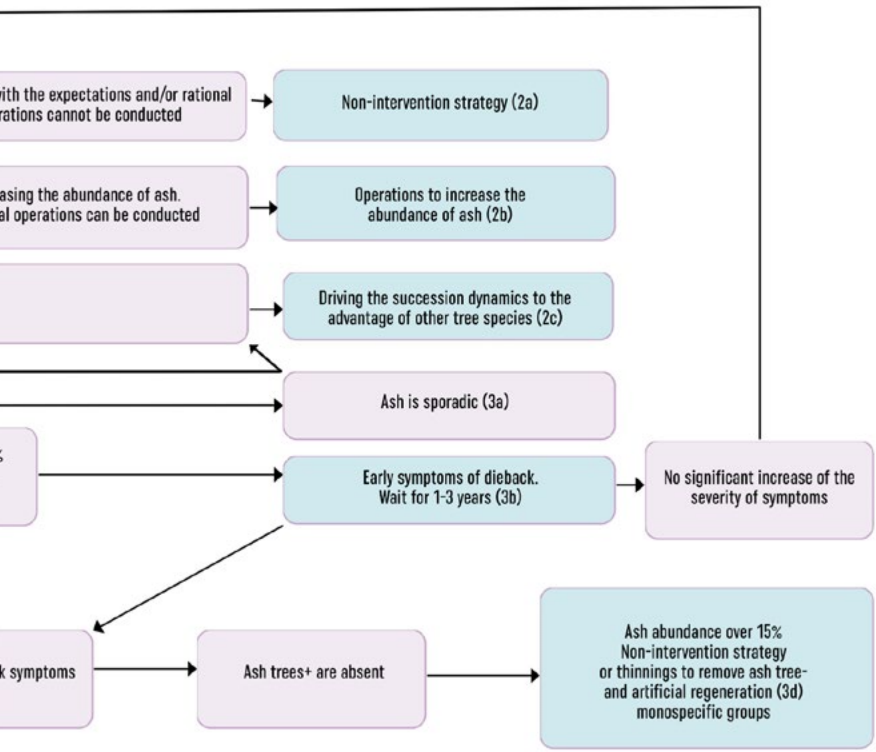

Ash not displaying dieback symptoms
2
Environmental conditions suitable to ash
Ash situation is in line w
silvicultural oper
Environmental conditions not suitable to ash
Environmental conditions can be improved
Interest in incre
Rational silvicultura
1
Environmental conditions cannot be improved
Rational silvicultural operations cannot be conducted
Non-intervention strategy (2d)
Ash abundance over 15%
or as present in large
monospecific groups
Ash displaying dieback symptoms
3
Ash trees+ are present
Severe ash diebac
Promote the vigor of ash trees+
and their regeneration
(natural or artificial).
Removal of ash trees- (3c)

***FIGURA 22***
*DIAGRAM FOR THE APPLICATION OF SILVICULTURAL AND PHYTOSANITARY GUIDELINES FOR ASH MANAGEMENT. THE ORANGE BOXES CORRESPOND TO THE DIFFERENT SETS OF ALTERNATIVE SCENARIOS AND POSSIBLE ACTIONS. THE PINK BOXES LIST THE CONDITIONS THAT SHOULD BE MET AT EACH STEP TO DEFINE A SUITABLE MANAGEMENT STRATEGY, THE LATTER SUMMARISED IN THE BLUE BOXES. THE NUMBERS INDICATED IN THE BOXES CORRESPOND TO NUMBERING OF THE OPTIONS PRESENTED IN THE MAIN TEXT. THE DIAGRAM SHOULD BE USED AS FOLLOWS: STARTING FROM BOX 1, SELECT AT EACH STEP THE ARROW THAT LEADS TO THE BOX THAT BEST DESCRIBES THE SITUATION OBSERVED IN THE FOREST.*



In addition to the guidelines described above, other aspects related to plant health should also be considered, when relevant.
For instance, if ash trees show symptoms of dieback, these trees could be inspected to check for the presence of diseases such as root rot. In fact, wood decay fungi causing root rots may pose serious threats to the mechanical stability of the tree, with potential damages to structures, infrastructures and people. Cutting trees affected by root rot or wood decay could be necessary when such damages may be likely, provided that an assessment of the tree stability has been previously conducted. Threats to the mechanical stability of the tree may not be particularly relevant if the risk of harm, injury and damage to people or anthropic structures is null or negligible. In fact, root rots and wood decay are a constitutive element of the forest dynamics. For example, dead trees degraded by wood decay fungi form necromass useful for the cycles of the soil organic matter and to create micro-habitats hosting different animal, plant and microbial species.
It should be noted that some of the proposed options for the management and conservation of ash trees may not lead to the desired outcome, due to unpredictable epidemiologic and disease dynamics including: the advancement of the invasion front, the accidental introduction of additional sources of inoculum, the massive production of apothecia of *H. fraxineus* at the litter level following favorable climatic and environmental conditions.

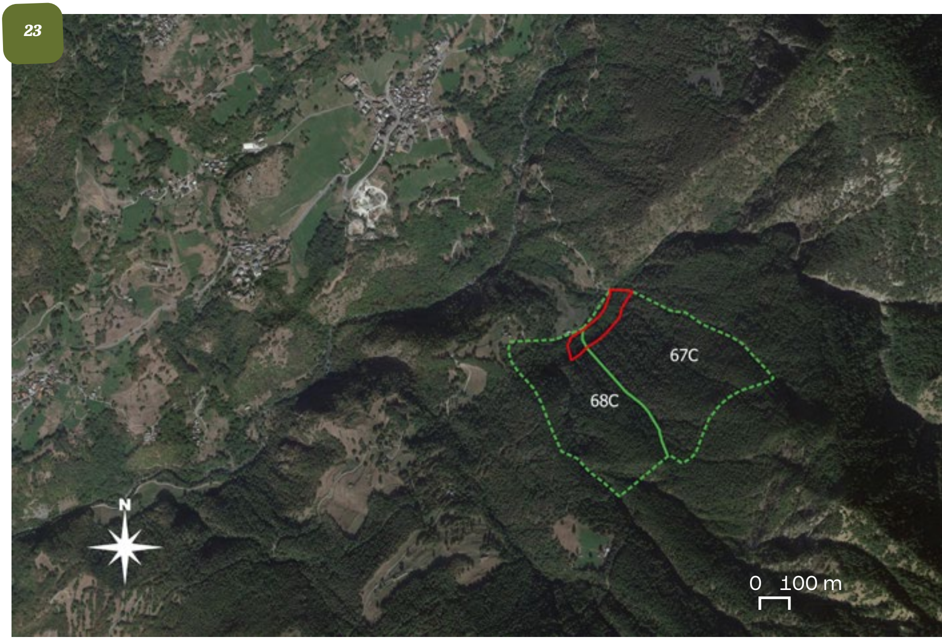
## APPLICATION EXAMPLES OF THE MONGEFITOFOR GUIDELINES FOR THE MANAGEMENT OF ASH DIEBACK

In the framework of the MONGEFITOFOR project, sites to host ***pilot*** sub-projects were selected to show the implementation and application of ***silvicultural*** and ***phytosanitary*** management ***guidelines***. For ash affected by dieback caused by *H. fraxineus*, forests stands called "Boc de La" and "Bois de Cretes" in the Municipality of Challand-Saint-Anselme were selected. Dendrometric, silvicultural and phytopathological observations, measurement or samplings were conducted in the above sites. The selected plots are located on a low-medium slope (elevation of approximately 900 m a.s.l.), with

NW aspect, in close proximity of an unpaved road that allows vehicle access. The vertex of the plot located near the road, NNO direction, is located at coordinates 402097, 5061665 (UTM WGS84 coordinate system) (**Figure 23**).
Next to the edge of the road, a strip consisting of ash trees (**Figure 24**) runs approximately along the NE-SW axis, for a length of roughly 250 m, a depth of around 60 m and an indicative topographic area of 1.6 ha. Ash tree growing in the above forest strip are young, although some elder trees of larger diameter (around 30-40 cm) are present. Ash is dominant in the NE portion of the forest stand, while towards the SW side it tends to mix with Scots pine, larch and some broadleaves.

***FIGURE 23***
*GENERAL MAP OF THE PLOTS (DASHED GREEN LINE) AND OF THE ASH VEGETATION STRIP (RED LINE) SELECTED IN THE MUNICIPALITY OF CHALLAND-SAINT-ANSELME FOR THE IMPLEMENTATION AND APPLICATION OF THE GUIDELINES FOR SILVICULTURAL AND PHYTOSANITARY MANAGEMENT OF DECLINING ASH TREES INFECTED BY HYMENOSCYPHUS FRAXINEUS. IN THE BACKGROUND, SATELLITE IMAGE OF THE PROJECT AREA (SOURCE: GOOGLE MAPS, 2023).*

23



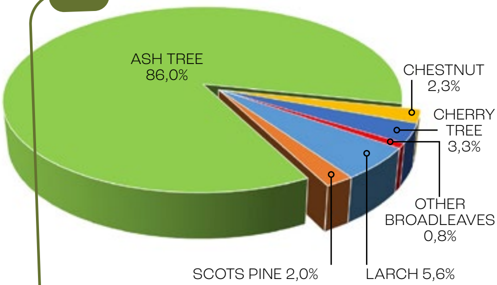
The plot is almost entirely located within a mesalpic pine forest with broadleaves, at the edge of forest patches with abundant alder to the west, maple and ash to the north. The forest stand can therefore be defined as mixed, with larch and Scots pine prevailing in height over the broadleaves including ash (the most abundant broadleaf), followed by cherry, chestnut, a few birches and some willows. Ash trees are mostly young, with diameters ranging between 8 and 15 cm, resulting in a low basal area to which broadleaves contribute reaching the 65% of the total. The specific composition, diameter distribution and quantification of the basal area and density of the forest stand are detailed below (**Figure 25** and **Table 2**).

24

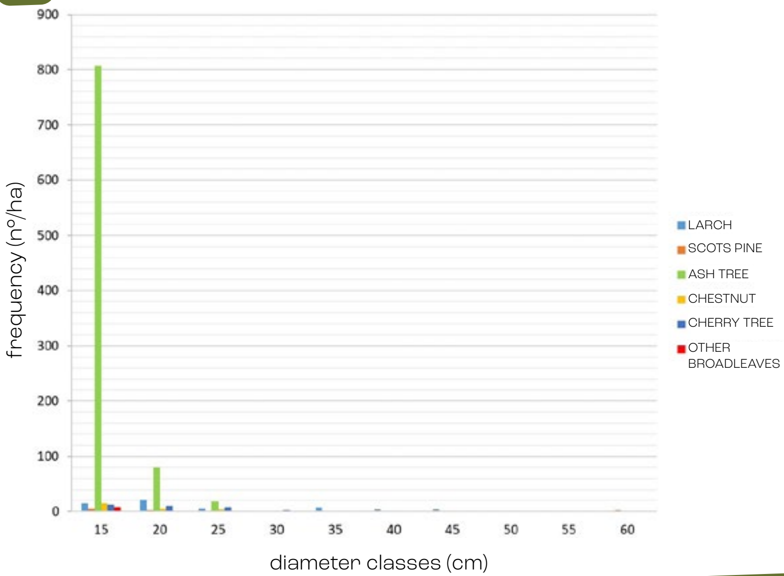
***FIGURE 24***
*TREES GROWING IN THE PLOT SELECTED FOR THE IMPLEMENTATION AND APPLICATION OF THE GUIDELINES FOR SILVICULTURAL AND PHYTOSANITARY MANAGEMENT OF DECLINING ASH INFECTED BY HYMENOSCYPHUS FRAXINEUS.*

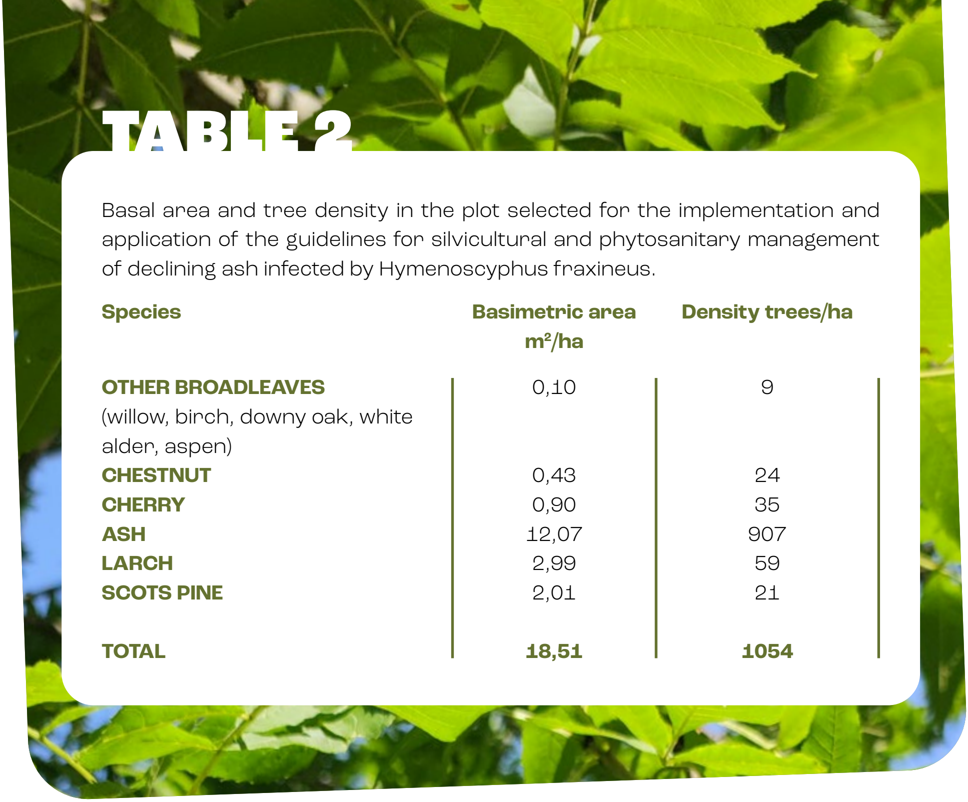
***FIGURE 25***
*PERCENTAGE DISTRIBUTION OF THE TREE SPECIES PRESENT (A) AND THEIR DISTRIBUTION BY DIAMETRICAL CLASS (B) IN THE PLOT SELECTED FOR THE IMPLEMENTATION AND APPLICATION OF THE GUIDELINES FOR SILVICULTURAL AND PHYTOSANITARY MANAGEMENT OF DECLINING ASH INFECTED BY HYMENOSCYPHUS FRAXINEUS.*

**25A**




**25B**





# TABLE 2

Basal area and tree density in the plot selected for the implementation and application of the guidelines for silvicultural and phytosanitary management of declining ash infected by Hymenoscyphus fraxineus.

| Species | Basimetric area m²/ha | Density trees/ha |
|---|---|---|
| **OTHER BROADLEAVES** (willow, birch, downy oak, white alder, aspen) | 0,10 | 9 |
| **CHESTNUT** | 0,43 | 24 |
| **CHERRY** | 0,90 | 35 |
| **ASH** | 12,07 | 907 |
| **LARCH** | 2,99 | 59 |
| **SCOTS PINE** | 2,01 | 21 |
| **TOTAL** | **18,51** | **1054** |

Silvicultural and phytosanitary operations were implemented by adapting the above guidelines to the situation characterizing the plot. In detail, the principles outlined in section 3 were considered consistent with the ash tree's health status.

Declining ash trees showed symptoms of dieback and either their abundance was at least 15%, or they were locally present in clusters of 5-10 trees where the forest stand was more differentiated in terms of specific composition. The symptoms of dieback were evident and their impact clearly visible, with an average crown transparency of more than 20%. In addition, several ash trees showing particularly severe symptoms of dieback were present. For the Challand-Saint-Anselme site, phytosanitary monitoring and laboratory analyses revealed an average crown transparency of 44% and a 20% incidence of *H. fraxineus*.

The spatial distribution of the symptoms was highly differentiated and a large number of ash trees+ was detected. Ash trees+ were asymptomatic or showed symptoms significantly milder that those displayed by the surrounding ash trees (ash trees-), with a crown transparency not exceeding 25%. The silvicultural operations were therefore outlined with the aim of preserving the presence of the species by improving the vigor of ash trees+. The forest stand showed eco-pedological characteristics suitable for the conservation of ash. The rational execution of silvicultural operations was technically and logistically feasible due to the presence of roads in the close proximity of the plot. Following the screening of the trees to remove, that were marked in the field with a visible paint spot (**Figure 26**), thinnings were carried out to regulate tree density in order to reduce intra- and interspecific competition and promote the vigor of the released ash trees.

The above operations were set up for demonstration purposes and are intended to serve as a practical example (**Figure 27**). For this purpose, the forest strip hosting ash trees was divided into 3 areas (A, B and C), each one attaining approximately 1/3 of the total area (roughly 5,000 m2 each). The 15%, 40% and 60% of the trees was felled in areas A, B and C, respectively (**Figure 28**), proceeding from SW to NE (**Table 3**).

26

***FIGURE 26***
*TREES TO BE FELLED ARE MARKED WITH A VISIBLE PAINT SPOT PRIOR TO THE IMPLEMENTATION OF THE SILVICULTURAL OPERATIONS TO PROMOTE THE VIGOR OF THE RELEASED ASH TREES+.*

27


28

Additional thinnings non exceeding the 5% of the total number of trees were allowed for technical and/or safety reasons (e.g. trees at risk of uprooting, with broken trunks, or representing an obstacle to the felling of neighboring trees). At the end of the thinning operations (**Figure 29**) the residues were chipped to remove potential sources of the pathogen infectious inoculum.

***FIGURE 27***
*ESTABLISHMENT OF THE PLOT WHERE SILVICULTURAL AND PHYTOSANITARY OPTIONS FOR THE MANAGEMENT OF DECLINING ASH TREES WERE SHOWN.*

***FIGURE 28***
*THINNING OPERATIONS AIMED AT REMOVING THE TREES PREVIOUSLY MARKED.*

# TABLE 3

Quantification of the silvicultural parameters describing the thinning operations conducted in the areas identified in the plot.

| | HARVESTED TREES | TOTAL TREES | HARVESTED OUT OF TOTAL TREES (%) | VOLUME HARVESTED (IN M3) | TOTAL VOLUME BEFORE THINNING (M3) | VOLUME HARVESTED (IN %) |
|---|---|---|---|---|---|---|
| **AREA A THINNING INTENSITY 15%** | 60 | 379 | 15,8% | 20,85 | 35,74 | 58,3% |
| **AREA B THINNING INTENSITY 40%** | 250 | 562 | 44,5% | 16,23 | 26,37 | 61,5% |
| **AREA C THINNING INTENSITY 60%** | 413 | 640 | 64,5% | 26,18 | 38,54 | 67,9% |

***FIGURA 29***
*THE FOREST STAND THE END OF THE THINNING OPERATIONS CONDUCTED IN THE PLOT. THE STUMPS OF THE ASH TREES REMOVED AND THE RELEASED ASH TREES ARE CLEARLY VISIBLE. TREE DENSITY WAS SIGNIFICANTLY REDUCED COMPARED TO THE INITIAL SITUATION (SEE FIGURE 24).*

**29A**

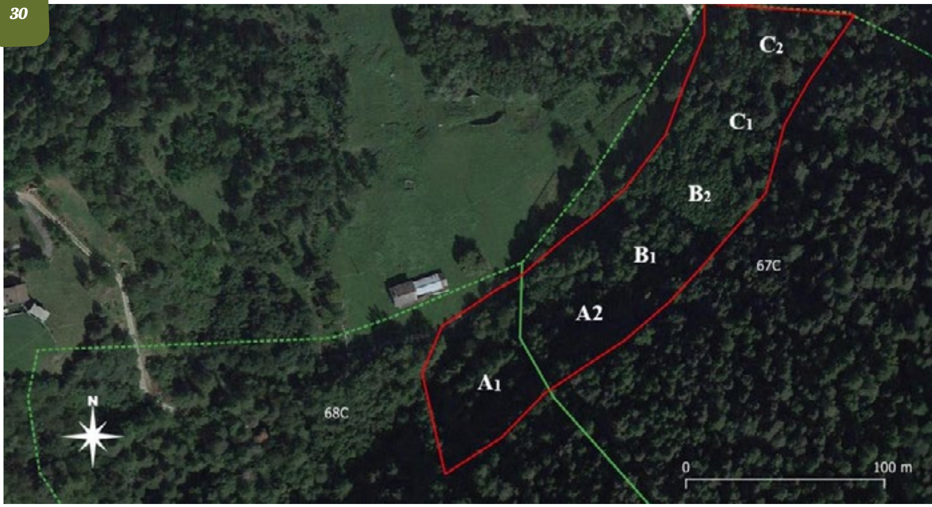
**29B**

Areas A, B and C were further divided into two sub-areas of equal size (approx. 2,500 m2) (**Figure 30**). In the first sub-areas (A1, B1 and C1, towards the SW) no further operations were conducted, while in the second (A2, B2 and C2, towards the NE) 9 vegetation-free zones (3 for each sub-area) of 5x5 m each were created. In each vegetation-free zone, 25 healthy ash seedlings were planted at a regular distance of 50 cm (artificial regeneration). The planting of the seedlings, as well as the other operations carried out in the site, were filmed by a professional reporter to produce and release thematic videos for the dissemination of the project results to forestry operators and stakeholders (**Figure 31**). The seedlings, approximately 50 cm in height, were taken from ash tree+ offspring obtained from other sites in Valle d'Aosta and supplied by a specialised nursery structure run by the Forestry Corps of the Autonomous Region of Valle d'Aosta.

***FIGURE 30***
*ZONING OF THE ASH VEGETATION BELT (RED LINE). IN EACH ZONE DIFFERENT SILVICULTURAL AND PHYTOSANITARY OPERATIONS WERE CONDUCTED. IN THE BACKGROUND, SATELLITE IMAGE OF AREA (SOURCE: GOOGLE MAPS, 2023).*

***FIGURE 31***
*PLANTING OF SEEDLINGS TO OBTAIN THE ARTIFICIAL REGENERATION OF ASH. AN OPERATOR MAKES AUDIO-VISUAL RECORDINGS OF THE OPERATIONS IN ORDER TO PRODUCE TECHNICAL AND INFORMATIVE THEMATIC VIDEOS.*

30




31

# CONCLUSIONS AND PERSPECTIVES

In the framework of the ***MONGEFITOFOR*** project, Italian and Swiss institutions and research bodies, supported by European Union funding - Territorial Cooperation Programme ***INTERREG*** V-A Italy-Switzerland 2014/2020, cooperated to monitor the health status of cross-border forests and designed sustainable strategies for their management and protection. In particular, this ***technical-scientific handbook*** is dedicated to the ***decline of ash trees associated with the dieback*** caused by the ***fungal pathogen Hymenoscyphus fraxineus.*** The handbook aims to summarise in a practical and concrete way the results obtained during the project, providing the user with ***silvicultural*** and ***phytosanitary guidelines for forest management***.

Thanks to the results obtained from the phytosanitary monitoring and the related diagnostic analyses performed in the laboratory, the presence of H. fraxineus was reported for the first time in the Aosta Valley. Moreover, the incidence and severity of the dieback affecting ash were assessed and quantified, and such results are of outmost relevance considering that ash is a forest tree species of great ecological, environmental, naturalistic importance. The decline of the species was widespread and characterised by a significant impact, and therefore it needs to be managed through a multidisciplinary approach that integrates traditional ***silviculture*** with ***plant pathology***. The key objective of the MONGEFITOFOR project was to outline this approach by creating suitable tools for the dissemination of ***knowledge*** and ***know-how*** relevant to the management of declining ash trees, for the benefit of owners, managers and administrators of forest resources, as well as for technicians, operators and for other stakeholders.

Looking forward, the MONGEFITOFOR project leaves the foundations for a more ***sustainable*** forest management of ash, providing technical and scientific tools whose application can contribute to increasing the ***resilience*** of the ***forest ecosystems***.

## ACKNOWLEDGEMENTS

This handbook has been produced in the framework of the activities of the ***MONGEFITOFOR*** project (Linee Guida per il **MON**itoraggio e la **G**estione delle **E**mergenze **FITO**sanitarie nelle **FOR**este delle Alpi centro-occidentali - Emerging Threats to Tree Health in Forests of the Central and Western Alps: Guidelines for Monitoring and Management - ID 540693), funded by the European Union through the Territorial Cooperation Programme INTERREG V-A Italy-Switzerland 2014/2020.

The Authors would like to thank Marianna Giraudo for her assistance in the laboratory, and the Agents, Officials and Personnel of the Forestry Corps of Valle d'Aosta who took part in the phytosanitary monitoring activities of ash, in particular Luigi Bionaz, Giacinta Bois, Gianluca Burgay, Alex Garda, Ettore Merlet, Corrado Montrosset and Alessandro Vuillermoz.

Special thanks go to the stakeholders that supported the MONGEFITOFOR project.


# ESSENTIAL BIBLIOGRAPHY


Bernetti, G. (1995). Selvicoltura speciale. Torino: UTET. ISBN 9788802048673

Bernetti, G. (2005). Atlante di selvicoltura. Dizionario illustrato di alberi e foreste. Bologna: Edagricole. ISBN 9788850646654

Camerano, P., Terzuolo, P.G., Varese, P. (2007). I tipi forestali della Valle d'Aosta. Arezzo: Compagnia delle Foreste.

Del Favero, R. (2004). I boschi delle regioni alpine italiane. Padova: CLEUP. ISBN 9788867870820

Ebone, A., Giannetti, F., Gonthier, P., Lione, G., Nicolotti, G., Petrella, F. Terzuolo, P.G. (2011). Querco-carpineti planiziali in deperimento: linee guida per la gestione. Regione Piemonte Direzione Opere Pubbliche, Difesa del suolo, Economia Montana e Foreste – Settore Politiche Forestali. ISBN 9788896046043

Ebone, A., Gonthier, P., Lione, G., Terzuolo, P.G. (2023). Scheda diagnostica di campo per la segnalazione dei sintomi di deperimento del frassino (Fraxinus excelsior) potenzialmente riconducibili al fungo patogeno Hymenoscyphus fraxineus. Regione Piemonte - Direzione Ambiente, Energia e Territorio – Settore Foreste. ISBN 9788896046098

Enderle, R., Stenlid, J., & Vasaitis, R. (2019). An overview of ash (Fraxinus spp.) and the ash dieback disease in Europe. CABI Reviews 14, 1-12. doi: 10.1079/PAVSNNR201914025

European and Mediterranean Plant Protection Organization EPPO (2013) PM 7/117 (1) Hymenoscyphus pseudoalbidus. Bulletin OEPP/EPPO Bulletin, 43, 449-461. Doi: 10.1111/epp.12061

Ferretti, F., Alberti, G., Badalamenti, E., Campagnaro, T., Corona, P., Garbarino, M., La Mantia, T., Malandra, F., Maresi, G., Morresi, D., Piermattei, A., Pividori, M., Romano, R., Salvadori, C., Sibona, E., da Silveira Bueno, R., Sitzia, T., Urbinati, C., Vitali, A., Pelleri, F. (2019). Boschi di neoformazione in Italia: approfondimenti conoscitivi e orientamenti gestionali. Rete Rurale Nazionale 2014-2020, Scheda n. 22.2 - Foreste, Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria. Roma. ISBN 9788833850153

Gellini, R., Grossoni, P. (1997). Botanica forestale. Vol. 2: Angiosperme. Padova: CEDAM. ISBN 9788813202606

Gonthier, P., Giordano, L., Sillo, F., Martinis, R., Pasi, V., Rettori, A.A., Tantardini, A. (2016). Sos cedri e frassini - Passaggio a Nord Ovest, Acer, 6, 25-29.
Google 2023. Google Satellite, Map data ©2015 Google, accesso effettuato in data 05/05/2023.

Kerr, G. & Cahalan, C. (2004). A review of site factors affecting the early growth of ash (Fraxinus excelsior L.). Forest Ecology and Management, 188, 225-234. doi 10.1016/j.foreco.2003.07.016

Lauber, K., Wagner, G., Gygax, A. (2001). Flora Helvetica. Bern: Haupt Verlag. ISBN 9783258077000

McKinney, L. V., Thomsen, I. M., Kjær, E. D., Nielsen, L. R. (2012). Genetic resistance to Hymenoscyphus pseudoalbidus limits fungal growth and symptom occurrence in Fraxinus excelsior. Forest Pathology, 42, 69-74. doi 10.1111/j.1439-0329.2011.00725.x

Ongaro, S., Lione, G., Isocrono, D. (2022). Composition and conservation value of epiphytic lichen communities on common ash in north-western Alps: a first assessment. Forests, 13, 1288. doi 10.3390/f13081288

Pignatti, S. (1998). I boschi d’Italia: sinecologia e biodiversità. Torino: UTET. ISBN 9788802052519

QGIS software versione 3.10.0 - A Coruña (2023). QGIS Development Team, QGIS Association. http://www.qgis.org.
QGIS plugin QuickMapServices versione 0.19.11.1. (2023).

Rigling, D., Hilfiker, S., Schöbel, C., Meier, F., Engesser, R., Scheidegger, C., Stofer, S., Senn-Irlet, B., Queloz, V. (2016). Le dépérissement des pousses du frêne. Biologie, symptômes et recommandations pour la gestion. Notice pour le praticien - Institut Fédéral de Recherches WSL - Birmensdorf, 57, 1-8.

Skovsgaard, J. P., Wilhelm, G. J., Thomsen, I. M., Metzler, B., Kirisits, T., Havrdová, L., Enderle, R., Dobrowolska, D., Cleary, M., Clark, J. (2017). Silvicultural strategies for Fraxinus excelsior in response to dieback caused by Hymenoscyphus fraxineus. Forestry 90, 455-472. doi: 10.1093/forestry/cpx012

November 2023
Printed by Tipolitografia Botalla s.r.l. - Gaglianico (BI) - Italy

This handbook has been produced in the framework of the activities of the MONGEFITOFOR project (Linee Guida per il MONitoraggio e la Gestione delle Emergenze FITOsanitarie nelle FOReste delle Alpi centro-occidentali - Emerging Threats to Tree Health in Forests of the Central and Western Alps: Guidelines for Monitoring and Management - ID 540693), funded by the European Union through the Territorial Cooperation Programme INTERREG V-A Italy-Switzerland 2014/2020.

Interreg
Fondo Europeo di Sviluppo Regionale
UNIONE EUROPEA
ITALIA SVIZZERA - ITALIE SUISSE - ITALIEN SCHWEIZ
MONGEFITOFOR

Région Autonome Vallée d'Aoste
Regione Autonoma Valle d'Aosta
DISAFA
Università degli studi di Torino
ti
WSL

PROGETTO GRAFICO: ANDREA DALLA FONTANA | ÉQUIPE ARC-EN-CIEL

ISBN
9791280561237