LIFE4FIR – Project LIFE18 NAT/IT/000164

"Decisive in situ and ex situ conservation strategies to secure the critically endangered Sicilian fir, *Abies nebrodensis*"

Report:
'Description of major potential diseases, pests and their antagonists, Action C1'

TABLE OF CONTENTS

1. Introduction

Scientific consensus and available evidence clearly indicate that natural and planted forests are experiencing severe challenges due to rapid climate change, severe weather events and increased pest and pathogen loads. In the framework of the Life4fir project various actions were planned to monitor the health state of both the natural population and natural regeneration of *Abies nebrodensis* and the plants raised in the local 'Piano Noce' forest nursery. Monitoring the health conditions through regular surveys is also aimed at evaluating the effect of the protection measures that will be implemented in the course of the project to the relic trees.

According to previous results described in the Report: 'Set up of protocols to investigate biotic and abiotic stresses of seedlings in the nursery' Action A1.3' (Annex 4 of the mid-term report) and in the Report: 'Map of initial *Abies nebrodensis* population and habitat, video hyperspectral inventory and health state, Action C1.4' (Annex 1 of the mid-term report), the most frequently disorders recorded both on adult plants and on seedlings were chlorosis, reddened needles, shoot blight and defoliation. Most of these symptoms reflect slight disease on plants and are mainly due to weak pathogens associated to environmental stresses which are common in the land site conditions site where the relic trees live. However, there are many factors influencing the balance of fungal/bacterial community on a small ecosystem. As regards *Abies nebrodensis*, gradual changes in climate, as well as climatic extremes such as drought, heat waves, hail, frost, strong light and injuries by wild herbivores may alter host-pathogen interactions, thereby promoting diseases caused by native or non-native pathogens or by previously harmless organisms (Desprez-Loustau et al., 2007). Another cause of emerging diseases is forest management and changes in land use which may alter the virulence of native pathogenic and endophytic species. For instance, degradation of forest ecosystems due to management intensification, irrational extensive grazing, excessive cutting of trees for timber, fires and erosion may contribute to increasing forest vulnerability to both native and non-native pathogens. Emerging diseases caused by native pathogens may also be enhanced by host-related factors such as a narrow genetic background, as in the case of *A. nebrodensis* (see results of Action A1.1).

For all these causes, the monitoring of the presence of potential disease agents can help in the future to cope with major constraints. This is of particular interest for the plants derived from the controlled crosses that will be planted in the reforestation plots and will represent re-diffusion cores of the species.

The purpose of this report is to compare the results on pests and pathogens encountered in the previous surveys with the existing literature, for possible emerging disease, and propose some good agricultural practices and solutions to maintain the healthy status of plants and seedlings, and to suggest possible interventions in case of future attacks.

2. Procedures

The project has planned to carry out phytopathological surveys on the natural population of *A. nebrodensis* to detect and monitor the occurrence of biotic or abiotic disorders and to eventually implement proper control measures (sub-action C1.4). In the natural population, most of the damages have been caused by herbivores in the basal part of the crown, or by weak pathogens causing shoot and twig blight favoured by environmental stresses. In the 'Piano Noce' nursery the most frequently recorded disorders were chlorosis and reddened needles (sub-action A1.3). Symptoms of "damping-off" often occurring in the nursery setting have not been observed and assays for *Phytophthora* disease were negative. Most adult trees showed disorders and symptoms in a limited portion of the crown (less than or equal to 15%), whilst few trees (namely the no. 28 and 31) were subjected to a greater general weakening that probably has favoured the action of opportunistic pathogens.

Among the biotic stress factors, we concentrated our assessment on fungal pathogens, according to preliminary surveys conducted in 2018 (when the project was being compiled) and according to results of the previous Life project (Conservation of *Abies nebrodensis* (Lojac) Mattei in situ and ex situ, LIFE00 NAT/IT/007228). For other biotic stresses, preliminary surveys did not show any particular symptom due to pests representing a threat for the *A. nebrodensis* trees. In this report the existing bibliography was consulted and compared to our assessment in loco.

3. Results

3.1 Fungi

Fungi (in a broad sense, including oomycetes) are the most frequent pathogens in forests and nurseries and are often characterized by complex life cycles. Fungal pathogens affecting the woody parts of trees (roots, stems, branches) usually persist for years in their host or in the rhizosphere (e.g. *Armillaria* species). Leaf fungi (e.g. rust or powdery mildew fungi), which have to re-infect leaves every year, may only persist in dormant buds, fallen leaves, and litter.

Because future climate extremes are expected to exacerbate the effects of biotic stresses on plants and further increase the virulence and diffusion of the existing parasites, here we report some of the fungal pathogens revealed during the first two years of survey, and their possible role as pathogenic agents, together with possible strategies for their management.

Molecular analysis of mycoflora associated with diseased needles in *A. nebrodensis* revealed that most of the fungal isolates found in the reddened or yellowed needles, belonged to taxa that have weak pathogenic activity, and to taxa that are usually saprophytes. Most of these fungi were found also in green needles sampled from the same *A. nebrodensis* trees, suggesting their endophytic behaviour. Endophytic fungi, however, often become pathogenic when their host suffers from other type of stresses (as for example *Alternaria alternata*).

In the diseased and healthy needles collected from *A. nebrodensis* relic trees in a recent survey conducted in 2020 within the Life4fir project (see the pertaining deliverable, Annex 1 to the Mid-term report, Action C1.4), almost the 80 % of the species identified by molecular analysis at IPSP-CNR were represented by two fungi: *Cytospora abietis* Sacc. (syn. C. pinastri Fr., anamorph of *Valsa abietis* (Fr.) Fr.) and *Rhizospaera macrospora* Gourb. & M. Morelet. (**TAB.1**)

taxon	No. of colonies		Frequency (%)	
	reddened needles	green needles	reddened needles	green needles
Cytospora abietis anam. of Valsa friesii	99	24	27.9	6.9
Grovesiella abieticola		1		0.3
Lachnellula caliciformis	4	9	1.1	2.6
Rhizosphaera macrospora	16	6	4.5	1.7
Phoma sp.	2	2	0.6	0.6
Penicillium murcianum	1	1	0.3	0.3
Apiognomonia errabunda	2		0.6	0.3
Tricharina sp.	2		0.6	
Crepatura ellipsospora	7		2.0	
Dothideomycetes	3	3	0.8	0.9
Phacidium sp.	1		0.3	
Coniothyrium dispersellum	2		0.6	
Phaeosphaeria sp.		3		0.9
Epicoccum nigrum	2		0.6	
Alternaria angustiovoidea		1		0.3
Other isolates	9	4	2.5	1.1

Tab. 1 Fungal taxa and their frequencies identified by molecular (ITS) and morphological characters on disordered and healthy needles of the natural population of *A. nebrodensis*

Cytospora spp. (the asexual form of *Valsa abietis*) are widely distributed and often occur as endophytes, saprobes or phytopathogens. They primarily cause canker and dieback diseases of woody host plants, leading to the growth weakness or death of host plants, thereby causing significant economic and ecological losses. Most of the times, they are reported as associated with blighted shoots and cankers of some conifers damaged by other biotic or abiotic stressors. In conifers, *Cytospora* canker commonly occurs in the lowermost branches of mature trees, and stops spreading to the trunk (Adams et al., 2005). Generally, in canker disease, *Cytospora* begins to infect through cracks and wounds in the bark. The wounds include pruning wounds, cold injuries, leaf scars, and branches with weakened shade. If perennial cankers originating from pruning wounds occur in places critical to the strength of the trees, they can be highly destructive (Biggs, 1989; Adams et al., 2006). Therefore, the occurrence of *Cytospora* canker and dieback diseases can be minimized by maintaining susceptible trees as strong as possible, and by removing dead and dying branches in the dry season. All unnecessary damage should be avoided. Moreover, the occurrence of *Cytospora* canker diseases is not only affected by the environment and distribution, but also by transmission (Fan et al., 2015b), which may act as potential inoculum sources for other hosts in natural and artificial environments.

In Europe, *Cytospora* canker was reported on fruit trees and deciduous trees, but not on conifers. Like many opportunistic pathogens, *Cytospora* sp. can colonize the host by establishing an endophytic relationship, i.e. without causing apparent damage. This behaviour was evidenced in the samplings carried out on *A. nebrodensis* in 2020, which allowed isolation of *C. abietis* also from green needles. Actually, *C. abietis* (syn. *C. pinastri*) has been reported as an endophyte in senescent needles of *A. alba* in Switzerland.

In a recent work of Pan et al. (2021), several *Cytospora* species reported on various hosts have been identified and classified, and new species have been found to pose a high risk of causing severe damage if exported to other suitable environments. No *Cytospora* species have been specifically reported on *A. nebrodensis*; however, due to the large host range in conifers, monitoring the crowns of the *A. nebrodensis* trees for cankers and other foliage disorders is recommended. The assessment of all *Cytospora* strains for their possible pathogenicity and aggressiveness in various environments is likewise very important.

According to EPPO (European and Mediterranean Plant Protection Organization) database, dieback of conifers is referred as *Cytospora abietis* as preferred name, and *Valsa abietis* as synonymous; the EPPO code for this pathogen is VALSAB.

Rhizospaera macrospora like many opportunistic pathogens including the above mentioned *Cytospora spp*., *Rhizospaera* sp. can colonize the host by establishing an endophytic relationship, i.e. without causing apparent damage. This behaviour was evidenced in the samplings carried out on *A. nebrodensis* in 2020, which allowed isolation of *Cytospora pini* and *R. macrospora* from both reddened and green needles, although the frequency of isolation in the latter needles was lower.

The genus *Rhizosphaera* has been considered as a little-known group of fungi associated with needle disease of conifers. A species of *Rhizosphaera*, *R. oudemansii*, was found associated with a needle cast of *Abies pinsapo* in Spain. Development of *R. oudemansii* in green needles of diseased branches, and the pycnidia production on yellowing needles also suggest a participation of this fungus in the *A. pinsapo* needle cast. The pathogenic character of *Rhizosphaera* species has not always well demonstrated. On *A. alba* needles, *R. oudemansii* was reported to behave as a specific primary saprophyte and occasionally as a weakness or opportunistic parasite. However, the fungus may act as a pathogen collaborating with other biotic or abiotic factors. Gourbiere & Morelet clarified the taxonomy of the genus and their cultural states and the ecology of *R. oudemansii* on *A. alba*. However, *Rhizosphera* sp. distribution is probably very little known since it has only been reported in The Netherlands, France, Germany and U.S.A. on needles of *A. alba, A. cephalonica, A. grandis, Picea glauca, P. omorica, Psudotsuga menziesii* and *Tsuga heterophylla.*

Both *Rhizosphaera* sp. and *Cytospora* sp., respectively associated with needle cast and cankers on various conifers, can act as weak pathogens, but they can also occur as endophytes. As regards *A. nebrodensis*, various weakening factors promoted the necrotrophic action of these two fungi: drought, high temperature and strong light during summer, late frosts, injuries due hail and to wild herbivores on twigs and branches.

Fig. 2. One week grown (in PDA plates) colonies of *Cytospora abietis* (left) and *Rhizospahera macrospora* (right), the two fungi most frequently isolated from reddened needles of *A. nebrodensis* (Life4fir, Action C1.4).

All the other fungal taxa were isolated from the needles with relatively low frequencies (from 3.6% to less than 1%). All are Ascomycetes (mostly in the asexual form), reported in bibliography as opportunistic (weak) pathogens, or saprophytes, or endophytes

Fungal taxa	**Percent of all obtained isolates**
Alternaria alternata	30
Cytospora sp. Anam. of *Valsa*	15
Allantophomopsis lycopicodina	11
Alternaria sp.	10
Coniothyrium aleuritis	9
Ceuthospora phacidioides	6
Pestalotiopsis funerea	5
Libertasomyces myopori	3
Dothydeomycetes sp.	3
Elytroderma deformans	3
Phacidium sp.	1
Foliophoma sp.	1
Diaporthe rudis	1
Angustimassarina sp.	1

Tab. 2. Relative frequencies of the fungal taxa isolated from reddened, blighted and chlorotic needles collected from the *A. nebrodensis* saplings raised in the Piano Noce nursery in 2020 (Life4fir, action A1.3)

Regarding the nursery, abiotic stress caused by environmental conditions or injuries can also predispose many growing seedlings to fungal attack. In the 'Vivaio Piano Noce' nursery we found that most of the fungal isolates obtained from disordered needles (affected by reddening and needle blight) were ascomycetes (mostly in the asexual form), reported in bibliography as saprophytes, opportunistic pathogens or endophytes (*Alternaria alternata*, *Cytospora* sp. (anam. of Valsa abietis), *Allanthomopsis lycopicodina*, *Alternaria sp*.,*Coniothyrium aleuritis*) (**TAB. 2**)

Alternaria spp. Fungi of the genus *Alternaria* are ubiquitous, occurring frequently in the organic matter and reported in numerous habitats and hosts, known for their saprophytic behaviour, although some species of *Alternaria* are weak pathogens. Many species are saprotrophs, plant pathogens or crop pathogens. Pathogenic *Alternaria* fungi cause diseases of various crop plants and are present on all continents. Already in 1960, Pomerlau and Nadeau named the fungi of *Alternaria* spp. as the cause of withering of 20-65% of seedlings of coniferous plants in nurseries. Likewise, James and Woo (1987) found that seedlings of conifers infected by *Alternaria alternata* withered away completely.

Among the other isolated fungi, *Allanthophomopsis lycopicodina* was reported from pine needles blighted by *Phytophtora* while *Coniothyrium aleuritis* (asexual form of *Dothideomycetes*) was reported as a leaf endophyte of conifers and as a pathogen on juniper.

3.1.1 Biological control:

To date, control of forest tree pathogens is mostly attempted through cultural practices and by using resistant plant material in commercial plantations. Direct chemical control of forest fungal pathogens has been widely applied in nurseries where impact of seedling diseases can be extremely high, and treatments can be easily carried out. In forests, the use of fungicides is less common and chemical control is only authorized for a limited number of pathogens.

Implementation of biological control measures is always problematic in forest ecosystems; emerging pathogens are particularly difficult to control because of the specificities of both the hosts and the pathogens.

A new frontier to successfully control forest pathogens is the application of locally selected biocontrol agents (BCAs) defined as a natural enemy, antagonist or competitor, or other organism used for pest control (International Standard for Phytosanitary Measures (ISPM) 3; FAO, 2006). The term 'biocontrol agent' means a beneficial microorganism capable of combating the attack of phytopathogenic organisms and, in the most modern definition, exert a beneficial effect on the host plant. The direct biocontrol action on plant pathogenic fungi is the result of mechanisms of mycoparasitism (formation of hyphal windings that kill the host), antibiosis (production of metabolites toxic to the pathogen) and competition (mainly nutrients).

To date, among the fungal biocontrol agents, *Trichoderma* spp. are being used most abundantly against plant pathogens. Several species of *Trichoderma* which produce volatile and non-volatile antibiotics and enzymes are antagonistic to phytopathogenic fungi and nematodes. *Trichoderma* spp. are free-living and abundantly present in the soil and rhizosphere and are mycoparasitic of several soilborne plant pathogens. It has also been exploited successfully as a biocontrol agent for controlling the foliar diseases of economically important plants. Strains of this fungus are effective against pathogens causing various diseases of the root region of plants, i.e. collar rot, foot rot, damping off, etc. In the rhizosphere, some strains of *Trichoderma* spp. release metabolites which improve the growth of seedlings, promoting resistance against abiotic stress. *Trichoderma* spp. have great potential against soilborne pathogens, and it may be able to hopefully replace chemical pesticides in the near future. For instance, the *Trichoderma* isolate T05, collected and isolated from a poplar (*Populus simoni* × *Populus nigra*) stand in the province of Heilongjiang, China, was tested for its efficacy in controlling *Cytospora chrysosperma* in vitro and *Cytospora* canker of poplar in the field. *Trichoderma* isolate T05 was identified as *Trichoderma longibrachiatum*. In a field trial, *Cytospora* canker on poplar trunks was controlled with efficacy similar to that of routine chemical control by applying a *T. longibrachiatum* preparation. Other *Trichoderma* isolates are currently commercialized for the control of *Cytospora* canker in peach orchards.

In conifers, records on biological control of pathogens are ascribed to the monitoring of seedlings in nurseries: for instance, several strains and species of *Trichoderma*, such as *T. viride, T. harzianum, T. longibrachiatum, T. polysporum*, and *T. atroviride*, have already been successfully applied for the control of *Fusarium circinatum* in Chilean and New Zealand forest nurseries. For this purpose, *Trichoderma* is produced by solid state fermentation (SSF) and inoculated into growing substrates and roots of *Pinus radiata* during the plant production process (Reglinski and Dick, 2005, Moraga-Suazo et al., 2011). Results have shown an improvement in the emergence and vitality of *P. radiata* seedlings when applied at the nursery stage. Specific trials should be conducted to evaluate the effect of *Trichoderma* on the vitality and growth of *A. nebrodensis* seedlings in the nursery.

3.2. Pests (insects)

Insect outbreaks are a recurrent natural phenomenon in forest ecosystems, expected to increase due to climate change. Individual tree detection is a necessary step towards identifying attacked trees and monitoring the spread of tree diseases in forest, as in the case of isolated and relic population of *A. nebrodensis*.

Several studies were conducted to study the reasons of the vulnerable status of the species, and visual inspection have been done for each single tree to record eventual threats.

Even though no serious insect-related threats seem to exist in the Mediterranean firs (including *A. nebrodensis*), they are usually damaged by *Cacoecia muriana*, *Chroristoneura murinana*, *Cryphalus piceae*, *Dioryctria aulloi*, *Ips vorontzowi*, *Mindarus abietinus*, *Phaenops cyanea*, *Pissodes picea*, *Pityokteines curviden*s, *Pityokteines spinidens* and *Xyloterus lineatus* (especially after dry periods). Seed insects of the genus *Megastigmus* are known to significantly reduce seed crops (Alizoti et al. 2011)

According to previous LIFE2000 NAT/IT/7228 project "Conservazione in situ ed ex situ di *Abies nebrodensis* (Lojac.) Mattei" (2002-2005), surveys on harmful insects on the relic trees of *A.*

nebrodensis revealed that only few damages have been attributed to insects. Two trees (namely tree no. 10 and 11) showed a slight drying of the young needles which slowed their growth. This symptom has been attributed to an attack by an aphid (*Mindarus abietinus* Koch, Hemiptera: Aphididae) that affect also other species of the genus *Abies* (*A. alba*, A. *nordmanniana*, A. *sibirica*, *A. concolo*r, etc.) causing some spots at the base the young needles, and their twisting and folding upwards. The aphid infestation, in some cases, was accompanied by the production of honeydew. Since this aphid was not particularly harmful and that attacks were substantially very sporadic and limited to few needles the implementation of control measures was not considered appropriate.
In recent surveys on plants (2020 and 2021) no signs of insect damages (honeydew, foliar waxes) have been found on the relic trees or in the seedlings and young plants raised in the Piano Noce forest nursery.
Considering that the balsam twig aphid *Mindarus abietinus* is reported as a major pest of economic importance for the Christmas tree industry and that the global warming during the 21st century could potentially increase local population densities and reinforce the pest status of this aphid in commercial fir, surveys should pay attention to this pest.
Because Sicily represents a place of great interest for the study of plants and animals, particularly for insects, some reports are available for the entomofauna. Among insects, scale insects (*Hemiptera*: *Coccomorpha*), are the most interesting due to the close connections with their host plants which they do not leave at all or almost at all for the duration of their life (MAZZEO et al., 2011). In Sicily there are 169 species of scale insects, which represent 42% of the Italian scale insects fauna. The species mostly belong to the families Diaspididae (43%), Pseudococcidae (20%) and Coccidae (17) (MAZZEO et al., 2011).
Three species of scale insects have been securely reported on *A. nebrodensis* relic population: *Dynaspidiotus abietis* (Schrank) (Diaspididae), *Nemolecanium graniforme* (Wunn) (GARCÍA MORALES et al., 2016), and *Parlatoria parlatoriae* (Mazzeo et al 2016).

- *Dynaspidiotus abietis* is a species of Nearctic and Palaearctic region. It has been recorded in many European countries and in the Mediterranean basin, infesting more than 26 species of plants which belong to the families: Aceraceae, Cupressaceae, Pinaceae and Rosaceae (Ben-Dov 2006). According to EPPO (European and Mediterranean Plant Protection Organization) Database, the code name is DYNAAB and is not listed on *A. nebrodensis*. According to literature, *Dynaspidiotus abietis* does not appear to be a serious pest of fir trees.

- *Nemolecanium graniforme*: This rare species is only present in seven European countries and always associated with host plants belonging to the family Pinaceae and the genus *Abies*. It has been found in *A. nebrodensis* only once in 1985.
- *Parlatoria parlatoriae* is listed among the scale insects that infest fir trees in Europe and has been associated with *Abies*, *Cedrus* and other conifers (Garcia Morales et al., 2016). This species is rare and has been recorded in Northern Italy on *Pinus nigra*. In 2016 it has been recorded in Sicily in Mount Scalone, Madonie Park, infesting the needles of *A. nebrodensis* trees.

3.2.1 Pest management

The artificial environment of the nursery, such as planting density, species or clone choice, and monoculture, can be favourable to pest development. To minimize damage, detecting and treating pests before they spread is essential. All the operations (from the cone scales removal to the transplanting of seedlings) have been done in the Piano Noce forest nursery, where it is essential to keep pest out of nursery. Together with the monitoring of potential fungal disease, pest prevention is highly recommended, as of course to take immediate action if pests are detected.

Here a list of good practices that can be used to prevent pest spread:

- making healthy stock and carefully monitoring the condition of seedlings and cuttings.
- If possible, keep new plant materials separate from the main growing area for a period of observation, to prevent bringing pests into the nursery.
- Provide the best possible growth conditions (e.g. nutrients, water, light, appropriate spacing and weed control) to raise healthy, vigorous and resistant plants.
- Test seed prior to planting to ensure good germination and seed health; and apply seed treatments, if needed.
- Keep new plant material isolated from main growing areas, where it can be monitored for pests without risk of them spreading to the whole nursery.
- Keep appropriate records that permit identification of sources of production material, and where it is grown and planted out, so that the source of any infestation/infection may be traced.
- Use soil or an inert growing medium that is free from insects, pathogens and weed seeds.
- Treat soil if necessary to kill pests before planting.
- Carry out regular monitoring to permit the early detection of pests.

- Use adhesive traps to detect the presence of insect pests and spore traps to detect fungal spores.
- Ensure irrigation water is free of pathogens and other contaminants such as pesticides, particularly if the water source is a pond, where water accumulates from infected or treated fields or is suspected to be contaminated. Simple filtration systems can be installed to sanitize infested water.
- Avoid leaving leaves wet, especially when watering at night, as this can allow pathogens to infect plants.
- Install screens or nets in plant production facilities to prevent insect entry or spread.
- Clean (thoroughly remove all soil and plant materials from all surfaces and crevices) and, if necessary, disinfect all tools, footwear and equipment before entering and before leaving the nursery area, especially if a pathogen is present.
- Clean and disinfect tools that are used for different operations within the nursery before and after use.
- Dispose of infested soil or growing media carefully so as not to contaminate new plants or soil.
- Collect and remove dead plants and debris every week to decrease the probability of infestation.
- Destroy or sanitize infested plant waste by burning, composting or treating with heat to kill the pest.

Once the plants are transplanted, or in the natural population, regular inspection to verify pest presence and impact are essential to monitor their health status and eventually plan the adoption of control measures.

3.3. Potential diseases (quarantine pathogens):

In the literature, few data are available for *A. nebrodensis* diseases, pests and pathogens. However, the European and Mediterranean Plant Protection Organization (EPPO), aiming at developing an international strategy against the introduction and spread of quarantine pests (including invasive alien plants) that damage cultivated and wild plants, has drawn up a list of quarantine species that could be referred as possible pathogens for *A. nebrodensis* (**Tab. 3**). Most of the fungal species listed, however, have been reported on other *Abies* species, and presumably cannot be considered of particular interest in the specific contest of *A. nebrodensis* in Sicily. For instance, the only securely reported pathogen on *A. nebrodensis* (referred as “major host” in Tab. 3) to be considered in the EPPO alert list is the fungus *Neonectria neomacrospora* (anamorph *Cylindrocarpon cylindroides*). The fungus *N. neomacrospora* has recently caused an epidemic outbreak in conifer species within the genus *Abies* in Denmark and Norway. The damages caused by *N. neomacrospora*, including dead shoot tips, cankers and dieback, red flagging of branches and potentially dead trees, have therefore caused concern about reduced quality and loss of trees in Christmas trees plantations. According to literature and EPPO database this fungus has been reported firstly in Norway and, from 2011 on, in other countries in North Europe. Field observations in experimental trials reported this fungus on firs (*Abies* spp.) in arboreta or Christmas trees plantations (Brodal et al. 2015). No direct observations of this pathogen have been reported in *A. nebrodensis* in its natural habitat, although it has been proven that the Madonie fir can be a host for this species by a study conducted on a clonal arboretum in Denmark (Brauner niellsen et al. 2017) and at the Bedgebury National Pinetum in England (Gonzalez et al. 2021). Probably the harsh and dry climate of the Madonie site is not conducive for the development and spread of this fungus.

TAB. 3 List of species that are of regulatory interest, possible host for *A. nebrodensis*, from EPPO Global Database (https://gd.eppo.int). The only “major host” is underlined. If known, tissue in which the species has been found is reported.

Species	*organism*	*type*	*tissue*
Chrysomyxa abietis (as Abies) (CHMYAB)	fungi	HOST	
Coniferiporia weirii (as Pinaceae) (INONWE)	fungi	HOST	non-squared wood
Gremmeniella abietina (as Abies) (GREMAB)	fungi	HOST	plants for planting
Heterobasidion irregulare (as Abies) (HETEIR)	fungi	HOST	squared wood
Melampsora medusae (as Abies) (MELMME)	fungi	EXPERIMENTAL	plants for planting
Melampsora medusae f. sp. deltoidis (as Abies) (MELMMD)	fungi	EXPERIMENTAL	cut flowers or branches
Neonectria neomacrospora (NECTMA)	<u>fungi</u>	<u>MAJOR HOST</u>	
Phacidium coniferarum (as Abies) (POTECO)	fungi	HOST	
Phytophthora cinnamomi (as Pinaceae) (PHYTCN)	fungi	HOST	
Acleris gloverana (as Abies) (ACLRGL)	animalia - insect	HOST	cut flowers or branches
Acleris variana (as Abies) (ACLRVA)	animalia - insect	HOST	cut flowers or branches

Species	***organism***	***type***	***tissue***
Chionaspis pinifoliae (as Abies) (PHECPI)	animalia - insect	HOST	
Choristoneura freemani (as Abies) (ARCHOC)	animalia - insect	HOST	
Choristoneura fumiferana (as Abies) (CHONFU)	animalia - insect	HOST	cut flowers or branches
Crisicoccus pini (as Abies) (DACLPI)	animalia - insect	HOST	
Dendrolimus sibiricus (as Abies) (DENDSI)	animalia - insect	HOST	cut flowers or branches
Dendrolimus spectabilis (as Abies) (DENDSC)	animalia - insect	HOST	
Dendrolimus superans (as Abies) (DENDSU)	animalia - insect	HOST	non-squared wood
Dryocoetes confusus (as Abies) (DRYOCN)	animalia - insect	HOST	cut flowers or branches
Gnathotrichus sulcatus (as Pinaceae) (GNAHSU)	animalia - insect	HOST	non-squared wood
Ips amitinus (as Abies) (IPSXAM)	animalia - insect	HOST	non-squared wood
Ips subelongatus (as Abies) (IPSXFA)	animalia - insect	HOST	
Leptoglossus occidentalis (as Abies) (LEPLOC)	animalia - insect	HOST	
Malacosoma disstria (as Abies) (MALADI)	animalia - insect	HOST	
Monochamus alternatus (as Abies) (MONCAL)	animalia - insect	HOST	
Monochamus impluviatus (as Abies) (MONCIM)	animalia - insect	HOST	
Monochamus marmorator (as Abies) (MONCMR)	animalia - insect	HOST	
Monochamus obtusus (as Abies) (MONCOB)	animalia - insect	HOST	
Monochamus saltuarius (as Abies) (MONCSL)	animalia - insect	HOST	
Monochamus scutellatus (as Abies) (MONCST)	animalia - insect	HOST	
Monochamus sutor (as Abies) (MONCSU)	animalia - insect	HOST	
Monochamus titillator (as Abies) (MONCTI)	animalia - insect	HOST	
Monochamus urussovi (as Abies) (MONCUR)	animalia - insect	HOST	
Orgyia leucostigma (as Abies) (HEMELE)	animalia - insect	HOST	
Polygraphus proximus (as Abies) (POLGPR)	animalia - insect	HOST	cut flowers or branches
Sirex ermak (as Abies) (SIRXER)	animalia - insect	HOST	
Sirex noctilio (as Abies) (SIRXNO)	animalia - insect	HOST	
Tetropium gracilicorne (as Abies) (TETOGR)	animalia - insect	HOST	plants for planting
Trichoferus campestris (as Abies) (HESOCA)	animalia - insect	HOST	

All insects and pathogens here listed have been found mainly in other geographical regions and/or on *A. alba* species; hence we assume that none of them can be currently found on *A. nebrodensis* relic population. Because forest pests can move between countries through many different pathways (including importation of logs, chips, solid wood packaging and propagative units), this list could be helpful in case of future introduction in Sicily of these insects.

4. Conclusions

Among the actions supporting the natural population of *A. nebrodensis*, the regular monitoring of the growth and the state of health of the relic trees is of particular importance. These, in fact, grow in very limiting pedo-climatic conditions, and in many cases they are isolated and exposed to bad weather. Abiotic stresses such as drought, high temperatures, high light intensity, late frosts, hail, wounds caused by the rubbing of the branches due to the wind and wild herbivores are constantly occurring. In addition, the effect of climate change further exacerbates the environmental stress on plants.

Despite the hard environment where the relic trees of the *A. nebrodensis* population live, their health state resulting from the recent surveys was not particularly poor. Most of the surveyed trees (26 out of 30) showed disorders on a relatively small portion of the crown, not exceeding 15%. On most trees, the observed symptoms were located in the lower part of the crown and are represented by branches and twigs drying out, needles reddening and branch defoliation due to environmental stresses to which the trees are subjected. Laboratory analysis and fungal isolations allowed to exclude the involvement of aggressive pathogens as the cause of the observed disorders. All isolated fungi are classified as weak pathogens, endophytes or saprophytes, whose development in the plant is associated with environmental disturbances to which the trees are subjected in their natural habitat. Based on the surveys carried out, the relict trees seem in a situation of substantial equilibrium with the surrounding environment. However, the worsening of environmental conditions could alter this equilibrium causing a weakening of the plants and favoring the action of opportunistic pathogens.

Even in the Piano Noce nursery there was a low incidence of disorders and mortality overall. The results of the observations, currently exclude the need to implement measures for the control pathogens, both in natural populations and in the nursery. However, regular monitoring appears as an important action to implement prompt measures against emerging diseases or in the case of exacerbation of the symptoms already reported.

Also, among insects, an aphid and some species of scale insects have been reported on the crowns of *A. nebrodensis* in the natural population in previous surveys (before the Life4fir project), but the observed disorders were extremely sporadic and of non-significant impact. However, considering that some of these insects, such as the aphid *M. abietinus*, can cause significant damage in some conditions and that global warming can favor the increase of populations, it should not be neglected in monitoring.

The use of Bioncontrol agents (BCAs) is promising for the sustainable control of pest and pathogens. Some *Trichoderma* isolates could usefully be tested in the nursery to evaluate their effect on the growth of seedlings and on their resistance to abiotic stress. Their use on plants of the natural population appears to be problematic, due to technical difficulties in the treatments and to regulatory restrictions related to the introduction of alien microorganisms, unless using locally selected isolates.

The EPPO reports *A. nebrodensis* as a potential host of a few quarantine insects and pathogens. In fact, they have been reported in some European regions on other fir species, or in nursery setting (i.e. production of Christmas trees), or in orchards or in experimental conditions. Although it is difficult to say how likely their real danger to the natural population of *A. nebrodensis* may be, it is a good practice to be aware of them for their rapid monitoring in case of introduction into Sicily.

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