



**LIFE  
4FIR**

**LAYMAN'S REPORT**  
**LIFE4FIR project**



LIFE18 NAT/IT/000164  
01/08/2019 - 31/07/2023

LIFE4FIR is a project co-financed with the contribution of the LIFE financial instrument of the European Union



Scattered *Abies nebrodensis* trees in their natural habitat inside the Madonna degli Angeli Valley



*Abies nebrodensis* tree in its natural habitat in the Madonie Regional Park inside the Vallone Madonna degli Angeli



Plantlets of the *Abies nebrodensis* natural regeneration

## THE LIFE4FIR PROJECT

The LIFE4FIR project had the general objective of developing and implementing in situ and ex situ strategies for the conservation and safeguard of *Abies nebrodensis*, an endemic species of the Madonie Park, located in the central-northern part of Sicily.

The population of the Madonie Fir is in fact made up of only 30 adult trees, distributed in an 84 hectares area within the Park and is classified as critically endangered and is included in the IUCN red list. The LIFE4FIR project has implemented a series of actions to counter the main threats affecting *A. nebrodensis*, improve its conservation status and reduce its risk of extinction, promote knowledge and awareness of environmental issues and develop a replicable model of good practices.

## A. NEBRODENSIS VULNERABILITY AND THREATS FACED

A series of threats makes this species highly vulnerable:

### Severe genetic erosion and fragmentation

The extremely small number of fertile trees and their fragmentation favours self-fertilization (autogamy). The decreased genetic variability can lead to a reduced ability of populations to adapt to the new challenges proposed by their environment, such as infectious diseases or climate change.

### The poor natural regeneration

Due to the largely rocky and superficial soils, the irregular flowering and the seedlings largely derived from self-fertilization, natural regeneration is very sparse and develop slowly.

### The uncontrolled wild herbivores

Fallow deer and wild boar populations have reached unsustainable levels and are difficult to control, causing damage to both adult plants and natural regeneration.

### Risk of hybridization with non-native firs

The presence of plantations of non-native firs, such as *A. alba* and *A. cephalonica*, set up in the past in the Park territory, represents a risk for the genetic integrity of *A. nebrodensis*, since the *Abies* species are interfertile and can give rise to introgression phenomena (hybridization) following the fertilization of the female cones with pollen of non-native firs.

### Reduced germination and survival of seedlings in nurseries

Open-pollinated seeds show a low germination rate due to the high percentage of empty seeds. Moreover, the resulting seedlings show a high mortality in the first few years, due to their slow growth and high vulnerability. It is therefore very laborious to obtain new seedlings to use for reforestation.

## THE OBJECTIVES OF THE LIFE4FIR PROJECT

- General objective has been to address the main threats affecting *A. nebrodensis* and improve its conservation status through the implementation of a series of in situ and ex situ measures.
- Support and protect the population of the relic adult trees and the natural regeneration in their natural habitat with the installation of a new system of fences against wild herbivores and human pressure, a video surveillance system and monitoring the health status of plants through phytosanitary surveys and multispectral analyses.



The new fence installed around the *Abies nebrodensis* trees

- Increase the genetic diversity of the progenies by promoting cross-pollination between the fertile adult trees and encouraging exogamy. Raise new selected seedlings in the nursery and use them for reforestation in suited areas to create new re-diffusion nuclei and restore the dynamic structure of the population.
- Ensure the conservation of the species also through ex-situ measures based on the creation of a clonal orchard and the launch of a seed bank and a cryobank.
- Promote the measures implemented for the conservation of *A. nebrodensis*, ensuring their sustainability and replication in the years to come, increase awareness and sensitivity towards environmental issues.

## RESULTS

### Protection of the natural population

A new fence system has been set up to protect the residual population of *A. nebrodensis*.

The LIFE4FIR Project planned the extension and strengthening of the fences around the *A. nebrodensis* trees to meet two basic needs: 1) most of the current fences showed signs of deterioration and were damaged, having lost much of their functionality; 2) seedlings of the natural regeneration were growing outside the perimeters of the old fences and needed adequate protection against wild herbivores and grazing. So, a new fence system has been installed to better protect the adult trees and the natural regeneration on a broader surface.

The new fences are 2m high and were made with 2.40 m long chestnut poles with a top diameter of no less than 7 cm, caulked for the lower 60 cm portion with cold tar. Overall, 1,800 chestnut poles, 2m spaced, 5,000 kg of galvanized iron wire, 3,750 m of wire mesh were used. Each fence is equipped with a 1.5 m wide gate entrance.

Overall, the total perimeter of the new fences amounts to 2,167 m, while the protected area covers more than 14,000 m<sup>2</sup>.



Female cones were protected with special bags for controlled crosses



Needle reddening and twig blight on the crown of the *Abies nebrodensis* tree due to environmental stress



A young female cone with pollen grains on its surface



Male cones just before their opening and pollen release

#### Installation of a video surveillance system

A video surveillance system has been installed as a deterrent and to control wildlife and abandoned livestock. The video surveillance system is based on 5 self-powered stations which were positioned in key points of the park in key sites of the *A. nebrodensis* range.

Through an LTE/4G router, the acquired images can be transmitted to cell phones via SIM cards. The images can also be saved in local SD memories. The system was then upgraded to allow the real time transmission of the images acquired via satellite.

The video surveillance points have been positioned in the places more frequently visited: at the entrance gate to the natural population area of *A. nebrodensis*; at the beginning of the path that runs through the Madonna degli Angeli Valley; near the trees #22 and #30, the first reached by the path.

#### *A. nebrodensis* health state monitoring

Monitoring the state of health of the natural population provided useful knowledge about the ongoing disorders and assisted in managing proper protection and conservation measures.

Biotic and abiotic stresses were monitored through visual inspections of the crowns and analysis of the fungal microorganisms involved. The acquired data were integrated with multispectral surveys (remote sensing). Surveys excluded the presence of aggressive pathogens. The trees showed a substantially good state of health, reporting only localized symptoms of needle reddening, needle cast and twig blight. These are due to environmental stresses and the involvement of opportunistic pathogens, indicating that the species tolerates conditions currently occurring in its habitat. Remote sensing highlighted a correspondence between the NDVI index which reports the state of the forest or single trees and the data obtained with visual inspections of the canopies.

Multispectral techniques provided reliable indications on the health state of the crowns of the *A. nebrodensis* trees and represent a promising tool for monitoring the physiological conditions of the population over time in relation to environmental stresses and climate change.

#### Increasing genetic variability of *Abies nebrodensis*

The main issue in the conservation of threatened small populations is that they have undergone a significant genetic erosion. Genotyping based on 120 SNPs was used to assess the genetic diversity and structure of the *A. nebrodensis* population. The Effective population size, a key parameter in population genetics to estimate the number of individuals that effectively contributes offspring to the next generation, was found to be as low as 6 and paternity tests revealed that the natural regeneration was affected by a rate self-fertilization exceeding 90%. To increase genetic diversity of the progeny, outbreeding was promoted by conducting controlled crosses among the 24 fertile plants of the population. Numerous cross combinations were carried out in 2020 and 2022 respectively, resulting in over 800 cones and 100,000 seeds obtained. Despite the high percentage of empty seeds and a significant seedling mortality rate, more than 4,000 selected seedlings were successfully obtained. These seedlings underwent mycorrhization, control of pathogens, and have been raised in the nursery following enhanced cultivation practices to prepare them for being outplanted in the new sites.

#### Raising of vigorous seedlings for reforestation

Production of vigorous seedlings for reforestation purposes has been pivotal to improve the conservation of *A. nebrodensis*. Seedlings derived from controlled crosses were raised in the nursery following optimized practices based on use of seed trays for germination and a standardized



A plantlet growing after being planted out



Seed samples conserved in the seedbank at -18°C



Inserting samples into the dewar for conservation in liquid nitrogen



A grafted plant with new shoots

substrate with adequate physical, chemical and organic features, selection of full seeds, control of pathogens, adequate watering, mycorrhization. This led to obtain a vigorous stock of 4,000 selected plants to be outplanted in the new repopulation nuclei.

#### Establishment of new reforestation plots

The genetically selected seedlings were used to create repopulation and re-diffusion nuclei, established in 10 areas of the park between 750 and 1,610 m above sea level. The new plots were obtained taking into account the ecological characteristics of *A. nebrodensis*, preferring sites with north, north-east exposure, under the cover of existing tree canopy and with the help of local brooms planted at the same time, so as to ensure adequate support and protection for the young fir seedlings. The new plots have previously fenced, to protect them from wild herbivores. A total of 3,500 seedlings were planted, applying shelters as an additional form of protection.

#### Ex-situ conservation measures

In addition to in-situ actions, the consortium has implemented a series of activities for the ex-situ conservation of *A. nebrodensis* germplasm. The seed bank and cryobank are running at the *Abies Nebrodensis Museum (MAN)* in Polizzi Generosa, and the clonal orchard has been established at the Piano Noce regional nursery.

To achieve the seedbank, protocols were developed for the selection of viable seeds using an X-ray procedure and conservation at -18°C. The cryopreservation of the germplasm required the development of procedures that guaranteed the long-term viability of pollen, zygotic embryos and embryogenic callus lines after immersion in liquid nitrogen at -196°C.

The clonal orchard is a collection of germplasm, but also a facility for the future production of improved seeds and has been set up thanks to an innovative grafting procedure that allowed the propagation of all 30 plants of the natural population.

#### Transfer of knowledge and experience gained

Workshops and replication and training events allowed to directly involve the most relevant stakeholders for a future replication and transfer of the proposed solutions, including authorities, policy makers, professionals in the field of nature and biodiversity, managers of protected areas and representatives of institutions, the scientific community.

#### Replication events

- 1<sup>st</sup> Replication event in Ronda (Spain), held the 7 November 2022.
- 2<sup>nd</sup> Replication event in Sicily, held the 18 May 2023.

#### Workshops and seminars

- 1<sup>st</sup> Italian Workshop on 'Protection of biodiversity' organized the 17 November 2021 in Castelbuono (Sicily), where local bodies and communities were involved.
- 2<sup>nd</sup> Italian Workshop on 'ex situ conservation strategies' the 17 May 2022 in Palermo.
- 3<sup>rd</sup> Spanish Workshop at the Faculty of Biology of the University of Seville held on 10 November 2022 'Innovative strategies for in situ and ex situ conservation of *Abies*'.
- 4<sup>th</sup> Italian Workshop on "Il monitoraggio fitosanitario e la conservazione in situ di *Abies nebrodensis*"; Palermo Botanic Garden, 28 November 2023.
- Online seminar on 'Measures to counteract genetic erosion and endogamy in endangered species: the *Abies nebrodensis* case study' held in May 29th 2024 held by for the formation



Visiting the planting sites of LIFE4FIR



One of the LIFE4FIR training events

of PhD students, research fellows and post-doc.

#### Technical courses

- Technical course on Updated procedures of propagation by seed and grafting of *A. nebrodensis* held in Sicily the 18 November 2021.
- Training course held the 2 June 2022 on *A. nebrodensis* disorders and threats for local people, environmental guards and technicians of public institutions.

In the course of the project a Best Practice Handbook, a Manual and an E-manual to correctly use the developed tools for in situ and ex situ conservation of the Madonie fir have been produced and spread.

#### Communication and disclosure

All the LIFE4FIR project partners contributed to communication, through online dissemination, organization of open days and guided visits, participation in fairs, with the aim of raising awareness among local communities and general public on the issues of nature and biodiversity conservation and on the Life program. More than 1,000 students from various parts of Sicily took part in visits to the in situ population.

The LIFE4FIR website and the social pages of the project are running. Updates of technical activities and results are here reported along with dissemination material produced: brochures, posters, Best Practice Handbook, Manual, E-manual, video and footages, general articles related to the project and scientific papers published. The dissemination material was also produced on physical supports and distributed together with the LIFE4FIR gadgets during participation to events, fairs, visits.

The beneficiaries also attended national and international conferences related to biodiversity conservation, plant translocation, biotechnology, genetics, bioengineering, botany, didactics.

### CONCLUSIONS

The LIFE4FIR project has certainly had an impact on multiple levels:

- On a technical level, it has demonstrated the validity of the solutions proposed for the protection of the endangered species *Abies nebrodensis*. The techniques and tools developed can easily be replicated and adapted for the protection of other threatened species, supporting the biodiversity conservation;
- On a social level, the project has contributed to increase awareness and knowledge of environmental issues and measures to support biodiversity funded by the European Union. It has also implemented procedures to replicate the proposed solutions, making knowledge and experience gained during the project available to the scientific community and those involved in the forest management and protection.
- On the legislative level, during the implementation of the project, new plans for monitoring, controlling and capturing the populations of fallow deer and wild boar, including the integral protection zone A of the Park where *A. nebrodensis* lives, were authorised by the Regional Department.

An After-LIFE plan has been prepared to depict the activities to carry out and continue after the end of LIFE4FIR. Dissemination and the transfer of knowledge and experience gained with the implementation of the project will continue, thanks to the activities of institutions and the participation in national and international events.



## LIFE4FIR'S RESULTS AT A GLANCE

3,6 ha	Reforested areas
4000	Outbred seedlings raised for reforestation and outplanted
500	Grafted plants from all the 30 <i>A. nebrodensis</i> trees
76%	Increment of the natural regeneration
300	People involved in training courses and workshops
200	People interviewed about the nature and biodiversity perception and the impacts of the project
>1000	Students visiting the <i>A. nebrodensis</i> natural site

## THE INVOLVED AREA: MADONIE REGIONAL PARK



Consiglio Nazionale delle Ricerche  
Istituto per la BioEconomia  
Dipartimento di Scienze Bio Agroalimentari



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