



LIFE4FIR – Project LIFE18 NAT/IT/000164

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

**“Report and map of the newly constituted clonal orchard”
Action C3.**

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REPUBBLICA ITALIANA

REGIONE SICILIANA
ASSESSORATO REGIONALE
DELL'AGRICOLTURA, DELLO SVILUPPO RURALE
E DELLA PESCA MEDITERRANEA


Parco delle Madonie


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1. Introduction

The clonal orchard is a specialized plantation created through the vegetative propagation of the 30 trees of the natural population of *Abies nebrodensis*. This structure is intended not only as a simple collection of germplasm, but also as a plantation assigned to the future production of improved seeds, characterized by greater genetic variability, as cross-fertilization between the different genotypes is promoted, as the plants are placed one close to the other.

The clonal orchard will also allow constant monitoring and study of individual genotypes with regards to growth, habit, phenology, etc., for scientific and educational purposes. In the future, when the plants reach maturity, the orchard will be used as a new source from which seeds or other propagation material can be collected, avoiding negative impacts on the natural population.

As first step, for establishing the clonal orchard, each single genotype of the natural population of *A. nebrodensis* was propagated vegetatively with the grafting technique.

Graftings planned for April 2020 and 2021 were not allowed by the Covid pandemic, so they shifted to April 2022: 469 graftings done; April 2023: 850 graftings done and April 2024: 295 graftings done.

2. Grafting propagation of *A. nebrodensis* trees.

Many species of conifers do not respond to cuttings or micropropagation, which give limited results, not sufficient to guarantee a suitable number of plants for reforestation purposes. In all these cases (including *Abies nebrodensis*) it is necessary to follow grafting propagation. The grafting technique that has been used in this Project is called “veneer-side grafting”, by far the most used in the nurseries for the grafting propagation of conifers.

2.1 A grafting protocol optimized for *A. nebrodensis*.

A Course was organized in November 2021, at the ‘Piano Noce’ forest nursery to train technicians and workers on the procedures of the proper grafting technique to follow for the propagation of *Abies nebrodensis*. The leaflet prepared for this event is reported in Fig. 1. The specific procedure is below summarized.

- Two weeks before grafting, 4-5 years-old, seed rootstocks (*A. nebrodensis* pot plants) were transferred to the greenhouse to force vegetative activity and root growth. The substrates were moistened, preventing them from being too wet. The lower 7-10 cm of the plant stems were kept clean by cutting any branches, needles and soil eventually present.

- The scions were collected at the beginning of April, when plants were still in winter rests, as soon as climatic conditions were suited depending on the presence of snow (Fig. 1). However, at the time of harvesting the plants still had closed buds.
- Scions were kept at 4°C in a refrigerated room. Grafting was carried out within three days after they had been collected. Rootstocks, scions and equipment were assembled in a comfortable workstation in the nursery. A team of 6 people (experienced grafters, plus helpers) was organized. The grafting knives were prepared extremely sharp and clean.
- The collected scions were terminal shoots, mainly taken from the lower third of the tree. The scions for grafting (10-15 cm long) were prepared by removing any needles in the lower half.
- All cuts on the scion and rootstock were made in one smooth motion. This yielded the best surface for mating the scion with the rootstock. The cut on the rootstock was as close as possible to the width of the scions, while still penetrating the bark of the rootstock.
- A downward, one angled cut was then made with the end of the scion to create a flap. The length of the cut was equal to the length of the cut made on the rootstock.
- The scion was then inserted into the “pocket”, created in the basal cut part of the rootstock; the side of the scion aligned with the cut surface of the rootstock. When the grafting was done properly, the scion remained perfectly inserted in the rootstock pocket, with a perfect alignment of the cut surfaces.
- The scions were then tied with a rubber strip to tighten the graft; the wrapped area of started and ended above and below the cuts. The grafted area was then covered with aluminium foil to prevent excessive drying (Fig. 2).
- As conifers require high humidity while the scion is healing, the grafted plants were covered with a transparent plastic bag.
- The grafted plants were moved back to the greenhouse, after cutting the upper part of the rootstock. The soil in the pots was periodically moistened, avoiding dripping. Particular attention was paid to avoiding dehydration of the soil, as this is a critical moment for the success of the grafts.
- Plants were exposed to sunlight, but direct and intense solar radiation was avoided.
- After 4-5 weeks, the plastic bag was removed and another third of the rootstock was cut, just above the insertion of the scion.
- Between mid and late summer, the elastic strip was removed to prevent it from excessively compressing the stem at the junction point (Fig. 2). The grafted plants were then moved outside to a shaded area of the nursery.

2.2 Results of the implemented grafting procedure

A survey carried out 4 months after grafting, showed that on average over 50% of the grafts were still alive, a result of absolute excellence for a species like *A. nebrodensis*.



Fig. 1. Leaflet of the course held in November 2021 by Life4fir, dedicated to grafting propagation of *Abies nebrodensis* (left); collecting scions from *A. nebrodensis* trees (right).



Fig. 2. Grafts with aluminium foil protecting the healing area from drying (left); a graft completely healed (right).

In November 2024, at the end of the project, 454 grafts were available for setting up the clonal orchard. Results for each mother tree are reported in Table 1 as number of obtained grafts and success rate (compared to the total number of grafts carried out).

ID Mother Tree	No. Total grafts carried out	No. Living grafts	Success (%)	No. grafts in the orchard
1	26	9	34,62%	8
2	82	15	18,29%	12
4	51	9	17,65%	8
6	64	22	34,38%	14
7	87	21	24,14%	14
8	80	17	21,25%	15
9	62	18	29,03%	14
10	92	11	11,96%	9
11	95	13	13,68%	11
12	83	24	28,92%	15
13	86	23	26,74%	14
14	61	27	44,26%	14
15	38	15	39,47%	12
16	71	18	25,35%	15
17	43	26	60,47%	17
18	49	14	28,57%	11
19	51	23	45,10%	14
20	23	12	52,17%	12
21	63	33	52,38%	15
22	66	17	25,76%	12
23	54	5	9,26%	4
24	28	8	25,00%	8
25	41	13	31,71%	8
26	35	16	45,71%	18
27	90	15	16,67%	14
28	28	4	14,29%	4
29	22	14	63,64%	12
30	21	4	19,05%	2
32	22	9	40,91%	7
tot	1614	454	31,05%	333

Table 1. Grafts carried out over three years (2022-2024), number of grafts alive in November 2024 (available for planting the clonal orchard) and success in percent, recorded for each mother tree.

3. Setting up the clonal orchard

The clonal orchard has been set up inside the ‘Piano Noce’ nursery in December 2024, a few weeks before the end of the project. Initially, the space dedicated to hosting the orchard had been identified next to the nursery. The space had been fenced for this purpose already a year before carrying out the planting. However, due to frequent damage to the fence caused by wild herbivores (especially fallow deer), it was decided to set up the plantation inside the nursery which is better protected by a sturdy fence. The area hosting the orchard inside the nursery is characterized by a rather uniform, but not too dense, tree cover of a mature stand of black pine (*Pinus nigra*). The area is located at 1043 m a.s.l. elevation, 37°50’43.59” N, 13°59’50.18” E, and at a distance of 2270 m from the main nucleus of the natural population of *Abies nebrodensis* in the Vallone Madonna degli Angeli. To obtain a good number of grafted plants, it was necessary to carry out three grafting campaigns, from 2022 to 2024. These allowed the missing or poorly represented mother plants to be propagated again in the next year. Planting in late autumn was considered more suitable for grafts, so as to avoid the hot and dry months of summer after being planted out.

In the same month of December, the position of the seedlings in the orchard was georeferenced using a GPS device. Each plant was catalogued with a progressive number, separately for each mother plant, recording its coordinates. In the right column of Table 1 it is reported the number of grafts planted out in the orchard per each mother tree.

For some mother trees, the number of grafts present in the orchard is lower than the 12 planned in the project. For some mother trees (Id. 1, 4, 10, 23, 24, 28, 30, 32) the number of grafted plants obtained was lower than expected. This was probably due to the lower biological quality of the scions taken from more stressed crowns. With the redundant grafts of other mother trees, it would have been possible to compensate for this discrepancy and reach the 360 grafts in the orchard (454 grafts are available). However, we believe that in the after-Life phase, by integrating the missing grafts with new graftings, in 2-3 years it will be possible to extend the clonal orchard by adding a new plot where all 30 genotypes of *A. nebrodensis* are evenly represented.

The presence in the orchard of grafts of some mother trees such as 28, 32, 23, 24, 25 is noteworthy and has a particular significance for the conservation of the *A. nebrodensis* germplasm. Among all the trees of the natural population, the aforementioned trees are the most exposed to the harsh environmental conditions that characterize the habitat of the species. The 28 and 32, in particular, show a bushy habit and their health state is poor.

Post planting cultivation cares will be essential to promote the growth and settlement of grafted plants. In the after-Life phase, regular irrigation (at least in the first two years), fertilization and manuring, weeding, replacement of failures have been planned to guarantee the success of the

plantation. The grafted plants must be supported with stakes for the first two years to favor a straight trunk.

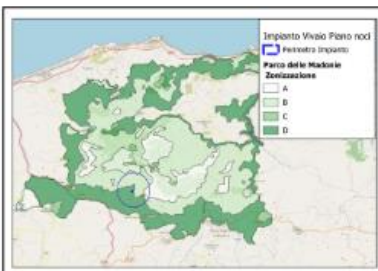
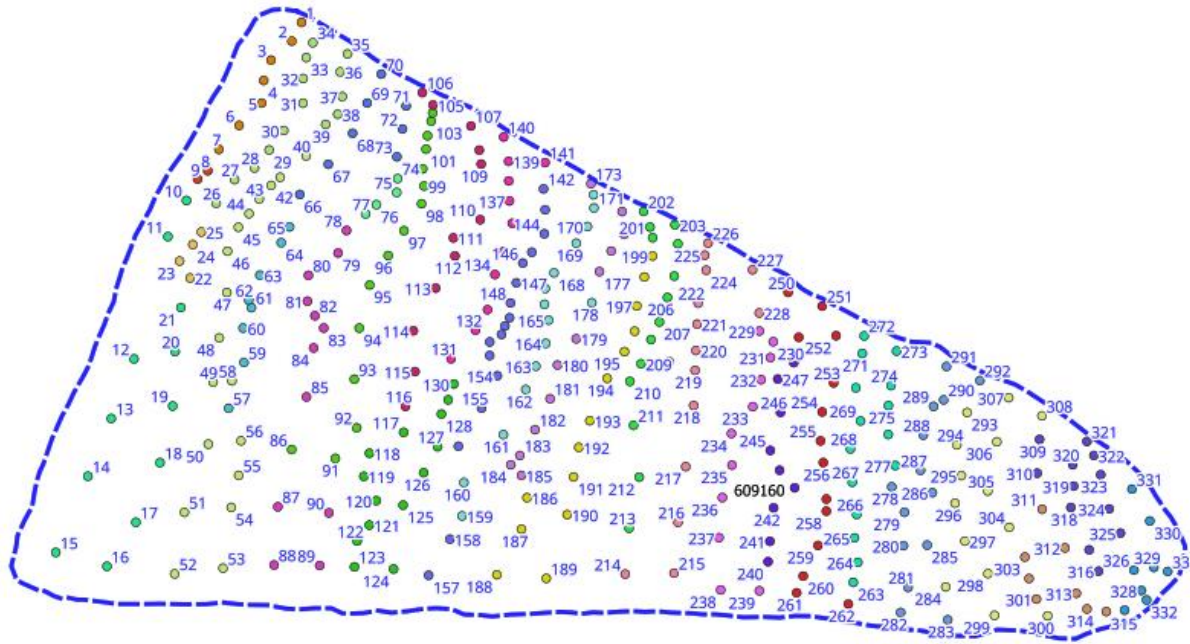


Fig. 3 Checking and labeling the grafted plants in the Piano Noce nursery, before being planted out.

Planimetria Impianto Vivaio Piano Noci
Scala 1:300

Numero Piantine a dimora per Pianta Madre

- Pianta Madre - 1 - n° 8
- Pianta Madre - 2 - n° 12
- Pianta Madre - 4 - n° 8
- Pianta Madre - 6 - n° 14
- Pianta Madre - 7 - n° 14
- Pianta Madre - 8 - n° 15
- Pianta Madre - 9 - n° 14
- Pianta Madre - 10 - n° 9
- Pianta Madre - 11 - n° 11
- Pianta Madre - 12 - n° 15
- Pianta Madre - 13 - n° 14
- Pianta Madre - 14 - n° 14
- Pianta Madre - 15 - n° 12
- Pianta Madre - 16 - n° 15
- Pianta Madre - 17 - n° 17
- Pianta Madre - 18 - n° 11
- Pianta Madre - 19 - n° 14
- Pianta Madre - 20 - n° 12
- Pianta Madre - 21 - n° 15
- Pianta Madre - 22 - n° 12
- Pianta Madre - 23 - n° 4
- Pianta Madre - 24 - n° 8
- Pianta Madre - 25 - n° 8
- Pianta Madre - 26 - n° 18
- Pianta Madre - 27 - n° 14
- Pianta Madre - 28 - n° 4
- Pianta Madre - 29 - n° 12
- Pianta Madre - 30 - n° 2
- Pianta Madre - 32 - n° 7



A

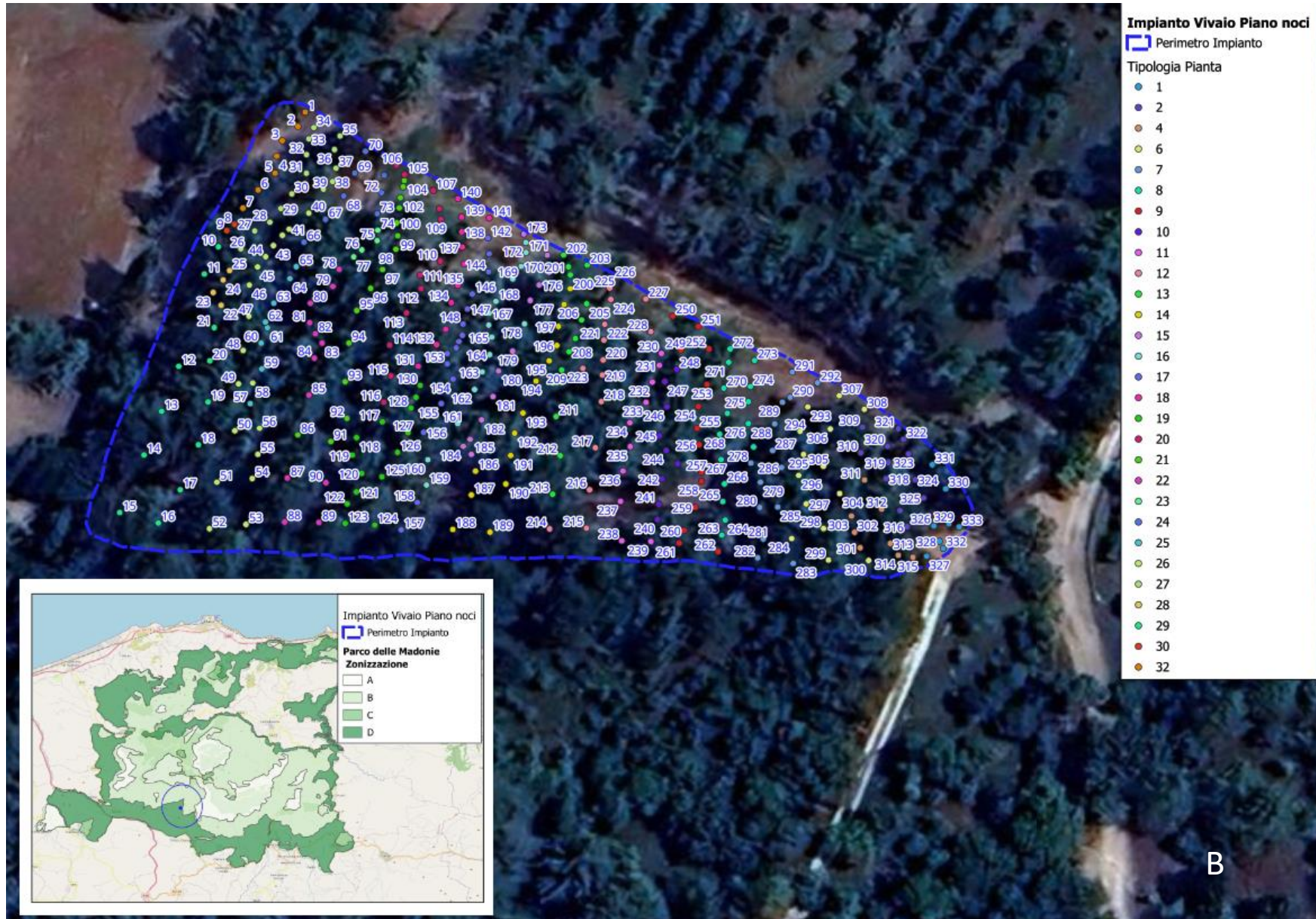


Fig. 4. A and B. Maps of the clonal orchard based on GPS survey of the grafted plants. Scale 1:300. In B) position of plants in the orchard are reported in a Google Earth image.

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