LIFE4FIR – Project LIFE18 NAT/IT/000164

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

"Map of final *A. nebrodensis* population and habitat video/hyperspectral inventory and 'health' state" - Action C1.

TABLE OF CONTENTS

1. Introduction

Being in a transitional zone, Mediterranean forest ecosystems are affected by mid-latitude and tropical processes and are vulnerable to climate changes. Extreme weather events may impact plant health, reducing plant growth and vigour, altering phenology, impairing physiological processes, and creating wounds that promote the attacks of pathogens and pests These effects may be even more detrimental for the endangered species living in altered and fragmented habitats.

To preserve endangered species, monitoring is essential to detect trends in species distribution through time, measure the impacts of threatening processes and evaluate the effectiveness of management responses.

As part of the action C1 'Support and preserve *Abies nebrodensis* in its natural habitat', the phytosanitary survey of *A. nebrodensis* population is one of the pivotal measures for *in situ* conservation of the species within the Life4fir project. Evaluating and monitoring the state of health of the natural population provides useful knowledge about occurring disorders and can assist in managing proper protection and conservation measures.

Some crown disorders, such as needle blight, have been observed to affect *A. nebrodensis* natural population for years, but generally they have not been described in detail and their real impact has remained unclear. Needle reddening and blight in *Abies* species have been reported since the beginning of the last century across Europe in natural populations, plantations and nurseries. In Italy, needle browning has been reported only locally and sporadically on *A. alba*.

In the framework of the Life4fir project it's useful to describe symptoms and investigate on the causes of the observed disorders and monitor their evolution in relation to the environmental conditions and taking into consideration the climate change. After the phytosanitary surveys of the trees in the natural population conducted in November 2019 and October 2020, a third survey was carried out in May 2023 to verify the evolution of the health conditions of the population over the years of the project. Ground inspections of the crowns of *A. nebrodensis* trees were accompanied by multispectral analyses with a drone to monitor any physiological and biochemical disorders at the whole crown level.

2. Procedure

2.1 Ecological context

The 30 residual trees (numbered from 1 to 32) representing the *A. nebrodensis* population grow between the vegetation belt dominated by *Quercus petraea* (Matt.) Liebl. and *Ilex aquifolium* L. and that one dominated by *Fagus sylvatica* L., on a prevailing north, north-western slope (Fig. 1). Beech forms wooded nuclei and groves, though they are fragmented. Soils are poor and shallow, on quartz-arenitic substrate, often extremely rocky and stony near ridges and screes. The range of Sicilian fir is extremely fragmented, and trees grow in different microclimatic and site conditions. Some trees are fully isolated, often growing on screes, ridges and bare soil, others grow near or inside beech groves or other broad-leaved formations.

The Walter and Lieth diagram summarize average and seasonal climatic conditions for Polizzi Generosa over three decades, from 1991 to 2020 (Fig. 2).

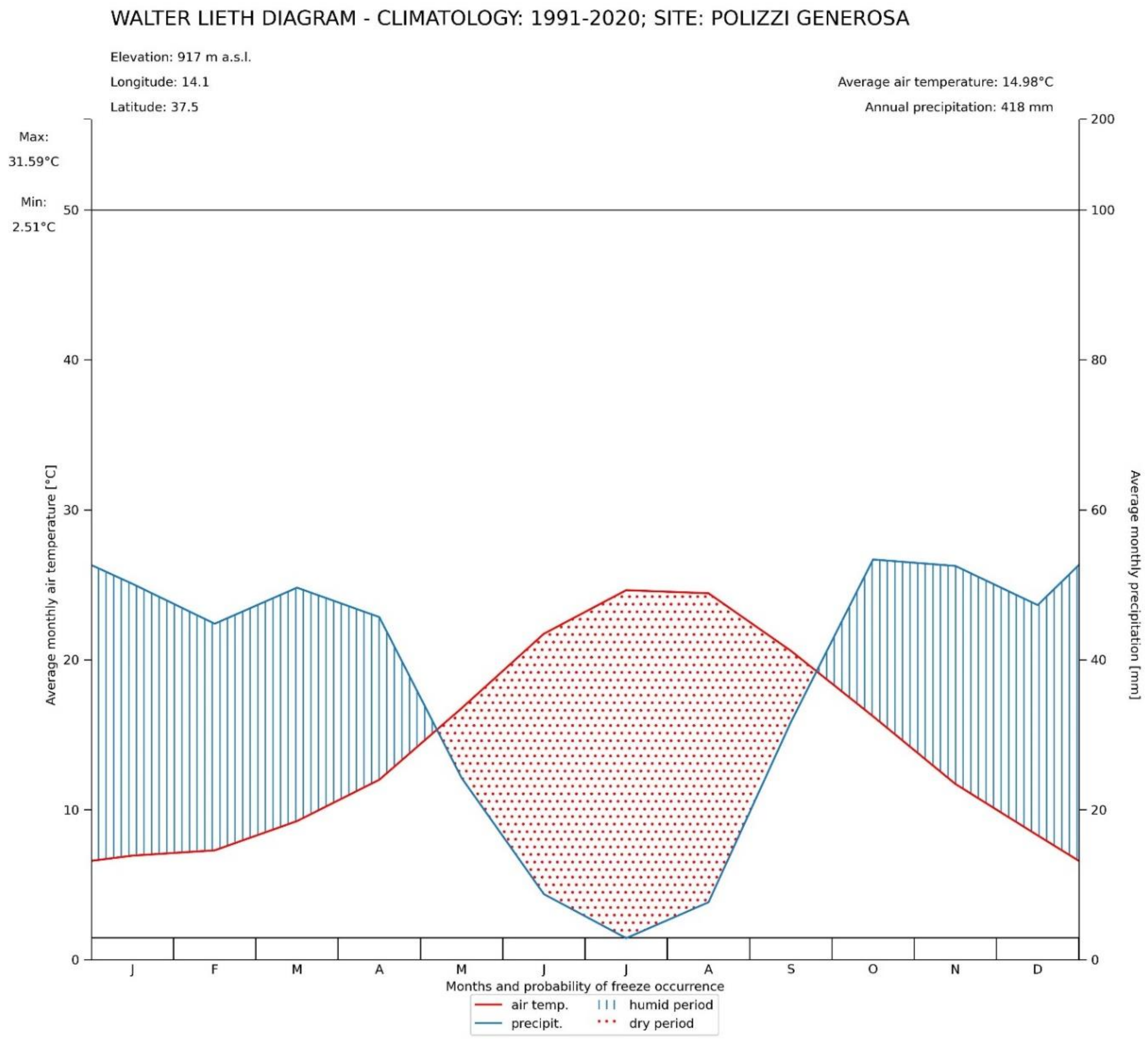

Fig. 1. The Walter and Lieth diagram for Polizzi Generosa.

Over this time period, the mean temperature was 15.0°C, the maximum mean temperature was 31.6°C, and the minimum mean was 2.5°C. The mean annual precipitation was 418 mm, with a 5-month dry period extending from May to September.

2.2 Assessment of health conditions of trees

Surveys were carried out in October 2020 and May 2023 on the trees of the natural population and in November 2019 in the nursery. Trees in the natural population were subjected to a careful visual examination to determine the occurrence and rate of foliage blight and needle reddening on the crowns of single trees. Impact (I) of disorders on the crowns was estimated and summarized by dividing the number of symptomatic twigs counted throughout the crown (n) by the crown surface meant as a cone lateral surface area (L), with a height equal to that of the tree trunk and with a diameter at the base equal to the diameter of the crown at its bottom ($I = n/L$). The resulting value was the number of reddened twigs per unit of crown surface (m^2).

2.3 Multispectral surveys

Physiological and biochemical disorders caused by biotic or abiotic stresses on trees influence the radiation absorbed or reflected by crowns. The visible and near-infrared radiation reflected by plants can be captured and measured by multispectral cameras.

Within the Life4fir project, a second survey on the *Abies nebrodensis* trees was conducted in August 2023 by using the drone DJI Phantom 4 Multispectral equipped with a digital camera and multispectral imagers. It was always connected to the ground station (D-RTK2) able to correct the position of each shot in real time. For the photogrammetric procedures, the Pix4D software was used. The drone is equipped with the following features:

Six sensors CMOS 1/2.9", including a RGB sensor and five monochromatic sensors for multispectral imaging acquisition. Each sensor has: 2,08 MP (2,12 MP in totale). Filters: Blue (B): 450 nm ± 16 nm, Green (G): 560 nm ± 16 nm, Red (R): 650 nm ± 16 nm, Red-Edge (RE): 730 nm ± 16 nm; NIR: 840 nm ± 26 nm. Lenses: FOV (visual field) 62,7; focal length 5,74 mm (equivalent to 35 mm, 40 mm), autofocus ∞. Aperture: f/2.2. ISO Interval RGB sensor: 200 – 800.

Finally, maps were suitably analyzed by capturing the pixels of the single crowns separately and processing the data obtained with a dedicated software to obtain a series of vegetation indices. The high reflectance potential of the leaves in the NIR allowed the evaluation of the defoliation of the forest through these sensors. The defoliation of forest decreases the reflectance of the NIR. The most commonly used remote sensing index that calculates the ratio of the difference and sum between the Near Infrared and Red bands of multispectral images is NDVI (Normalized Difference Vegetation Index), obtained as:

$$NDVI = \frac{\mathrm{NIR} - \mathrm{RED}}{\mathrm{NIR} + \mathrm{RED}} \quad (1)$$

This parameter evaluates the state of the forest or single trees and is based on the photosynthetic activity of plants and the radiation reflected. The values of this parameter may range between -1 and +1, being values from -1 to 0 water, values close to 0 barren areas and the closer the values get to 1, the healthier is the forest, with values approaching 0.8 resulting generally in good physiological conditions of the crown.

Variation of the vegetation indices was studied in relation to the spread of disorders observed with ground inspections of the crowns.

Using the formula (1), vegetative vigour images of the sampled crowns were produced based on the NDVI index.

3. Results

3.1 Tree health surveys in the natural population

Adult trees of the natural population were subdivided into five groups depending on the standardized number of reddened and blighted twigs per square meter of crown surface (I) (Tab. 1). Surveys showed that 7 out of 30 trees reported no disorders (group 1); 9 trees showed an I value ranging from 0.01 to 0.1 (group 2); 7 trees from 0.11 to 0.50 (group 3); 4 trees from 0.51 to 1.00 (group 4); 4 trees showed an I > 1.00 (group 5). In the group 5 with the greatest impact of symptoms (I > 1.00), reddened twigs showed a thorough distribution along the tree canopy depth, while generally symptoms remained confined to the lower part of the crown in the other groups of trees.

Table 1. Adult trees of *A. nebrodensis* were subdivided in five groups based on the number of reddened and blighted twigs per unit of crown surface (Impact, I). L: crown surface meant as a cone lateral surface area. Grey shades indicate groups of trees showing a different I rate.

Tree n.	Trunk height (m)	Crown diameter (m)	L (m^2)	Elevation a.s.l. (m)	Position	I	Group of trees
20	9.2	2.9x3.2	44,7	1480	Within a beech grove	0	Group 1 No symptoms
22	12.0	5.90	56,0	1400	Within a beech grove	0	
27	10.0	7.4x6.7	117,4	1597	Isolated	0	
29	10.5	4.2x4.9	76,8	1468	Within a beech grove	0	
30	1.76	1.7x1.6	5,2	1400	Under oaks	0	
32	1.96	1.7x1.8	5,7	1449	Within a beech grove	0	
21	11.6	8.5x7.7	156,3	1433	Within oak grove	0.03	Group 2 0.01 < I > 0.1
8	11.0	7.7x6.9	132,9	1577	Near broadleaves	0.04	
17	10.7	8.7x7.3	143,5	1488	Isolated	0.04	
19	5.5	4.6x4.9	44,7	1487	Margin of a beech grove	0.05	
13	11.1	9.5x9.2	176,9	1567	Isolated	0.05	
2	14.4	9.2x9.1	217,2	1526	Isolated	0.06	
14	7.2	6.5x6.8	82,1	1556	Near oak trees	0.06	

26	6.0	3.8x3.6	36,5	1599	Within a beech grove	0.08	
7	5.7	5.3x5.5	53,5	1603	Near an oak tree	0.09	
10	7.5	7.5x7.3	97,2	1525	Isolated on a ridge	0.11	Group 3 0.11 < I > 0.5
18	7.6	5.2x4.5	60,1	1503	Near beech trees	0.13	
15	8.5	5.7x5.2	76,4	1539	Near oak trees	0.17	
16	5.3	7.7x4.8	59,8	1488	Isolated on shallow soil	0.18	
11	8.2	5.1x4.6	65,1	1520	Isolated on a ridge	0.20	
4	0.85	2.1x1.5	3.5	1639	Under a bigger *A. nebrodensis* tree	0.19	
6	7.8	7.2x6.3	90,1	1639	Isolated	0.26	
23	7.5	4.6x4.3	54,7	1673	Isolated on a ridge exposed to strong winds	0.33	
24	3.1	3.8x4.1	22,8	1705	Isolated on a ridge exposed to strong winds	0.53	Group 4 0.51 < I > 1.00
25	3.3	3.5x1.6	14,2	1705	Isolated on a ridge exposed to strong winds	0.71	
1	6.5	6.4x7.6	81,2	1651	Isolated and exposed to strong winds	0.74	
12	8.5	6.8x8.4	111,2	1604	Isolated on a scree exposed to strong winds	1.28	Group 5 I > 1.00
31	1.25	1.4x1.3	2,9		Small, damaged by wild herbivores	1.39	
9	1.8	3.6x3.6	14,4	1617	Isolated on rocks exposed to strong winds	2.43	
28	0.48	1.3x1.1	1,4	1586	Small, damaged by wild herbivores	2.96	

In general, the extent of symptoms observed was related to the environmental conditions that trees are facing at a microclimate and site level. Trees were subdivided in five groups based on the rate of reddened and blighted twigs observed per unit of crown surface. Trees belonging to group 1 (reporting no symptoms on the crown) are all located within beech groves where they benefit from better edaphic conditions due to a deeper soil layer and a greater amount of organic matter in the soil, and where they are also protected from strong winds. An increase in the number of symptomatic twigs was generally observed with the occurrence of more severe conditions which the trees were subjected to, depending on their topographic and site position. Trees of the fourth group (showing a needle reddening impact between 0.51 and 1.00) for example are isolated near ridges, such as trees no. 1, 24, and 25, where they are exposed to strong winds, storms, hail. Finally, trees no. 28 and 31, in the group 5, showed a very stunted growth and advanced decline, due to repeated injuries caused by wild herbivores leading to needle and twig blight.

In Fig. 2, the conformation of the Madonie range and the topography of the territory are appreciable and show how woods (appearing as dark green tree formations) are strictly located on the north facing slopes. It's not different with the *A. nebrodensis* population, as the main nucleus is located in the Vallone Madonna degli Angeli, while 4 trees (1, 23, 24 and 25) are isolated near ridges.

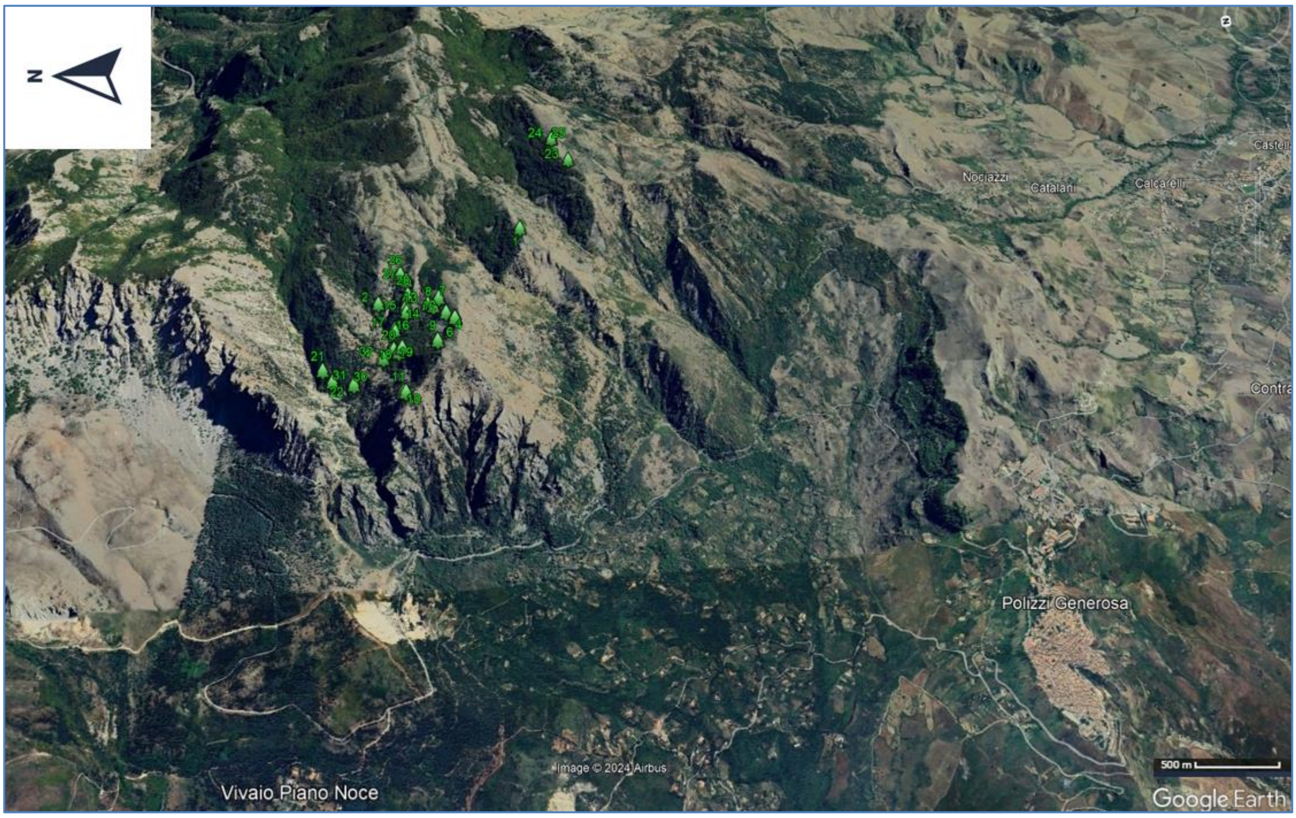

Fig. 2. Position based on UTM coordinates of each of the 30 adult trees in the natural range of *Abies nebrodensis* in relation to the topography of the territory and exposure based on a Google Earth map. Position of Polizzi Generosa and Vivaio Piano Noce in regard to the *A. nebrodensis* natural population are also appraisable.

3.2 Multispectral surveys

The infrared spectral reflectance depends on water and chlorophyll absorption in the leaf. There are various shades of vegetation due to type, health, leaf structure and moisture content of plants. Vegetation stands out in red and jumps out as brighter because green vegetation readily reflects infrared light energy, with healthier vegetation being more vibrant in colour (due to the higher amount in chlophyll and water).

Based on this principle, an evaluation procedure was developed that included an object-based analysis of each individual crown. The workflow adopted involved, for each tree, the delimitation of the canopy by means of on-screen photo manual interpretation, the extraction of the pixels using the NDVI image and the statistical inference analysis, both at the level of the individual plant and at the level of the groupings defined by the tree health symptomatology.

This methodology also allowed to derive detailed statistics for each individual plant and for groupings based on health status (Table 2-3).

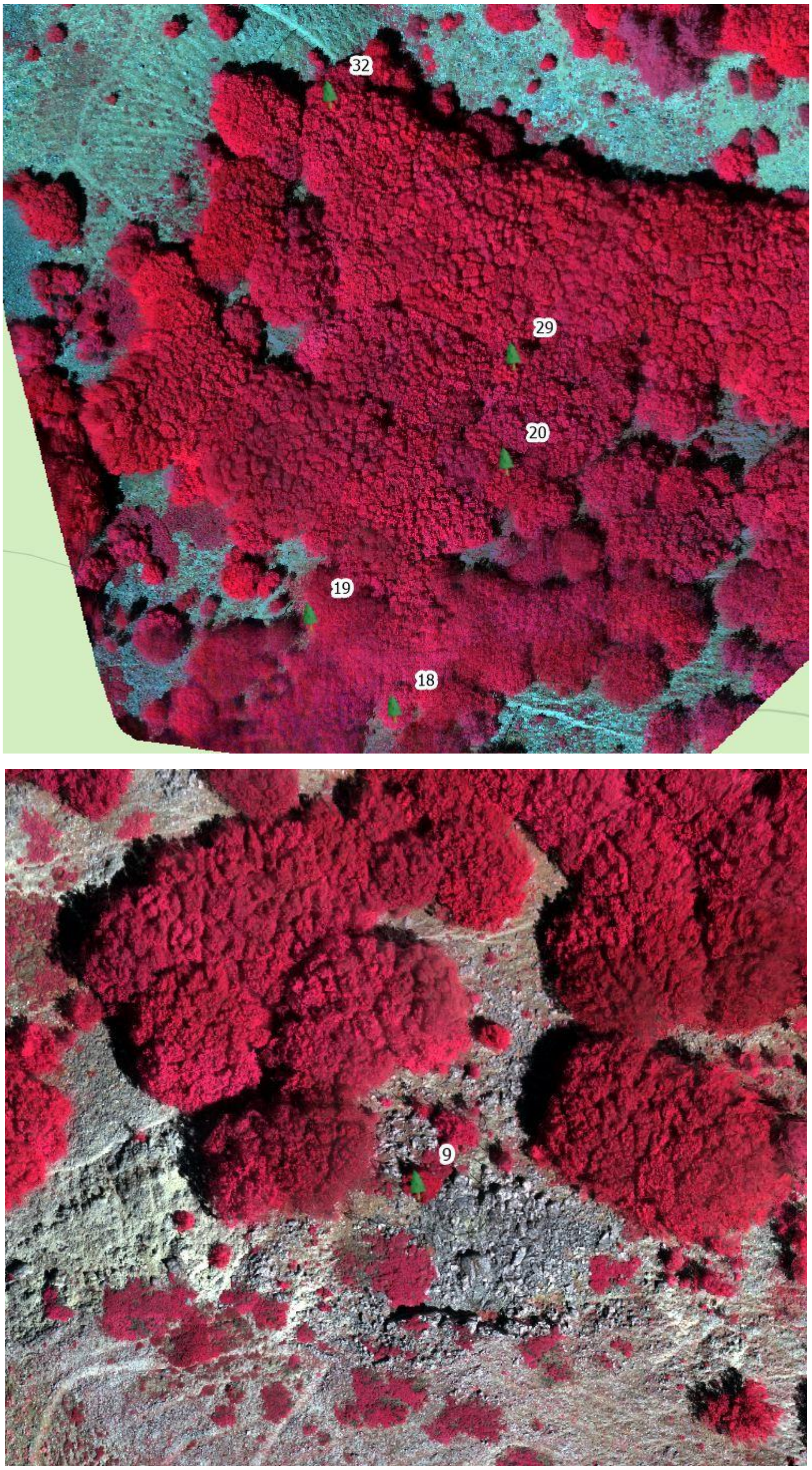

Fig. 3-4: infrared maps of portions of the main nucleus of the *A. nebrodensis* population, including the trees no. 18, 19, 20 and 29 growing inside the beech grove, and the tree no. 9 isolated near ridges.

In Fig. 3 and 4, infrared images of some portions of the area inside the Vallone Madonna degli Angeli where the main nucleus of the *A. nebrodensis* population is located are reported. In particular, Fig. 3 includes trees no. 18, 19, 20, 29 and 32, located inside the beech forest, while Fig. 4 includes tree no. 9 located in a ridge position on rocky and bare soil.

Table 2. Mean NDVI obtained for each sampled tree based on the number of pixels captured from the multispectral images. Count: number of pixels per plant; mean: mean NDVI index for each sampled plant; sem: standard error; median: median value; min: minimum value; max: maximum value; std: standard deviation; q0.25: first quartile; q0.75: third quartile.

NDVI									
Id	**count**	**mean**	**sem**	**median**	**min**	**max**	**std**	**q0.25**	**q0.75**
10	53268	0,787482	0,000169	0,798444	0,703937	0,853821	0,038998	0,771017	0,815736
11	58464	0,795112	0,00014	0,797641	0,716699	0,877324	0,033746	0,776934	0,81709
12	64106	0,7282	0,000501	0,78581	0,489917	0,891358	0,12679	0,685576	0,816015
13	85986	0,807757	0,00015	0,824454	0,720391	0,883541	0,043905	0,791438	0,838803
14	36720	0,840124	0,00014	0,844435	0,77854	0,906577	0,026908	0,826553	0,858563
15	14472	0,835163	0,000226	0,842047	0,774989	0,884496	0,02715	0,822731	0,854559
16	87584	0,808901	0,000159	0,822207	0,714158	0,917977	0,04706	0,79059	0,841545
18	64262	0,807369	0,000166	0,81197	0,71186	0,910195	0,042077	0,786236	0,835819
19	13024	0,850615	0,000165	0,852685	0,807731	0,897005	0,018828	0,841209	0,863527
20	5874	0,839726	0,000353	0,838146	0,779711	0,898709	0,027056	0,824335	0,854084
23	15768	0,691408	0,00127	0,755542	0,335022	0,904247	0,159452	0,618804	0,807991
24	24180	0,695446	0,001067	0,76049	0,330986	0,900209	0,165989	0,623242	0,81808
27	40098	0,798567	0,000184	0,810351	0,72131	0,886939	0,036913	0,783455	0,824885
29	8008	0,840228	0,000214	0,839921	0,793026	0,887036	0,019133	0,82828	0,851782
6	35156	0,792262	0,000197	0,802739	0,717779	0,877253	0,036886	0,777795	0,817806
7	28900	0,817105	0,000303	0,834455	0,715922	0,913681	0,051543	0,796797	0,850713
8	52704	0,822948	0,000138	0,832367	0,757035	0,882865	0,031623	0,810182	0,845614
9	20172	0,732334	0,00107	0,802412	0,444063	0,89406	0,151928	0,682095	0,840783

Table 2 shows the average value of the NDVI index (third column on the left) obtained for each of the 18 trees analyzed with the multispectral surveys. The values range from a minimum of 0.69 for trees no. 23 and 24 to a maximum of 0.85 obtained for tree no. 19. The first two are isolated, near a ridge, on rocky soil and are exposed to severe weather, such as wind, hail, and storms. Tree no. 19 is instead located inside a beech grove, protected from environmental extremes and where the soil fertility is greater.

Table 3. Mean NDVI calculated for three groups of trees, distinguished by the spread of disorders observed on the crowns with ground inspections. Healthy: no symptoms observed; Medium (I): trees with 0.11<I>1.00, i.e. with less than one symptomatic twig per square meter of crown surface; High (I): trees with an impact I>1.00, i.e. with more than one symptomatic twig per square meter of crown surface.

	NDVI								
	count	mean	sem	median	min	max	std	q0.25	q0.75
Health state									
Healthy	239886	0,82941	5,69E-05	0,83322	0,71598	0,92801	0,02788	0,81585	0,84744
High (I)	90697	0,77291	0,000287	0,79754	0,33099	0,90020	0,08652	0,75236	0,82704
Medium (I)	297931	0,80638	8,61E-05	0,81166	0,33512	0,92946	0,04701	0,78879	0,83218

In Table 3, the trees were subdivided into three groups based on the frequency of reddened and blighted twigs observed on the crowns, as represented by the I index in Table 1. In particular, the 'Healthy' group included trees with an I index ranging from 0 to 0.1, with crowns showing no symptoms or with very sporadic symptoms. The 'Medium' group included trees with 0.11<I>1.00, i.e. with less than one symptomatic twig per square meter of crown. The 'High' group included trees with an I>1.00, i.e. with more than one symptomatic twig per square meter of crown surface.
The mean NDVI value calculated for the three groups was found to vary according to the health status of the trees expressed in terms of number of symptomatic twigs per unit of crown surface, and was 0.77 for the 'high' group, 0.80 for the 'medium' group and 0.82 for the 'healthy' group. With a t-test, difference among the means obtained for the three groups was evaluated ($p < 0.05$) (Fig. 5).
Figure 5 shows as boxplots the variation among the three groups of trees of a series of vegetation indices, including NDVI. All the calculated indices, except Red Edge, were higher for the 'Healthy' group and lower for the 'High' group, with significant differences between the three groups ($p \leq 0.05$).
In the plot of Fig. 6, position of the trees is defined by the reflectance values in the red and infrared bands reported on the x and y axis, respectively. The same plot shows the straight lines that start from the origin of the axes and express defined NDVI values as reference. In the graph it is possible to observe that the values obtained for the *A. nebrodensis* trees analyzed are all well above the value of 0.6 which represents the threshold below which plants are suffering.
Most of the trees have an NDVI value between 0.75 and 0.85 which indicates good health condition. The trees on which the crown inspections have highlighted a higher frequency of symptomatic twigs have NDVI values just below 0.7.

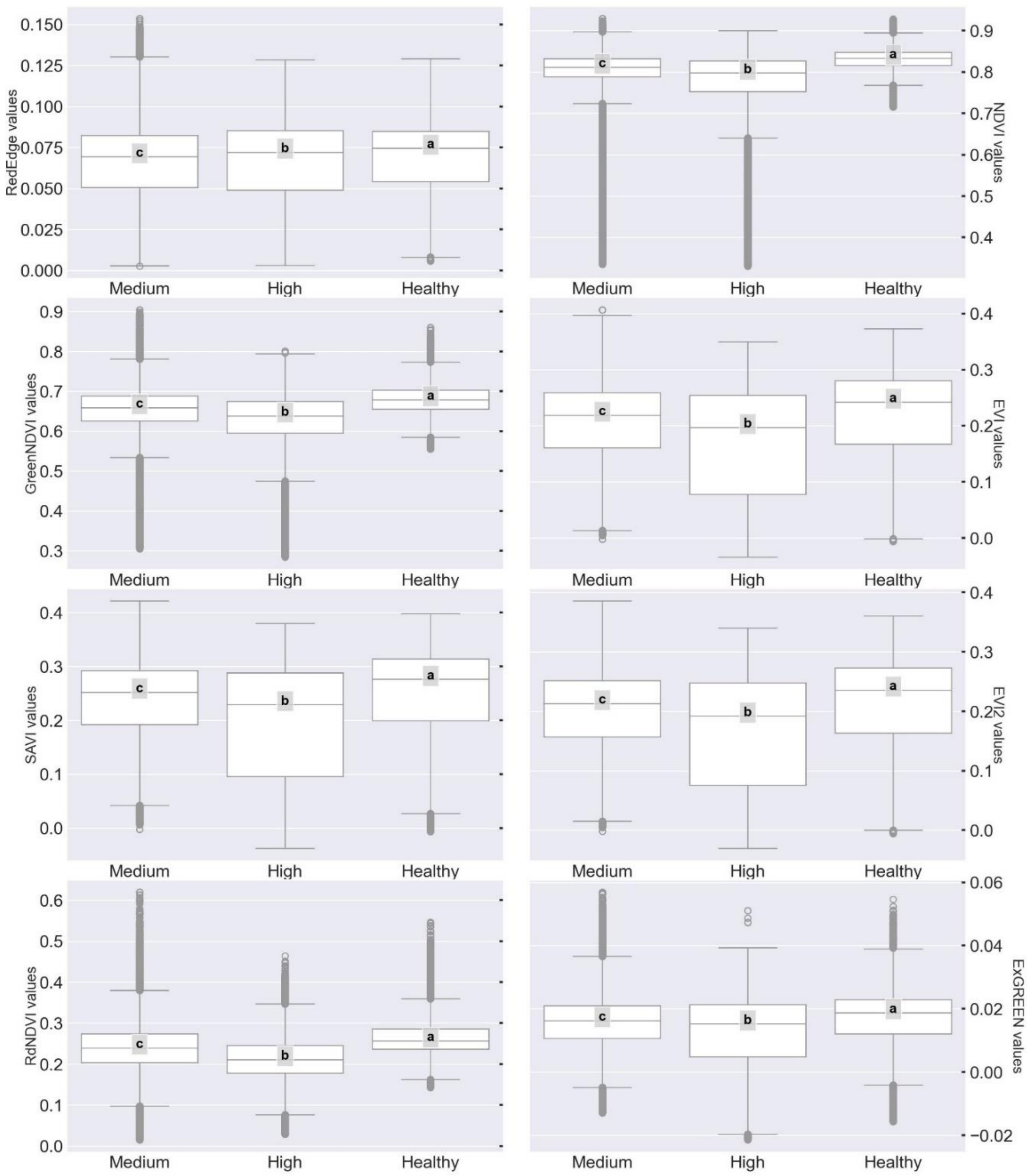

Fig. 5. Vegetation indices obtained for the three groups of trees. Healthy: no symptoms observed; medium: trees with 0.11<I>1.00, i.e. with less than one symptomatic twig per square meter of crown; high: trees with an I>1.00, i.e. with more than one symptomatic twig per square meter of crown surface.

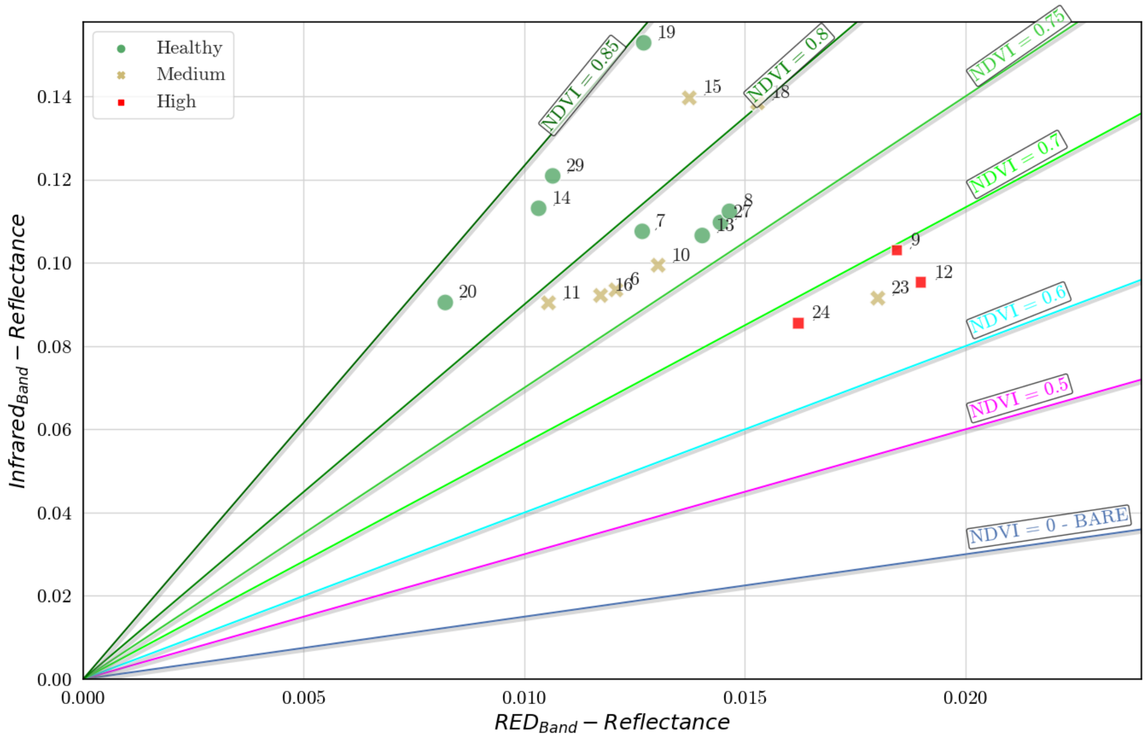

Fig. 6. Each *A. nebrodensis* tree is positioned in the plot based on the mean infrared and red reflectance. Green marker: healthy trees; yellow x marker: trees with medium impact I of symptoms (0.11<I>1.00), i.e. with less than one symptomatic twig per square meter of crown surface; red markers: high impact I of symptoms (I>1.00), i.e. with more than one symptomatic twig per square meter of crown surface.

4. Conclusions

- During the Life4fir project, *A. nebrodensis* trees were monitored to assess their health conditions. Monitoring was carried out by conducting close crown inspections, with description of the observed disorders and evaluation of their spread in relation to unit of crown surface. These observations were accompanied by multispectral surveys through which the reflectance of the crowns was evaluated in various bands of the spectrum, as expression of their physiological state, water content and photosynthetic activity. This was synthetically represented by NDVI. Some other vegetation indices were calculated.
- Crown inspections of the trees in the natural population highlighted that the most common symptoms are needle reddening and needle cast with subsequent defoliation of twigs and branches in the outer and lower part of the crowns. With few exceptions, disorders did not affect the upper two-thirds of the crowns. In general, the extent of symptoms observed was related to the environmental conditions that trees are facing at a microclimate and site level. Trees were separated into five groups based on the rate of reddened and blighted twigs

observed per unit of crown surface. Trees belonging to group 1 (reporting no symptoms on the crown) are all located within beech groves where they benefit from better edaphic conditions due to a deeper soil layer and a greater amount of organic matter in the soil, and where they are also protected from strong winds. As far as the other groups are concerned, an increase in the number of symptomatic twigs was generally observed with the occurrence of more severe conditions which the trees are subjected to, depending on their topographic and site position. Trees of the fourth and fifth groups showing a needle reddening impact between 0.51 and 1.00 or higher are isolated near ridges, where they are exposed to strong winds, storms, hail or are subjected to repeated injuries caused by wild herbivores.

- The Walter and Lieth diagram summarize average and seasonal climatic conditions for Polizzi Generosa over three decades, from 1991 to 2020. Over this time period, the mean temperature was 15.0°C, the maximum mean temperature was 31.6°C, and the minimum mean was 2.5°C. The mean annual precipitation was 418 mm, with a 5-month dry period extending from May to September.
- NDVI of single trees ranged from 0.69 to 0.85 and is well above the value of 0.6 that represents a threshold of reduced vigour of plants. Most of the plants analyzed showed values ranging between 0.72 and 0.85, denoting overall good physiological conditions, despite the severe environment that characterizes the habitat of the species.
- The mean NDVI values showed to be related to the spread of reddened and blighted needles per unit of crown surface. The three groups of plants arbitrarily distinguished based on the impact of the disorders on the crown, showed significant differences in the NDVI, which increased as the number of observed disorders decreased. Other calculated vegetation indices including, GreenNDVI, RdNDVI, SAVI, EVI, EVI2, ExGREEN showed to vary similarly to NDVI and were related to the spread of disorders on the crowns.
- Based on the findings of the ground-based phytosanitary inspections and with multispectral analyses, site and microclimatic conditions influence both the impact of crown disorders and tree physiology in terms of water content and photosynthetic activity.
- In light of the observations made and the data obtained, multispectral techniques with drones seem to be able to provide reliable indications on the health state of the crowns of the *A. nebrodensis* trees and represent a useful tool for monitoring the physiological conditions of the population over time in relation to environmental stresses and climate change.

5. References

- Frascella, A., Barberini, S., Della Rocca, G., Emiliani, G., Di Lonardo, V., Secci, S., & Danti, R. (2024). Insights on the fungal communities associated with needle reddening of the endangered Abies nebrodensis. *Journal of Plant Pathology*, 1-15.
- Frascella A, Della Rocca G, Barberini S, Emiliani G, Secci S, Lambardi M, et al (2022) Innovative In Situ and Ex Situ Conservation Strategies of the Madonie Fir Abies nebrodensis. Sustainability 14(19): 12643.
- Giorgi F, Lionello P (2008) Climate change projections for the Mediterranean region. Global and planetary change 63(2-3): 90-104.
- Palahi, M, Mavsar R, Gracia C, Birot Y (2008) Mediterranean forests under focus. International forestry review 10(4): 676-688.
- Jactel H, Koricheva J, Castagneyrol B (2019) Responses of forest insect pests to climate change: Not so simple. Current Opinion in Insect Science 35: 103–108. https://doi.org/10.1016/j.cois.2019.07.010
- Garza G, Rivera A, Venegas Barrera CS, Martinez-Ávalos JG, Dale J, Feria Arroyo TP (2020) Potential effects of climate change on the geographic distribution of the endangered plant species Manihot walkerae. Forests 11(6): 689.
- Giorgi F, Lionello P (2008) Climate change projections for the Mediterranean region. Global and planetary change 63(2-3): 90-104.
- Pasta S, Sala G, La Mantia T, Bondì C, Tinner W (2020) The past distribution of Abies nebrodensis (Lojac.) Mattei: results of a multidisciplinary study. Vegetation history and archaeobotany 29(3): 357-371. https://doi.org/10.1007/s00334-019-00747-0
- Pasta S, Troia A (2017) Abies nebrodensis. In: Pasta S, Perez-Graber A,Fazan L, de Montmollin B (eds) The top 50 Mediterranean island plants UPDATE 2017. IUCN/SSC/Mediterranean plant specialist group, Neuchâtel (Switzerland). http://top50 .iucn-mpsg.org/
- Raimondo FM, Schicchi R (ed) (2005) Progetto LIFE Natura n°LIFE2000NAT/IT/7228. Rendiconto sul progetto LIFE Natura "Conservazione in situ ed ex situ di Abies nebrodensis (Lojac.) Mattei". Tipolitografia Luxograph, Palermo. http://www.parcodelle madon ie.it/webdo c/doc/life.pdf

Authors

Roberto Danti, Gianni Della Rocca, Sara Barberini, Giovanni Emiliani, Arcangela Frascella, Stefano Secci @ IPSP – CNR.;

Lorenzo Arcidiaco @ IBE - CNR.

Peppuccio Bonomo @ Ente Parco delle Madonie;

Rosario Schicchi @ Unipa - Saaf.