LIFE4FIR – Project LIFE18 NAT/IT/000164

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

Report: Map of initial *Abies nebrodensis* population and habitat, video hyperspectral inventory and health state (Action C1.5)

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REPUBBLICA ITALIANA
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DELL'AGRICOLTURA, DELLO SVILUPPO RURALE
E DELLA PESCA MEDITERRANEA

Parco delle Madonie

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SAAF
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1. Introduction

Monitoring the health state of natural forest areas provides basic knowledge about occurring threats, as well as proper protection and conservation measures. Moreover, the collected information may provide clues to understand the influence of disturbances, such as climate change and invasive species on forest ecosystems. The impacts of climate change on forests have already been observed in Europe and there is concern that tree mortality will increase due to physiological stress, insect outbreaks and wildfires driven by future climate change. The finding that Europe's most vulnerable forests are located in the Mediterranean indicated that, due to warmer temperatures caused by climate change, Mediterranean forests will be at risk of forest fires, pest outbreaks and desertification.

The action C1 of the Life4fir project 'Support and preserve *Abies nebrodensis* in its natural habitat' relies on a series of activities aimed at the preservation of the relic trees of this unique natural population by monitoring and controlling their threats (in situ conservation). This deliverable reports the outcomes and information obtained by the surveys conducted within the sub-action C1.5 'Spatial and health analysis of *A. nebrodensis* natural population using drone technology'.

In addition to the threat posed to plants by wild herbivores (expecially fallow deers and boars), symptoms and disorders have been sporadically observed over the years on the crowns of *A. nebrodensis* trees. 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. The project has planned to carry out phytopathological surveys on the natural population of the Madonie fir to detect and monitor the occurrence of biotic or abiotic disturbances and to eventually implement proper control measures. 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.

Surveys on plant health were based on the visual examination the single trees alongside multispectral analysis to monitor eventual physiological and biochemical disorders at the whole crown level.

2. Procedures

2.1 Health surveys of trees and monitoring

Phytopathological surveys were carried out in November 2019 and in October 2020. Trees were subjected to a careful visual examination to evaluate their state of health based on observations of the crown shape and transparency, turning foliage, presence of declining, desiccated or damaged parts, occurrence of lesions. Disorders observed on the crowns were separately described, recording the type of affected organ (trunk, branches, twigs, shoots, needles), the portion of the crown involved, ideally divided into three parts along the longitudinal axis (lower, intermediate and upper third), the direction: north, south, east and west, the impact in terms of percentage of damaged crown.

Laboratory analysis were conducted on the collected samples through: observations under stereomicroscope, isolation and culture of fungal colonies and their genetic characterization through PCR amplification and sequencing of target loci.

This allowed to identify the fungal microflora associated with the observed disorders and eventually detect the occurrence of harmful pathogens.

2.2 Samplings and fungal isolations

Samples of affected needles and twigs were taken from the affected crowns for further observations and analysis in the laboratory. With the aid of a stereoscope, samples were observed to define in detail the signs of colonization by pathogens (reaction tissues, fruiting bodies of fungi, etc.) and used for the in vitro isolation of fungal microorganisms. Isolations were performed from needles (reddened, entirely or partially necrotized) and from blighted shoots. Isolations were repeated in 2020 from both affected and green needles.

For surface sterilization, needles and shoots were first immersed in a 70% ethanol solution for 1 min and then in a sodium hypochlorite solution (4-5% active chlorine) for 4 min. Then they were rinsed with sterile water and dried with sterile filter paper. The needles were cut into 3-5 mm fragments and placed in Petri dishes containing PDA (Potato Dextrose Agar). Plates were incubated at 24° C in the dark for 3 weeks. Each week the plates were checked twice to assess the progressive development of the fungal colonies growing out from the fragments and to arrange for their subculturing.

The colonies obtained were grouped into morphotypes based on their cultural characteristics (mycelium and reproductive structures). Morphotypes were then caharcterized by sequencing specific regions of genomic DNA, known for their diagnostic value (ITS1-ITS4).

To this end, fungal colonies representing each morphotype were grown on a sterile cellulose membrane placed on Potato Dextrose Agar medium (BD Difco) 2% in Petri plates. Plates were incubated in the dark at 25°C for about 10 weeks and the mycelium was scraped out and lyophilized. Samples were maintained at -20°C in sterile tubes up to genomic DNA extraction. Extraction was carried out with the 'NucleoSpin' Plant II kit (Macherey Nagel GmbH & Co. KG) using on 20 ng of lyophilized mycelium. Polymerase chain reaction (PCR) amplification of the template DNA was performed using the primers ITS1 and ITS4. Amplicons were then purified and sequenced. For identification, the resulting sequences were blasted against available sequences from the GenBank database.
A table of fungal taxa isolated from the plated samples and their respective isolation frequencies was then obtained.

2.3 Multispectral analysis by drone technology

Mediterranean forest is currently facing an important number of biotic and abiotic stresses. Climate change is altering meteorological patterns and extreme weather events are getting more frequent. The loss of the forest is encouraging the use of new technologies to detect areas where these different sources of stress are making an impact on the forest. Drones are going to be used to evaluate the health of the forest using different high-resolution images. Biotic or abiotic stresses on trees involve their physiological and biochemical disorder which in turn modifies the radiation absorbed or reflected by the crown. Multispectral cameras will measure the visible and near-infrared radiation reflected by plants.
Monitoring of the *Abies nebrodensis* trees and the surrounding vegetation 'health' traits (spectral indices) by remote sensing technology based on the use of a drone (UAV - Unmanned Aerial Vehycle) equipped with a digital camera and a hyperspectral imagers, has been performed the 8-9 October 2020. A second drone survey will be repeated at the end of the project to compare the health state of the habitat after the implementation of the planned protection measures.
Two cameras were used: 1) RGB conventional camera. The images taken were geolocated. An orthophoto of the terrain and Digital Elevation Model was made to find correlations between the topographic traits and the development of stresses. 2) Multispectral Camera obtained 4 simultaneous images, on for each band: Red, Red Edge, Green, Near Infrared. Using these captured images, reflectance maps were created. Different vegetation indices

(NDVI) will result from the combination of these reflectance maps. These indices will be specifically designed to produce a value that indicates the amount or vigour of the vegetation. The frames (in RGB and in the spectrum of green, red, red edge and near infrared) were aligned and, through the Structure from Motion (SfM) procedure, the cloud of points was created. This was used to obtain the orthophoto and the production of Digital Terrain Models (DTM) and surfaces (DSM), through the procedure of triangulation.

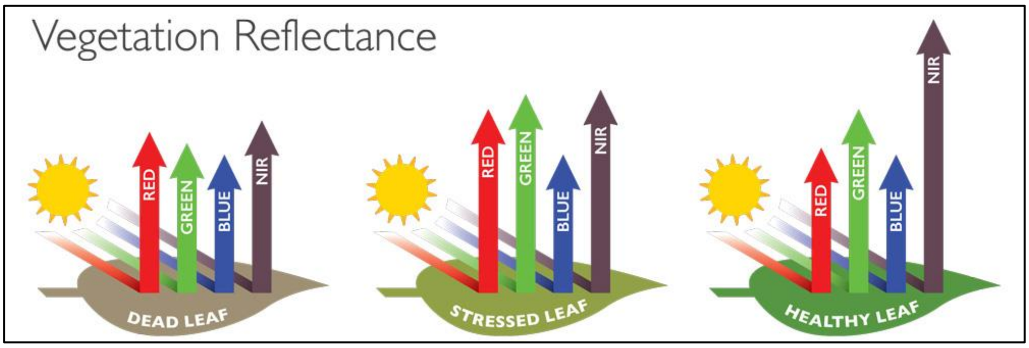
For the multispectral images only, detected in the RED (Red), REG (Red Edge), GREEN (Green), NIR (Near Infrared), the reflectance maps were produced after appropriate calibration of the camera made by means of the light sensor mounted on the drone and using the known reflectance values obtained from the calibration panel.

Finally, these maps were suitably analyzed for the production of a NDVI map (Normalized Difference Vegetation Index). The high reflectance potential of the leaves in the NIR allowed the evaluation of the defoliation of the forest through these sensors. The 50% of the radiation is reflected by the forest canopy if the forest is healthy. The defoliation of forest decreases the reflectance of the NIR.

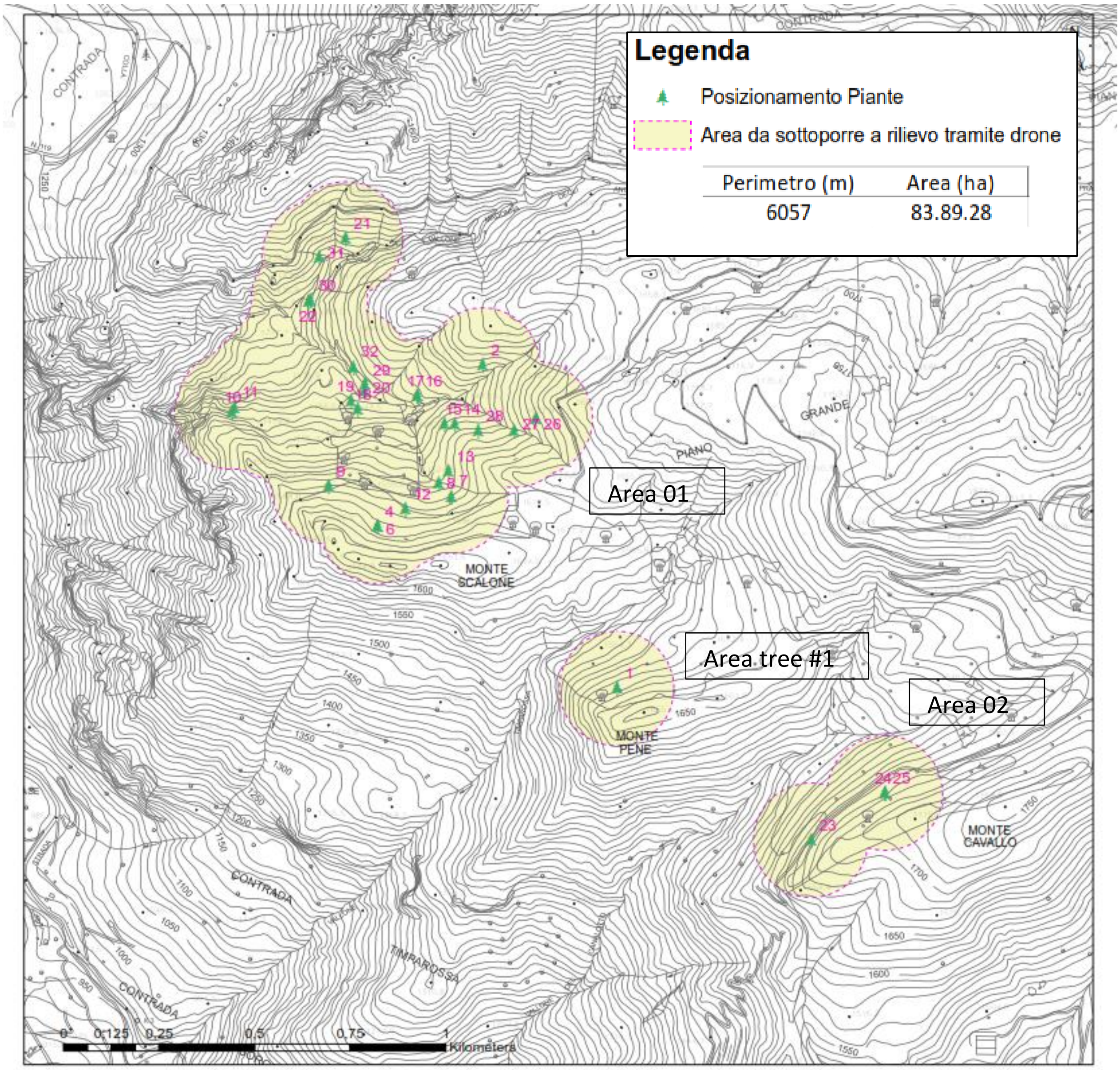
With the NIR and the RED band the normalized difference vegetation index NDVI is calculated as:

$$NDVI = \frac{NIR - RED}{NIR + RED}$$

This parameter evaluates the state of the forest and is based on the photosynthetic activity of the plant 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.



Data were graphically reported through the use of a legend bearing colors in shades of red, yellow and green.



Ubicazione piante della popolazione naturale

N° inventario	Lat	Long	N° inventario	Lat	Long
1	37° 50' 5,598" N	14° 1' 46,864" E	18	37° 50' 29,108" N	14° 1' 18,820" E
2	37° 50' 32,969" N	14° 1' 32,152" E	19	37° 50' 29,797" N	14° 1' 18,088" E
4	37° 50' 19,178" N	14° 1' 21,031" E	20	37° 50' 30,708" N	14° 1' 19,509" E
6	37° 50' 19,178" N	14° 1' 21,084" E	21	37° 50' 43,574" N	14° 1' 17,339" E
7	37° 50' 21,799" N	14° 1' 28,928" E	22	37° 50' 38,279" N	14° 1' 13,392" E
8	37° 50' 22,932" N	14° 1' 27,540" E	23	37° 49' 52,866" N	14° 2' 7,808" E
9	37° 50' 22,513" N	14° 1' 15,769" E	24	37° 49' 56,957" N	14° 2' 15,737" E
10	37° 50' 28,756" N	14° 1' 5,352" E	25	37° 49' 56,956" N	14° 2' 15,655" E
11	37° 50' 29,174" N	14° 1' 5,561" E	26	37° 50' 28,488" N	14° 1' 37,860" E
12	37° 50' 20,695" N	14° 1' 24,016" E	27	37° 50' 27,468" N	14° 1' 35,608" E
13	37° 50' 23,913" N	14° 1' 28,601" E	28	37° 50' 27,377" N	14° 1' 31,716" E
14	37° 50' 27,949" N	14° 1' 29,217" E	29	37° 50' 31,249" N	14° 1' 19,558" E
15	37° 50' 27,933" N	14° 1' 28,123" E	30	37° 50' 38,351" N	14° 1' 13,645" E
16	37° 50' 30,253" N	14° 1' 25,137" E	31	37° 50' 41,985" N	14° 1' 14,521" E
17	37° 50' 30,326" N	14° 1' 25,203" E	32	37° 50' 32,715" N	14° 1' 18,259" E

Fig. 1. Map of the *Abies nebrodensis* population. Position of each of the 30 adult trees is indicated by UTM (Universal Transverse Mercator) coordinates reported in a 1:10000 topographyc map of the Sicily Region. The three areas in yellow are those surveyed by drone flight..

Table 1. Summary table decribing the disorders and symptoms observed on the crowns of the 30 adult trees of the *Abies nebrodensis* population.

Phytosanitary survey **Date: 20/11/2019**							
***Abies nebrodensis* natural population**							
Tree no.	**Crown transparency**	**Turning foliage (%)**	**Affected organ**	**Position in the crown**	**Direction (N, S, E, W)**	**Percentage of damaged crown**	**Description**
1	0	-	60-70 small shoots	Lower and intemediate thirds	N prevailing	10	Shoots with reddened needles
		-	branches	Lower third	All	5	Branches showing mechanical injuries and desiccated due to wild herbivores
2	0-20	-	5 twigs 1° order	Lower third	S-O	10-15	Dried out and defoliated, no due to shading
			Twigs 1° order	Lower third	All		Chlorosis and small needles
			Branch Twigs 2 order < 5 cm	Lower third Lower third	NE E	2 1	October 2020: distal portion (50 cm) defoliated October 2020: reddened needles
4	0-20	-	Twig 1° order	Thorough	All	5	Desiccated due to shading
6	0-20	-	Twigs 2° order	Lower third	All	9	Distal portion
	0-20	-	Twig 1° order	Thorough	All	4	5-6 branches distal portion (100 cm) dried out
			Twigs 2 order	Lower third	N (downhill)	3	October 2020: distal shoots reddened
			Branch	Lower third	N (downhill)	2	October 2020: 50 cm distal defoliated + reddened needles
7	0-20	-	Twig 1° order	Lower third	O		Branch dried out distal portion (100cm)
	0-20	-	N° 2 twigs 2° order	Lower third	N	< 1	Distal shoots with reddened needles
	0-20	-	Twig 1° order	Lower third	O		Twigs dried out, distal part 20-30 cm
	0-20	-	Branch 1° order	Lower third	N	1	Wound due to friction with the soil
	0-20	-	Branch 1° order	Lower third	S	1	Wounds due to herbivores

Phytosanitary survey Date: 20/11/2019							
***Abies nebrodensis* natural population**							
Tree no.	**Crown transparency**	**Turning foliage (%)**	**Affected organ**	**Position in the crown**	**Direction (N, S, E, W)**	**Percentage of damaged crown**	**Description**
8	0-20	-	N° 2 twigs 1° order	Lower third	S	1	Branches with wounds due to herbivores
			Terminal shoots	Lower third	N	<1	Reddened shoots
9	0-20	-	Twigs 1° e 2° order	thorough	All, N prevailing	10	Distal portions dried out
10	0-20	-	Twig 2° order	Lower third	All	< 1	Distal part with reddened needled
	0-20	-	Branch 1° order	Lower third	S-O	< 1	Branch broken dried out in its distal part (40 cm)
	0-20	-	Branch 1° order	Lower third.	N-E	1	Branch drying and defoliating, also wounds by herbivores
11	0		Twigs 2° order (60 - 70)	Lower and intermediate thirds	All, N prevailing	1	Distal part with reddened needles
	0		N° 3 twigs 1° order	Lower third	N	1	Branches dried out at 1-1,5 height from the groundm
12	10 mainly in the upper part	About 30%	Twigs 2° order	thorough	All	10	Distal part with reddened needles, 2-10 cm in length
			Twigs 1° order	thorough	All	10	Distal portion (100 cm) dried out
			Twigs 1° e 2° order	thorough	All	10	Yellowed distal portion up to 30-40 cm
13	0-20	-	Twigs 2 order	Lower third	SE (uphill)	< 1	inner twig reddened (20 cm)
			Twigs 2 e 3 order	Lower third	SE (uphill)	<1	5 twigs reddened and defoliated close to the ground
14	0-20	-	7 branches 1° order	Lower third	NE-N-O	5	Branches with wounds in the distal 1-2 m due to herbivores

Phytosanitary survey Date: 20/11/2019							
Abies nebrodensis **natural population**							
Tree no.	**Crown transparency**	**Turning foliage (%)**	**Affected organ**	**Position in the crown**	**Direction (N, S, E, W)**	**Percentage of damaged crown**	**Description**
	0-20	-	11 twigs 2° order	Lower third	All, N prevailing	< 1	Reddened needles in the distal 2-10 cm part
15	0-20	-	7 twigs 2° order	Lower third	All	< 1	Outer crown
	0-20	-	Twig 1° order	Lower third	S	1	Twig dried out in the distal 30 cm, close to the ground
	0-20	-	Twig 1° order	Lower third	O	1	Distal part (20 cm) with reddeded needles
16	0-20		2 twigs 2° order	Lower third	S-O	< 1	Reddened needles
	0-20		Twigs 2° order	Lower third	All		Small xeromorph needles
	0-20		15 twigs 2° order	Lower third	All	< 1	Outer twigs with reddened needles, expecially S-O direction (
17	0-20	-	N° 4 twigs 2° order	Lower third	All	< 1	Distal part with reddened needles
17			Branch	Lower third	W	1	Twigs dried out are inserted in a branch damaged by herbivores
18	0-20 (a S-E 20-40 e 40-60)	-	Branches and twigs	Lower and intermediate thirds	S-E	20	Three branches and twigs dried out due to shading by beech trees
	0-20	-	N° 3 twigs 1° order		N-O	2	Branches dried out at 1 m height from the ground
19			N° 2 branches 1° order	Lower third	E	2	Twigs dried out due to injuries by herbivores
			Twigs 2 order	Lower third	SE		Two twigs with reddened needles

Phytosanitary survey Date: 20/11/2019							
Abies nebrodensis **natural population**							
Tree no.	**Crown transparency**	**Turning foliage (%)**	**Affected organ**	**Position in the crown**	**Direction (N, S, E, W)**	**Percentage of damaged crown**	**Description**
20	25	-	Crown	Lower and intermediate thirds	S	40	Tree with half of the crown defoliated
	25	-	Branches 1° order	Lower third E 1/3 Int.	N-E and N-O	10	Branches dried out
21	0-20	-	Branches 2° order	Lower third	All, SE prevailing	4	Distal half of branches is defoliated also those not shaded
			Twigs 2 order	Upper third		<1	October 2020: two reddened twigs
22	0-20		Branches and twigs	Lower third	N-S-O-E	20	Twigs long-dried out due to shading
23	0-20	-	Twigs 2° order	Thorough	All	5-7	Distal portions with reddened needles, uo to 20 cm in length, small needles
24	0-20	-	Twigs 1° order	Lower third E 1/3 Int.	S (uphill)	5	Twigs broken due to herbivores
	0-20	-	Twigs 2° order	Lower third	S (uphill)	3-4	Distal portions (2-10 cm) with reddened needles, wounds due to herbivores
25			Twig 1° order	Thorough	N	2	Twigs 10-20 cm long dried out and defoliated, slight damage by herbivores
26	20		Branch, twigs	Lower third	All	5	Branches and wigs dried out up to 2 m height from the ground due to shading
	20		Twigs 1° order	Lower third		1	Two twigs dried out due to rubbing
			Twigs 3 order	Lower third	S (uphill)	1	Three reddened twigs due to rubbing

Phytosanitary survey Date: 20/11/2019							
***Abies nebrodensis* natural population**							
Tree no.	**Crown transparency**	**Turning foliage (%)**	**Affected organ**	**Position in the crown**	**Direction (N, S, E, W)**	**Percentage of damaged crown**	**Description**
27	0-20	-	Twigs 1° and 2° order	Lower and intermediate thirds	All	2	Inner twigs dried out due to shading
28	50	0-20	Crown	Thorough	S (uphill)	50	Thorough damage due to herbivores
	50	0-20	Twigs 1° e 2° order	1/3 upper	All	20	Defoliated branches and twigs, reddened needles
29	25-30	-	Chioma	Lower third		40	Crown dried out up in the lower half due to shading by the adjacent beech
30			Twig 2° order	Intermediate third.	E	1	Small healing wound on the trunk, daughter of the PM 22, first cone produced in 2019 (empty)
			Twig 2° order	Lower third	E	1	
			Twig 1° order	Lower third	N-E	0	Healing wound close to trunk
			Trunk	Lower third	E	0	Wound due to friction, broken twig
31		60-80 e >80	Branche di 1° e 2° order	Thorough	All	60	Branches dried out, declining
32			Trunk	Half height		15	Four branches dried out, with wounds due to herbivores

3. Results

3.1 Position of the A. nebrodensis trees

The georeferencing of the 30 adult trees of the natural population of *A. nebrodensis* allowed to determine the exact position of trees and the actual distribution of the species in the Madonie range.

The population is spread over an area of 84 hectares. The extant trees are subdivided into three nuclei. The main nucleus is made up of 27 plants, distributed along the Vallone Madonna degli Angeli on the north-western slope of Mount Scalone, on an area of approximately 41 hectares. Within this group, the farthest trees are the no. 10 (and 11) and the no. 27 which are 720 m far towards W-E direction, while in the N-S direction the maximum distance (about 700 m) is between the trees no. 21 and no. 4-6. The second nucleus consists of the sole tree no. 1, near the top of Monte dei Pini; while the third nucleus consists of trees no. 23, 24 and 25 (with the latter two located about 200 m apart from the former) on the eastern slope of Monte Cavallo. This nucleus of three plants, located at the south-eastern end of the population, is about 1.9 km from the farthest plant, the no. 21, along the SE-NW direction.

3.2 Tree health surveys

Table 1 reports a comprehensive decription of disorders and symptoms observed on the surveyed *A. nebrodensis* trees. In table 2 trees were subdivided based on the proportion of affected crown.

Twenty-six out of 30 adult trees of the natural population of *Abies nebrodensis* reported disorders and symptoms limited to a portion of the crown less than or equal to 15%. For 11 of these trees, damage appeared as a number of little scattered spots and affected 1% of the foliage, while 15 trees showed disorders limited to 2-15%. Two trees, the no. 12 and the no. 18 showed disorders on a significant portion of the crown (between 16 and 50%), while the no. 28 and no. 31 showed signs of a long-standing decline, with more than 50 % of foliage damaged or missing.

Percentage affected crown	No. of trees	Id. Trees
0-1	11	8, 10, 13, 15, 16, 17, 20, 22, 26, 29, 30
2-15	15	1, 2, 4, 6, 7, 9, 11, 14, 19, 23, 24, 25, 27, 32
16-50	2	12, 18
> 50	2	28, 31

Table 2. Trees were subdivided based on the percentage of affected crown in four classes.

Localization of symptoms in the crown	No. of trees	Id. Trees
Lower third	13	2, 7, 8, 10, 13, 14, 15, 16, 17, 19, 22, 26, 29
Lower + intermediate	7	1, 11, 18, 20, 24, 27, 30
intermediate	1	32
thorough	8	4, 6, 9, 12, 23, 25, 28, 31
Lower + upper third	1	21

Table 3. Trees were grouped based on the localization of symptoms in the crown (ideally subdivided in thirds along the stem axis).

Type of symptom	Number of affected trees	Id. trees
Reddened shoots and twigs	13	1, 2, 6, 7, 10, 11, 12, 14, 15, 16, 17, 23, 24,
Defoliation due to excessive shading	4	20, 22, 26, 29
Wounds due to herbivores	9	1, 7, 8, 10, 14, 19, 24, 26, 30
Small leaves and chlorosis	3	2, 12, 16
Desiccation of twigs and branches	16	6, 7, 9, 10, 11, 12, 15, 18, 20, 21, 22, 25, 27, 28, 31, 32

Table 4. Main symptoms detected and their distribution among the 30 *Abies nebrodensis* trees.

In the trees of the first group, with the least impact of disorders, the prevailing symptom was the reddening of the outer branches and twigs and of the terminal shoots, dotted in the lower part of the canopy. These symptoms are often accompanied by wounds on the lower branches caused by wild herbivores (7, 8, 10, 26) or due to the rubbing with the ground caused by the wind. Plants no. 20, 22, 26, 29, despite showing the lower part of the foliage withered due to the shading exerted by the surrounding beech trees, are growing well and exhibited good vegetative conditions, benefiting of better edaphic conditions.
Most of the trees in this first group showed symptoms limited to the lower part of the crown, due to the effect of herbivores, or to the high temperatures reached by the bare soil and the strong light during the sunny days of the summer months (Table 3).
The group of trees showing a percentage of affected crown between 2 and 15% was the most represented. In this group, the most frequent symptoms were the total or partial desiccation of twigs and branches, due to the detrimental action of wild herbivores or to environmental stresses. The presence of reddened twigs in the outer crown is another symptom frequently observed in this group of plants. Some trees of this group, such as no. 1, 9, 11, 23, 24, 25, are located near ridges, exposed to strong winds, where the environmental conditions are

certainly hard. On these trees the symptoms are distributed at different heights throughout the crown (Table 3). Three trees showed more than 20% of damaged crown. Among these, the no. 12 grows in a site very exposed to strong winds, in the middle of a scree, where the site conditions are certainly poor, both from an atmospheric and edaphic point of view. This tree is subjected to a general weakening that probably favor the action of opportunistic pathogens. The no. 18 is affected by the shading exerted by the surrounding beech trees and by the poor soil conditions. The trees no. 28 and 31 showed a very stunted growth and advanced decline, due to the repeated action of wild herbivores. The attempt to improve their growth conditions requires the restoration of an adequate protective system of fences, to prevent herbivores from approaching.

As regards the frequency of the main symptoms (Table 4), the most commonly observed were: the reddening of the twigs and shoots (in the outer crown) and the partial or total desiccation of twigs and branches. Other symptoms found are the presence of wounds on the distal part of branches and on the stem, the defoliation in the lower crown due to conditions of excessive shading, and small needles with chlorosis, limited to a few twigs or branches.

3.3 Fungal isolations

Tables 5 shows the fungal taxa isolated in 2019 from the needles affected by yellowing, reddening, partial or total necrosis. Table 6 reports the fungal taxa isolated from green and affected needles sampled in 2020. Almost the 80 % of the obtained colonies are represented by 2 taxa: *Cytospora abietis* Sacc. (syn. *C. pinastri* Fr., anamorph of *Valsa abietis* (Fr.) Fr.) and *Rhizospaera macrospora* Gourb. & M. Morelet (see also Fig. 2).

taxon	no. isolations	Frequency (%)
Cytospora abietis anam. of *Valsa abietis*	86	34.7
Rhizospahera macrospora	52	21.0
Thysanophora penicllioides	11	4.4
Alternaria alternata	9	3.6
Rhizosphaeria pini	4	1.6
Coniothyrium aleuritis	3	1.2
Alternaria ethzedia	3	1.2
Foliophoma sp.	2	0.8
Penicillium sp.	1	0.4
Lemonniera sp.	1	0.4
Dendrophoma pleuropsora	1	0.4

Table 5. Frequency of isolations (percentage of plated samples colonized by a given fungus) of the fungal taxa grown out in vitro from the needles sampled in 2019.

Species of the genus *Cytopsora* were reported as agents of cankers on broadleaves and conifers. As canker-causing fungus, *C. abietis* is reported on many species of firs, spruces and on Douglas-fir. Infections are due to spores released from cankered branches and spread by rain, wind, insects, birds or man, and typically occurs through wounds, first infecting and killing the bark and eventually spreading to kill the entire branch. *Cytospora* spp. are mainly opportunistic, being favoured by trees weakened by drought, late frost or growing in bark killed by other pathogens. Damage is usually seen on older, larger trees.

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	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

Table 6. Frequency of isolation of the fungal taxa grown out in vitro from affected and green needles sampled in 2020.

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 behavior was evidenced in the samplings carried out on *A. nebrodensis* in 2020, which allowed isolation of *Cytospora pini* from green needles. The same behavior was shown for *Rhizosphaera macrospora* which was isolated from both reddened and green needles, although the frequency of isolation in the latter was lower.

Actually, *C. abietis* (syn. *C. pinastri*) has been reported as an endophyte in senescent needles of *A. alba* in Switzerland. In the same study, senescent needles were also colonized *R. macrospora*, while *T. penicilliodies*, a fungus isolated from *A. nebrodensis* with a frequency of 4.4% (see Table 5), was reported as a colonizer of litter needles, as a typical saprophyte. 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*.

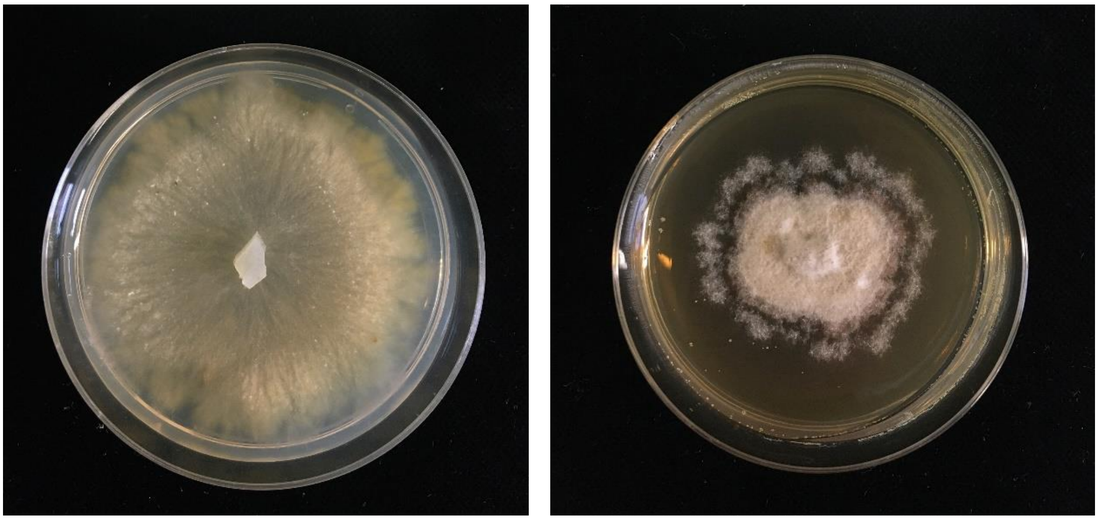


Fig. 2. One week grown (PDA plates) colonies of *Cytospora abietis* (left) and *Rhizospahera macrospora* (right). the two fungi most frequently isolated from reddened needles.

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 be occur as endophytes.

As regards *Abies nebrodensis*, various weakening factors promoted the necrotrophic action of these two fungi: drought, high temperature and strong light during summer, late frosts, injuries due to wild herbivores on twigs and branches. The next surveys should monitor the eventual presence of cankers on twigs and branches that *Cytospora* could cause. Up to now, disorders in the crowns due to cankers have never been observed on *A. nebrodensis*.

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. *Alternaria alternate*, *Epicoccum nigrum* and *Phoma* sp. are ubiquitous fungi reported in numerous habitats and hosts, known for their saprophytic behavior, although some species of *Alternaria* are weak pathogens.

Coniothyrium sp. (*Dothideomycetes*) is reported as a leaf endophyte of conifers and as a pathogen on juniper; *Foliophoma* sp., isolated from olive leaves attacked by other pathogens; *Lemonniera* is another *Dothideomycetes* found as a saprophyte in dead bark of conifers and in decaying, dead and rotting leaves of various broadleaves; *Dendrophoma pleurospora* was reported from branches of oaks in Georgia and from leaves and trunks of grapevines affected by esca disease.

3.4 UAV Multispectral survey

At the beginning of October 2020 the survey of the *Abies nebrodensis* natural site was carried out by a drone (UAV – unmanned aerial vehicle) equipped with a multispectral camera. During the drone flight panoramic shots of the habitat and of the individual trees of the *Abies nebrodensis* population were acquired (Fig. 3).

Fig. 3 Shots captured by drone of the trees no. 8, 12, 16-17 and 10-11 from left to right and top to bottom.

RGB ortophoto

The 3D reshaped DTM (Digital Terrain Model) (Fig. 4) of the area where the natural population of *A. nebrodensis* shows the distribution of trees in relation to the morphology of the territory. Most of the trees that are part of the main nucleus are located along the N-NW slope of Monte Scalone and only a few plants, such as no. 21, 22, 30 and 31 are located on the opposite side of the Madonna degli Angeli Valley. In Fig. 4 it is also possible to observe the position of the remaining separated trees, the no. 1 isolated near a southern ridge of Monte dei Pini and the no. 23, 24 and 25 on the eastern side of Monte Cavallo. The image also shows the latter 4 trees are located along ridges at higher elevation (between 1650 and 1700 m a.s.l.) and are very exposed to the prevailing winds.

Figure 4. 3D reshaped Digital Terrain Model of the *A. nebrodensis* population distributed along the Vallone Madonna degli Angeli, Monte dei Pini e Monte Cavallo.

Fig. 5 reports the orthophoto of area no. 1 including the main nucleus of the *A. nebrodensis* population. An ortho-photo (ortho-image) is an image which has been “corrected” for the geometric distortions (different projection, lens / sensor distortion, relief) so that it can be used as a map and to measure true distances. This is fundamental for: using the image with other maps in a Geographic Information System; combining the image or the results of its processing (e.g. classification) with other maps; comparing the image or the results of its processing with points representing the ‘ground truth’.

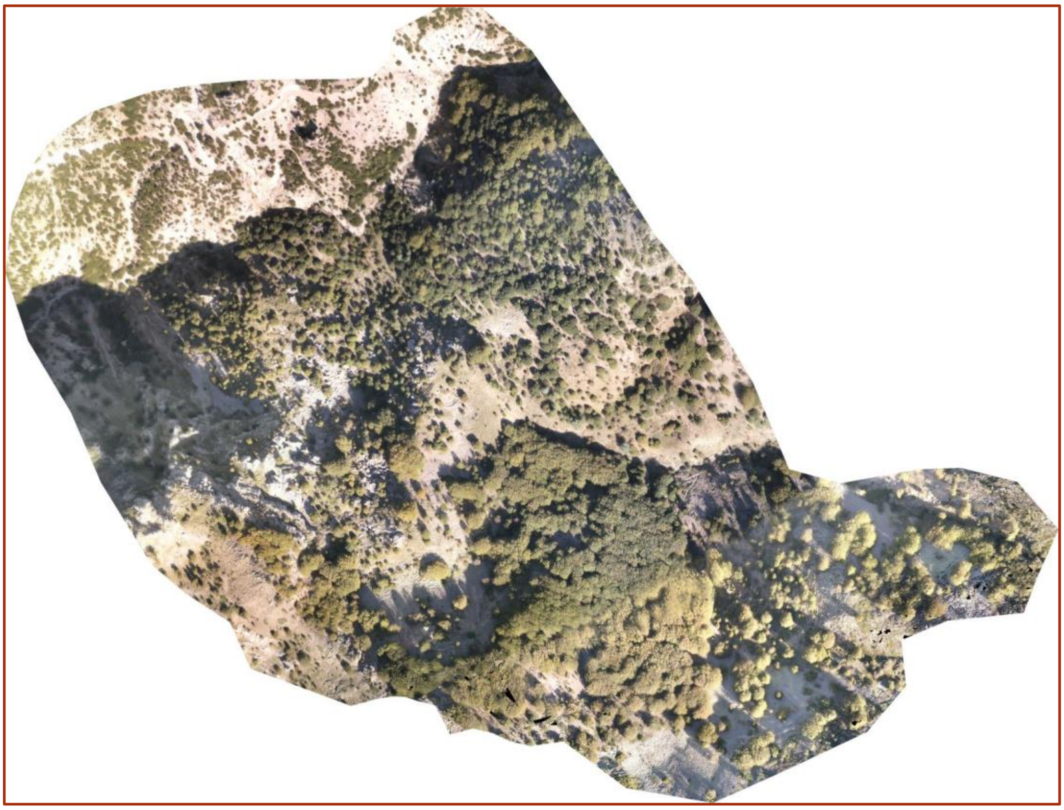


Fig. 5 Ortophoto (RGB) of the Area no. 1 including the main nucleus of the Abies nebrodensis population.

Fig. 5 shows the discontinuity of the vegetation in the *A. nebrodensis* habitat, reported as "9220: beech forests with *A. nebrodensis*" in the Habitat Directive. In the higher belt, tree species are mostly represented by beech (*Fagus sylvatica*) which forms the wooded nuclei of larger extension, though they are fragmented. In addition to the beech, the English oak (*Quercus robur*), the holly (*Ilex aequifolium*), the mountain elm (*Ulmus glabra*), the Turkey oak (*Q. cerris*) are sporadically found. At the lowest elevations in the map there are discontinuous formations of holm oak (*Q. ilex*). The Madonie fir is extremely fragmented at elevations ranging between 1400 and 1700 m a.s.l. Some trees are fully isolated, often growing on screes, others grow near or inside beech groves or other broad-leaved formations. In this case, firs benefit from better edaphic conditions due to presence of this broad-leaves, even if they may be affected by excessive shading.

The RGB image shows that a substantial part of the area is made up of bare soil, or covered by grasslands or bare rocks.

Fig. 6 Ortophoto of the area 02, including the trees no. 23, 24 and 25 of the Abies nebrodensis natural population.

Fig. 6 represents the orthophoto of area 02 where trees no. 23, 24 and 25 are located. The image shows a very exposed ridge area, where the vegetation is made up of fragmented formations of beech and shrubs. The partion covered by bare soil and cushion plants is prevailing.

Infrared maps

The infrared image (see Fig. 7) shows in red the reflectance of the cover canopy of the trees. The spectral reflectance is based 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).

Vegetation emerges in shades of red, soils vary from dark to light browns, or can appear yellow or green or grey, depending on their composition. Hardwood trees generally will appear light red than Coniferous as needles have a darker response comparing to the leaves.

The infrared band shows various hues of red colours which represent the differences between the reflection of the trees. In general, profound red hues (purple) represent broadleaves or/and healthier vegetation (greater amount of chlorophyll), whereas the lighter reds indicate sparsely vegetated or grasslands areas.

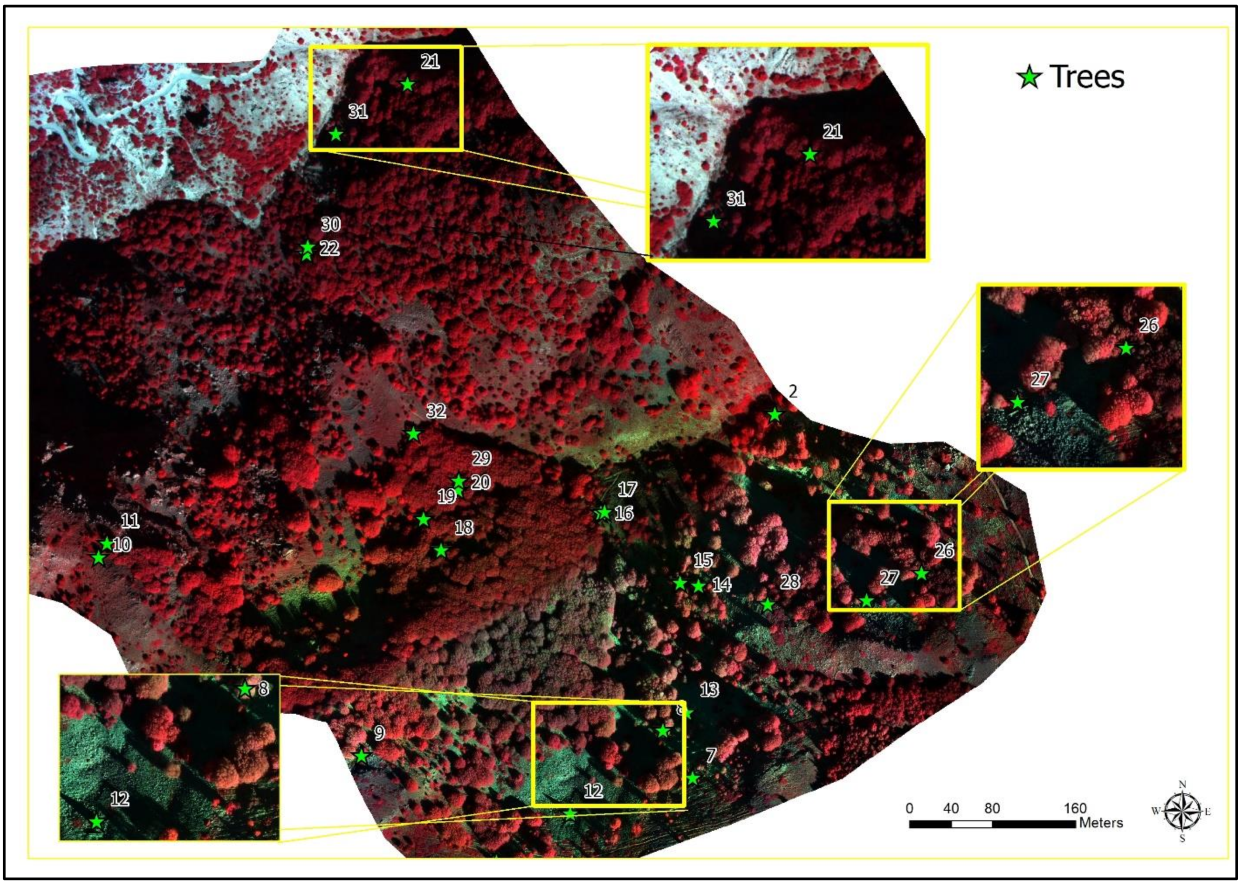


Fig. 7. Infrared map of the area 1, including the main nucleus of the *A. nebrodensis* population, with some zoomed sites where A. nebrodensis trees can be distinguished.

High resolution images allow portions of interest to be zoomed in for detailed analyses. In Fig. 8 and 9, for example, three areas where some *A. nebrodensis* trees are located have been zoomed in. From the enlarged images it is possible to carry out observations and reflectance analyses of single crowns of the fir trees and to make inferences on their physiological state. Maps will allow for a comparative analysis between the different trees in relation to site conditions. Furthermore, a second drone survey that has been planned for the end of the project will allow to monitor the vegetative conditions of trees over time in relation to the climatic trend and the measures that will be implemented in the meantime.

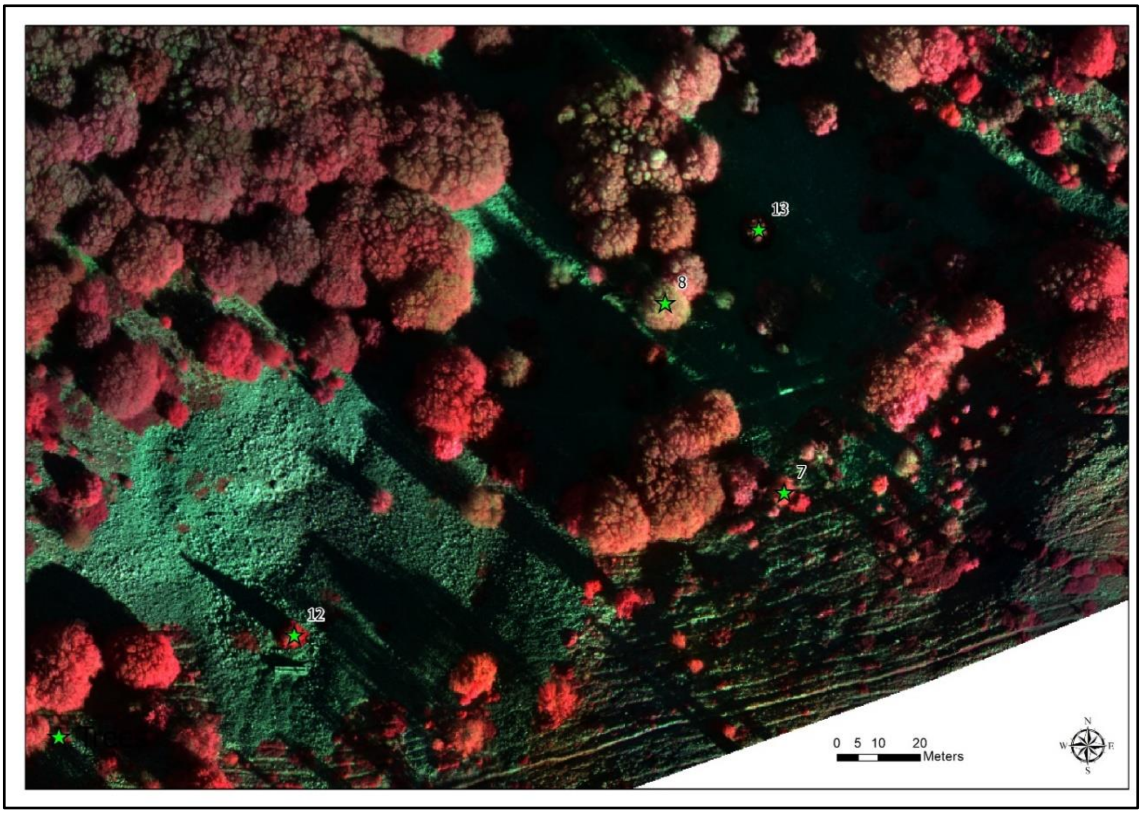


Fig. 8. Detailed infrared map of the area 01 including the trees no. 8, 12 and 13.

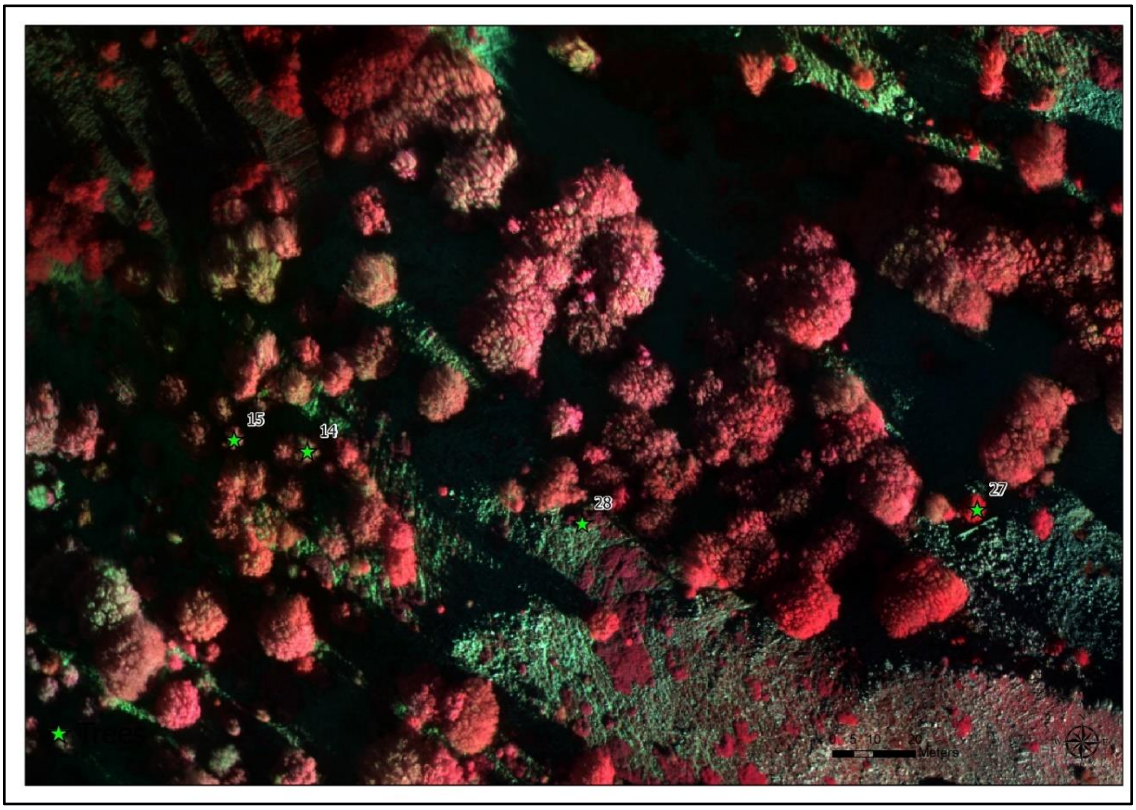


Fig. 9 Detailed infrared map of the area 01 including the trees no. 14, 15, 27 and 28

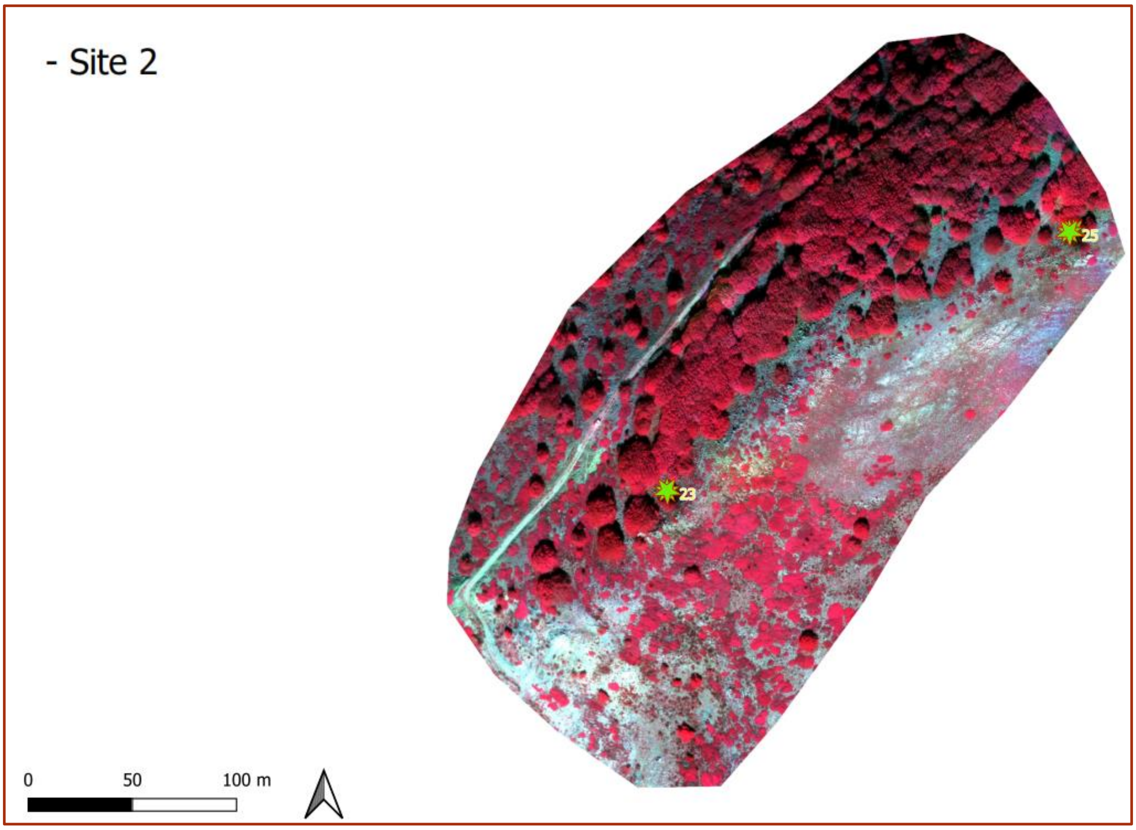


Fig. 10. Infrared map of the area 02 (site 2) of the A. nebrodensis natural population, including the trees no. 23. 24 and 25.

Similarly, Fig. 10 represents the infrared image of area 02 of the *A. nebrodensis* population. which includes trees 23, 24 and 25. In the different hues of red of trees, shrubs and grass can be appreciated. The possibility of enlarging in detail the portions where the firs are located will allow comparative analysis to be carried out.

NDVI maps

A vegetation index, such as NDVI, is an indicator that describes the greenness, the relative density and health of vegetation for each picture element, or pixel, in a drone image.
NDVI values range from +1.0 to -1.0. Areas of barren rock, sand, or snow usually show very low NDVI values (for example, 0.1 or less). Sparse vegetation such as shrubs and grasslands or senescing plants may result in moderate NDVI values (approximately 0.2 to 0.5). High NDVI values (approximately 0.6 to 0.9) correspond to dense vegetation such as that found in temperate and tropical forests or crops at their peak growth stage.
The NDVI of the site 01 (Fig. 11) ranged between -0.4 and +0.61. The NDVI map shows the areas where the index is higher than 0.5 (red and orange areas) that correspond to the presence of dense vegetation are rather limited. The areas in yellow and light green with NDVI ranging between 0 and 0.3 prevail. They represent bare soils or soils with grasslands or discontinuous

shrubs vegetation. Actually, the survey carried out at the beginning of October, at the end of the vegetative season, has captured the foliage of many deciduous trees in the senescence phase.

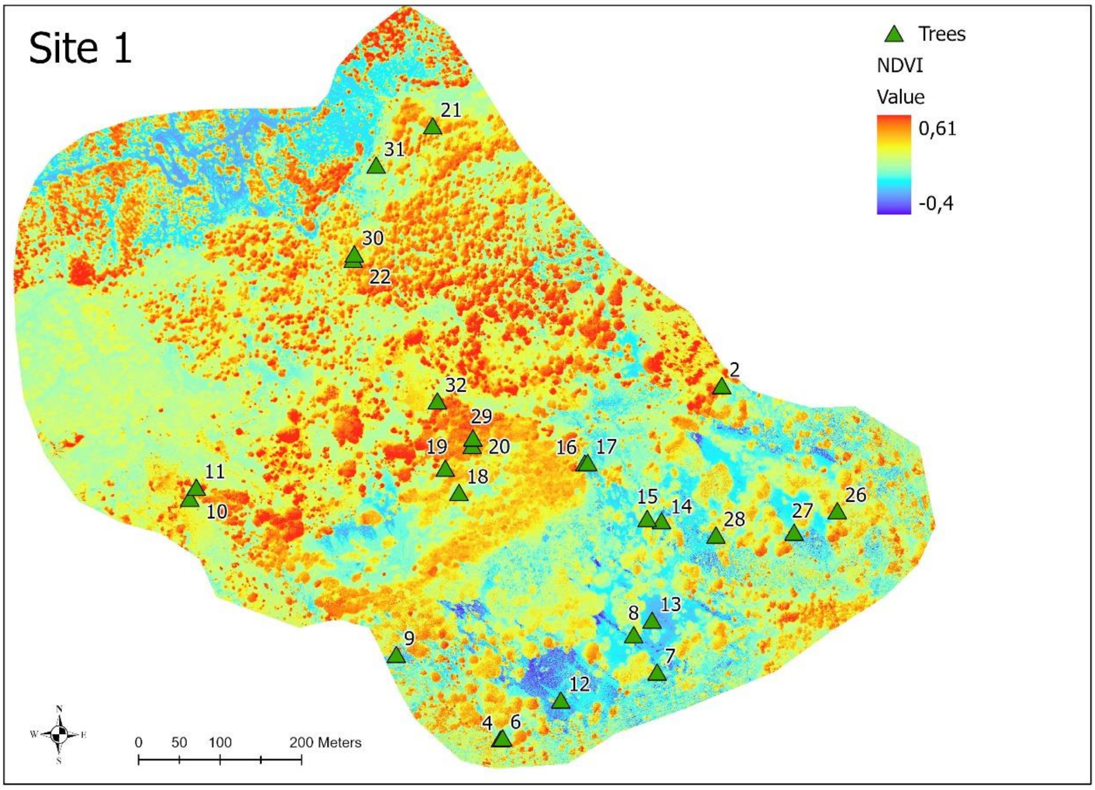


Fig. 11. NDVI map of the area 01 including the main nucleus of the A. nebrodensis population.

The multispectral images captured of the *A. nebrodensis* area area will be used to carry out an environmental analysis on a statistical basis. The maps obtained will be processed to conduct a geographic investigation regarding the distribution of the trees and the evaluation of their vegetative state based the NDVI values extracted from each single crown. Based on these values, an ANOVA analysis will be performed on each tree in to evaluate whether it is possible to infer their vegetative state, possibly studying their relationship with environmental conditions (elevation, exposure, isolation, etc.) through a spatial autocorrelation analysis. When analyzed through time, NDVI can reveal where vegetation is thriving and where it is under stress, as well as changes in vegetation due to human activities such as deforestation, natural disturbances such as wild fires, or changes in plants' physiological state.

4. Conclusions

Phytosanitary surveys were carried out in November 2019 in October 2020. Trees were subjected to a careful visual inspection to evaluate their vegetative and health condition based on observations of the crown shape and transparency, turning foliage, presence of declining, desiccated or damaged parts, occurrence of lesions.

Considering the hard environment where the relic trees of the *A. nebrodensis* population live, their health state resulting from the survey 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%. Two plants, the no. 12 and no. 18, had symptoms extended to 20-30% of the crown. Only the trees no. 28 and 31 showed an advanced decline, mainly due to the destructive action of wild herbivores.

Most of the observed symptoms are located in the lower part of the crown and are represented by branches and twigs drying out, needles reddening and defoliation due to environmental stresses to which the trees are subjected, such as summer drought, late frosts, the high temperatures reached by the bare rocks during summer and the intense solar radiation, in addition to the damage caused by wild herbivores, in particular fallow deers that have massively reproduced in the Park territory in the last years. Protection of *A. nebrodensis* trees requires the timely implementation of the new fencing system (traditional, with a 2 m high mesh, plus an additional external electric fence) as planned in the project. It would be advisable at a political level, that the authorities in charge plan new measures in accordance with the local hunting associations to reduce and maintain the population of fallow deers at a sustainable size.

The 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.

The analysis of the fungal microflora isolated from symptomatic needles and twigs (reddened and dryed out) showed the clear prevalence of two species of Ascomycetes, *Cytopospora abietis* and *Rhizosphera macrospora*. These two fungi are known as weak pathogens on conifers weakened by other stressors. Both were isolated also from green needles of *A. nebrodensis*, showing the ability to colonize living tissues as endophytes, without causing apparent damage. Actually, both were reported as endophytes in senescent silver fir needles in a study carried out in Switzerland. It is therefore not surprising to find them in symptomatic and healthy needles of *A. nebrodensis*, though this represents their first report on conifers in Italy.

The multispectral maps obtained by the drone represent a sound basis for analyzing the vegetative and physiological state of the *A. nebrodensis* trees in the natural population and of the habitat vegetation. The information obtained with remote sensing on the physiological and vegetative state of individual trees will be validated based on a cross comparison with the data obtained in the phytopathological inspection.

The maps obtained will be useful for conducting a comparative investigation of the health state of the single trees in relation to environmental conditions through a spatial autocorrelation analysis. By carrying out a second drone survey before the end of the project, multispectral maps will be also useful to monitor the evolution over time of the health state of trees as a function of climatic fluctuations and of the measures that will be implemented meanwhile.

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