SAM FIX

SAving Mediterranean Forests from Invasions of Xylosandrus beetles and associated pathogenic fungi

LIFE SAMFIX PROJECT

SAving Mediterranean Forests from Invasion of Xylosandrus beetles and associated pathogenic fungi

WHY LIFE SAMFIX?

In September 2016 in the Italian National Park Circeo the first massive outbreak of ***Xylosandrus compactus*** and ***Xylosandrus crassiusculus*** was discovered in a European natural ecosystem.

Infested trees showed damage such as desiccation, branch dieback and shoots breakage. Before the Circeo outbreak, ***X. compactus*** had already been identified in other Italian Regions such as Campania, Tuscany and Liguria and had most recently emerged in France in Saint-Tropez and Saint-Jean-Cap-Ferrat and in the Botanical Garden Villa Thuret of Antibes. ***X. crassiusculus*** was instead identified for the first time in central-northern Italy and then in France at Mont Boron near Nice, in the biological reserve Ile Sainte Marguerite near Cannes and in Spain at El Pla de les Clotxes in the region of Valencia.

The alarming damage to the Circeo promontory, the increasing detections of both species of ***Xylosandrus*** along the Tyrrhenian coast in or near natural parks, and the consequent vulnerability of many species of Mediterranean vegetation, gave us reason to combine the scientific expertise we had acquired to test and implement protocols and tools to deal with the threats, to gain knowledge to better understand the pathways of spread of these bark beetles, the causes of damage and the risks to ecosystems; and to share the results with other stakeholders.

This booklet brings together the work done and the results obtained, in order to spread awareness and knowledge, with the ultimate goal of safeguarding and protecting Mediterranean forests from invasions by *Xylosandrus compactus* and *Xylosandrus crassiusculus*.

This project is co-financed by the LIFE programme, since 1992 the main financial instrument of the European Union through which projects related to the environment, climate change and nature conservation in Europe are funded. The project coordinator is the Circeo National Park, while other project beneficiaries are the Lazio Region, Terrasystem s.r.l., Università degli Studi della Tuscia, Universidad de Alicante, l'Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement and Ville d'Antibes Juan-les-Pins.

SAMFIX
SAving Mediterranean Forests from Invasions of Xylosandrus beetles and associated pathogenic fungi

WHAT ARE XYLOSANDRUS BEETLES?

Xylosandrus compactus

Xylosandrus crassiusculus

Xylosandrus compactus *(black twig borer)* and ***Xylosandrus crassiusculus*** *(granulate ambrosia beetle)* are highly polyphagous parasites that can infest many tree and shrub species.
They originated in Asian regions and then spread to other parts of the world, probably through the plants and timber trade.

These beetles, which host symbiotic fungi, dig tunnels in young branches ***(X. compactus)*** and trunks ***(X. crassiusculus)*** of trees.
Infested trees can show wilting, branch deformation, breakage and general decay.

Since many species of plants growing in the Mediterranean region are considered possible hosts, widespread desiccation of the Mediterranean maquis could occur if no containment intervention is put in place.

AMBROSIA BEETLES AND FUNGI

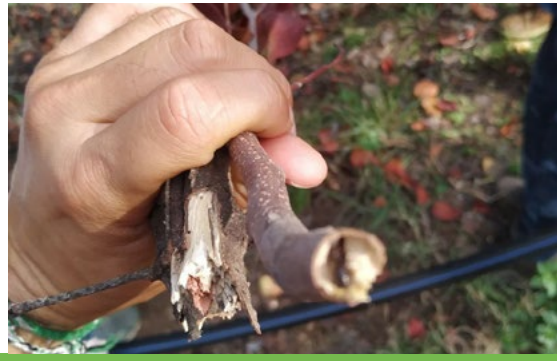
Xylosandrus species establish symbiosis with fungi. Some are brought in a particular structure called mycangium located between head and abdomen, and represent the source of nourishment for the different stages of insect development inside the tunnels, as ***Xylosandrus*** species are unable to draw enough nourishment from the woody tissues. A vast and biodiverse community of other fungi is associated in other parts of the insect's body and some of them, such as the Fusarium, are jointly responsible for the damage visible on the plants.
The results of the investigations conducted within the **LIFE SAMFIX** project indicate that other potentially pathogenic fungi, some of which reported for the first time in Europe and/or associated with the main insect hosts in other areas of the world, are part of the fungal community associated with the insect that includes a total of more than 200 fungal taxa.
This evidence confirms the need for accurate monitoring of the fungal community carried by the insect, to be implemented both in the propagation corridors of ***Xylosandrus*** species, for example nurseries, and in natural areas of invasion, in order to prevent potential epidemic phenomena.

THE PROJECT
THE OBJECTIVES

LIFE SAMFIX aimed to protect the forests of the Mediterranean basin against invasions by ***Xylosandrus*** beetles, which pose a serious threat to plant ecosystems in the Mediterranean scrubland and beyond.

This aim was to be achieved through the following specific objectives:
- establish in and around 6 European detection sites in or close to natural parks effective protocols for prevention, early warning and rapid response, eradicating or containing current infestations and preventing future expansions;
- extend prevention and early warning protocols to 8 natural parks located in the surroundings of these sites to prevent expansions;
- disseminate knowledge on pathways and risks, and prevention, early detection and rapid response protocols amongst Mediterranean and EU bodies, networks and experts engaged in IAS policies and amongst natural parks managers to facilitate adoption of phytosanitary measures and replication of protocols to preserve Mediterranean forests and maquis landscapes.
- involve citizens and local stakeholders in Citizen Science activities and make them aware of the protection of the natural environment against these alien species that represent a threat to native forest biodiversity.

Project activities were focused on approximately 42,179 ha of natural and semi-natural environments, of which 40,180 ha of relevant habitats in Natura 2000 sites.
In addition to field tests of the experimental protocols, through laboratory activities we tried to understand the identity of symbiotic fungi and their role in the damage caused to infested plants; as well as the factors and pathways of introduction starting from the comparison of bark beetle DNA. In addition, the impacts on ecosystem services and functions were analysed as well as the impact of awareness raising activities on the target groups. Finally, the available tools have been enriched with an App to report damage and/or presence and with an intelligent trap.



TRAPPING PROTOCOLS

Trapping is the main technique for early and continuous collection of information on the presence of insects and can also be used to attract or repel them, for containment purposes. However, it was necessary to understand which lures and which type of traps would be most effective; and how they should be positioned to achieve the most valid results.
For these verifications, experimental trapping networks were installed over the years in various project sites, testing various configurations and analysing first the best lures and traps; and then the best positioning.

FIELDWORK

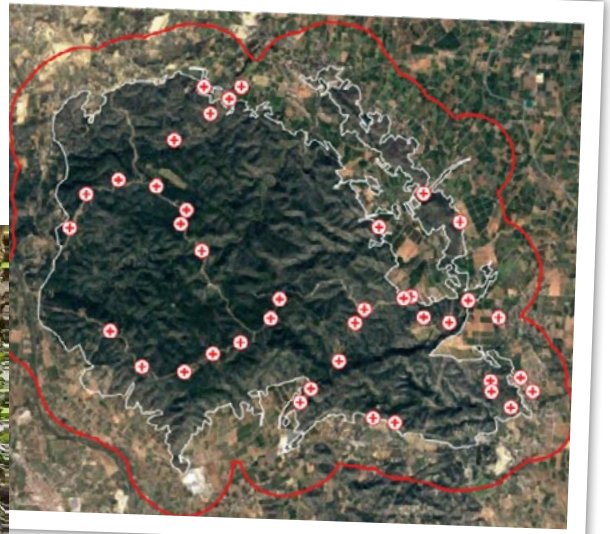
In all project sites, networks were installed during the insect flight season, from March / April to October / November.
Traps were regularly monitored: every 2/3 weeks samples were collected and provided to the scientific team for laboratory analyses, while lures were changed every 6 weeks.

CIRCEO NATIONAL PARK AND SURROUNDINGS:

Networks were installed in the park areas with the highest presence of ***Xylosandrus*** species: in the Circeo Promontory localities Quarto Freddo and Quarto Caldo, in the lowland forest and in nurseries located within the territory of the National Park.

Trapping results showed a decreasing level of ***Xylosandrus*** infestation of the Park, currently considered very low and without evident damage to the flora. However, the insect is still present in several areas, so monitoring actions are still required.

Linear transept trapping network in Circeo National Park, 2021.



Trapping network in El Tello and surrounding.

EL TELLO AND SURROUNDINGS:

Experimental networks were installed in the El Tello forest and its surroundings, where the first species of ***Xylosandrus crassiusculus*** in Spain had been detected. After reports of further presences in Náquera, in the northern part of the Valencian region, experiments were also carried out there.

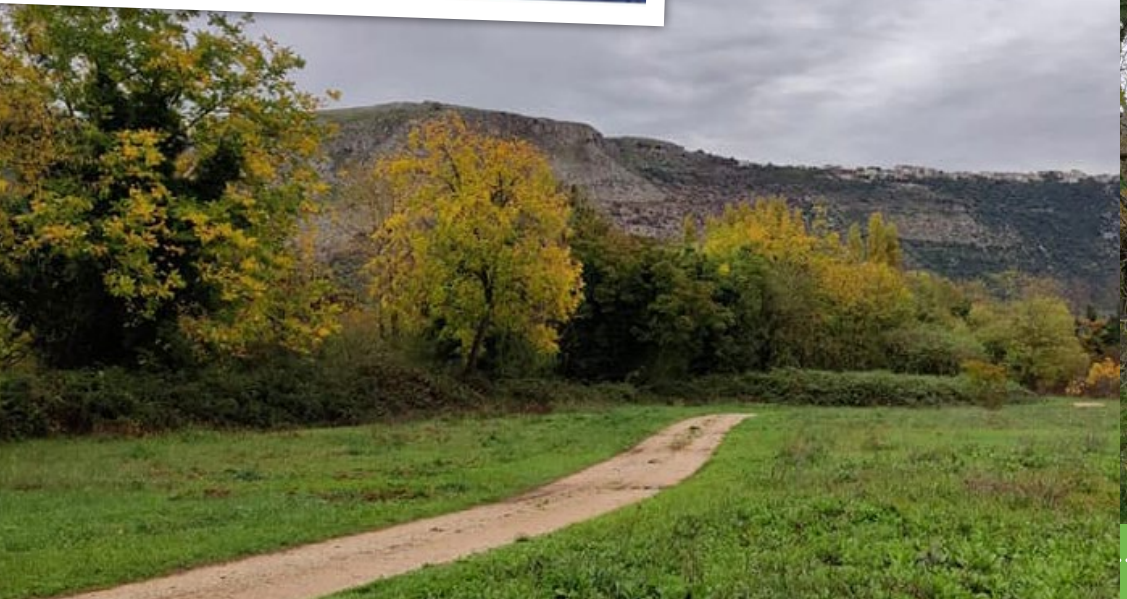
The latest results showed that the only ***Xylosandrus*** species present in Spain until recently did not spread further in El Tello. However, its presence was ascertained in other areas of the region, only on carob tree. Since 2019, ***X. compactus*** has also been introduced into the country.

ANTIBES, ILE STE MARGUERITE AND NICE COTE D'AZUR

Experiments were mainly carried out in areas with greater ease of access to allow constant monitoring, namely in Antibes, in its botanical garden Villa Thuret, in Bois de la Garoupe, and in 2021 also in one of its squares. Other networks have been installed for preventive and monitoring purposes in Ile Ste Marguerite, Cornices de la Riviera, Mont Boron and surroundings.

Also in France the latest monitoring results showed a decrease of ***Xylosandrus*** species abundance in the sites where they were initially found and a very low level of damage, while expansion to neighbouring areas - e.g. on the edge of Cornices de la Riviera and other places in the city of Antibes - did not stop.

Trapping networks in Antibes, Ile Ste Marguerite and Nice Cote d'Azur in 2020.



THE VALIDATED PROTOCOLS

Various combination of lures were tested, with the final result that a combination of 4 compounds, i.e. quercivol, α-copaene, Ethanol and α-pinene was found to be the most effective for capturing the different species of ***Xylosandrus*** and obtaining information on their abundance and spread.

As regards the kind of traps, both the so-called multifunnel traps and the cross-panel traps were found equally effective as long as they are black or black/dark green. However, it has been noted that multifunnel traps are easier to manage.

The various tests to analyse the best positioning of the traps had two purposes:

- early warning and abundance monitoring;
- containment.

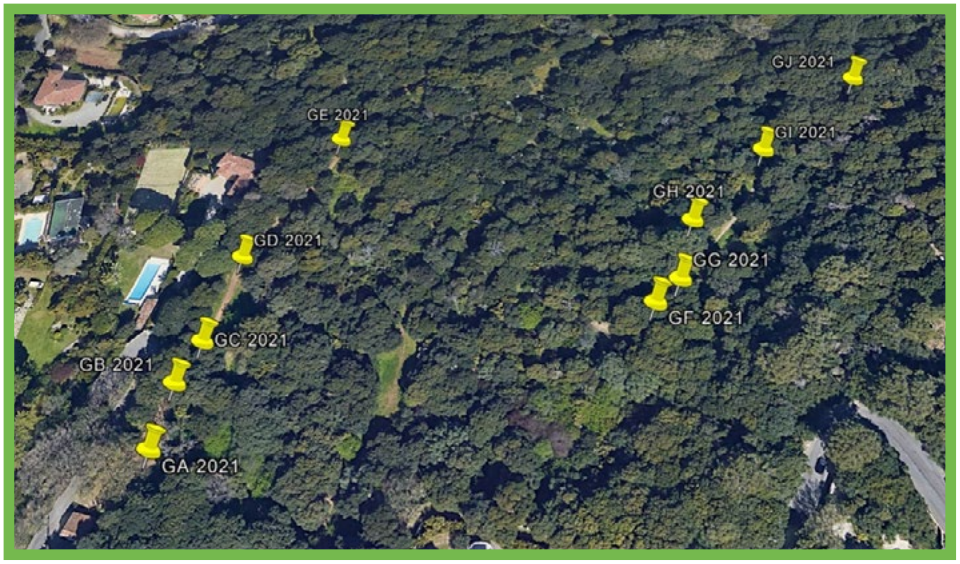
As regards the first purpose, it has been possible to establish that the realization of transects, spacing the traps about 20 meters apart, offers the best results, avoiding that insects get confused between two traps or that some areas remain uncovered.

MULTIFUNNEL TRAPS

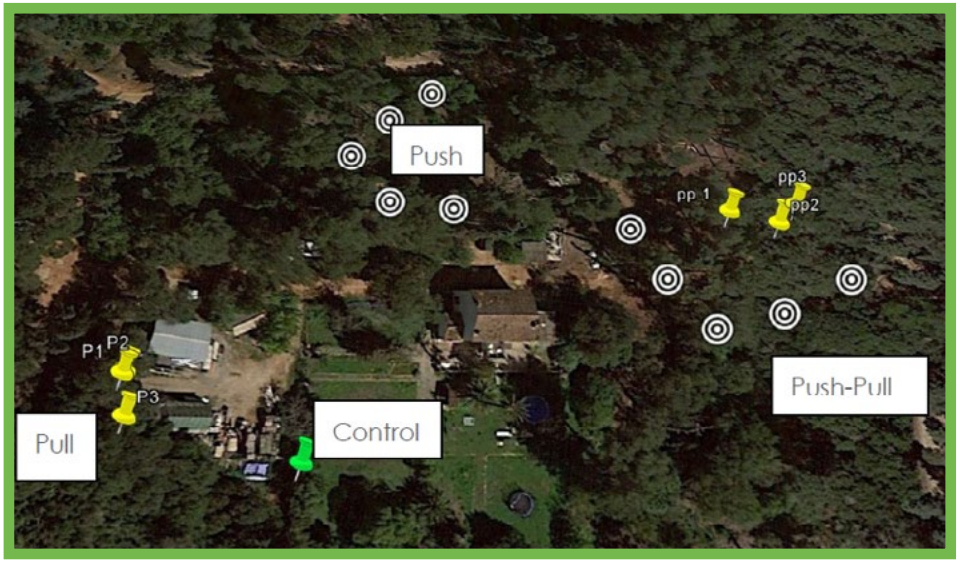
CROSSVANE TRAPS

Two protocols were tested for containment: the “mass trapping”, that is the installation of many traps with attractive lure around the area from where to eliminate the insects; and the “push & pull”, where a repelling product such as Verbenone is placed in the center of this area in order to push the insects towards the edge where attractive traps are deployed to attract (pull) and capture them.

Unfortunately, despite these protocols are notoriously effective with other scolytid species, the too low level of infestation observed in the tested areas in both France and Italy did not allow to validate with certainty their efficacy for ***Xylosandrus*** species, not offering statistically reliable data. Future experiments have to be carried out in highly infested areas in order to validate this method.



Tests of transepts at increasing distance, Boi de la Garoupe, Antibes (2021).



Push and Pull in Villa Thuret, 2021, with 4 configurations: Pull, Push, Push-Pull, and nothing.

X-TRAP

The **X-Trap** has been successfully developed and tested within trapping networks, to facilitate the collection of data from traps, especially in impervious areas.
It is a "smart" trap that allows for the real-time recognition of daily catches and the count of specimens of ***Xylosandrus***.

The X-Trap is equipped with a GPS detector and a digital camera, adding to the functionality of a traditional trap that of acquiring high resolution images of the captured insects, that are sent to a central server for subsequent processing. The energy required for operation in the field and data transmission is provided by solar panels, making the system ecological and sustainable.

The recognition and counting of specimens belonging to the species of interest of the genus ***Xylosandrus*** is carried out by means of computational algorithms based on deep neural networks, now widely used in various contexts including some of ecological and environmental interest.
Deep neural networks, trained in recognition through examples of images classified by expert entomologists of the **LIFE SAMFIX** project, have shown high recognition capabilities of ***Xylosandrus*** specimens. Automated and real-time counting of ***Xylosandrus*** specimens, recognized by the algorithm, will provide useful information for the prediction and prevention of infestations.

Regularly updating the samples used for training will improve the performance of the algorithm by making the prevention and monitoring capabilities robust even to future changes.

> TO FACILITATE THE COLLECTION OF DATA FROM TRAPS, ESPECIALLY IN IMPERVIOUS AREAS, THE X-TRAP HAS BEEN SUCCESFULLY DEVELOPED AND TESTED IN TRAPPING NETWORKS.

REPLICATION AREAS

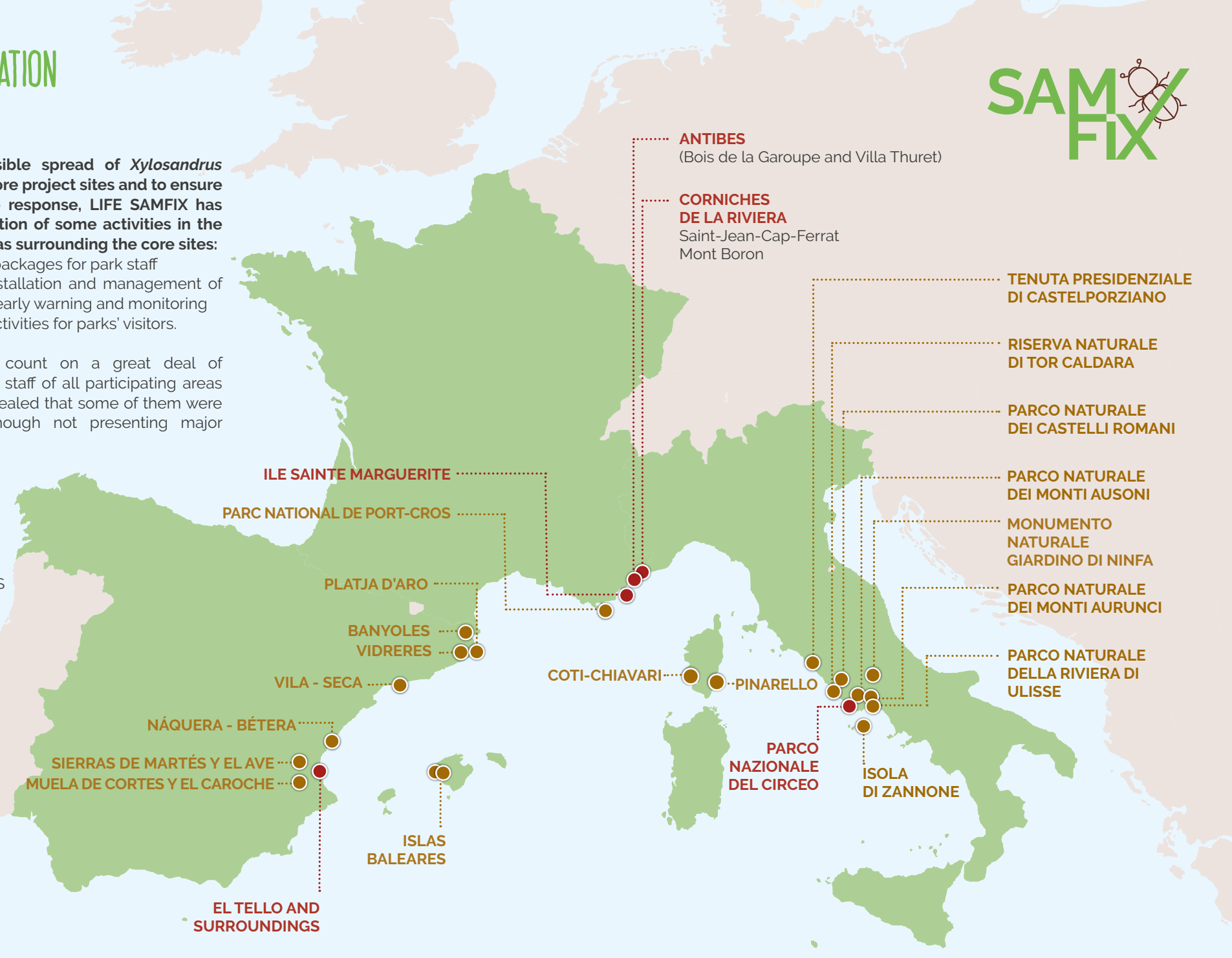
To prevent the possible spread of *Xylosandrus* species outside the core project sites and to ensure a rapid and effective response, LIFE SAMFIX has promoted the replication of some activities in the protected natural areas surrounding the core sites:

- events and training packages for park staff
- assistance in the installation and management of trapping networks for early warning and monitoring
- awareness-raising activities for parks' visitors.

The activities could count on a great deal of commitment from the staff of all participating areas and, unfortunately, revealed that some of them were already infested, although not presenting major damage.

LEGEND

- CORE SITES
- REPLICATION SITES



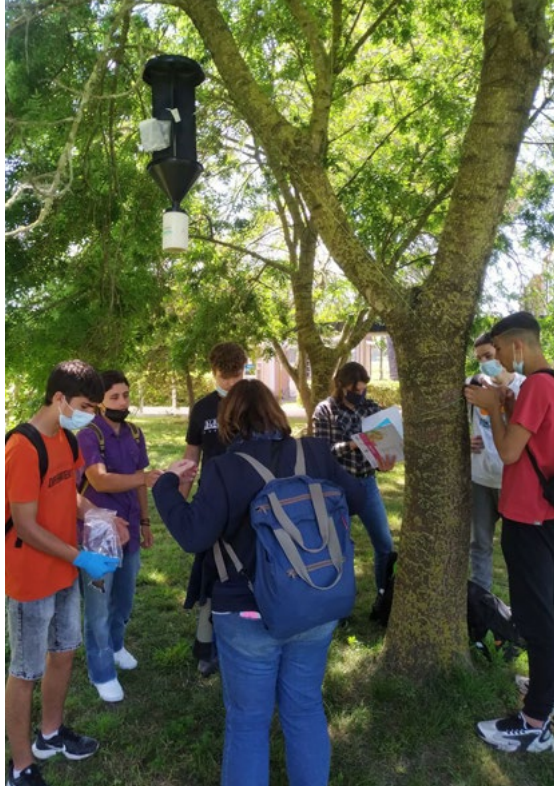
IN ITALY, the activity was planned in the following 6 Lazio Regional Parks: Riviera di Ulisse Natural Park, Monti Aurunci Natural Park, Monti Ausoni Natural Park, Castelli Romani Natural Park, Tor Caldara Nature Reserve.
During the project, other sites, both public and private, asked to be involved and have been addressed, thus covering all areas of particular naturalistic value in southern Lazio:

- Castelporziano Natural Reserve
- Ventotene and Zannone Islands
- Pantanello Oasis
- Ninfa Garden

Thanks to the trapping networks, in 2020 the presence of ***X. crassiusculus*** was detected in the Natural Parks Monti Ausoni and Monti Aurunci; and ***X. compactus*** in the latter. In 2021 some specimens were also reported in the Castelli Romani, Tor Caldara and Riviera di Ulisse Parks. However, the monitoring results here also showed a low number of ***Xylosandrus*** captured and no visible damage to plants.
In all the Regional Parks, as well as in Ninfa Garden, guided tours and educational events with schools were organised to raise public awareness.

IN SPAIN, two protected areas are located near to the first detection spot of ***Xylosandrus crassiusculus***: Muela de Cortes y El Caroche and Sierra de Martés y el Ave. They have been subjected to prevention activities through staff trainings and to monitoring and containment activities through the installation of traps for early warning. In September 2021, ***X. crassiusculus*** was found in 4 traps placed in Sierra de Martés y el Ave, although no attacks on plants were recorded.
The managers of the areas involved have been adequately trained and, for the summer of 2022, intensive monitoring activities have been planned in the area. Furthermore, following reports from private owners in Náquera and Bétera and from the Plant Health Department (DARP) of the province of Tarragona, the Spanish **LIFE SAMFIX** team provided assistance and information on both monitoring the infestation and removal and pruning of infested plants. Similar activities targeted Mallorca after the first detection of ***X. compactus*** in a private garden in 2019.

IN FRANCE, the park selected for replication of preventive activities was Port Cross National park. Initially, the **LIFE SAMFIX** team focused on the neighbouring coast, training staff and installing traps in nurseries and other public and private areas, because a westbound spread was taking place along the coast of the Cote d'Azur. Subsequently, the trapping network was extended to Porquerolles Island, close to the park, and then to the interior of the park. No species or signs of damage were found.
Meanwhile, following the detection of ***X. crassiusculus*** specimens north of Nice, trappings were deployed in more northern areas as an early warning tool to prevent infestation of the Mercantour National Park.
Finally, following a report in Corsica, the French team also provided there assistance and organised the monitoring of the infestation.



COLLECTION AND DATA ANLYSIS

LIFE SAMFIX has an on-line platform dedicated to the collection and processing of data on the areas monitored in Italy, France and Spain.
The platform provides information on the impacts of the invasion of ***Xylosandrus*** species in the monitored areas, and shows the results of the monitoring, mitigation and communication activities implemented by the partners.

THE PLATFORM IS FED WITH DATA FROM VARIOUS SOURCES:

- remote sensing activities through satellite sources, which offer an overview of the vegetation health in all areas;
- data collected in the field by park operators and project staff and entered manually;
- data provided through the "SAMFIX Agent" App, a mobile application which collects reports and provides users with a wide range of information on insects and the damage they cause.

The platform allowed to continuously monitor the level of invasion of ***Xylosandrus*** species, define risk zones and produce maps, analyses and reports, then made available on the platform itself.

The activities based on remote sensing and on data collected on the ground allowed to produce risk maps and assess the development of the invasion in the target areas over time. Risk maps indicate stress in the vegetation, which can result from pest attack. Stress due to other causes, such as water shortage, can sometimes foster the establishment of conditions favourable to the spread of pathogens.
Maps were also produced to understand which types of vegetation are most susceptible to pest attacks.

SYMBIOTIC FUNGI

Two specific analysis protocols were designed, tested and validated: one for the characterization of fungi associated with ***Xylosandrus*** species; and one to evaluate their pathogenicity for attacked plants. From the captured insects, but also from the trunks and branches of infected trees, fungi were extracted and analysed in the laboratory.

The results showed that several fungal species are associated with the Ambrosia beetle species. The fungi most abundantly present among those isolated from ***X. compactus***, ***X. germanus*** and ***X. crassiusculus*** were pathogenic. During the invasion process, Ambrosia beetles modulate their associated fungal community, including exotic species and native / local or recently acquired species. This modulation in the fungal community structure is likely influenced by the host community and environmental conditions.

The symbiotic activity of insects and associated pathogenic fungi could have an unexpected impact on a wide range of host species.
The discovery of new associations between insects and pathogenic fungi highlights the risk of new interactions with invasive alien species in nurseries that could evolve into stable associations. Such events are favored by the wide range of hosts of ***Xylosandrus*** species ranging from exotic to native European plants, that could facilitate hosts movement of associated fungi.

> THE RESULTS SHOWED THAT SEVERAL FUNGAL SPECIES ARE ASSOCIATED WITH THE AMBROSIA BEETLE SPECIES.

DOWNLOAD THE SAMFIX AGENT APP

Google Play

FUNGI ISOLATION

WHERE DO THEY COME FROM AND WHICH ARE THEIR FAVORITE HOSTS?

The comparison between the genetic structure of the species found in the various project areas and of species in the areas of origin and other areas where the species have spread, showed chronologies of contrasting invasion paths:
X. compactus invasion history in Europe was straightforward, with a single invasion in Italy, followed by intercontinental dispersion. The proximity with specimens from Shangai suggests that it could be the donor area. However, Shanghai could have been invaded in the past, with the trade of ornamental trees within China for example.

X. crassiusculus invasion history in Europe is more intricate, and our results suggest several independent invasions. One invasion from Japan or the USA in Italy, which acted as a source for the invasion of Spain. An invasion from the Shanghai area in south-eastern France, which spread to south-western France and Slovenia. Another invasion of Italy and south-western France, potentially from Japan. In Slovenia, we found specimens which do not relate to the other sampled specimens, suggesting a fourth invasion from an unknown locality.

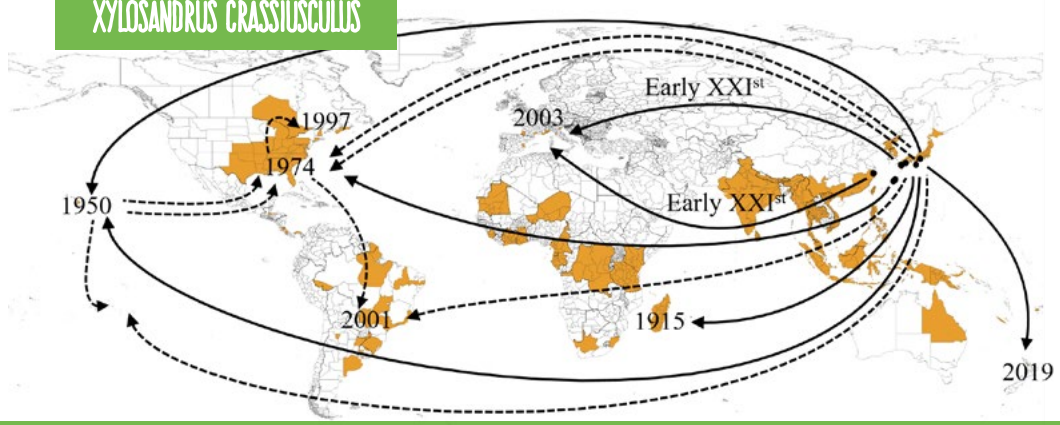
The presence of samples with shared genetic traits in Europe and Shanghai for both species, suggests that the Shanghai region may have been a major source of invasion. Our analyses showed that the climate was already favourable at the beginning of the XXth century, evincing that recent invasions were not directly enabled by recent climate change. These are in fact mainly human-mediated, presumably through ornamental plant trade, known to be the biological invasions' main pathway worldwide. However, our models predict an increase in northward suitability in the future, which could impact invasion dynamics.

Xylosandrus species are highly polyphagous: at least 220 host species belonging to 62 different families are known worldwide for ***X. compactus***. They infest woody, arboreal and shrubby plants, mainly broad-leved trees, although occasionally recorded also on conifers (***X. germanus*** on pine and spruce and ***X. compactus*** on common cypress).

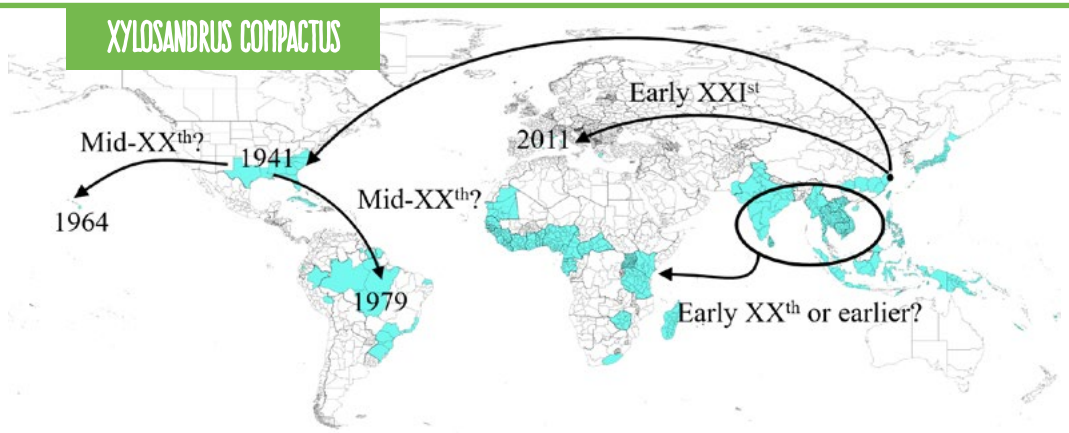
The main hosts of economic importance are crops of intertropical areas such as coffee *(Coffea canephora)*, tea *(Camelia sinensis)*, cocoa *(Theobroma cacao)*, tropical and subtropical fruit trees such as macadamia *(Macadamia ternifolia)*, lyxes *(Litchi chinensis)*, mango *(Mangifera indica)* and avocado *(Persea americana)*. In countries where ***Xylosandrus*** species were introduced many other species belonging to the genera *Acacia, Acer, Azalea, Castanea, Celtis, Cornus, Eucalyptus, Ficus, Hibiscus, Khaya, Liquidambar, Magnolia, Malus, Ostrya, Platanus, Swietenia* and *Vitis* can be attacked. In Italy and France, ***Xylosandrus compactus*** has been reported on many species of trees and shrubs including (in alphabetical order and not by preference): *Acer* spp., *Alnus* spp., *Arbutus unedo, Azalea* spp., *Banskia* spp., *Camelia* spp., *Castanea sativa, Celtis australis, Ceratonia siliqua, Cercis siliquastrum, Citrus aurantifolia, Citrus limonum, Cornus sanguinea, Corylus avellana, Cycas* spp., *Evonymus* spp., *Fraxinus ornus, Gardenia* spp., *Laurus nobilis, Laegerstroemia* spp., *Liquidambar styraciflua, Liriodendron tulipifera, Magnolia* spp., *Morus alba, Olea europea, Phillyrea* spp., *Pistacia lentiscus, Pittosporum* spp., *Platanus* spp., *Prunus laurocerasus, Punica granatum, Quercus ilex, Quercus robur, Rhododendron* spp., *Ruscus aculeatus, Tilia* spp., *Ulmus* spp., *Viburnum tinus, Buxus sempervirens*. However, for the moment attacks of ***Xylosandrus crassiusculus*** appeared mostly restrained to *Ceratonia siliqua, Cercis siliquastrum and Olea europea.*

In Spain ***X. crassiusculus*** attacks have been reported only on *C. siliqua,* and ***X. compactus*** on *C. siliqua, L. nobilis, L. styraciflua, P. lentiscus and Corylus avellana.*

XYLOSANDRUS CRASSIUSCULUS



XYLOSANDRUS COMPACTUS



PEST AND PATHWAY RISK ANALYSIS

With all the data available, a useful document was provided to the phytosanitary authorities at regional, national, Mediterranean and European level for risk analysis on the basis of which they can decide on any precautionary measures to be taken.

THE IMPACT OF XYLOSANDRUS ON ECOSYSTEMS

LIFE SAMFIX assessed the impact of the invasions of ***X. compactus*** e ***X. crassiusculus*** on ecosystem services of the habitats in monitored areas, and developed guidelines to reduce vulnerability.

To this end, an inventory and cartography of ecosystem services in the El Tello area was created and the impacts on xylophagous and pollinator communities were analyzed. The results of the analyses show no evidence of impacts in host communities that have caused losses in ecosystem services. Rather the opposite: ***X. crassiusculus*** has been found in more stable communities. This is probably because the insect has found an empty ecological niche that has allowed its inclusion without however bringing a measurable impact.

To evaluate the impact of the presence of ***Xylosandrus*** species on ecosystem functions, 10 experimental sites were selected at the Circeo National park in two different ecosystems, which have been monitored since 2019. The evaluations showed that the tree communities most prone to drought are usually more vulnerable to decay caused by additional biotic factors, which in turn accelerate soil degradation (the death of a tree has a great impact on the emission of CO_2 from the soil) and thus the productivity of the entire ecosystem.

SAMFIX COMMUNICATION

- SAMFIX results were shared with experts and end users in numerous meetings, seminars and conferences, both nationally and internationally, presenting the acquired knowledge and validated best practices. In addition to its presence at events organised by other projects, networks or organisations, the partnership delivered 4 national conferences, an international final conference and a satellite event in a conference for entomologists;
- 18 technical and scientific articles have been published in national and international journals;
- several training events were organised, targeting specific groups of interested parties (park staff, nursery owners and gardeners, etc.) and a variety of materials were made available on the project website to facilitate the replication of best practices in other areas that might need them;
- local citizens and tourists visiting the sites were sensitised and informed through educational meetings, walks and guided tours in the Parks, and encouraged to provide their reports through the SAMFIX Agent App;
- project updates were disseminated through the Facebook, Twitter and LinkedIn pages and information distributed through brochures and posters;
- three short videos were released, respectively in Spanish, French and Italian, providing an overview of the species and the damage caused and focusing on the activities implemented in each country;
- during the project, the activities intersected with those of other 8 European projects, with the transnational European and Mediterranean Organization for the Protection of Plants (EPPO) and with a project funded by the French Ministry of Agriculture.

While monitoring, prevention, containment and awareness raising continue according to the needs in the various areas that have adhered to the developed protocols, the **LIFE SAMFIX** team remains at the disposal of the staff of other parks, plant protection services and private managers of nurseries, gardens or natural areas, to offer them technical or scientific support whenever needed.

To this end, training courses, protocols and guidelines can be downloaded from the web site **www.lifesamfix.eu** and the e-mail *xylosandrus@parcocirceo.it* remains active.

SAMFIX

SAving Mediterranean Forests from Invasions of Xylosandrus beetles and associated pathogenic fungi

INFORMATIONS:

Project code and acronym: LIFE17 NAT/IT/000609 LIFE SAMFIX
Duration: 48 months (start date: 01/07/2018 – end date: 30/06/2022)
Total budget: 2,844,675 €
EU contribution: 1,706,805 €
Coordinating beneficiary: Ente Parco Nazionale del Circeo (IT)
Associated beneficiaries: Ville d'Antibes Juan-les-Pins (FR); Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement (FR); Regione Lazio – Direzione Regionale Capitale naturale, parchi e aree protette (IT); Terrasystem srl (IT); Università degli Studi della Tuscia (IT); Universidad de Alicante (ES)

CONTACTS:

E-mail: xylosandrus@parcocirceo.it
Address: Via Carlo Alberto, 188, 0416 Sabaudia (IT)
Telephone number: +39 0773512240

SAMFIX

SAving Mediterranean Forests from Invasions of Xylosandrus beetles and associated pathogenic fungi

This project is co-financed by the European Union's LIFE programme through Grant Agreement LIFE17 NAT/IT/000609.

Project start: 01/07/2018 / **Project end:** 30/06/2022