Action C5.
Extention of the prevention
and early warning system to other Natural Parks

Deliverable: Final prevention, early warning and rapid response protocols

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SUMMARY

The Asian black borers belonging to the genus *Xylosandrus*, ambrosia beetles native to south-East Asia, were recorded for the first time in Europe in central Italy. Currently we assist to a progressive dispersal of these invasive species in many European Mediterranean countries (France, Spain, Slovenia and Greece), both in agricultural and urban surroundings and in natural protected areas. The SAMFIX project, co-funded by the LIFE programme, aimed to establish effective protocols for prevention, early warning and rapid response, eradicating or containing current infestations and preventing future expansions. They were trialed in and around 6 European detection sites in or close to natural parks and extended to 8 natural parks located in the surroundings of these.

The developed knowledge on pathways and risks, and prevention, early detection and rapid response protocols are disseminated 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.

This document aims to provide information and generalized protocols that can be easily applied in natural parks, as well as in their agricultural or urban surroundings, to prevent infestations, provide for early warning, monitor diffusion and contain damage due to the spread of *Xylosandrus* spp..

The main features of the biology and ecology are here reported, and the infestation symptoms and the produced damages are described as well. An updated list of the potential host-plants is also provided. Protocols for the pest monitoring and control are suggested and lastly references are provided to obtain more information and tools.

1. BIOLOGY AND ECOLOGY OF *XYLOSANDRUS* SPP.

Xylosandrus are xylem beetles – i.e. they develop deep in the wood, unlike most European bark beetles which are corticicolous – with intimate obligatory symbiosis with associated fungi which allow them to develop very quickly in the woody tissues of the host, notoriously poor of nutritive substances. The damage that results to the plant is due to the combined action of pathogenic fungi, transmitted by the insect, and the tunnels excavated by adults during colonization of the host tree. Most of these insects attack plants in stressful conditions caused by drought, heavy pruning, transplant damage, extreme weather events such as heat waves or frosts, attacks of other pests or pathogens, or any other factor which can somehow reduce the plant vigor. In the Mediterranean basin, adults are active between mid-March and end of September, albeit with variations linked to trends in local and seasonal climatic conditions. Only females are able to fly and attack new plants, which are chosen among potential host trees in stressed conditions. Females usually penetrate twigs and small branches with thin, smooth and slightly suberified barks, of shrubs, young plants or in the peripheral portion of the foliage of large plants. The species may attempt to penetrate even larger drums but in such situations often the colonization hole aborts. In Sicily and Spain, however, cases of infestation are also reported on trunks of carob trees over 30 cm in diameter. Colonization by the female occurs through a circular 0.7-0.8 mm diameter entry hole,usually hollowed out on the underside of the branch.

Once entered the host, the female proceeds with the excavation in the wood. At this point the entrance gallery widens to form a characteristic breeding chamber which has a diameter of a few mm. The reproductive system of *Xylosandrus* spp. is in fact of the "xylematic chamber" type, with a regular maternal gallery penetrating into the plant orthogonal to the fibers of wood for a few millimeters and then enlarging into a small elongated chamber: the breeding chamber. Here the eggs are laid: fertilized eggs will give birth to females, unfertilized eggs to males. The larvae develop and pupate inside the chamber. Like in all lignicolous (xylematic) species of beetles, larvae do not dig tunnels and do not feed directly on wood but on symbiont fungi that grow on the walls of the breeding chamber.

Such fungi are introduced into the host plant by the female during the colonization phase of the twigs. Compared to insects that feed directly on xylem tissue, this food makes the development of larvae much faster and impressively increases the number of plants potentially susceptible to the insect, which just needs a host to grow the symbiotic fungi. At the time of wood colonization the female releases into the breeding chamber the spores of the fungi carried on its body in a special structure called mycangium or mycetangium. The fungal spores find ideal humidity and temperature conditions in the chamber excavated by the female, where they germinate and produce a white fungal mycelium that will feed both the larvae and contribute to the subsequent sexual maturation of the newly formed adults. The larvae develop gregariously in the chamber feeding on fungi during about 20-25 days, after which they metamorphose first into pupae and then into new immature adults. Such tender adults have a gregarious behavior and remain a few days inside the breeding chamber continuing to feed on fungi inside the host tree for their sexual maturation. After mating among siblings, the few small males present are unable to fly and die in the tunnel. The females, instead, leave the plant host through the entrance hole initially dug by their mother, imbuing themselves again with the spores of the symbiont fungus, ready to colonize a new host. In the Mediterranean, the whole development cycle from egg to adult completes in about 4-6 weeks in relation to the climatic conditions and to the different seasons. Generally the first attacks are observed starting from end of March, with average air temperatures reaching about 18-20° C, and last until the end of September. Therefore, in favorable climatic conditions there are numerous generations per year (3-5). Overwintering concerns the adults of the late- summer generation and takes place within the host plant where the insects have developed in summer.

2. HOST PLANTS

Xylosandrus spp. are highly polyphagous species on woody plants, trees and shrubs, mainly broad-leaved trees, although they have been reported also on conifers (*X. germanus* on pine and spruce, and *X. compactus* on common cypress). At least 220 host species belonging to 62 different families are known worldwide. The main hosts of economic importance are crops of the 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* spp. was 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 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 France and Italy. In Spain, attacks have been reported in *Ceratonia siliqua, Laurus nobilis, Liquidambar styraciflua,* and *Pistacia lentiscus.*

3. DAMAGES AND SYMPTOMS

Since colonization and reproduction of *Xylosandrus* spp. mainly affects plants in severe difficulties, these species are defined - like many other species belonging to this family - “parasite of weakness” as they are generally unable to colonize healthy and vigorous plants except in conditions of high population density. *Xylosandrus*' attacks show different types of damage ranging from reduced plant growth caused by the destruction of twigs, shoots and branches, to the death of trees, shrubs and hedges heavily and repeatedly infested. In case of infestations occurring in nurseries, in urban parks and gardens or in areas of high touristic value, major importance concerns also the aesthetic damages caused by the drying of the tree canopies or portions of them, with the consequent costs related to tree management. *Xylosandrus* spp. can cause severe infestations in coastal areas following very intense winter colds, especially if accompanied by strong winds. Also prolonged droughts and heat waves may be at the origin of the outbreaks of these ambrosia beetles. Significant damage is recorded with increasing frequency in central-southern Italy, north-eastern Spain, Majorca and in southern France, where Mediterranean scrub stands, plant nurseries, forest and ornamental plants, and urban parks and gardens are attacked and damaged in a few weeks. These insects trigger infestations from groups of weakened plants; in many cases these are single small events of modest importance, which become extinct spontaneously. However, if the stressing conditions weakening the stands persist for a long time, the populations of these invasive insects may outbreak with particular intensity.

A symptom of the population increase consists in the drying of a large number of shoots and canopies of hedges, trees and shrubs. The symptomatology of the attacks is quite characteristic and presents a consistent pattern. Most of the affected plants show evident drying out phenomena of shoots, twigs and small branches or canopy portions, with subsequentperipheral redness of the foliage.

Infestation in laurel

Another symptom is the presence of small circular colonization holes on the newly lignified shoots, which usually occur on the lower portion of small branches for *X. compactus*. Conversely, both *X. germanus* and *X. crassiusculus* attack larger branches and trunks with the characteristic emission of a white cylinder of wood dust. In the case of widespread attacks on most of the foliage, the drying out of the canopy has repercussions on the general health of the entire plant, causing important economic and aesthetic damage on ornamental plants in the nursery sector. The dying of branches and twigs appears on average after 7-10 days from colonization, with an initial browning of the cortical tissues close to the female's entry hole. Within a few days, this browning spreads upwards, affecting the entire upper portion of the branch with simultaneous appearance of cortical necrosis and a progressive wilting of the leaves, although they will not fall down. The affected twigs dry out and are often broken by the wind falling to the ground. It is also common to observe a chromatic alteration of the wood with browning or bluing that spreads from the breeding chambers of the insect and results from the development of its symbiotic fungi. Overall, a rapid and generic decline of trees, with reddened foliage and peripheral desiccation can be observed. Heavily infested plants die progressively and quickly become susceptible to attacks by other pests and pathogens. In addition to the symbiotic fungi that are essential for larval feeding but not pathogenic to the plant, such as *Ambrosiella xylebori* and *Ambrosiella macrospora, Xylosandrus* spp. may vector phytopathogenic fungi, such as *Fusarium solani*, *Geosmithia pallida*, and *Epicoccum nigrum*. Thus, plant damage results from the combination of the mechanical action of wood excavation of insect galleries and the pathogenic action of the associated fungi. Finally, other pathogens and bacteria can penetrate in woody tissues autonomously through the insect entry holes, involving physiological stress resulting in vegetative decline that may become irreversible, leading to the death of the plant.

4. MANAGEMENT INDICATIONS

Aggressiveness and polyphagy made *Xylosandrus* spp. exotic quarantine species in some countries, for instance *X. compactus* in Morocco (since 2018) and Israel (since 2009) and recommended as such in South America (OIRSA). *Xylosandrus crassiusculus* is a quarantine pest in Morocco and Tunisia and recommended as such in Turkey and in South America (COSAVE). The European agency for the protection of plants (EPPO) had included since 2009 *Xylosandrus crassiusculus* and since 2017 *Xylosandrus compactus* in its Alert List for the countries of the European Union, to draw the attention of member states on the potential economic or ecological risk they represented for their territories. However, they were removed in respectively 2021 and 2020 given the rather large invaded areas in the Mediterranean Basin, kept on the data sheets but not qualified as recommended for regulation as quarantine pest, leaving the management to the discretion of countries. France, for example, has performed a specific assessment of the associated phytosanitary risk to the potential introduction of *Xylosandrus* spp. in its country. In this regard, the management of populations of *Xylosandrus* spp. is based on the realization of simultaneous and integrated actions, as illustrated below.

4.1 Preventing infestations

The protection against infestations of *Xylosandrus* spp. must be primarily preventive, trying to keep the plants in the best vegetative conditions possible, avoiding for example excessive trauma or damage to canopy and roots, ensuring the necessary quantity of water during the drier and warmer seasons, and providing adequate fertilization. A specific surveillance of the general health conditions of trees and hedges is also required to provide, where possible, their improvement through emergency irrigations, targeted fertilizations and soil aeration. In case of a total or partial replacement of hedges and urban trees which aesthetic functions are compromised by severe attacks of *Xylosandrus* spp., the use of plant species not included in the list of potential hosts is recommended. The use of different multiple species would also reduce the risk of possible future infestations by others parasites that would affect only a part of the new planted plants.

In the forest, a sound silvicultural management is pivotal to reduce abiotic stress and thus infestation risk. Dying trees of high-risk species should be removed and a forest thinning carried out to improve the growing conditions. Acting on groups of plants through silvicultural interventions in order to limit intraspecific competition, will reduce biotic and abiotic stresses and therefore the risk of infestation.

4.2 Early warning and population monitoring of *Xylosandrus* spp.

A precise picture of the epidemiological situation of the populations of *Xylosandrus* spp. requires a continuous monitoring based on attractive traps. In this respect, the setting of traps baited with attractive volatile represents one of the most effective tools to know which species occur on the territory, where they are present in the territory (geographical distribution), their numerical consistency (population density), the period of the year in which the flights occur (phenology), the temporal variations in abundance within the year (voltinism) and between years (population dynamics). To this aim, it is necessary to dispose of adequate traps and specific attractive volatiles to bait the traps.

The monitoring of *Xylosandrus* species can be performed using various types of black traps, usually multi-funnel or cross-vane traps. Although having the same effectiveness, multi-funnel traps are more discreet, more manageable, more resistant to the wind and take up less space for winter storage than cross-vane traps. Therefore, information coming from traps is very useful to plan specific control strategies required to contain the pest.

A novel kind of trap, particularly useful in impervious areas, is the X-trap, which has been designed and tested during SAMFIX. It consists of a solar-powered "smart" trap that allows for the real-time recognition of daily catches and the count of specimens of *Xylosandrus* spp.. 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 recognition by trained algorithms. It will soon become available on the market and meanwhile prototypes can be asked for at Terrasystem Srl.

4.3 Trap positioning and activation

Traps have to be set out in early spring (by February- March) by hooking them a couple of meters above the ground on stakes, poles, fences, or on large branches of susceptible trees. SAMFIX trials showed that the optimal distance between traps to ensure the best detection of *Xylosandrus* spp. is of 20-30m. Whenever possible, it is better to deploy the traps in clearings, open spaces in the forestor along the forest edges in order to facilitate the aerial dispersion of attractants and simplify their periodic checks, avoiding to set them up in the core of dense forests. Traps should be geo-referenced and made identifiable with a unique specific code reported on the trap collection cup. Traps have to be fixed also at the bottom to avoid wind damage and the "flag" effect. They will then be activated with dispensers releasing specific volatiles attractive for *Xylosandrus*.

SAMFIX trials showed that a mixture of quercivorol, a-copaene, ethanol and (-)a-pinene provides the best results in the interception of *Xylosandrus*species. These volatiles are supplied with ready-to-use dispensers which only need to be fixed on the trap. Quercivorol (1ml) and a-copaene (2 ml) can be obtained as bubble cups, whereas Ethanol UHR (100 ml with 96% purity; release rate 2 g/day at 20°C) and (-) a-pinene (25 ml with 98 % purity; release rate 0.3 g/day at 20°C) can be obtained as diffuser packs. Although expected by the suppliers to last 60 days, pragmatic observations led to consider that the efficacy of these doses disappear long before under the Mediterranean climate during summer. Thus, all of these doses are to be replaced after 6 weeks. The attractants spread through the dispenser which, once extracted from its container, must not be cut or pierced. The dispensers must be fixed externally to the trap at about half of its height. Quercivorol, a-copaene and ethanol must be positioned in the center of the trap, while a-pinene at the base.

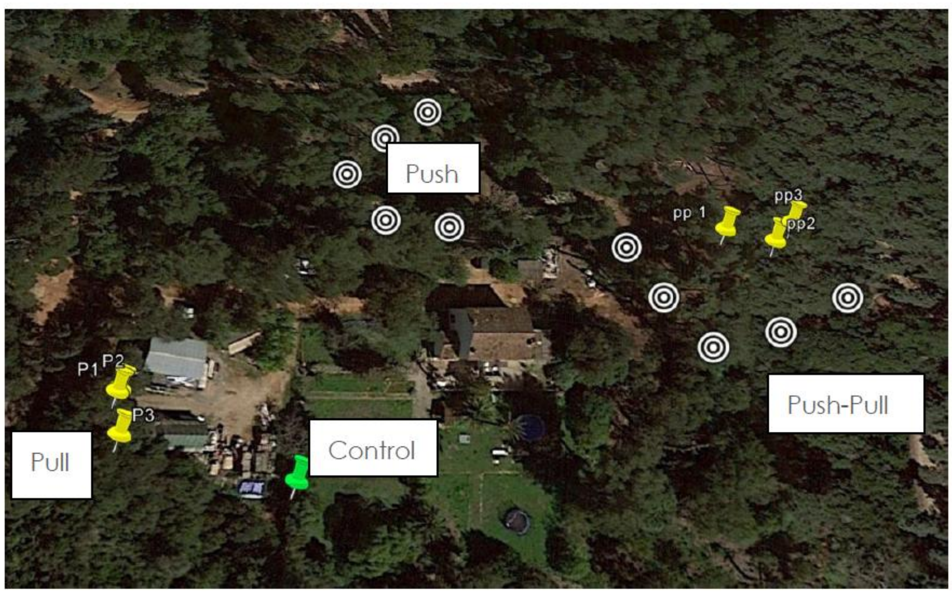
If the function of your trapping network is to monitor the species occurrence, and not to control them, it is not necessary to set up a large number of traps. In this respect, the density of the traps installed in the monitored area may not be particularly high, and ranging about 3-4 traps per 100 hectares. For an early detection of the insect arrival in a *Xylosandrus*- free natural area and for the monitoring of its populations, it is suggested to settle a linear transect including one trap every 20m along the edge of the area.

Monitoring will have to continue until the end of October of each year and the traps will have to be checked and emptied every 2-3 weeks. While checking the traps it will be necessary to turn the contents of the collector into a jar with an internal label reporting the number of the trap and the collection date written in pencil. The samples have to be stored in the freezer at -10 °C until the identification and count of the trapped insects. Flight curves can then be built at the end of the monitoring period by counting the captured insects per date of trap check.

Traps set up following this protocol are not aimed at the control or containment of ongoing infestations, but only at the interception of new exotic species or the collection of information relating to the flight period and population density of already know species. The suggested traps and attractive substances are not dangerous for humans and pets, although in some cases they can also accidentally capture non-target species of insects.

4.4 Techniques for containment

The possible application of push-pull tactics to reduce Xylosandrus populations is still under investigation. Push and Pull techniques are designed to protect already-infested stands from further larger infestations. They rely on the combined action of beetle repellents and beetle attractants in which the repellents are pushing away the beetles out of a stand whilst attractive traps deployed at the periphery of the stand are capturing the repelled insects. Their application must start from early spring on, before the first flight of female beetles. Dispensers of Verbenone, an anti-aggregation pheromone produced by micro-organisms in many female bark beetles, could be used as repellents. The dispensers are stapled on tree trunks (or tied to) every 20 m in several linear rows, the number of rows depending on the stand size and structure. The attractive traps, baited with the attractants detailed above, are deployed at 15-20m off the stand edge, in a linear row with one trap every 20-30 m. Repellent and attractants have to be replaced every 6 weeks. The protective effect is assessed by the differences in beetle damage on shoots, twigs and trunks in the stand between the starting and the end of the application. Unfortunately, although such a method is notoriously effective for other scolytid species the trials carried out in France and Italy during the SAMFIX project were not yet able to validate the efficacy of this method for *Xylosandrus* spp. because of the too low level of infestation observed in the tested areas. Whereas the damage level decreased following the push & pull treatment, it simultaneously decreased in the untreated areas, thus preventing any reliable statistical treatment of the data. Future experiments have to be carried out in highly *Xylosandrus*- infested areas in order to validate this method as a management tool for the future.



Example of a Push and Pull experiment, with 4 configurations: Pull, Push, Push-Pull, and nothing.

4.5 Phytosanitary sanitation

In case of ongoing infestations it will be imperative to act as soon as possible on the infested plants or their parts by proceeding to their destruction (sanitation) in order to eliminate the insect broods developing in the wood. This type of action is of major importance and provides excellent results in case of localized infestations, when canopy pruning or tree cut is manually practicable in a short time. Under conditions of severe and heavy infestations involving more than 50% of the canopy, hedges or bushes, pruning become instead particularly expensive, difficult to implement in short time and with uncertain results. Pruning must also be conducted in periods consistent with the life cycle of the *Xylosandrus* species, as removing branches already abandoned by the insects is useless from a phytosanitary point of view. In this regard, infested plants remain asymptomatic for many days and symptoms appear only about a couple of weeks after the females have penetrated the host tissues. Consequently, the temporal window available to locate the infested branches and proceed with an effective pruning is very narrow. In this context, the phytosanitary sanitation - especially on evergreen plants - is instead especially useful if carried out in winter on the overwintering generation. This solution gives the time needed to implement the operations, reduces physiological damage to plants that are in winter diapause, if done correctly has a significant impact on the insect population density, and allows to "clean up" the infested vegetation thus facilitating the monitoring of new attacks that eventually should appear in the following spring. All the material resulting from the pruning of the phytosanitary sanitation will have to be promptly destroyed by burning or, where regulations prohibit fires, by cheeping.

5. MORE INFORMATION

Training materials, presentations and other publications can be found at https://www.lifesamfix.eu/

In the same website, access is provided to the SAMFIX data platform, directly accessible at http://x-platform.lifesamfix.eu/samfix/cms/dashboard.php.

The App "SAMFIX agent", suitable for Android mobile phones, can be downloaded from Google Play Store.

These tools support early warning and monitoring through:

- the gathering and exploitation of different data and maps that are integrated in a dynamic ICT environment, in order to produce relevant information for different categories of users;
- supporting the data collection on beetles and damages presence inside or outside the project parks areas by the use of the x-app, sharing the outputs also for other activities.
- engaging citizens, raising awareness about the *Xylosandrus* threats by citizen science activities, through gamification.