

## Guidelines for Pest and Pathways Risk analysis of *Xylosandrus compactus* and *Xylosandrus crassiusculus*

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Authors: Massimo Faccoli, Andrea Vannini, Alain Roques, Diego Gallego, Stefano Speranza, Carmen Morales Rodriguez, Teddy Urvois, Noelia Molina

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Contact information:

E-mail: [xylosandrus@parcocirceo.it](mailto:xylosandrus@parcocirceo.it)

Tel. +39 0773512240

[www.lifesamfix.eu](http://www.lifesamfix.eu)

## Summary

*Xylosandrus compactus* and *X. crassiusculus* are two ambrosia beetles native to Asia, recently recorded for the first time in Europe as invasive insects. This document was elaborated within the LIFE SAMFIX project and makes use of the findings of its experimentations and studies. It provides for guidelines for pest and pathway risk analyses of *Xylosandrus spp.*

To this end, firstly the species origin, distribution and pathways of arrival are described. Thereafter, the main features are reported of the biology and ecology and particular attention is dedicated to the associated fungi populations. An updated list of the potential host-plants is given and the infestation symptoms and produced damage are described as well. Lastly, protocols for population management and pest control are suggested.

A list of references for further information completes the document.

## 1. Introduction

Recently, the species *Xylosandrus compactus* and *X. crassiusculus*, species of Asian origin, have been introduced and rapidly spreading in many European countries, locally causing significant damage to various tree and shrub species used as ornamental plants in urban contexts, in the forest nursery sector, in plant plantations for wood production and in natural forest formations.

*Xylosandrus* spp. are small beetles with dark black, glossy, winged females, and smaller reddish, rare and wingless males (Dole & Cognato 2010). The larvae have the characteristic “C” shape of the bark beetles, creamy-white, legless, hairless, with a brown head. The pupa, of the same length as the adult, is creamy-white.

## 2. Species origin, distribution and pathways of arrival

*Xylosandrus compactus* and *X. crassiusculus* are both described as native to eastern tropical Asia. Yet, as many invasive species mostly studied in their invaded range, the boundaries of their native range are not well defined. They are widely distributed in the intertropical regions of Africa, Asia, South America and Oceania, and were also introduced in Northern America, the Pacific islands (Rabaglia *et al.* 2006) and more recently in Europe. *X. crassiusculus* is also present in New Zealand since 2019 (Ministry for Primary Industries 2019) and has been detected several times in Australia in the last two decades (Hoppe *et al.* 2020).

Ambrosia beetles are minute species living most of their lives in galleries dug in their host tree (see 3. Biology and ecology). Therefore, they can easily be transported unnoticed with their host species and introduced in new geographic areas with live plant trade. In addition to live plants, ambrosia beetles have been reported on several types of wooden commodities: round wood with or without bark, sawn wood, wood chips, hogwood and processing wood residues and wood packaging material (e.g. pallets) (Grousset *et al.* 2020). *X. compactus* mostly colonizes branches of small

diameters (see 3. Biology and ecology); it is thus unlikely to travel in round wood, saw wood or wood packaging material. Conversely, *X. crassiusculus* colonizes large branches and trunks and was reported on hardwood ties (Labonte 2010), logs and sawn wood (Hulcr & Stelinski 2017) and wood packaging materials (Netherlands Food and Consumer Product Safety Authority 2018).

### *Xylosandrus compactus*

*Xylosandrus compactus* was found for the first time in Europe in 2011 in central Italy (Garonna *et al.* 2012), probably arrived through international trade in nursery material. In just a few years it has spread along the Tyrrhenian coast (2012 in Tuscany and Liguria, 2016 in Lazio and Sicily), to then reach the northern hinterland (2015 in Lombardy), and finally the Adriatic coast (2018 in Emilia Romagna, and 2019 in Veneto). In 2015 it reached France (Côte d'Azur) from Liguria (Chapin *et al.* 2016). In 2019 it was found on the Spanish island of Mallorca (Balearic Islands) on a carob tree promptly treated to attempt eradication (Leza *et al.* 2020, EPPO 2020). In July of the same year *X. compactus* was also found in southern Greece on carob, laurel, olive, Judas tree and shrubs belonging to the genus *Rhamnus* (Spanou *et al.* 2019), thus becoming a problem at the European level. Finally, in 2020 it was found in Corsica in 2020 (Département de Santé des Forêts 2020) and in continental Spain in the Tarragona province (EPPO 2021).

As part of the LIFE SAMFIX project, *Xylosandrus compactus*' invasion history was studied using two genetic markers (Urvois *et al.* 2021). The two genetic markers showed consistent results and revealed at least three independent invasion events worldwide: one in Africa, one in the American-Pacific region, and one in Europe. Indeed, the absence of genetic diversity in Europe suggests a single introduction in Italy, followed by human-mediated dispersion between different regions and countries, probably with live plant trade. However, the absence of genetic diversity in Europe prevents us from concluding the invasion history in Europe. The study showed that specimens with similar characteristics were found in Shanghai, suggesting that Shanghai could be the donor area of the invasion. However, it is likely that the haplotype is not native to Shanghai and was instead introduced to Shanghai from another locality, possibly elsewhere in

China. The situation is similar for the American-Pacific region, where the absence of genetic diversity suggested a single introduction to North America followed by dispersion and bridgehead effect (where an invaded area serves as a source for another invasion) to the Pacific Islands and South America. The specimens were closely related to the European and Shanghai ones, suggesting a recent divergence and potentially similar or geographically close origins. The very limited sampling in Africa and the native area did not allow to draw conclusions regarding the invasion history in Africa. A second study aimed at modelling *X. compactus*' potential distribution (i.e. identifying areas suitable for its establishment) according to current and future climate according to several climate change scenarios (Urvois *et al.* 2021). The study mainly focused on Europe, and revealed that *X. compactus* could colonize new areas, such as the Western part of the Iberian Peninsula, Western France and most Mediterranean islands. The study also predicted an impact of climate change on *X. compactus*' potential distribution, with an increase in suitability toward Northern Europe. Some of the busiest ports of entry in Europe could thus become suitable for *X. compactus* in the future, which could increase its establishment probability, allow invasion from new origins and modify *X. compactus*' invasion dynamics.

### *Xylosandrus crassiusculus*

*Xylosandrus crassiusculus* was found for the first time in Europe in 2003 in Italy in the Tuscany region (Pennachio *et al.* 2003), before spreading to other Italian regions (Liguria in 2007 (EPPO 2009), Veneto in 2009 (EPPO 2010) and Cuneo in 2018 (Dutto *et al.* 2018). In France, it was first detected in 2004 in the Southeast (Provence-Alpes-Côte d'Azur (Roques *et al.* 2014)) but was then reported westwards in Southern (Occitanie) and Southwestern France (Nouvelle-Aquitaine) in 2018. It was also detected in Slovenia in 2017 (Kavčič 2018) and Malta in 2021 (EPPO 2021). In addition, it was also intercepted several times in the Netherlands during the last decades, in wood packaging from Vietnam and China (Netherlands Food and Consumer Product Safety Authority 2018).

*X. crassiusculus*' invasion history was also studied as part of the LIFE SAMFIX project, using the same protocol as for *X. compactus*, with two genetic markers.

As far as Europe concerns, this invasion history 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.

### 3. Biology and ecology

*Xylosandrus* spp. are ambrosia beetles, i.e. they evolved intimate and obligatory symbiosis with associated ambrosia fungi that allow the larvae to develop very quickly in the woody tissues of the host, notoriously poor of nutrients. They develop deeply in the wood, unlike most of the European bark beetles, which are corticolous.

The damage that results to the plant is therefore due to the combined action of pathogenic fungi transmitted by the insect (see next paragraph) with the direct action of digging tunnels in the vascular tissues by adults at the time of colonization of the host tree. Most of these insects attack plants exposed to chronic and/or temporary stressing factors such as drought, severe pruning, transplant damage, extreme weather events such as heat waves or frost, attacks by other pests or pathogens, or any other factor that can somehow weaken the plant and lower its immune defenses.

In southern Europe, where *X. compactus* and *X. crassiusculus* occur, adults are active between mid-March and late September, although with variations linked to local and seasonal climatic trends. Only the females are able to fly and attack new plants, which are chosen among the potential hosts in situations of suffering. *X. compactus* 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 canopy of large plants. Those most frequently affected are 1-3 year old twigs up to a maximum diameter of 4-6 cm. The species can try to penetrate even larger stems but in

such situations the colonization hole often aborts. In Sicily, however, cases of infestation successes have also been reported on carob trees trunks and branches of diameters greater than 80 cm and 30 cm (Gugliuzzo *et al.* 2016) and more recently on trunks of plane trees in Southeastern France (Roques, personal observations). Females of *X. crassiusculus* are able to infest even the trunks of large trees, but were also recently observed in areca nut kernel (Thube *et al.* 2022).

Colonization by the female occurs through a small circular entrance hole with a diameter of 0.7-0.8 mm, usually dug on the lower side of the branch. Once inside the host, the female proceeds with the excavation and the entrance gallery widens to form a characteristic breeding chamber. The reproductive system of *Xylosandrus* is in fact of the "xylem chamber" type (Faccoli 2015), with a regular maternal gallery that penetrates the plant orthogonal to the wood fibers for a few millimeters and then widens to form a small elongated chamber (breeding chamber). The eggs are laid in the breeding chamber: females will be born from the fertilized eggs, and males from the unfertilized ones. The breeding chamber has a diameter of a few millimeters and develops longitudinally for a few centimeters where the eggs are laid, the larvae develop and finally the pupae appear. As in all ambrosia beetle species, there are no larval galleries as the larvae never dig into the wood and do not feed directly on the plant tissues, but feed by browsing symbiont fungi introduced into the host by the mother during the host colonization and that grow on the internal walls of the breeding chamber. This mechanism makes the development of the larvae much faster and dramatically increases the number of potential plants susceptible to the insect, which only needs a host in which it can grow symbiont fungi. At the beginning of the colonization, the female releases in the breeding chamber the spores of the fungi transported on her body in a special structure called mycangium or mycetangium. In the chamber dug by the female, the spores of the fungus - finding ideal humidity and temperature conditions - will germinate and then produce the white fungal mycelium that will serve as food for the larvae and for the subsequent sexual maturation of the newly formed adults.



The larvae develop gregariously in the chamber, feeding on the fungi for about 20-25 days. Reached maturity they face the metamorphosis from which the pupae and then the new immature adults appear. The immature, tender and pale adults remain gregariously for a few days in the breeding chamber, continuing to feed on fungi inside the host in order to reach sexual maturity. After mating between siblings, the few males present - smaller and wingless - die in the gallery while the females leave the plant through the entrance hole initially dug by the mother, getting smeared again with spores of the symbiotic fungi and ready to colonize a new host. The new adults do not disperse in the environment in an active way but tend to remain on the same plant or on neighboring ones.

In southern Europe, the entire development cycle from egg to adult is completed in about 4-6 weeks in relation to the climatic trend and the different seasons. Generally the first attacks are observed starting from April, with the reaching of average temperatures of about 18-20°C, and then continue until the end of September (Gugliuzzo *et al.* 2019). In Italy there are at least three generations a year, while in the central-southern regions there may be four. Wintering is entrusted to the adults of the late-summer generation and takes place inside the host plant in which the insects have developed.

## 4. Associated fungi

*Xylosandrus* species is commonly associated with fungi. Some of these fungi, such as *Ambrosiella* sp., are considered true mutualists and supply the diet for the larvae in the galleries (Bateman *et al.*, 2016). True mutualists are typically carried in the mycangia and provide the diet for larvae through so-called fungus-farming (Hulcr and Stelinski, 2017). Others may be pathogenic and contribute to symptom developments on host plants. Among these many *Fusarium* spp., often members of the *Fusarium solani* complex (FSSC), considered stable associates and that are involved in necrosis development on infested twigs/branches (Bateman *et al.*, 2016; Bosso *et al.*, 2012;

Vannini *et al.*, 2017). Symbiosis plays a critical role when the insects attempt to invade a new habitat. Understanding the ecological factors that influence the adaptation of an organism in a new environment and the uptake of new microorganisms are the key to explain the mechanism of biological invasions. Right one of the most complex examples of symbiosis is the one between ambrosia beetles and ambrosia fungi. Nevertheless, it should be considered that different kind of fungal species are associated to different species of ambrosia beetles and this is the reason why some of the fungal species associated to some beetles are not found in association with *X. compactus/crassiusculus* or vice versa, as there is the difference between fungal species that are associated to an exotic or native ambrosia beetle species. After the introduction of an exotic species such as *Xylosandrus*, in a new environment, there is a series of biotic and abiotic forces that greatly influence the community of organisms in association with the insect. It is considered that forest habitats strongly influence the diversity of fungal species associated with the exotic ambrosia beetles. The absence of adaptation of exotic species could limit their establishment in a new environment. However, gaining microorganisms native to the invaded environment may support the exotic species to overcome these ecological barriers (Rassati *et al.*, 2019).

The fungal community associated with *X. compactus* and *X. crassiusculus* is biodiverse and functionally heterogeneous and includes true nutritional mutualists as the results of coevolution with the insect (i.e. *A. xilebori*, *A. roeperi*), stable commensal fungi, and occasional contaminants. The persistence of the association can be determined by different factors including the ecology of the single fungal taxa. It can be speculated that the ecological adaptation of the fungal taxa identified on the insects on a wide range of hosts and substrates, including bark and wood for some taxa such as *Fusarium*, *Geosmithia*, *Sarocladium*, might facilitate the interaction with the beetles and the opportunistic colonization of their galleries as commensals. Such behavior was claimed by Bateman *et al.* (2016) for the FSSC members associated with *X. compactus*, which were demonstrated to be present consistently on the surface and abdomen of the insect, but in a limited amount in mycangia. Thus, those taxa more

adapted to the environment they interact with, in terms of host range and competitiveness for space and nutrients, have the chance to be consistently represented over several generations of the insect. However, during the progress on invasion and the interaction with new hosts/habitats, additional species can meet the insect and, depending on their saprotrophic/pathogenic lifestyle, be differently represented in the mycoflora of the galleries. Summarizing, it can be assumed that during the progress of invasion less adapted species will decrease in abundance over the generations and disappear with time, while some well-adapted fungal species will become more stable associates. The clustering of fungal communities with the insect host plants found during this project supports the hypothesis of the environmental acquisition of fungal taxa during insect invasion (Rassati *et al.*, 2019).

Fungi typically live in highly diverse communities composed of multiple ecological guilds. "Plant-pathogens" have been the most abundant fungal-guild present on the isolated fungi from *Xylosandrus crassiusculus* and *X. compactus*. Of particular interest is the finding of several plant pathogens associated to different parts of the insect and having as hosts the family of Fagaceae or other tree species. For example: *Cryphonectria parasitica*, the causal agent of chestnut blight, isolated from *X. crassiusculus* or *Pestalopsis biciliata*, the causal agent leaf blotch symptoms on Eucalyptus. The genus *Fusarium* and the species complex *Fusarium solani* were isolated from both *Xylosandrus* species. *Fusarium solani* is a symbiotic fungus cultivated in tunnels of host plants by the female insect, which is attracted to volatiles from *F. solani* (Egonyu, 2017). Furthermore, members of genus *Fusarium* have been reported in association with other ambrosia beetles, and they are often reported as pathogenic to the host tree and other woody crops (i.e., avocado) in Sicily (Gugliuzzo *et al.*, 2020). Species belonging to the genus *Fusarium* have diverse ecological functions as they can act as saprophytes, endophytes and animal and plant pathogens. It is important to mention that the genus *Fusarium* includes important plant pathogens that affect both forest and agricultural species by producing different types of wall-degrading enzymes (e.g., cellulases, glucanases and glucosidases) and mycotoxins such as beauvericin

and fumonisins (Bezoz, 2018; Sharma, 2018). *Geosmithia pallida* is a species native to Europe (Lynch *et al.*, 2014) and one of the most diffused fungal species in Mediterranean maquis, closely associated with alien species. *G. pallida* appear to be more a no specific commensal. It was reported from other plant-insect interactions, such as *Castanea sativa* and the Cynipidae wasp *Dryocosmus kuriphilus* (Morales-Rodríguez *et al.*, 2019), *Carya illinoensis* and *Quercus laurifolia* with *Pseudopityophthorus minutissimus* (Huang *et al.*, 2019) or associated with *X. compactus* at the National Park of Circeo (Vannini *et al.*, 2017). But it can also behave as a plant pathogen, for instance, *G. pallida* have been reported in the literature as a causal agent of foamy bark canker in *Quercus agrifolia* in California in association with *Pseudopityophthorus pubipennis* (Lynch *et al.*, 2014). According to the literature, *G. pallida* was accidentally introduced from Europe, like an alien pathogen of live oaks in the United States (Lynch *et al.*, 2014).

Many fungal species are considered as cryptogenic, which means they are most likely alien but with unknown origin, as they are poorly represented in alien species databases and unfortunately there is poor knowledge of their biogeography. For example, species as *Paraconiothyrium archidendri* reported on Asia, *Acremonium roseolum* on Asia and South America or *Geosmithia* sp. 21 on USA; which roles should be further investigated.

## 5. Host plants

*Xylosandrus* spp. are highly polyphagous species infesting woody, arboreal and shrubby plants, mainly broad-leaved trees, although occasionally recorded also on conifers as reported for *X. compactus* in Lazio where the insect was found on common cypress (*Cupressus sempervirens*) (Phytosanitary Service of the Lazio Region 2014). More than 200 potential host trees belonging to at least 60 different families are known worldwide for *X. crassiusculus* and *X. compactus*. The main hosts of economic importance are crops of the intertropical belt such as coffee (*Coffea canephora*), tea

(*Camelia sinensis*), cocoa (*Theobroma cacao*), tropical and subtropical fruit trees such as macadamia (*Macadamia ternifolia*), liche (*Litchi chinensis*), mango (*Mangifera indica*) and avocado (*Persea americana*). In the countries where *Xylosandrus* have been 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 Southern Europe, *Xylosandrus* have been reported on many tree and shrub species including (in alphabetical order and not by insect preference): *Acer* spp., *Alnus* spp., *Arbutus unedo*, *Azalea* spp., *Camelia* spp., *Castanea sativa*, *Celtis australis*, *Ceratonia siliqua*, *Cercis siliquastrum*, *Citrus aurantifolia*, *Citrus limonum*, *Cornus sanguinea*, *Corylus avellana*, *Evonymus* spp., *Fraxinus ornus*, *Gardenia* spp., *Laurus nobilis*, *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* (Garonna et al. 2012, Phytosanitary Service of the Lazio Region 2014, Vannini et al. 2017).

## 6. Damages and infestation symptoms

Since the colonization and reproduction of *Xylosandrus* mainly affects stressed plants or plants in critical vegetative conditions, these species are defined as "weakness parasite" as they are generally unable to colonize healthy and vigorous plants except in conditions of high population density. In principle, the attacks of *Xylosandrus* cause different types of damage ranging from the reduction of plant growth caused by the destruction of twigs and branches on the crowns, to the death of heavily and repeatedly infested trees, shrubs and hedges. In the case of infestations occurring in nurseries, urban environment or in highly touristic areas, not least is the aesthetic damage represented by the drying of the canopies or their portions with consequent increased costs related to the garden and park management.

*Xylosandrus* can cause serious infestations in coastal areas as a result of very intense winter colds, especially if accompanied by storm surges and strong winds. Prolonged droughts and heat waves can also be at the origin of the bark beetle swelling. Serious damage is recorded with increasing frequency in central-southern Italy, France and Spain, where Mediterranean scrub forests, forest and ornamental plant nurseries and urban greenery present in parks, gardens and avenues are attacked and damaged in a few weeks (Gugliuzzo *et al.* 2019, Vannini *et al.* 2017, Contarini *et al.* 2020). Important economic damage has also recently been recorded in tourist resorts in Lombardy (Lake Garda) and in the Veneto (Venetian coast) where *X. compactus* infested hedges and trees in tourist resorts.

These insects tend to trigger outbreaks of infestation to the detriment of weakened groups of plants; in many cases these are infestations of modest importance, which disappear spontaneously. However, if the weakening conditions of the surrounding stands persist for a long time, there may be a demographic explosion of these insects with particularly intense infestations. A sign of the increase in populations is the observation of a large number of reddened and dried shoots on the foliage of hedges and shrubs, and of trees. The infestation symptoms are quite characteristic and present a constant pattern. Most of the affected plants show evident phenomena of desiccation of shoots, twigs and small branches or their portions, with subsequent peripheral redness of the crown. On the neo-lignified shoots there is also the presence of small circular colonization holes, usually located on the lower portion of the branch.

In case of widespread attacks on most of the foliage, the drying of the young twigs has repercussions on the general health of the entire plant, causing significant aesthetic damage to ornamental and economic plants in the nursery sector. The drying of the affected twigs appears on average 7-10 days after colonization, with an initial browning of the bark tissues adjacent to the female's entrance hole. Within a few days, this browning extends upwards, affecting the entire upper portion of the twig with the simultaneous appearance of bark necrosis and a progressive wilting of the leaves which, however, will not detach from the twigs. The affected twigs dry up and are often

broken by the wind falling to the ground. It is common to observe a chromatic alteration of the wood with browning or bluing due to symbiotic fungi that develop in the wood starting from the insect breeding chambers. This results in a rapid and generic decline of the trees, with reddened foliage and subsequent peripheral desiccation. Heavily infested plants wither progressively and quickly, becoming susceptible to attacks by other parasites and pathogens.

The damage is caused by the mechanical action of excavating the tunnels inside the twigs to which the pathogenic action of some fungi associated with the insect would also be added (Vannini *et al.* 2017). In addition to symbiotic fungi of *Xylosandrus*, such as *Ambrosiella xylebori* and *Ambrosiella macrospora*, fundamental for larval feeding but not pathogenic for the plant (Francke-Grossman 1952), the insects can also be a vector of phytopathogenic fungi, such as *Fusarium solani*, *Geosmithia pallida*, *Epicoccum nigrum* (Ngoan *et al.* 1976, Hara & Beardsley 1979, Daehler & Dudley 2002, Bambara 2003, Dixon *et al.* 2003, Vannini *et al.* 2017). Finally, other pathogenic fungal parasites and bacteria can penetrate the woody tissues autonomously through the insect entrance holes (Brader 1964), causing physiological stress with consequent vegetative decline which in the most serious cases can become irreversible, leading to death the plant.

## 7. Population management

Following the aggressiveness and polyphagia of *Xylosandrus* spp., and in order to draw the attention of member countries to these parasites which could represent an economic or ecological risk for their territories, the European and Mediterranean Plant Protection Organization (EPPO) has included *Xylosandrus crassiusculus* and *X. compactus* in the "Alert List" in respectively 2009 and 2017. They were then removed in respectively 2021 and 2020, kept on the data sheets but not qualified (yet) as recommended for regulation as quarantine pest. *Xylosandrus crassiusculus* is a quarantine pest in Morocco and Tunisia and recommended as such in Turkey and in

South America (COSAVE), whereas *X. compactus* is a quarantine pest in Morocco and Israel and recommended as such in south America (OIRSA).

The management of *Xylosandrus* populations is based on the simultaneous and integrated implementation of various actions, as illustrated below.

#### Plant management and infestation prevention

The control of the *Xylosandrus* infestations have first of all to be preventive, trying to keep the plants in the best possible vegetative conditions, avoiding for example trauma or excessive damage to the foliage and roots, guaranteeing the necessary water supply during the drier seasons, and providing adequate support fertilization. It is also necessary to carefully monitor the general health conditions of trees and hedges, providing, where possible, for their improvement through emergency irrigation, targeted fertilization and soil aeration. Deciding for a total or partial replacement of hedges or urban green already seriously compromised by the attacks of *Xylosandrus*, the substitution with plant species not included in the list of potential hosts of the parasite is strongly recommended. The use of many different species would also allow the distribution of the risk of possible future attacks by other parasites that would affect only a part of the new plants.

Where protected areas are at stake and naturally managed forests, prevention should focus on risk reduction by informing and stimulating neighbouring farmers, nurseries, public green managers and garden-owners to accurately apply prevention, early warning and rapid reply measures, to avoid dispersion towards the natural forest stands.

#### Population monitoring

A continuous monitoring of the *Xylosandrus* populations is required in order to have precise information about their epidemiological condition. This can be done using multifunnel or crossed panels black traps. Although having the same effectiveness, the multifunnel traps are more discreet, more manageable, more wind-resistant and take



up less space for winter storage. In these contexts, traps are very useful not only for a rapid evaluation of the spread of the insect in new areas, but also for quantifying the density of its populations and the relative variations between seasons and years. Finally, traps allows for the precise identification of the flight periods of females and therefore the targeting of both location and timing of the interventions necessary to contain the parasite. The traps should be exposed in early spring (late March) by hooking them a couple of meters above the ground on stakes, poles, fences, or on large branches of susceptible trees. They must also be fixed at the bottom to avoid wind damage and the "flag" effect. The optimal distance between traps to ensure the best detection of *Xylosandrus* spp. is of 20-30 m. Whenever possible, it is better to deploy the traps in clearings, open spaces in the forest or 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. They will then be activated with special dispensers that release an attractant into the air. Ethanol is a substance that has been shown to be attractive to *Xylosandrus* (Burbano *et al.* 2012), but the results of the SAMFIX project showed that a better attractiveness is obtained when other dispensers releasing volatiles of plant origin ( $\alpha$ -pinene,  $\alpha$ -copaene and quercivorol) are combined with ethanol. The ethanol dispenser will be fixed externally to the trap at about half of its height whereas the other dispensers have to be fixed on the second funnel from the basis of the trap. The monitoring will have to continue until mid-october and the traps will have to be checked and emptied each 2 or 3 weeks, counting the captured insects and keeping the information so that the flight curves can be reconstructed at the end of the monitoring. Lures need to be replaced periodically, usually between once a month to 6 weeks (but this depends on the brand). It is not necessary to set up a large number of traps as their function is mainly for monitoring and not for control. The traps and the recommended attractive substances are not dangerous for humans and pets.

### Sanitation felling

In case of ongoing infestations, it will be necessary to act as soon as possible by sanitation felling, i.e. the destruction of the infested material in order to eliminate the insect broods still developing in the plants. This type of action is of priority importance and obtains excellent results against small and located infestations or isolated trees, when their cut and removal or the pruning of the infested twigs and branches affects a small part of the crown and is manually practicable quickly. In southeastern France, the predominance of short-distance dispersal from local outbreak sites rather than long-distance events strongly supports the expected effectiveness of rapid local sanitary measures in controlling further *Xylosandrus* spp. expansion. In conditions of severe and heavy infestations that involve more than 50% of the foliage of plants, hedges or bushes, pruning becomes particularly expensive, difficult to implement and with uncertain results as it causes damage to the plant (physiological and aesthetic) sometimes worse than that caused by the insects. Sanitation felling have to be carried out within the times compatible with the development of the insect, as removing plants already abandoned by the insects is useless from a phytosanitary point of view, and in the case of large infestations that develop on large areas this is not always possible. Moreover, the main colonization symptoms appear only after about a couple of weeks from the penetration of the female, or only a few weeks before the emergence of the new adults, so the time available to identify the infested plants and proceed with effective sanitation felling is considerably reduced. On the other hand, the sanitation felling carried out in winter against the generation overwintering inside the plants colonized in late summer is particularly effective. Effective winter felling and pruning allows having the time necessary to carry out the control, reduces physiological damage to plants that are in winter diapause, and significantly affects the density of the insect population. It also allows removing all the dried part of the vegetation, which facilitates the monitoring of new attacks eventually restarting in the following spring. All the sanitation felling material has to be promptly destroyed.

### Push and Pull

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, shall be used as repellents. The dispensers must be 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, will be deployed at 15-20m off the stand edge, in a linear row with one trap every 20 m. Repellent and attractants will be replaced every 6 weeks. The protective effect can be assessed by the differences in beetle damage on shoots, twigs and trunks in the stand between the starting and the end of the application. The recent application of such techniques in several sites of France and Italy gave results difficult to be conclusive due to the low density of the beetles in the treated sites but the final beetle damage was usually lower than before the application.

### Chemical control

Specific chemical control can be carried out in order to contain infestations. However, against this group of parasites there are no curative chemical treatments of infested plants, not even with the use of systemic insecticides. Experimental control trials conducted in Spain through endotherapeutic treatments carried out through injections of abamectin-based products into the stem of already infested trees did not provide satisfactory results, as two months after the treatment there were still larvae active in the wood (Leza *et al.* 2020). The tree colonization by insects alters the lymphatic flows which prevent the lymph from circulating normally in the woody tissues, carrying the active substances of the injected insecticide products. Moreover, *Xylosandrus* spp. live and develop deeply in the wood, feeding on fungi, and they are therefore difficult to reach with normal insecticide treatments.

However, it is possible to control the adults during their emergence from the infested plants or during the first colonization phase of new healthy hosts, in order to reduce the insect populations and protect still healthy plants. Chemical protection treatments can only be performed in confined and easily accessible environments (nurseries, hedges, private gardens) as part of a chemical control program against adults based on treatments carried out by spraying insecticides with atomizers the under-foliage of small plants, shrubs and hedges. In this regard, it is advisable to use insecticide products distributed in water solution acting by contact or ingestion, and having a good killing capacity towards adults but a low persistence in the environment. The periods of treatment shall be calibrated on the basis of the data obtained through the monitoring conducted with the traps.

The infestation containment can therefore be achieved through a series of direct and indirect actions aiming to improve the vegetative conditions of potential host plants and to contain the insect through mechanical and chemical control interventions. Careful visual surveillance of the general health conditions of plants and continuous monitoring of populations by attractive traps are necessary in order to assess the population density of the parasite and the success of any future control interventions.

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