Deliverable: Management Protocols: prevention, early warning, eradication and containment of *Xylosandrus crassiusculus* in El Tello and surroundings. Second Update

Due date: 31/01/2021

Delivery date: 31/01/2021

Prepared by: Diego Gallego, Noelia Molina, Estefanía Micó, Andreu Bonet, Susana Bautista, Hugo Mas and Eduardo Pérez-Laorga.

With support of:

Universitat d'Alacant
Universidad de Alicante

GENERALITAT VALENCIANA

Revision history

Version n°	Date	By whom	Concerns
Rev.1	08/05/2020	Diego Gallego, Noelia Molina, Estefanía Micó, Andreu Bonet, Susana Bautista, Hugo Mas and Eduardo Pérez-Laorga.	First protocol version after a year of sampling and field prospections. These protocol will revisable after second year of sampling and prospections.
Rev.2	31/01/2021	Diego Gallego, Noelia Molina, Estefanía Micó, Andreu Bonet, Susana Bautista, Hugo Mas and Eduardo Pérez-Laorga.	Second protocol version after two years of sampling and field prospections. These protocol will updated after third year of sampling and prospections.

Table of content

1. Introduction

Xylosandrus crassiusculus was detected for first time in Iberian Peninsula in the residential estate "El Pla de les Clotxes" (Benifaió, Valencia) in October 2016, affecting 4 carob trees, located in a recreational zone with natural vegetation. *X. crassiusculus* is an invasive species included in the Alert List of EPPO, together with *X. compactus*. *Xylosandrus* genus is composed by about 54 species, widely distributed worldwide, mainly in tropical and temperate zones. *Xylosandrus* is included in the Ambrosia Beetle group, a group of Scolytinae that have established a symbiosis with saprophytic fungi, whose spores are transported in special organs called micangia. Adults females usually bore live woody vegetables, entering into the xylem and digging galleries. Those galleries are upholstered by the fungus, which is consumed by insect's larvae and adults. This behavior is known as xylomycetophagia. These insects are considered a concern due to their ability of transporting and inoculating fungi directly into xylem in many woody and non-woody plant species, wath suposes a high risk of entrance of pathogens or oportunist fungus. In adition, *X. crassiusculus* has invaded 55 countries of the tropical and temperate world, favoured by its high polyphagia, capable of attacking 29 agricultural and forest hosts. Fifteen of this 29 hosts are present in El Tello and surroundings, 6 in natural areas an 12 in residential and crops areas. However, currently, *X. crassiusculus* has only been detected attacking carob trees in the region. And its external symptoms may be confussed with damages made by the widely extended black rat (*Rattus rattus*), in this respect*X. craussiusculus* presence only could be clearly identified by a detail observation of the trunks and branches, searching fringed holes, exudate drops or dust strings.

After the second year of SAMFIX trapping and prospections (2020), and taken into account the limitations imposed by COVID19 restrictions, it is posssible to conclude that *X. crassiusculus* flight period starts from the end of April and early May, until the end of November or early December, with two peaks of polulation (sencond half of May or early July and first half of September), being the second one more important (Figure 1). Most of the captures have been located arround the first detection area (east of the study site, in the municipalities of Benifaió and Picassent). Althought some specimens have been captured in nord-western area (Monserrat municipality) at the end of September of 2019. Also one especimen was collected y a trap located near "El Balcón de Montroi" (Montroi), during the main fligh peak, so we suspect that this could be the true dispersive flight (Figure 2).

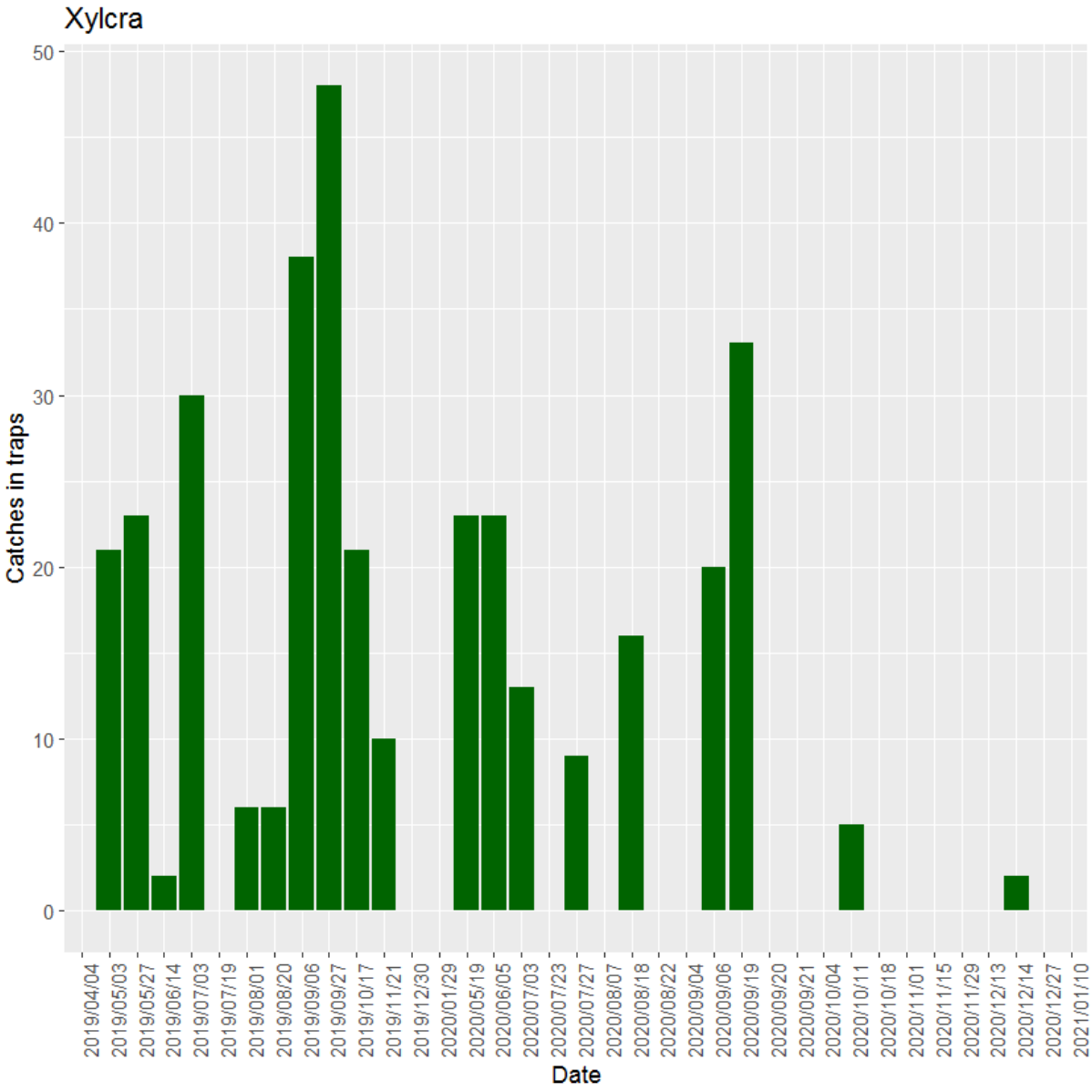

Figure 1: Flight period of *X. crassisusculus* in Valencia in 2019 and 2020.

Figure 2: Distribution of *X. crassiusculus* in the SAMFIX core area "El Tello and Surroundings" and the Replication Area "Sierras del Martés y del Ave", in 2019 and 2020. Arrow indicates distance between the possible dispersion events detected and the core area of el Tello; A: detected in fall 2019, B: detected in fall 2020.

At the moment, the unique host affected by *X. crassiusculus* in Spain is *Ceratonia siliqua*, the carob tree. Only 17 carob trees have been detected in the study area (Figure 3), and none of them has died after the beetle attack. In fact, no recurrent attacks have been observed on the same tree across the years, with the unique excepction of a very big carob tree that presents old atack evidences in a big branche and an active atack in another branch at the opposite side of its canopy. All damaged trees occurs at the extended or buffer area, and all of them are whether in abandoned old crops of carob trees, whether in young carob trees born in road ditchs or abandoned crops.

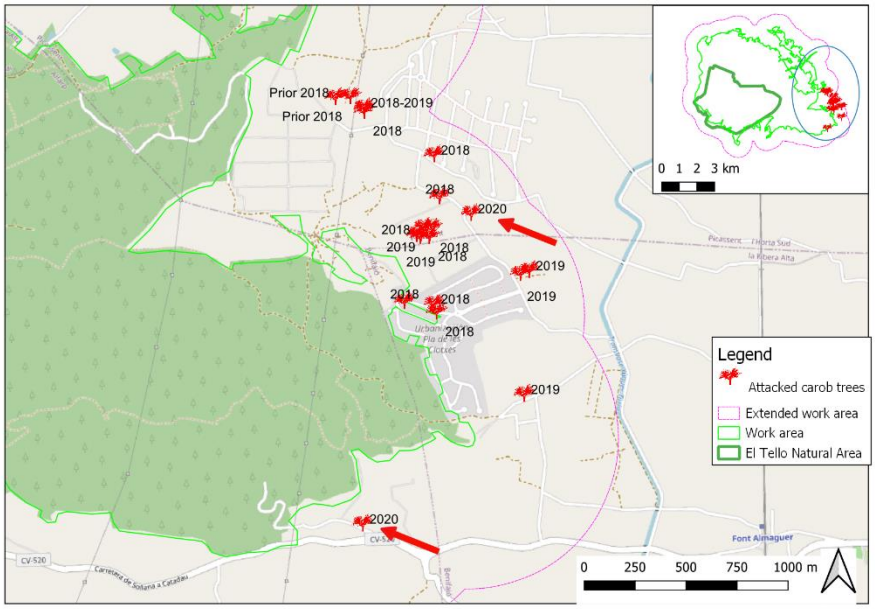

Figure 3: Affected carob trees by *Xylosandrus crassisusculus* in El Tello and Surroundings after 2018, 2019 and 2020 (marked by red arrows) prospections. Date correspond to the year of report of active symptoms. Prior 2018 indicates attack evidences without active symptoms in the first prospection in 2018.

No unmistakable external symptoms of *X. crassiusculus* attacks was defined. It can be confussed with a widspread carob damage: branche dessecation caused by bark gnawings of black rat (*Rattus rattus*) (Figure 4) . *X. craussiusculus* attacks only could be identified unambiguously by a detailed observation of the trunks and branches, searching for fringed holes, exudate drops or dust strings (Figure 5).

Figure 4: External symptoms of *X. crassiusculus* attacks and black rat gnawings. Picture at bottom left shows a gnawing detail.

Figure 5: Unmistakable symptoms of *X. crassiusculus* attack in carob trees. Top: White dust strings and exudate drops. Bottom: multiple holes and fringed holes (red arrows)

The work area of "El Tello and Surroundings" includes 3100 ha, with 2 public mountains (Monte V036 Monte Aledua and Monte V3044 Monte de Picassent), the vegetation microreseve "Lloma del Tramussar", the SIC ES5234005 "Sima del Águila", and the Municipal Nature Area "El Tello". In 2020 works will extended to two replication sites, SIC ES5333011 "Sierras del Martés y el Ave", and SIC ES2533040 "Muela de Cortes y el Caroche". At least 1080 ha of natural habitats have occurences of carob tree specimens, mainly originated by abandoned crops in 1970's decade. Its distribution covers a great part of the core area of "El Tello and Surroundings" (Figure 6), as more or less dispersed trees mainly into mediterranean scrubs or Aleppo pine forests. At the in 2020, no attacks have been detected in natural areas.

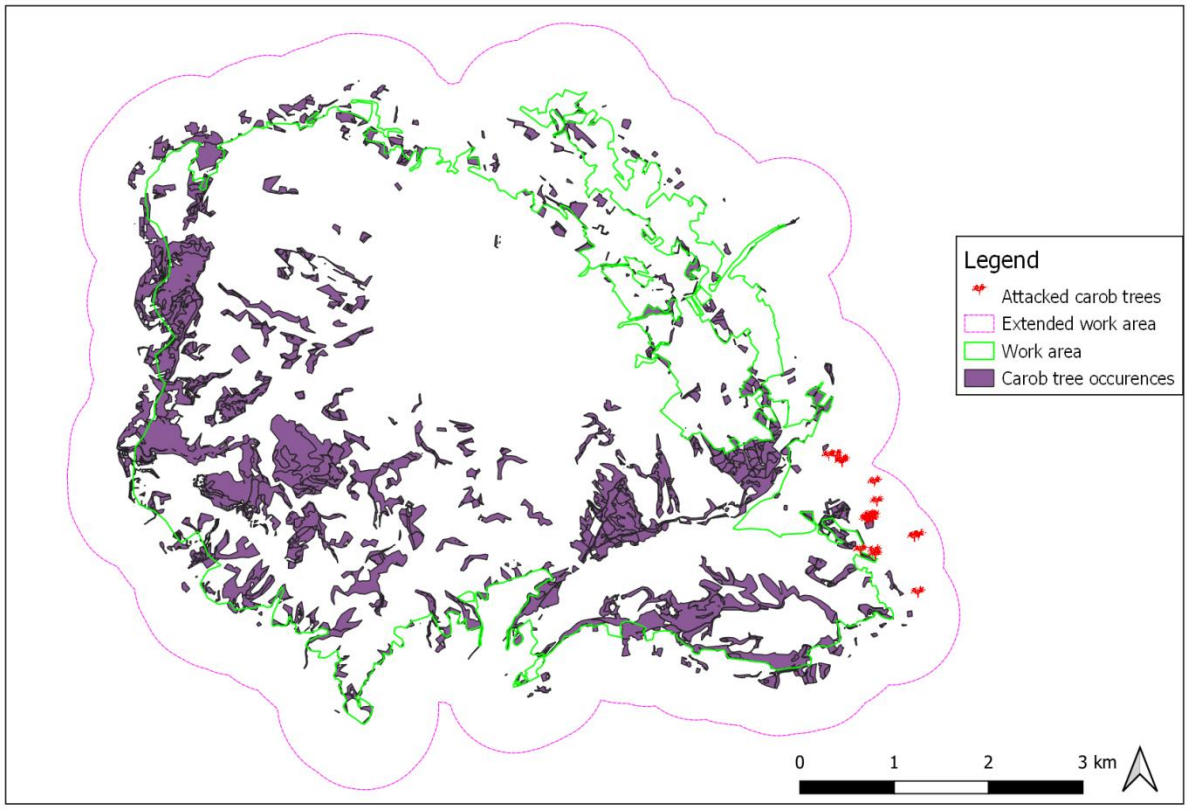

Figure 6: Distribution of *Ceratonia siliqua*, the carob tree, in the core SAMFIX area "El Tello and Surroundings".

2. Aim of the document

This document is aimed to establish the basis of management protocols rearding prevention, early warning, eradication and containment of *Xylosandrus crassiusculus* in El Tello and surroundings for 2021.

This document also includes incidences on C3 Actions due to the COVID19 restrictions.

3. Monitoring and experimental mass trapping actions in 2021

.- Monitoring trap network.

In 2021, the 40 trap installed in 2019 (Figure 7) will be maintained baited with generalist lure alpha-pinene and ethanol, with aim of monitoring dispersion of *X. crassiusculus* in the core area. Traps was activated and revised monthly since January to end February. Biweekly revissions was programmed for starting half March to end November, but this was interrupted since 15/03/2020 to 19/05/2020 for the restrictions imposed by the Spanish COVID19 quarantine. After this date, revissions of the traps have been carry out more or less irregulary due to COVID19 restrictions, although the total trapping period cover since 19/05/2020 to 14/12/2020.

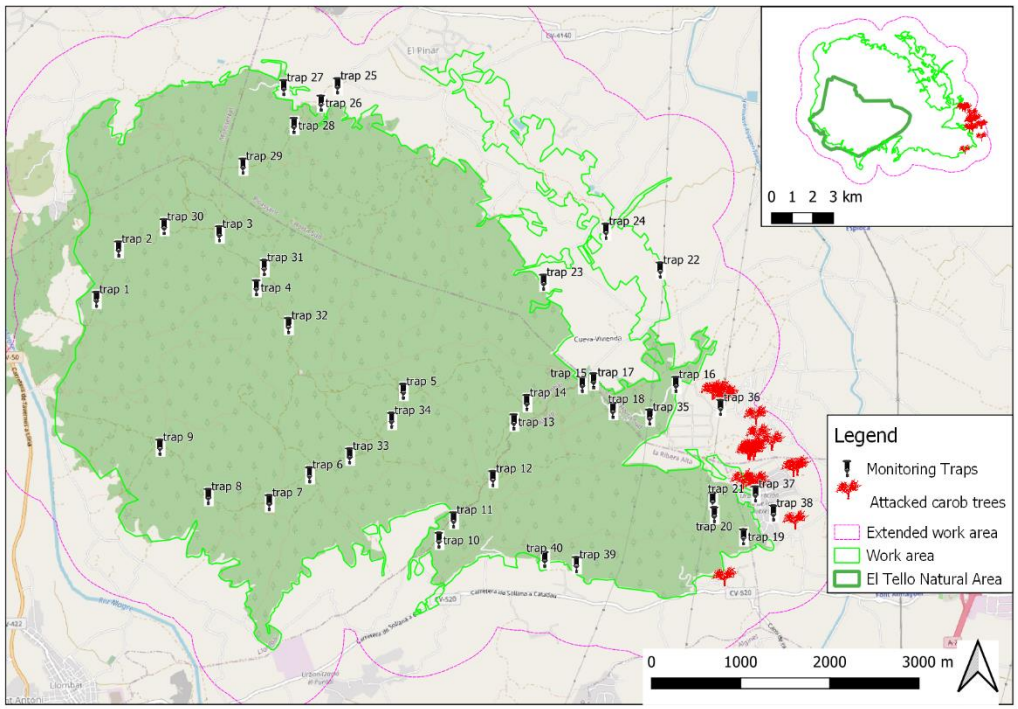

Figure 7: Monitoring trap network of El Tello and surroundings.

.- Experimental mass trapping.

An experimental mass trapping action was installed in 2020. A network of 10 trap aimed to massive catching of *X. crassiusculus* were placed in ubications showed in Figure 8. Traps were be baited with a multilure composed by alpha-pinene, ethanol, quercivorol and alpha-copaene (FourC). Traps must were installed end of March and revised biweekly to end November. This action was also interrupted since 15/03/2020 for the restrictions imposed by the Spanish COVID19 quarantine. Finally, traps were installed 13/07/2020.

Results of catches shows significant differences bewteen traps baited for monitoring (TwoC) versus traps baited for mass trapping (FourC), near tree times (Figure 9). So this lure is potentially a good tool for to reduce populations of this invasive species.

In 2021, mass trapping network will be maintained and revised like in 2020.

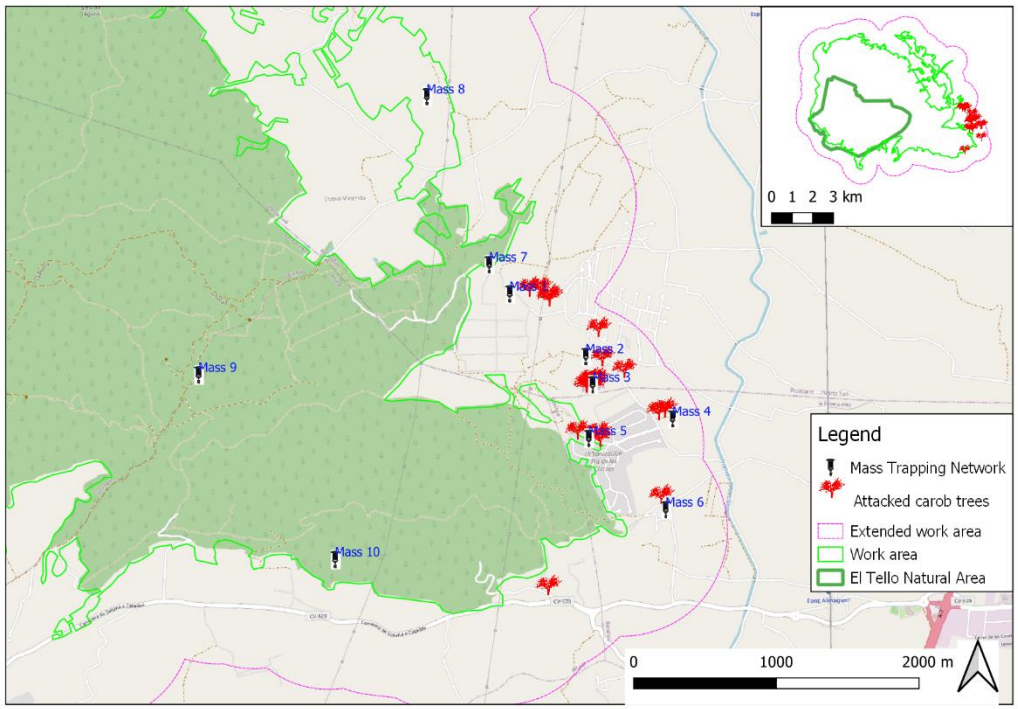

Figure 8: Mass trapping network installed in 2020.

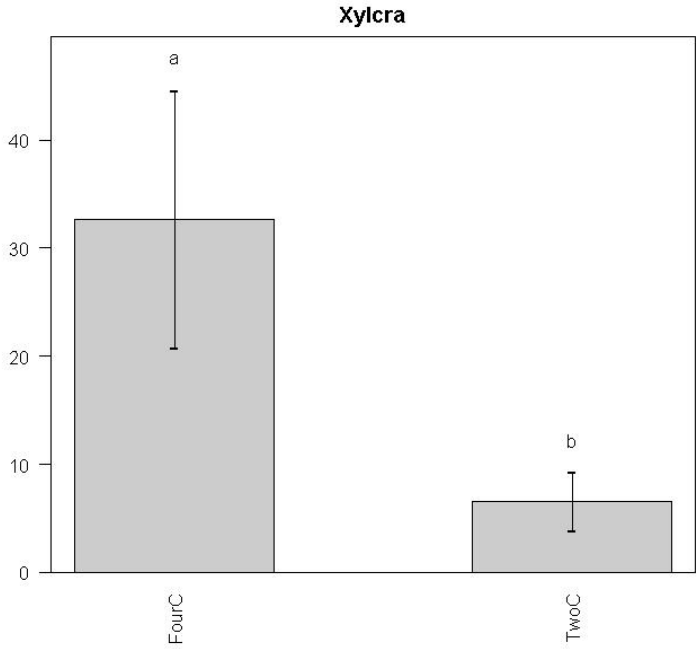

Figure 9: Cumulate captures of *X. crassiusculus* in traps baited for mass trapping (FourC) and for monitoring (TwoC).

.- Experimental Push&Pull.

In spring 2021 a Push&Pull experience will be developed in El Tello and Náquera. Combined mass trapping lures and repellants (verbenone), will be used for estimate their ability for control of *X. crassiusculus* populations.

An experimental design, agree between all SAMFIX partners, will be installed in El Tello, near the attacked carob trees area. Four square blocks of 20m x 20m with a buffer of at least 20m between successive blocks will selected.

In first half of March (before first emergence of female beetles), all attacked trees per block will be selected. On each of these plants, exit holes and other visible damage on trunk, on 10 branches and on 10 last year shoots will be estimated.

Just after this estimation, the following experimental design will be implemented, using Verbenone as repellent and the combination of the 4 attractive components (Ethanol, a-pinene, quercivorol, a-copaene) as attractant and insecticide in the collector:

.- Block 1- Push-pull (VA): a Verbenone dose in the center of the block stapled on a trunk and 4 traps baited with attractants at the periphery of the block.

.- Block 2- Push (V)- Test of repellent efficacy: a Verbenone dose in the center of the block stapled on a trunk, and no attractant at the periphery.

.- Block 3- Pull (A)- Test of trapping efficacy: 4 traps baited with attractants at the periphery of the block, no verbenone.

.- Block 4- control: no repellent, no attractant.

Traps of blocks 1 and 3 will be collected every 3 weeks until July (first dispersal flight supposed ended), counting *X. crassiusculus* specimens collected.

Repellents, attractants and insecticide will be replaced every 6 weeks in blocks 1, 2, and 3.

Resulting damage per block will be estimated similarly as before: by checking attacked trees per block.

.- Experimental X-traps

Along spring 2021, a field trying of X-traps will be developed in Naquera, due to the availability for to install the traps in private gardens without risk of theft or vandalism.

.- A new outbreak of *X. crassiusculus* detected in Náquera.

A new outbreak of X. crassisuculus has been detected in "Partida dels Plans", Náquera, near Valencia, at 35 linear km of El Tello's outbreak. Detection has been possible thaks to an alerta of an participant of National Simposium of Xylosandrus (Valencia, February 2020) that saw a picture of a carob tree with compatible simptoms in a Facebook group of trees' friends. The Spanish SAMFIX team visited the site 23/07/2020 and confirmed an strong attack in a big an old carob tree (Figure 10) located in a private garden in a residential area bordered with natural area. The inspections of the area report 10 carob tree affected (Figure 11). Two traps baited with alpha-pinene, ethanol, quercivorol and alpha-copaene for mass trapping have been installed. Traps have been revised biweekly by the owner of the tree.

Figure 10: Massive attack of *X. crassisusculus* to a big and old carob tree in Náquera, July 2020.

The owner notice us that the tree is died in 06/09/2020. We visit the area 10/09/2020 and confirm the dead of tree, being the first report of a tree by *X. crassisusculus* since 2016 in Spain. Nonetheless, a clear resprouting has been observed after the die of the last branch.

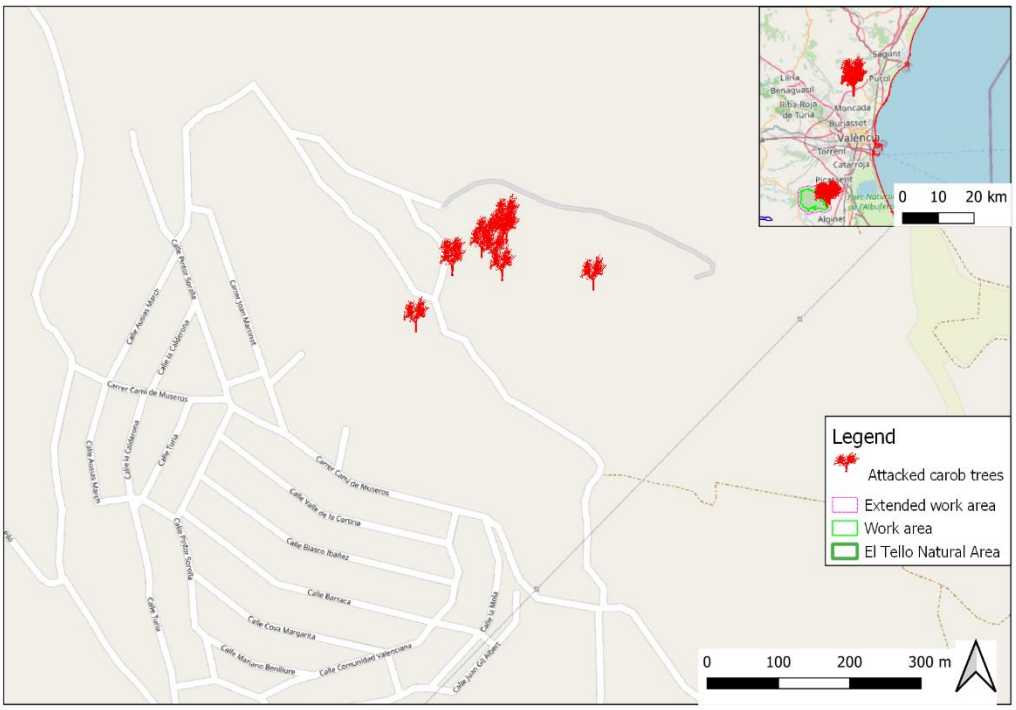

Figure 11: Location of affected carob trees in Náquera, July 2020.

4. Stage of invasion of *Xylosandrus crassiusculus* in Spain. New event or unsuccessfully event?

The first step of this plan is to define the stage of invasion of *X. crassiusculus* in Spain. This includes a date estimation of start date of the invasion and the putative pathway of entrance.

At the present we know:

1.- *X. crassisusculus* has been detected in Iberian Peninsula in two areas: the SAMFIX core work area "El Tello and Surroundings", covering arround 5800 ha; and an small area of 4 ha in Náquera. 43 km separe both areas.

2.- Dispersion ability of X. crassiuscus in Spain is still unknown, but some insects have been captured with traps more than 5 km far away from the core of the populations in 2019 and, at 14 km in 2020 (Figure 2). Both in coincidence with the peak of the flight (September). No colonized trees have been detected in this area.

3.- At the core area El Tello, the number of detections of new attacked trees since 2018 to 2020 is very low: 13 in 2018, four in 2019 and 2 in 2020. Only a recurrent attack on a same tree has been detected. All trees in a area of arround 160 ha. So, the infestation ability is low in the working area. In Náquera 10 carob trees has been attacked in 2020, covering four ha of surface.

Considering that a putative first invasion event occurred in 2016 or 2015, in a point near, between or into the residential areas of "El Pla de les Clotxes" and "Sierramar", possible by introducton of an alive plant for gardening. The relation between El Tello and the second outbreak of Náquera remain unknow yet.

The spread of the invasion was very slow, so called "silent invasion", possibly because of the possibility that carob tree is the unique suitable host in the area, since we have evidences of a possible dispersive fligth between 14 to 5 km far away from the core area, under assumtion that of all attacked trees of the area have been detected. So, erradication actions could be still considered.

5. Aggresivity of *Xylosandrus crassiusculus* in Spain.

Until September 2020, *X. crassiusculus* doesn't seem an aggresive species. Attacked trees do not die, only dead branches has been observed. The unique record of dead trees was observed in 2016 in "El Pla de les Cotxes", Benifaió, and a second report in 2020 in Náquera. The mortality rate could be stablished in a 3%. So, no generalized dead of trees occurred. After dead both trees started a clear resprouting from the stumps or tree basis (Figure 12 and Figure 13).

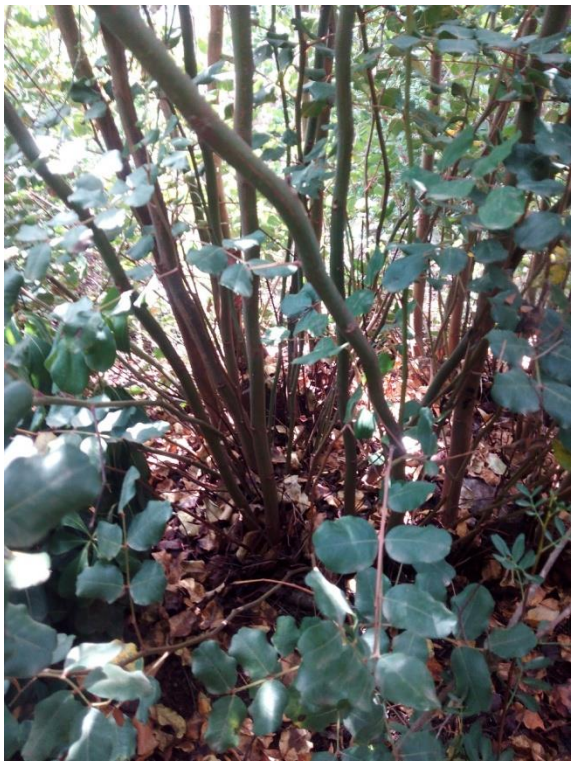

Figure 12: Resprouting from two stumps of *X. crassiusculus* attacked and killed carob trees in "El Pla de les Clotxes"

Figure 13: Resprouting from the basis of the carob tree died by *X. crassiusculus* in Náquera.

With our observations we can explain the attacking process of *X. crassiusculus* on carob trees in Spain as showed in Figure 14. Females select and attack trees along spring and summer, during

the first peak of the flight. The breeding generation emerges at end of September (second and main flight peak) and find other trees to spend the winter and breed a new generation that will emerge next spring. So, species behaves as bivoltine in the working area.

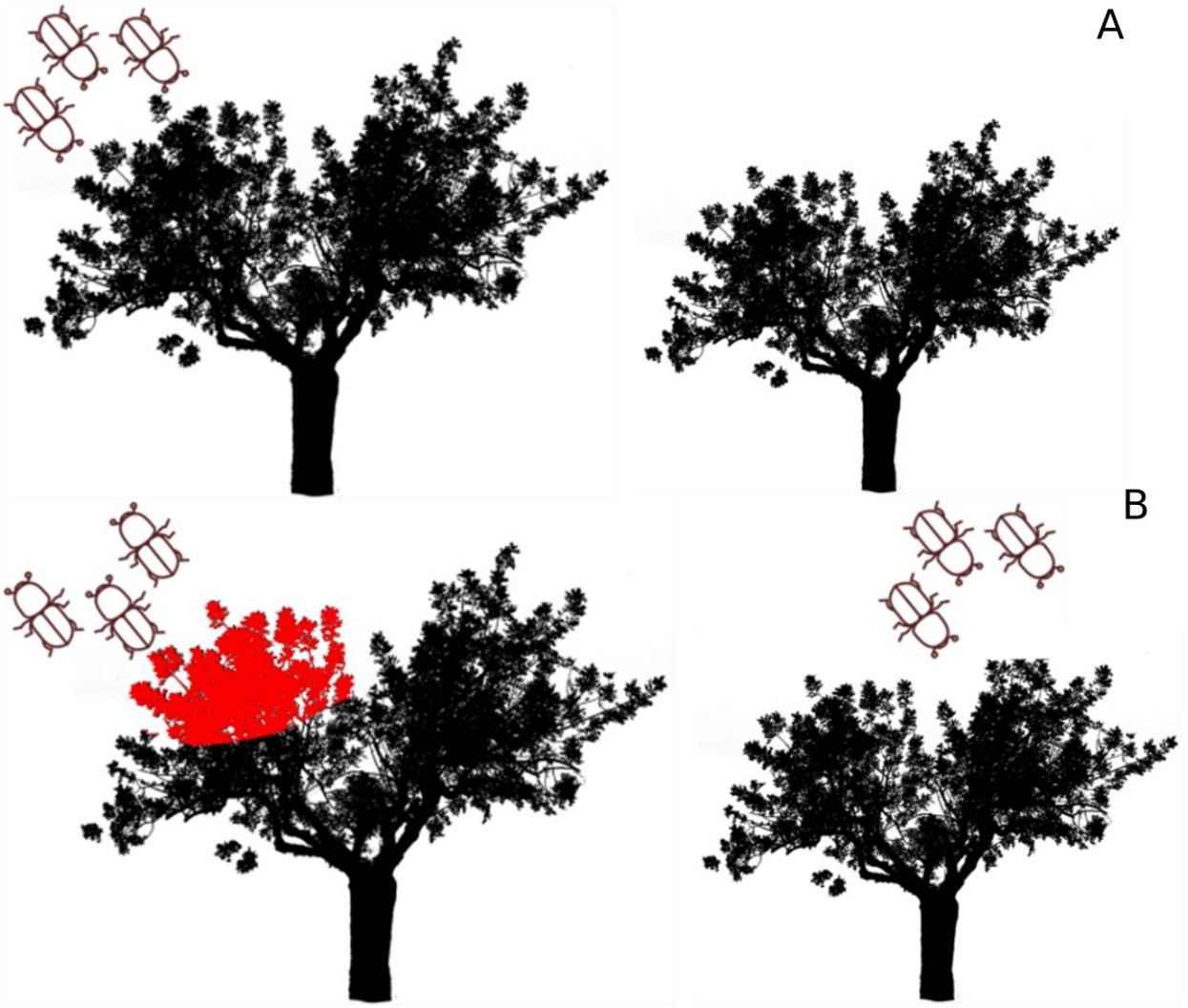

Figure 14: Scheme of possible attack process of *X. crassiusculus* to carob trees in a bivoltine behavior. A: Females select and attack trees along spring and summer. B: Breeding generation emerges at end of September and find another tree to spend the winter and breed a new generation that emerges in the next spring.

6. Action protocols for monitorization and eradication/containment of *X. crassisusculus* in Valencian Community.

a. Monitoring actions

Occurence and widespreading of *X. crassiusculus* in Spain need to be monitored. Monitoring actions are mainly two: capturing adult females with baited traps; and attacked trees detection by a direct and detailled inspection. These actions will be done in two ways:

1.- **Intensive actions** in the core area and in replication areas (trapping and tree inspection) will be carried out by SAMFIX actions until the beginning of 2022, when Life project will finish.

2.- **Extensive actions** willl be carried out in Valencian Region by Gerenalitat Valenciana resources:

.- Systematic trapping network MUFFET, composed by15 traps covering the forest area of Valencian Community; and RAT, composed by 8 traps located near ports, airports and timber industries in Valencian Community.

.- Permanent inspections by the Environmental Agents. Annual training sessions will be imparted under SAMFIX Actions until the end of the Project. This actions were interrupted in 2020 and possibly in 2021 due to COVID19 restrictions. On line formation must be considered.

b. Eradication/Containment actions

1.- Control of *X. crassiusculus* consists in massive trapping actions and destruction of infested vegetable material before the breeding generation emergence. Results of experiences of mass trapping in 2020 open possibilities for an effective contention or eradication by use of the lure composed by alpha-pinene, ethanol, quercivorol and alpha-copaene.

2.- Destruction of vegetable material infested by *X. crassiusculus* before breeding emergence is the more efective action for reducing insect population levels. Unfortunately, several reasons have made impossible to execute these actions until the present:

a.- The temporal window for acting is narrow, only between Agoust and early Sebtember, when unequivocal symptoms of active attacks can be detected. Action requires a forest crew equipped with tools for pruning and chiping *in situ* the vegetable material. Pruning can be carried out by common and or machine tools, but a chipper is so common tool, specially for big branches. Plus, it is a big machine that requires be towed. So, the availability of these staffs of and material endowments of Generalitat Valenciana throughout August and mid-September must be taken into account.

b.- Adminsitrative jurisdiction is the main problem to execute control actions. All infected carob trees occurred in privete gardens, private plots in abandonded crops, and in some cases in private recreative areas or ditches of private roads. In all situations, trees are out of the jurisdiction of Forest Healt responsibles (Forest Administrations), but within the jurisdiction of Plant Healt of Agriculture Administration. Taken into account that carob tree is a very marginal crop in Valencian Community, so, their pests has scarce or null importance versus other pests as the Quarantine species *Xylella fastidiosa* (an important outbreak is being managed currently at the region). Thus, no action on this affected trees has been carried out until the present.

7. Conclusions

This second plan summarices the status of knowledge about *Xylosandrus crassiusculus* in the both areas of this invasive species in Spain: El Tello and Surroundings and Náquera, Valencia. The plan, written under SAMFIX project framework, by collaboration between Responsibles of Forest Health of Gerenalitat Valenciana and the SAMFIX team the University of Alicante, analises the uncertainties and knowledge gaps, and proposes actions for monitoring and erradication or control. Plan will be updated annualy, accordingly the SAMFIX development, in order to incorporate results and recommendations.