Action C3.
Implementation and management of prevention, early warning, eradication and containment protocols in El Tello and surroundings

Revised local management protocols and plans – third update

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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.
Rev.3	28/02/2022	Diego Gallego, Noelia Molina, Estefanía Micó, Andreu Bonet, Susana Bautista, Hugo Mas and Eduardo Pérez-Laorga.	Third and final protocol version after three years of sampling and field prospections. Includes results of Mass trapping and Push&Pull experiments.

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, which poses 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 third year of SAMFIX trapping and prospecting (2021), it is possible to conclude that the flight period of X. crassiusculus begins from the end of April and the beginning of May, until the end of November or the beginning of December, with two flight peaks (second half of May or early July and first half of September), being the second most important, but not in 2021, which is more discreet. (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 specimen was collected in 2020 in 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), 14 km far of attacked trees. In 2021 no specimens have been collected out of core area, but a possible displacement of populations is observed in central core area (Figure 2), although no attacked trees were detected around these traps.

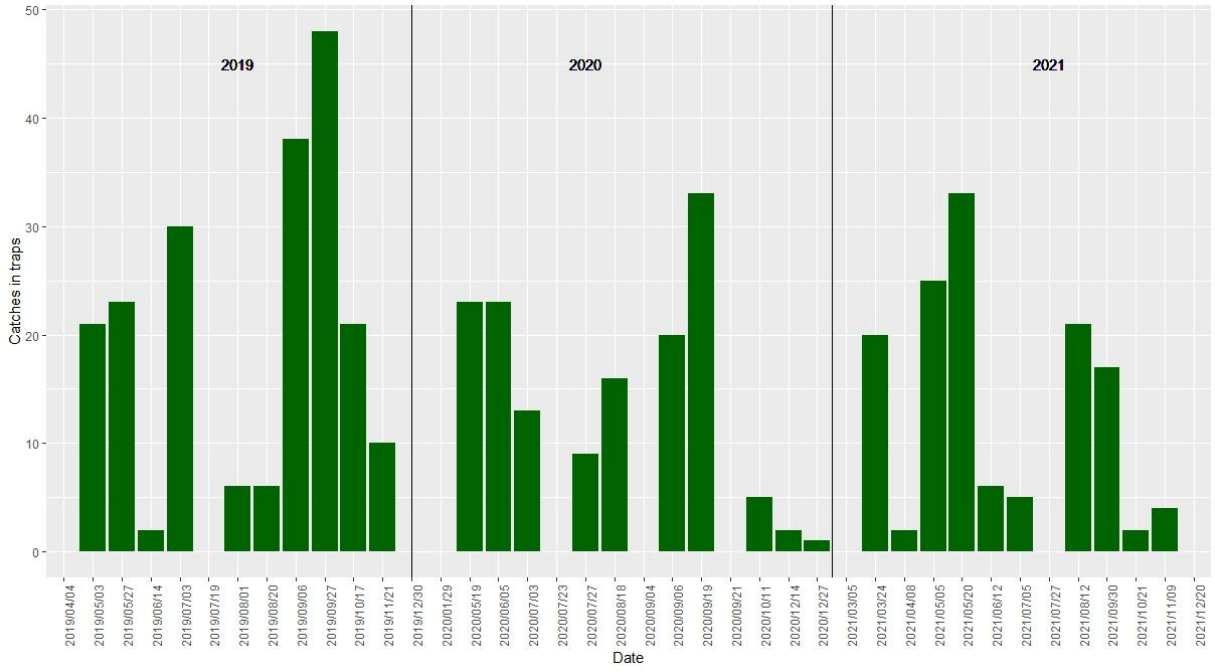

Figure 1: Flight period of *X. crassisusculus* in "El Tello and surroundings" since 2019 to 2021.

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 (top left), 2020 (top right) and 2021 (bottom left). Yellow circle indicates a possible dispersion event detected at 14 km of known attacked trees in 2020

At the moment, the unique host affected by *X. crassiusculus* in Spain is *Ceratonia siliqua*, the carob tree. Only 27 carob trees have been detected in the core area (Figure 3) from 2016 (or prior 2018), 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 attack 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.

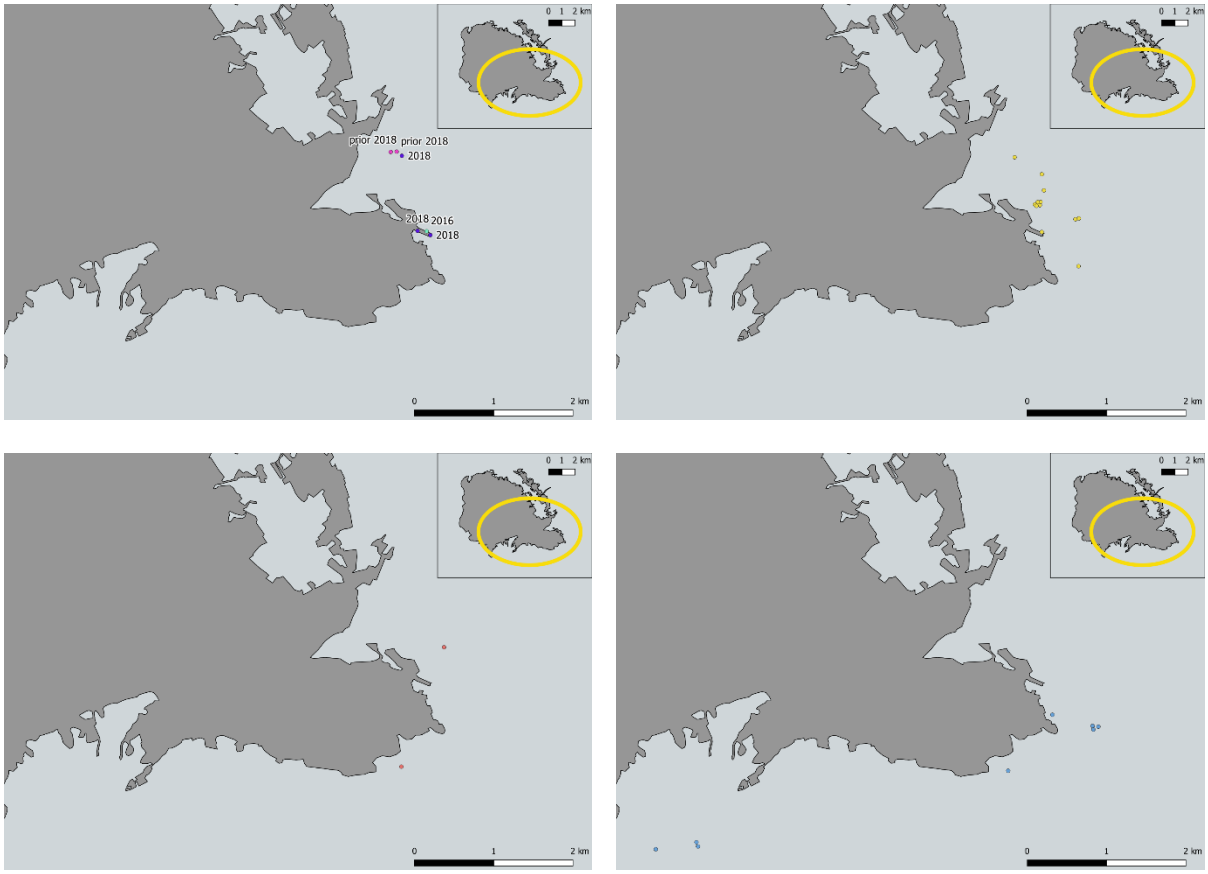

Figure 3: Affected carob trees by *Xylosandrus crassisusculus* in El Tello and Surroundings detected by yearly prospections: 2018 (top left), 2019 (top right), 2020 (bottom left), 2021 (bottom right). Prior 2018 indicates attack evidences without active symptoms in the first prospection in 2018. 2016 indicates the attacked tree reported in Gallego et al. (2017)

No unmistakable external symptoms of *X. crassiusculus* attacks was defined. It can be confused with a widspread carob damage: branche desiccation 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 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. In 2020, no attacks have been detected in natural areas.

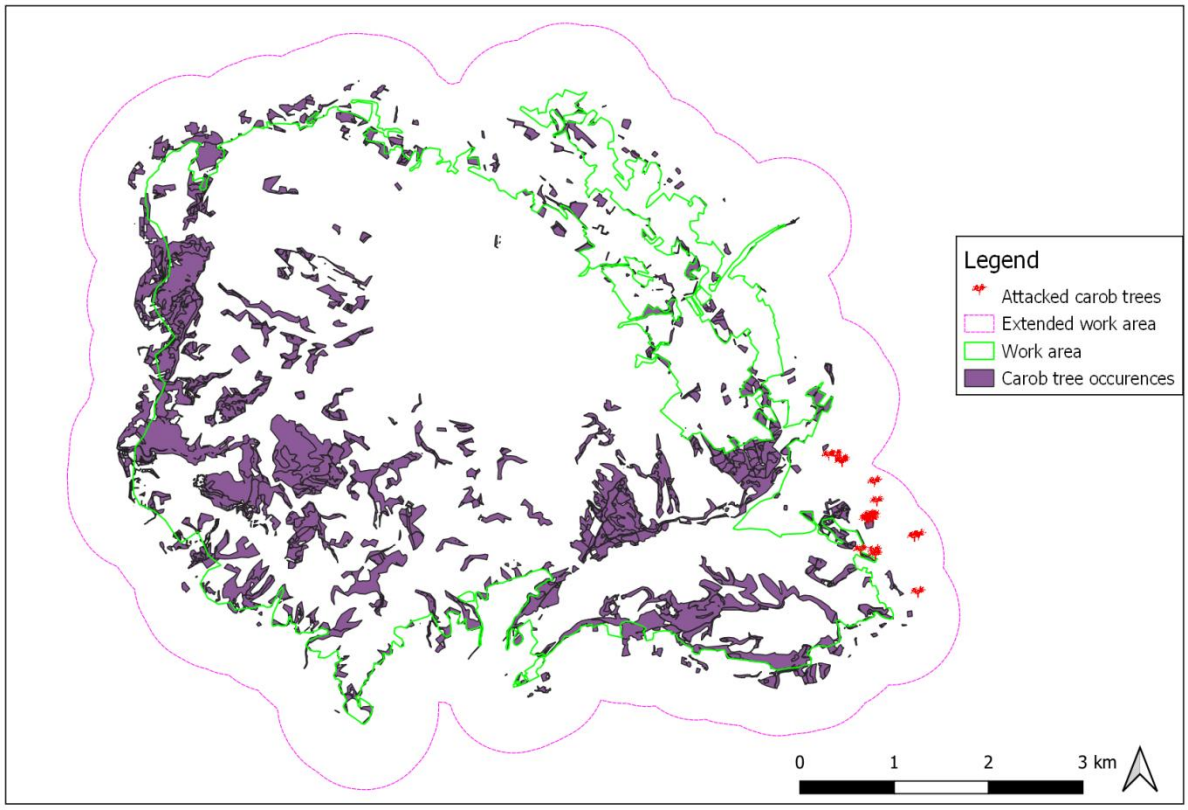

Figure 6: Distribution of *Ceratonia siliqua*, the carob tree, in the core SAMFIX area "El Tello and Surroundings".

2. Aim of the document

This document is aimed to establish the basis of management protocols regarding prevention, early warning, eradication and containment of *Xylosandrus crassiusculus* in El Tello and surroundings for 2022 and after the end of SAMFIX project in June 2022.

3. Monitoring and experimental mass trapping actions in 2022

.- Monitoring trap network.

The trap network of 40 traps installed in 2019 (Figure 7), baited with generalist lure alpha-pinene and ethanol, will remain active until end 2022, with the aim to monitor population and dispersion of *X. crassiusculus* in the core area. Traps will continue to be active and revised monthly since January to end February. Biweekly revisions was programmed to start from the half of March to end November.

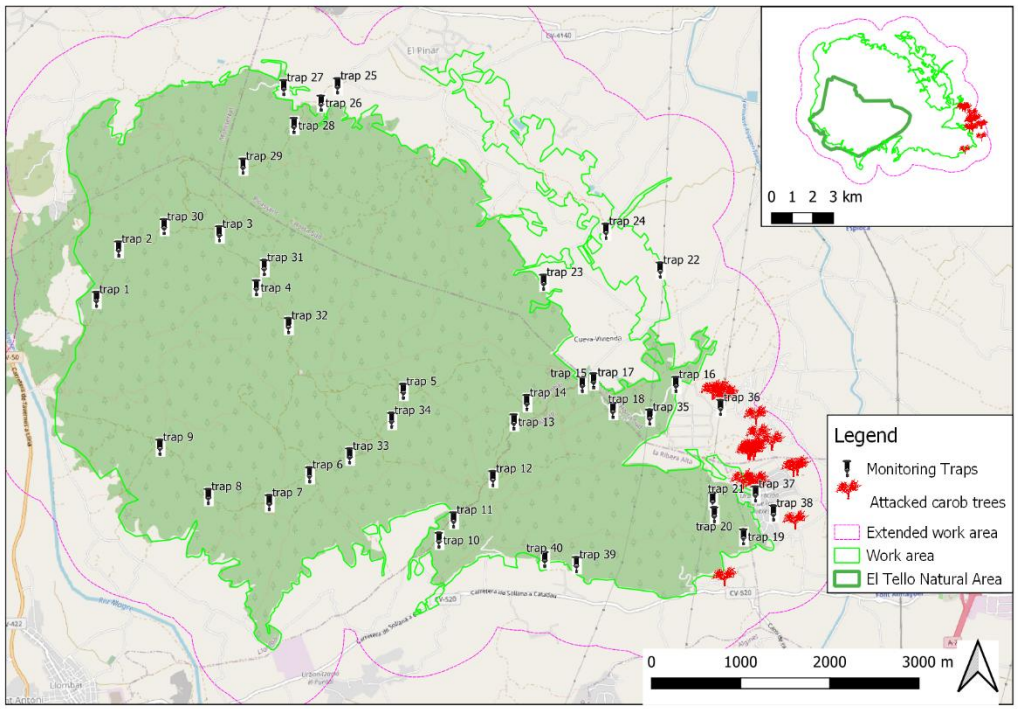

Figure 7: Monitoring trap network of El Tello and surroundings.

.- Experimental mass trapping.

An experimental mass trapping action was carried out in 2020 and 2021. A network of 10 trap aimed to massive catching of *X. crassiusculus* were placed in 2020 in ubications showed in Figure 8. Traps were baited with a multilure composed by alpha-pinene, ethanol, quercivorol and alpha-copaene (FourC), and revised biweekly since end March 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 show significant differences bewteen traps baited for monitoring (TwoC) versus traps baited for mass trapping (FourC) in 2020. Captures in traps baited with FourC are near tree times higher than TwoC (Figure 9, left). However, in 2021, no significant differences were detected (Figure 9, rigth) , due to an increasing in captures in TwoC bait, althought values of FourC are very close between both years. So this lure is potentially a good tool to reduce populations of this invasive species.

In 2022, mass trapping network will be maintained and revised like as in 2021.

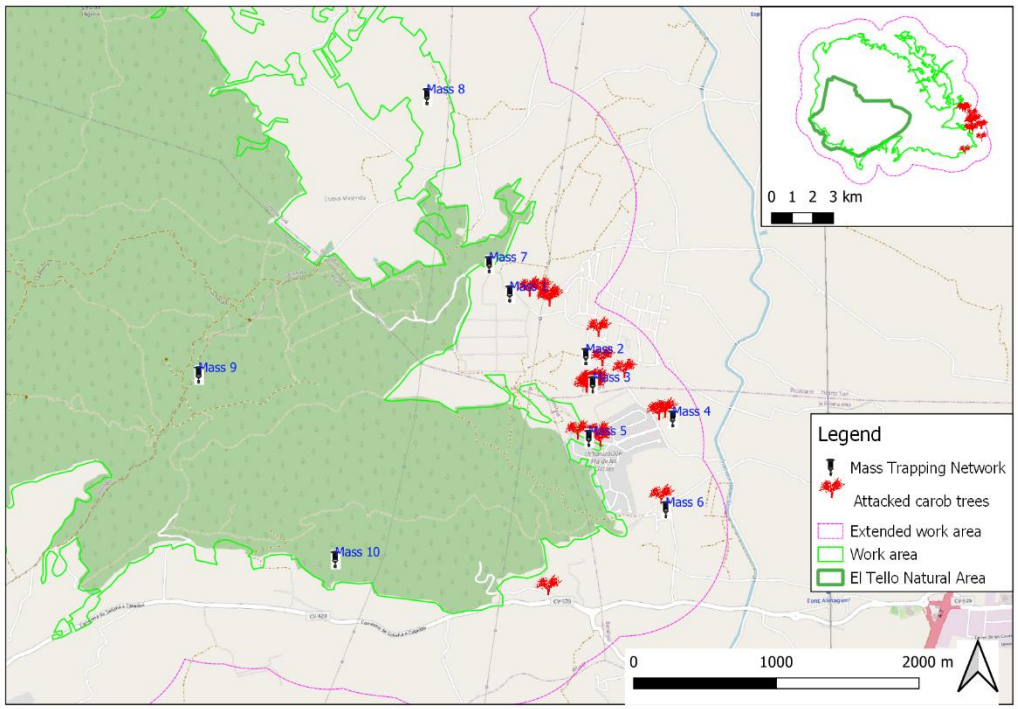

Figure 8: Mass trapping network installed in 2020.

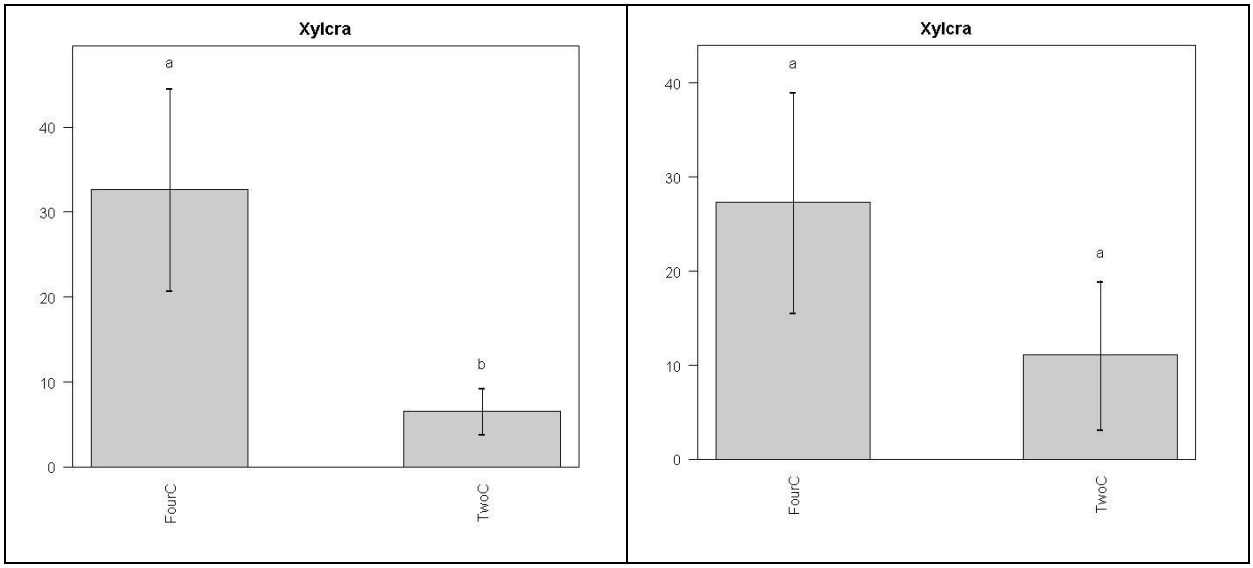

Figure 9: Cumulate captures of *X. crassiusculus* in traps baited for mass trapping (FourC) and for monitoring (TwoC), in 2020 (left)and 2021 (rigth).

.- Experimental Push&Pull.

In spring 2021 a Push&Pull experience was developed in Náquera, by combination of mass trapping lures (pull) and verbenone pouches with repellent activity (push) installed on the trees. The aim of this experience was to value its potential to reduce *X. crassiusculus* populations and their damages on the trees. Seven carob trees were visually inspected to discard *X. crassiusculus* attacks in 27 April 2021, after that, verbenone pouches were installed as "push" element (Figure 10, left pannel) the trees. Six "pull" traps, baited with FourC lure were installed around (Figure 10, right pannel). Previous, since beginning January 2021, two control traps baited for mass trapping were installed into the "push" area (Figure 10, right pannel). The experience being continued in 2022, in order to validate the results.

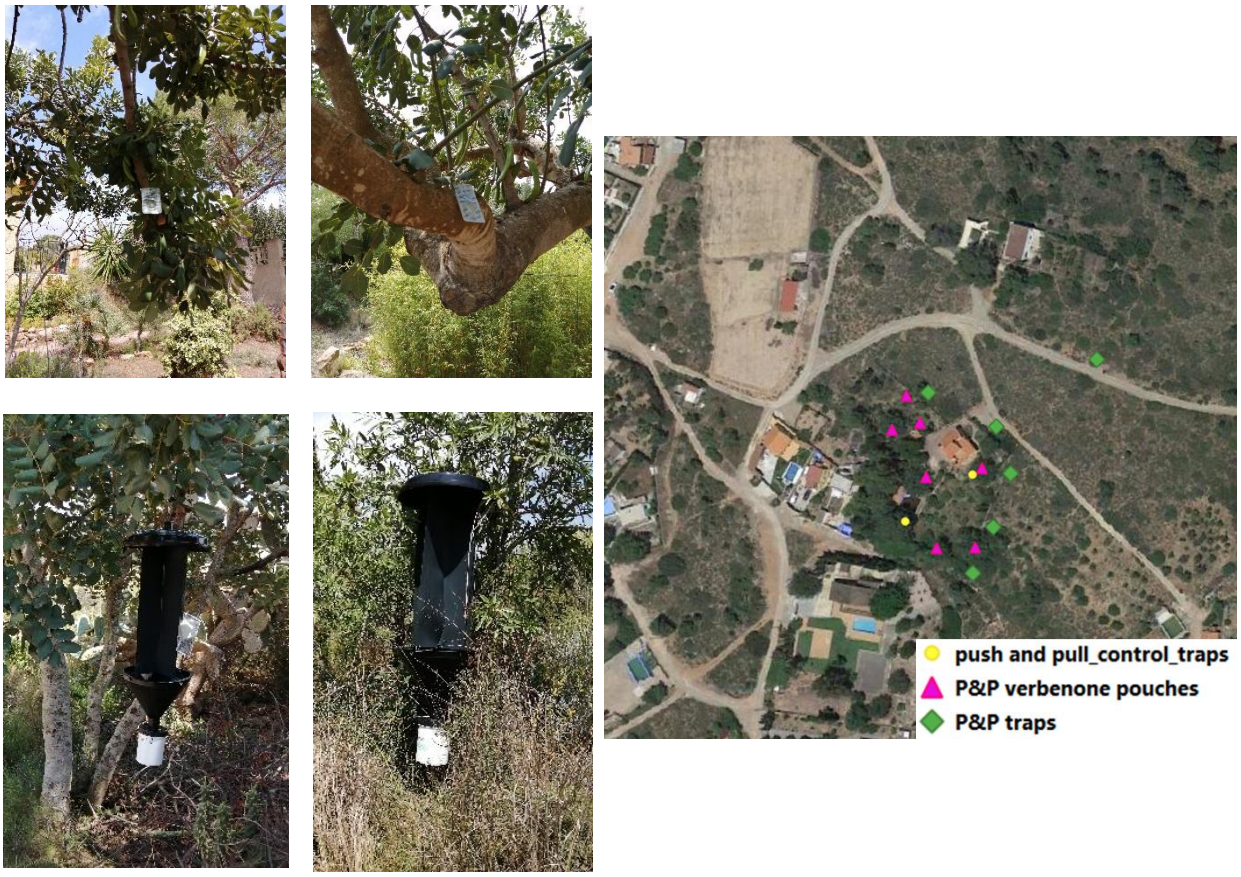

Figure 10: Left panel: Verbenone pouches installed in "push" trees (top), and "pull" mass trapping traps (bottom). Right panel: distribution of "push" repellent trees (with pouches), "pull" traps and control traps.

Very positive results were obtained from the experiment. No attacks have been detected in "push" trees. No captures in control traps were recorded after installation of "push" area, whereas "pull" traps collected all specimens (Figure 11). These results evidence the efficient action of verbenone as repellent, with the displacement of captures outside of the "push" area.

Figure 11: Results of captures of *X. crassiusculus* in control traps (called 1 and 2) and "pull" traps (called PAP). Vertical line indicates the installation date of verbenone pouches in "push" trees.

.- Experimental X-traps

Seven X-traps have been installed between 27/04 and 18/06/2021, six in the core area of El Tello and one in Náquera (Figure 12). All Xtraps were located into private and fenced areas, witch owners facilitated our access for installation and periodical maintenance actions.

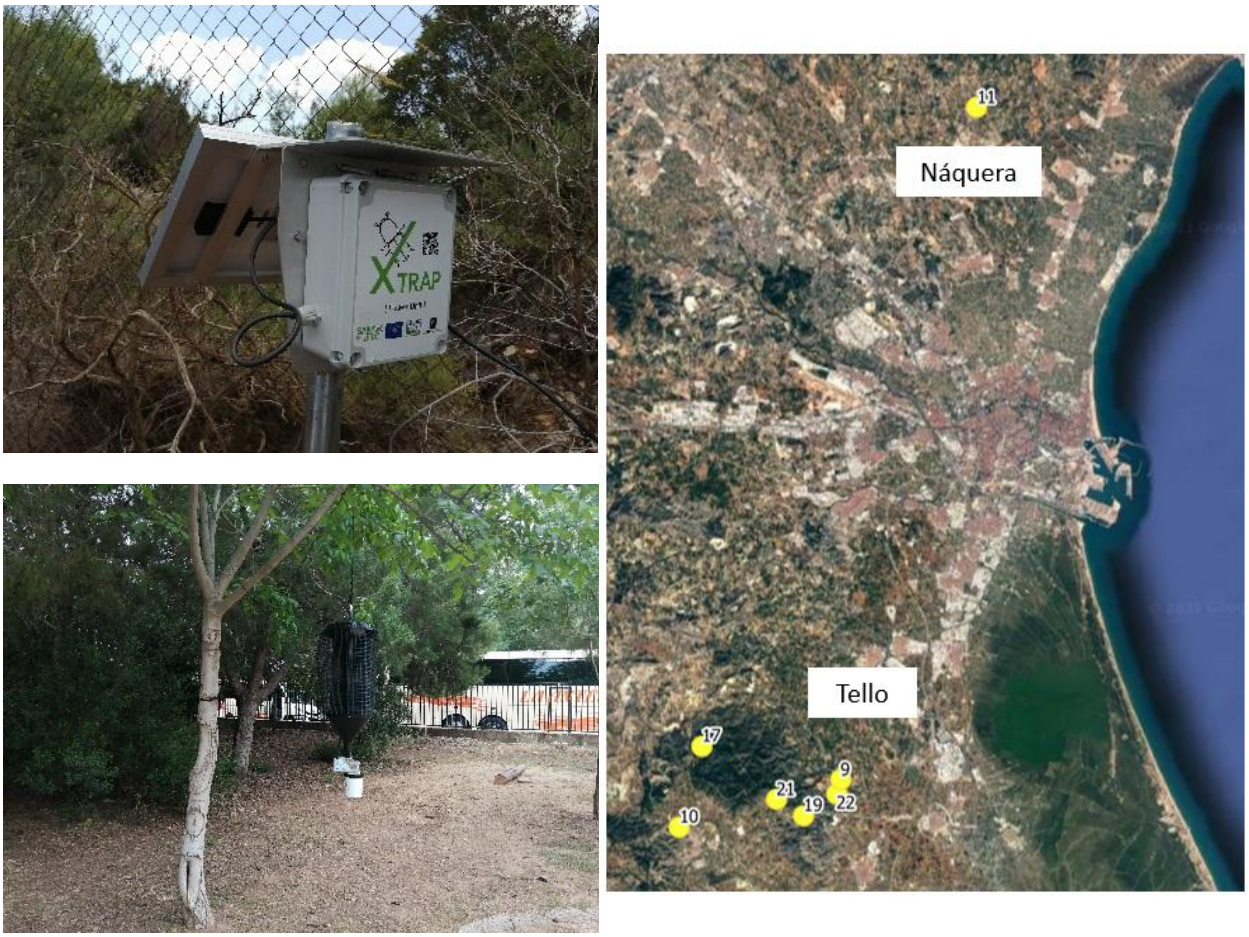

Figure 12: Unit power of Xtrap22 installed inside a fenced water tank of "Pla de les Clotches" residential area (top left); Xtrap10 installed inside the Scholar Centre "La Malvesia" (bottom left); Location of Xtraps installed in Spain (Rigth).

Xtraps remained operative since installation to December 2021. Along this period, we carry out the calibration of captures of X. crassiusculus, by comparison between pictures and cumulates captures in the collection jar of the each Xtrap (Figure 13).

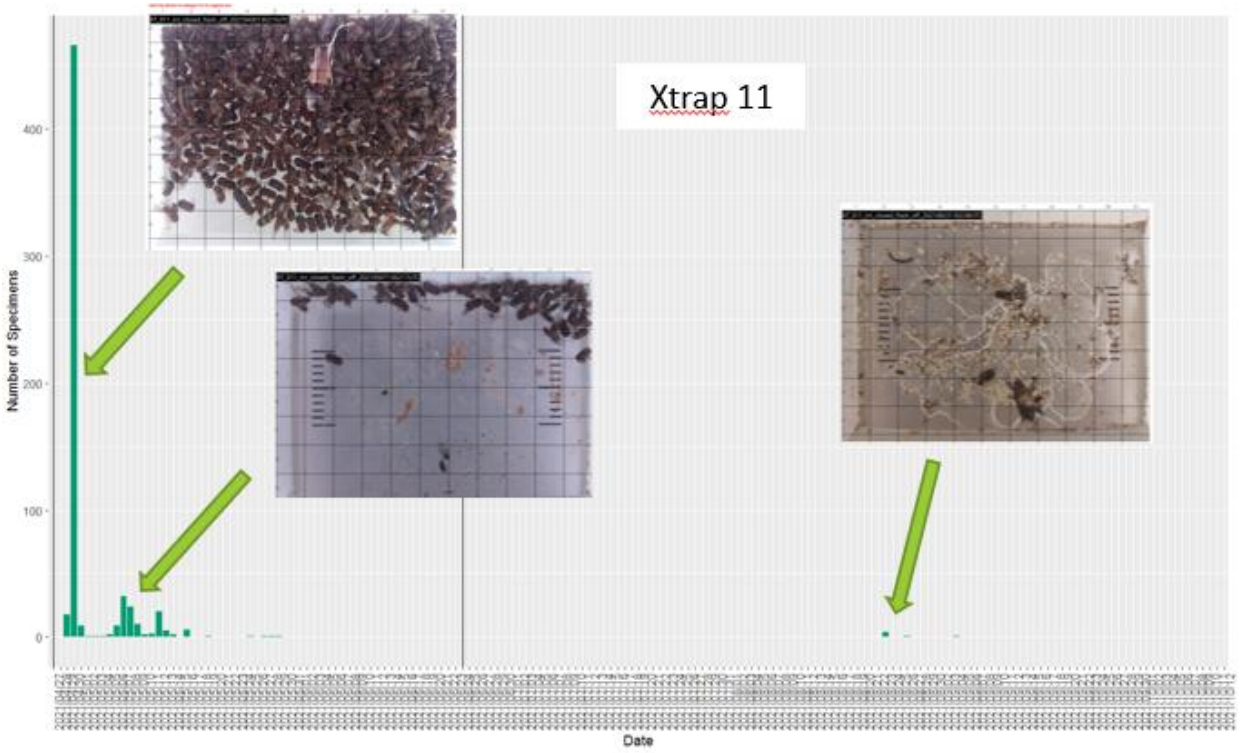

Figure 13: Example of calibration of captures in Xtrap11 (installed in Náquera). Top left photo correspond to a capture peak of more than 500 specimens of *X. crassiusculus* in late April.

At the present we are in process of labeling all photos in order to done material for machine learning process aimed to automatic identification of *Xylosandrus* species. We hope that these technology can be available for early identification of these species as soon as possible.

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

A new outbreak of X. crassiusculus has been detected in "Vertiente de Mont-Ros", Bétera, near Valencia, at 35 linear km of El Tello's outbreak, and 5 km of Náquera outbreak. Detection has been possible thanks to an alert of a participant of Second National Simposium of *Xylosandrus* (online April 2021). The Spanish SAMFIX team visited the site 15/09/2021 and confirmed an attack in a big an old carob tree located in a private garden surrounded by crops and forests. The inspections of the area report only a single carob tree affected, between 17 carob trees of the parcel. It is remarkable that the tree died as consequence of the attack, like the tree in Naquera in 2020 (Figure 14).

Figure 14: Location and only single tree attacked and died by *X. crassiusculus* in Bétera, September 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 the area of Náquera-Bétera at 43 km north of core area (Figure 15).

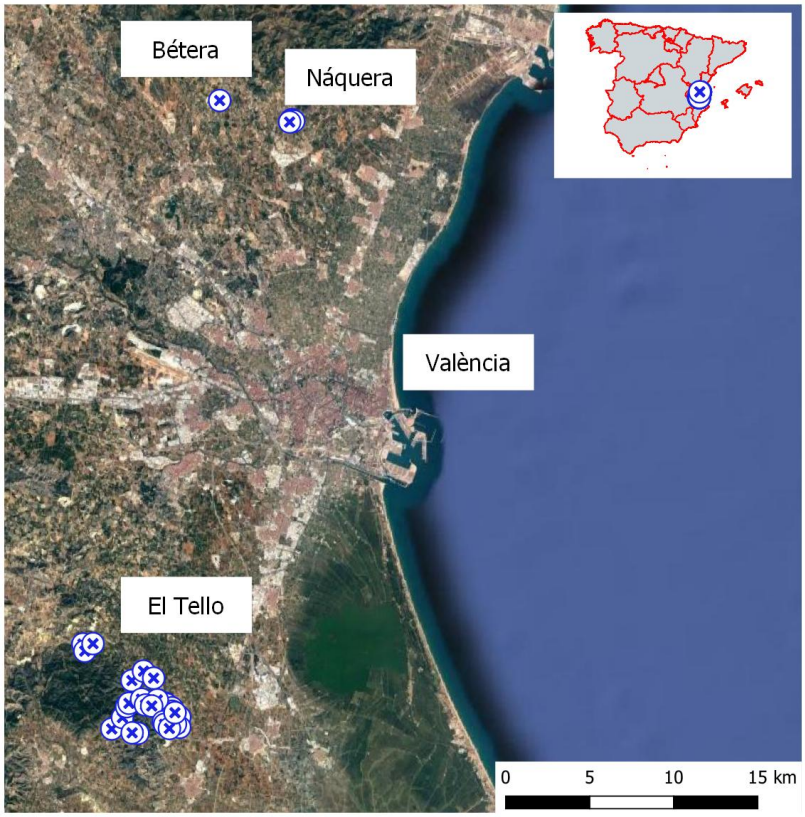

Figure 15: Known distribution of *X. crassiusculus* in Spain.

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 of September. No colonized trees have been detected in this area.

3.- A possible displacement of *X. crassiusculus* population into the core area is plausible, according to the results of 2021 trapping, althoug no attacked trees were detected in prospections (Figure 2).

4.- At the core area of "El Tello", the number of detections of new attacked trees since 2018 to 2020 is very low: 13 in 2018, four in 2019 and Two in 2020. In 2021 eight attacked carob trees have been detected. Total area of attacked trees incrases varies from 42 ha in 2018, 84 ha in 2019, 38 ha in 2020 and 90 ha in 2021.

5.- No recurrent attack on the same tree has been detected. So, the infestation ability is low in the working area.

6.- In Náquera 10 carob trees has been attacked in 2020, covering four ha of surface. In 2021, only tree carob trees has been affected by attack of *X. crassiusculus*, covering 0.2 ha.

A putative first invasion event might have been occurred in 2016 or 2015, near, between or into the residential areas of "El Pla de les Clotxes" and "Sierramar", possibly by a single or a few infested alive plants for gardening. The relation between El Tello and the second outbreak of Náquera an Bétera remains unknow yet.

The spread of the invasion is very slow, so can be called "silent invasion". We have evidences of a possible dispersive fligth between 14 to 5 km far away from the core area, under assumtion that all attacked trees of the area have been detected. So, eradication actions could be still considered.

5. Aggressivity of *Xylosandrus crassiusculus* in Spain.

Until September 2020, X. crassiusculus doesn't seem an aggresive species. Attacked trees do not die, only dead branches have been observed. The unique records of dead trees was observed in 2016 in "El Pla de les Cotxes", Benifaió, in 2020 in Náquera and in Bétera in 2021. The mortality rate could be established near 3%. So, no generalized dead of trees occurred. After dead, both trees Benifaió and Náquera, started a clear resprouting from the stumps or tree basis (Figure 16 and Figure 17). The resprouting of Bétera's tree was unrevised.

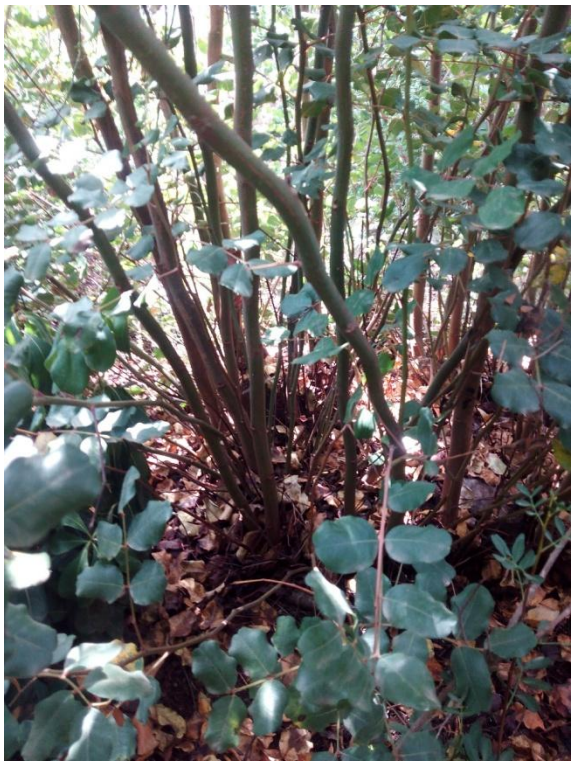

Figure 16: Resprouting from two stumps of *X. crassiusculus* attacked and killed carob trees in "El Pla de les Clotxes"

Figure 17: 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 18. Emerged females after wintering, select and attack trees for

breeding along spring and summer, during the first peak of the flight. The new generation emerges at end of September (second and bigger flight peak) and find other trees to spend the winter and breed a new generation that will emerge in the next spring, after wintering. In fall, death branches and damage evicences are more easely detectable that in former months. So, species behaves as bivoltine in the working area.

Figure 18: Scheme of possible attack process of *X. crassiusculus* to carob trees in a bivoltine behavior. A: Emerged females from wintering generation, select and attack trees for breeding along spring and summer. B: The new generation emerges at end of September and find another tree to spend the winter and breed a new generation that will emerge in the next spring, after wintering. Red indicates death branches by insect activity.

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 end of 2022, six moths after the LIFE project finishes. Starting 2023 a new trapping network will be desinged and implemented in core area.

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

.- Systematic trapping network MUFFET, composed by 15 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 activities were interrupted in 2020 and possibly in 2021 due to COVID19 restrictions. Possibly, training activities will be restarted after fininsh of COVID19 restrictions.

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 and 2001 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 effective 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 chopping *in situ* the vegetable material. Pruning can be carried out by common and/or machine tools, but a chipper is a common tool, specially for big branches. Plus, it is a big machine that requires be towed. So, the availability of the staff 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 final document summarices the status of knowledge about *Xylosandrus crassiusculus* in these areas in Spain: El Tello and Surroundings and Náquera-Bétera, Valencia. The document, 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 eradication or control. Actions and protocols have beenupdated annualy, accordingly to the SAMFIX development, in order to incorporate results and recommendations.