Action C4

Implementation and management of prevention, early warning, eradication and containment protocols in Antibes, Ile Ste Marguerite and Nice Côte d'Azur

Deliverable:

Revised local management protocols and plans - Third update

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SUMMARY

The 2021 trappings were conducted with the same designs and protocols as in 2020 but could be deployed earlier in the season in order to be capable of trapping the first generation of the beetles. These trappings confirmed the presence of both *X. compactus* and *X. crassiusculus* all over the core areas of Corniches de la Riviera, Antibes and Cannes- Ile Sainte Marguerite. However, populations of both beetles appeared to remain rather at a very low level in the Corniches de la Riviera Park. In addition, damage seemed to decrease in previously colonized areas such as Mont Boron. Expansion towards the north in the Upper Valley of Var river may be problematic in the future for the National Park of Mercantour. Both species were also widespread in the core areas of Antibes and Cannes but with rather limited densities, too. The natural expansion towards the West seemed to stop at Théoule/Mer at this moment. However, trappings revealed that public as well as private nurseries and recycling centres were likely to generate a dissemination of infested plants on a much larger scale. Mass trappings and Push & pull experiments aiming at testing methods for managing *Xylosandrus* which were carried out at Antibes and Ile Sainte Marguerite gave contrasting results, difficult to be interpreted because of the low densities of the target populations. Anyway, these tests provided interesting insights for the development of beetle control methods. The trap transects deployed at Antibes- Bois de la Garoupe for defining optimal trap density also captured a limited number of the target beetles but allowed to consider a distance of ca. 20m between two traps as the most effective.

This document lays down the protocols agreed for the management of the lure baited trapping networks during 2022 in Corniches de la Riviera, Antibes and Ile Sainte Marguerite. It has been developed on the basis of the results of analyses of data collected during the 2021 campaign and takes into account the conclusions on selected lures and traps.

1. Lure and trap design 2021

The trapping 2021 design was similar to the one defined in 2020. It used black multifunnel traps baited with 4 compounds: Ethanol + (-) a-pinene + quercivorol (1ml) + a-copaene (2 ml). The exact design is shown in Figures 1.

Ethanol UHR (100ml with 96 % purity; release rate 2 g/day at 20°C) (Figure 1A) and (-) a-pinene (25 ml with 98 % purity; release rate 0.3 g/day at 20°C) (Figure 1B) were obtained from Econex (Spain) as diffuser packs whereas Quercivorol and a-copaene were obtained as bubble cups (Figure 1C-D) from Synergy Semiochemicals Corp. (Burnaby, BC, Canada). 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 were to be replaced after 6 weeks.**

The **position of the doses** on the trap were as follows: the pack of ethanol tied to the middle of the trap (Figure 1) and the bubbles of quercivorol and a-copaene as well the pack of a-pinene tied to the 2nd funnel from the bottom.

Figure 1: Trapping design used in 2021

2. Lure-baited trapping networks 2021

Based on the previous trapping results and the capture of one specimen *Xylosandrus crassiusculus* in 2020 in the Park of Corniches de la Riviera, the geographic coverage of the trapping network of the Park has been extended in 2021 to the north towards the Col d'Eze and towards Vésubie, including new sites within these parks but also around the park in order to survey possible expansion towards it. Trappings also considered the core area of Mont Boron and the closeby municipal nursery of Nice but also the Upper Var Valley to survey a possible expansion towards the National Park of Mercantour. Thus, traps have been deployed at 11 sites (one trap per site), including 4 sites within the parks and 7 at their vicinity, whilst visual surveys of beetle damage were carried out in 3 additional sites. The trapping results are presented at Table 1.

Sites	*X. compactus*	*X. crassiusculus*
Menton	2	26
La Turbie	**2**	**3**
Grande corniche- Forna	**1***	**0**
Grande corniche- Mont Bastide	**0**	**2***
Grande corniche- Plateau Justice	**0**	**15***
Mont Boron	**destroyed**	**destroyed**
Villefranche- Château	0	4
Nice- Albert1er	0	0
Nice-Municipal Nursery	12	30
Var Valley- Plan du Var	0	7
Var Valley- Le Broc	4	5
Upper Var Valley- Puget-Théniers	0	1*

*Table 1: 2021 trapping results in and around Corniches de la Riviera and Mont Boron (in bold the sites within the parks; * Newly detected infestation)*

Unfortunately, vandalisms continuously affected the traps at Mont Boron and prevented any beetle captures. In the Corniches de la Riviera, *Xylosandrus compactus* seemed to expand gradually (2 sites newly infested) but at low density (1-2 beetle per trap). However, its presence was more important in the Nice municipal nursery. At Mont Boron, visual surveys confirmed a low density (8 shoots attacked on 10 carob trees) but

similar surveys in urban parks of downtown Nice revealed larger damage, especially on ornamental olive trees.

Unlike *X. compactus*, *X. crassiusculus* was trapped all over the Corniches de la Riviera at medium density and expanded in the upper valley of Var river, up to Puget-Théniers in the direction of Park of Mercantour. At Mont Boron, visual surveys tended to show a decrease in damage on previously highly-infested carob trees. *X. crassiusculus* was also trapped in numbers at the Nice municipal nursery. Thus, this nursery may be considered as a hotspot for the dissemination of both beetles with the plantings of infested ornamentals in town-managed areas as well as along roads circling the Corniches de la Riviera Park.

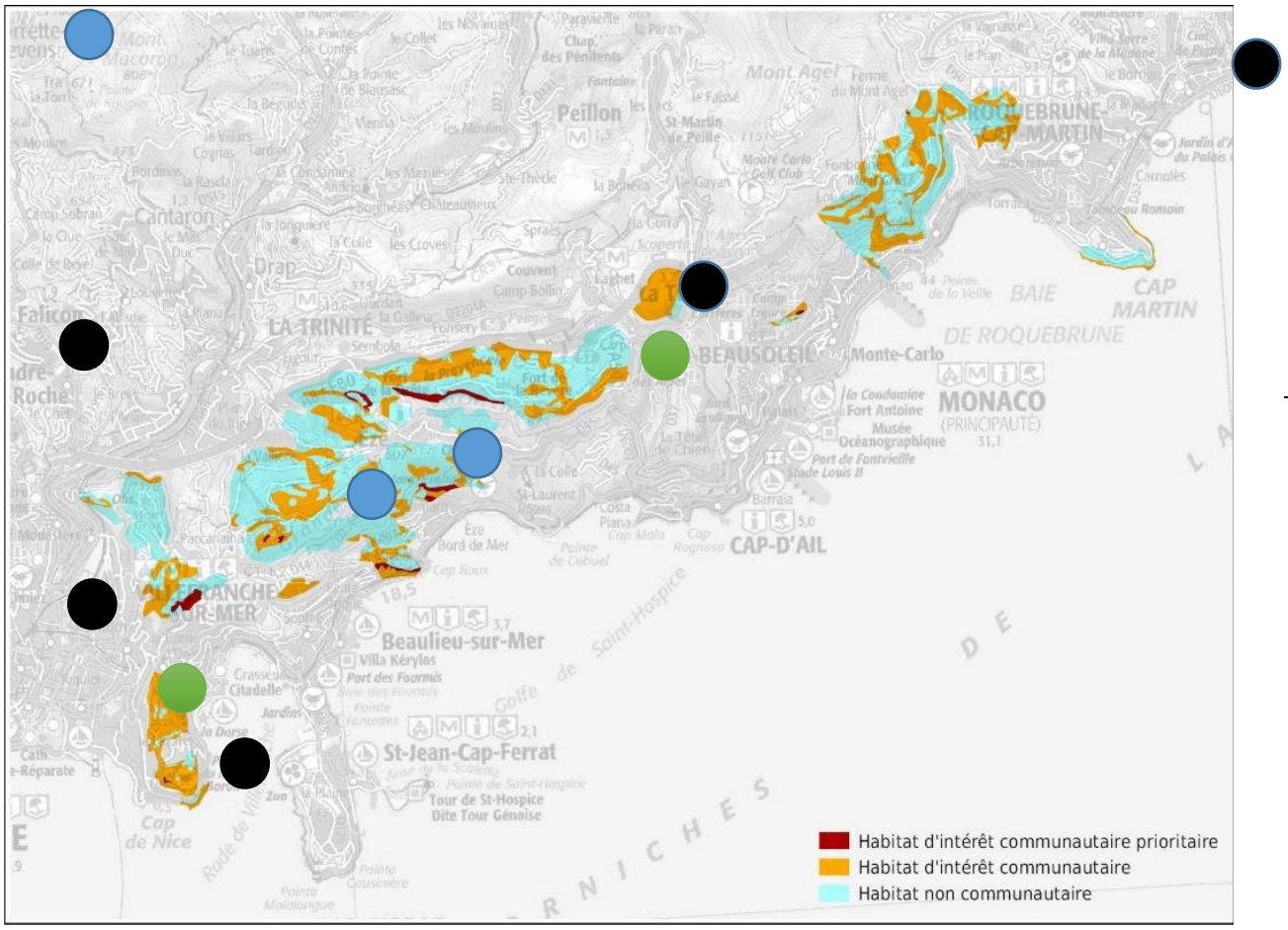

Figure 2: Presence in 2021 of the Xylosandrus species in the Park of Conriches de la Riviera and its vicinity (Black: both species; green: Only X. compactus; Blue: only X. crassiusculus)

The second trapping network concerned the core areas of Antibes and Ile Ste Marguerite. Thirteen sites were equipped with 38 traps, taking into account traps used in other experiments (but baited with the same lures) carried out at Antibes- Villa Thuret and Bois de la Garoupe and at Ile Ste Marguerite (cf 3).

Sites	*X. compactus*	*X. crassiusculus*
Antibes- recycling centre	4*	1*
Antibes- Delaunay square	0	0
Antibes- Villa Daurane	2*	5*
Antibes- Vilmorin square	5	0
Antibes Villa Thuret	**21**	**51**
Antibes Bois de la Garoupe	**7**	**6**
Cannes- Ile Sainte Marguerite	**20**	**52**
Cannes- Croix des Gardes Park	4	3
Vallauris- Paradou Park	3	10
Mandelieu- jardin Désirade	2*	0
Mandelieu- Rubino nursery	0	0
Théoule- Estérel Park	1*	2*

*Table 2: 2021 trapping results in and around the core areas of Antibes and Ile Sainte Marguerite (in bold the sites within the parks; * Newly detected infestation)*

Finally, 22 traps were deployed within the core areas of Antibes and Cannes. The others concerned close-by regional parks of Croix des Gardes (Cannes), Paradou (Vallauris) and Estérel (Théoule s/mer) but also places which may contribute to beetle expansion; i.e., a nursery, a wood recycling center, 2 urban squares and 2 private gardens located at the vicinity of Bois de la Garoupe. Table 2 presents the results of the trappings.

Both species were trapped in numbers (>50 for *X. crassisuculus* and >20 for *X. compactus*) in the core areas of Bois de la Garoupe and Ile Sainte Marguerite, but their distribution appeared more scattered in the western part of the area, stopping at the Estérel Park of Théoule/Mer. Smart traps were deployed at Villa Thuret and two other sites of Antibes and did not show significant differences in captures compared to those by usual multifunnels.

Damage surveys on 4 plots of the core areas revealed a larger damage by *X. compactus* than by *X. crassiusculus* at both Villa Thuret and Ile Ste Marguerite but these damage remained rather low (an average of 20 to 50 damaged shoots on 10 susceptible trees and shrubs surveyed per plot). A trap deployed at a wood-recycling center in Antibes captured several beetles and led to consider this place as a possible hub for *Xylosandrus* spread. The trappings additionally confirmed the presence of the other exotic ambrosia beetle, *Amasa truncata*, everywhere eucalypts plantations existed in the core area.

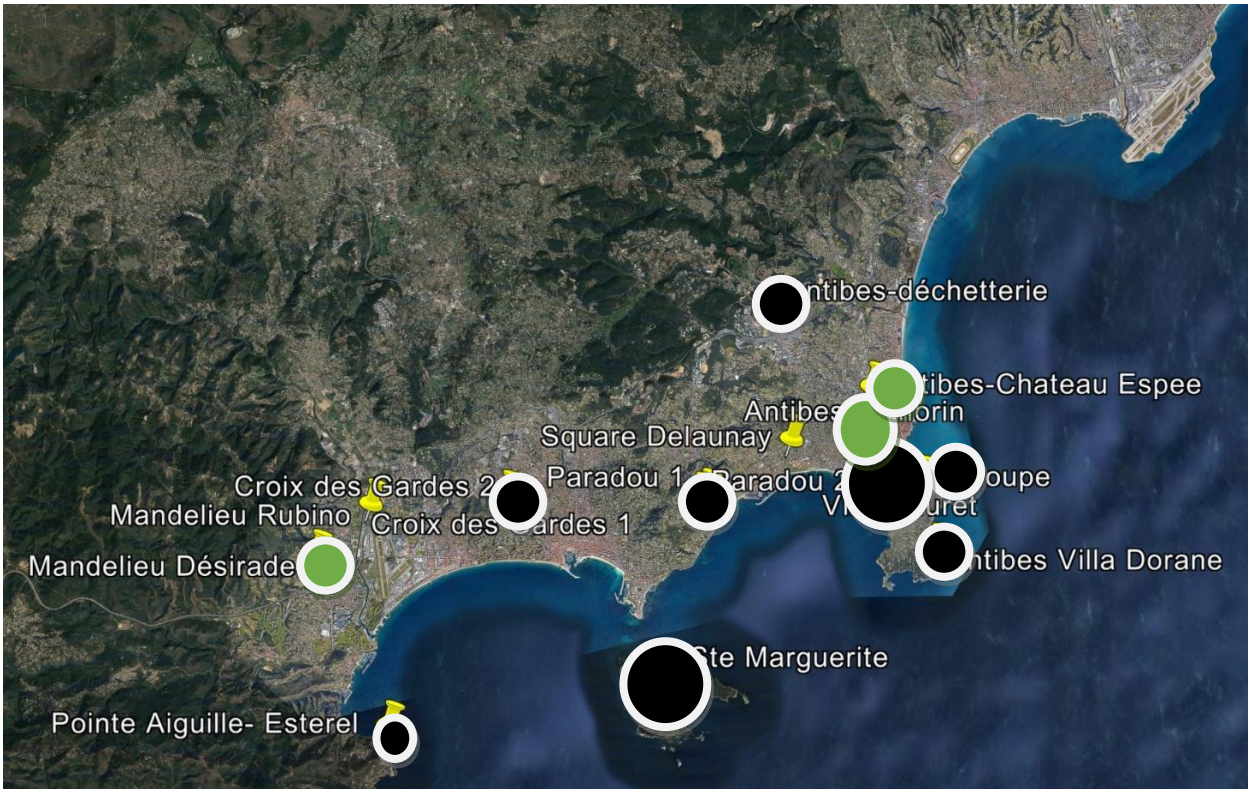

Figure 3: Presence in 2021 of the Xylosandrus species in the core areas of Antibes and Ile Sainte-Marguerite and their vicinity (Black: both species; green: Only X. compactus)

3. Control methods implemented in 2021

3.1. Mass trapping:

A massive attack of plane trees by *X. compactus*, with damage on trunks as well as on branches, has been observed in 2020 on trees planted around several public squares in the cities of Juan les Pins and Antibes. One of these sites (Vilmorin square in Juan Les Pins) was used for a tentative mass trapping experiment in 2021. The square, ca 50m x 100m hosts 12 plane trees around its limits. At each corner of the square, a trap baited with the 4-component combination was hung on a plane tree for a total of 4 traps on the square. The results were disappointing since only 5 beetles were trapped in total. However, a survey of the infestation carried out in February 2022 did not reveal any development of damage on the trunk of these plane trees nor new shoot infestation on oleander and roses. It may be possible that the beetles could not achieve their development in plane trunks and thus the population decreased.

3.2. Push and Pull

Two "Push and pull" experiments using the repellent compound verbenone were carried out at Villa Thuret and Ile Sainte Marguerite. At each site we selected 4 square blocks of 20m x 20m with confirmed presence of *Xylosandrus* spp., leaving a buffer row of at least 20 m between successive blocks. Before the experiment, the extent of damage was measured by selecting 10 trees at random (following a zigzag step) per block and counting the number of exit holes on the trunk and on 10 branches and on 10 last year's shoots per tree. At each site, each block was assigned to one of the following modalities:

- *Modality 1- Push-pull*: a dose of Verbenone in the center of the block stapled on a trunk and 3 traps baited with the attractants at the periphery of the block
- *Modality 2- Control*: no repellents, no push attractants
- *Modality 3- Pull - Trapping efficiency test*: 3 traps baited with attractants on the periphery of the block, no verbenone
- *Modality 4 –Push- Repellent efficacy test:* a dose of Verbenone in the center of the block stapled to a trunk, and no attractant at the periphery

Figure 4 presents the experimental design at Villa Thuret, which was set up on 15 March at Villa Thuret and Figure 5 the one at Ile Sainte Marguerite, which was set up on 7 April. Verbenone doses as well as the attractive lures were changed every 6 weeks from the

onset of the experiment. Trapped insects were collected every 3 weeks. Both experiments stopped on 18 October, and at this time the same plants surveyed at the onset of the experiment were checked again for damage development. Given the very low attacks observed for *X. crassiusculus* at both sites by early 2021, the analyses essentially concentrated on *X. compactus*.

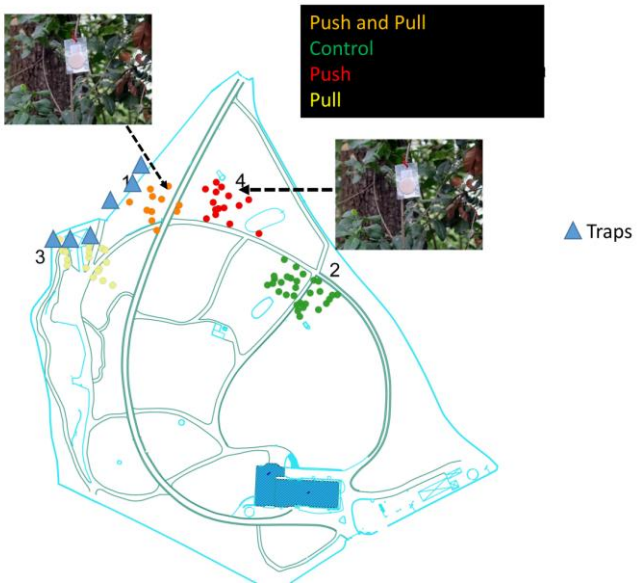

Figure 4: Design of the push and pull experiments at Villa Thuret (colored spots correspond to the surveyed trees and shrubs)

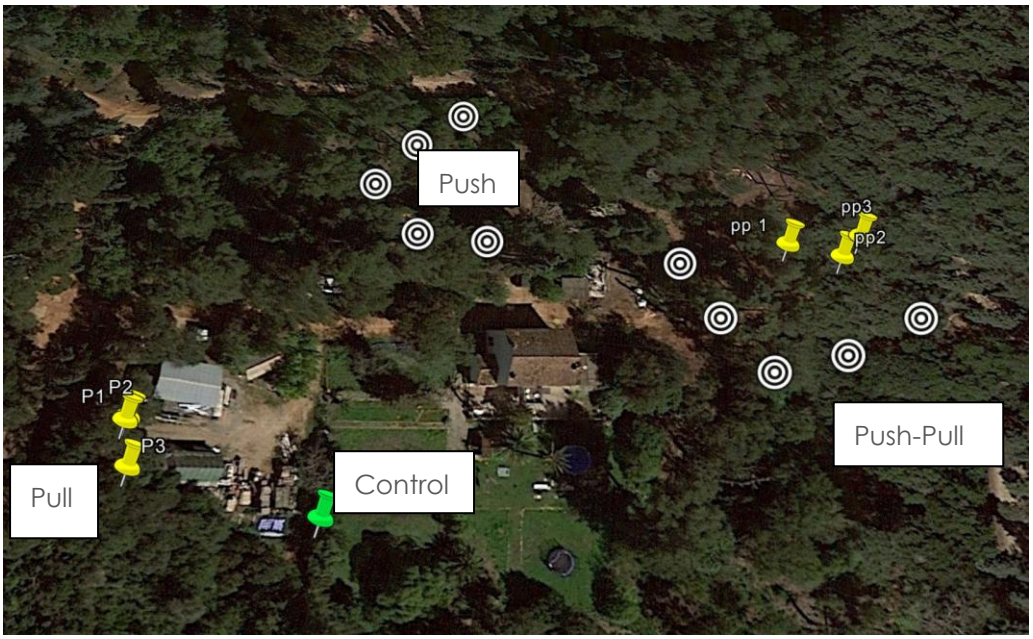

Figure 5: Design of the push and pull experiments at Ile Sainte Marguerite (circles correspond to verbenone doses and yellow pins to attractive traps)

At Villa Thuret, only a few (<20) *Xylosandrus* beetles were trapped with no difference between the Push-Pull and the Pull modalities but the Pull modality captured significantly more other scolytids than the Push-Pull one (average of 46 vs 12 per trap and period of 3 weeks- Figure 6).

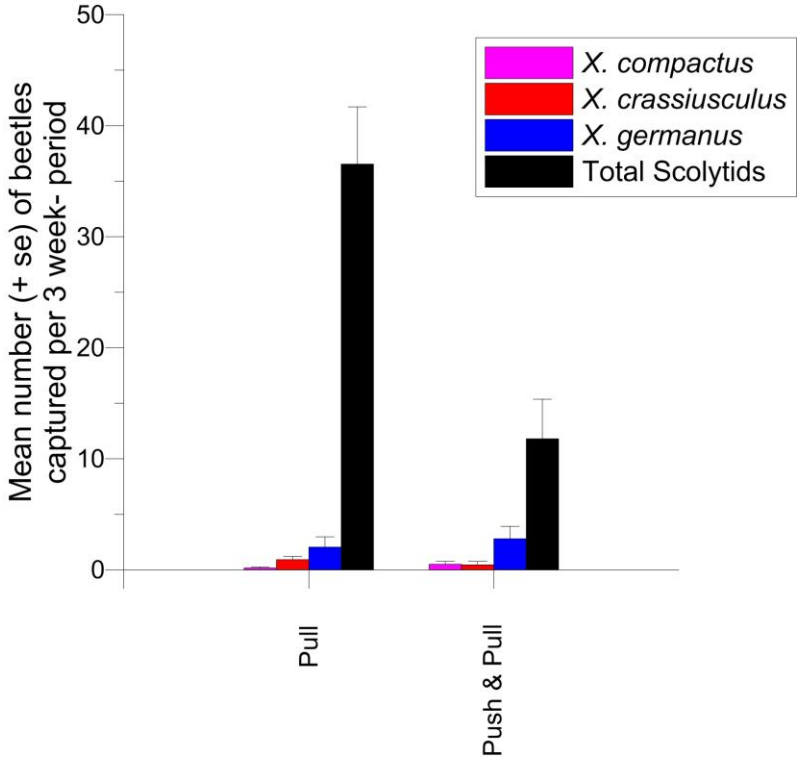

Figure 6: Captures of Xylosandrus spp. and other scolytids by the attractive traps used in the modalities Push and Pull and Pull at Villa Thuret

The comparison of the development of the damage on shoots by *Xylosandrus compactus* between the onset and the end of the experiment did not reveal any significant differences among any of the treatments, most of the surveyed trees and shrubs having shown a decrease in the number of entrance holes, especially the control ones (Figure 7). Actually, the damage seemed too low in all modalities to get any statistical significance. For *X. crassisuculus*, the damage was even lower with only 1 tree showing entrance holes on the trunk in all modalities except the push one which included 4 infested trees with at beginning of the experiment in 3 modalities and 4 in the push modality. At the end of the experiment, only one more tree was infested in the

push and pull modality and 2 more trees in the push modality, but none in the two other ones.

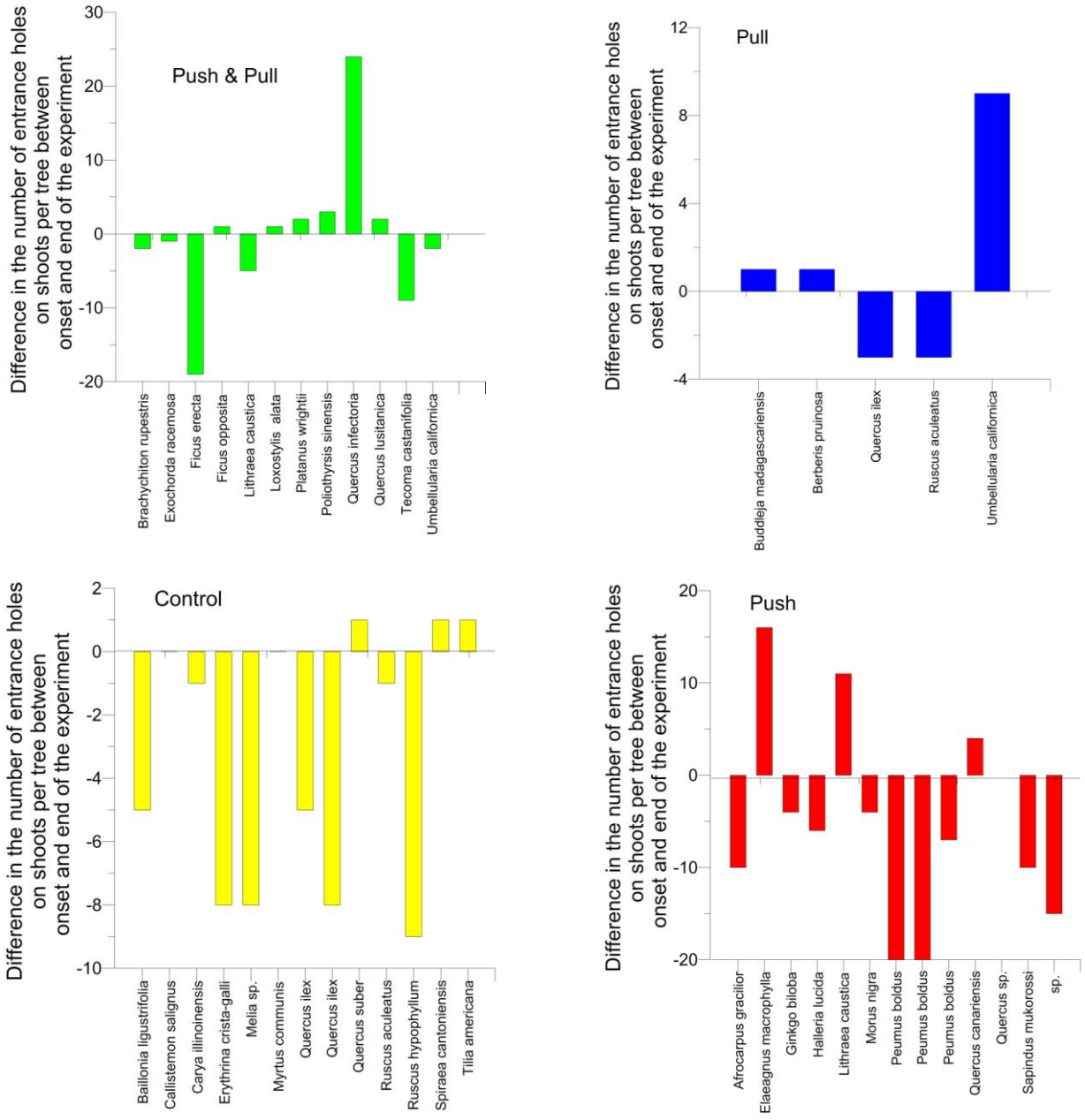

Figure 7: Differences observed in the development of shoot damage by X. compactus on trees and shrubs at Villa Thuret between the onset and the end of the experiment according to the 4 treatment modalities

The results were similar at Ile Sainte Marguerite with very low captures of each *Xylosandrus* species (<15). However, the total captures of scolytids were much more important at this site and the Push-Pull modality significantly more effective in trappings than the Pull one (average of 290 vs 60 per trap and period of 3 weeks; Figure 8).

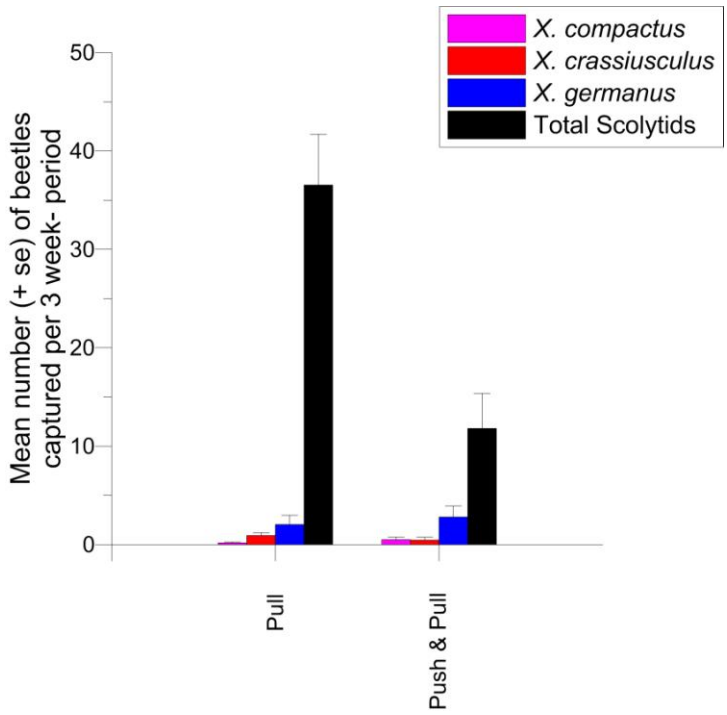

Figure 8: Captures of Xylosandrus spp. and other scolytids by the attractive traps used in the modalities Push and Pull and Pull at Ile Sainte Marguerite

How to explain such differences in total catches of bark beetles between the two sites for the two modalities, "Push and Pull" and "Push"? Differences in starting population level at each block ? Anyway, the Push and Pull combination on Sainte Marguerite has led to the displacement of hundreds of bark beetles, showing thus its effectiveness for species others than *Xylosandrus* spp. The identification of these bark beetles are in progress and is interesting for purposes of controlling other bark and ambrosia beetles.

Similarly as in Villa Thuret, at Ile Sainte Marguerite the number of shoots damaged by *X. compactus* decreased between April and October in all modalities except in the Pull (1 tree over 6) and Push (2 trees over 11) ones (Figure 9). For *X. crassisusculus* only two *Cercis siliquastrum* were attacked at the beginning (1 in Pull, 1 in control) and no change was observed later for any modality. Thus, it appeared difficult to draw any conclusion on the effectiveness of the treatments.

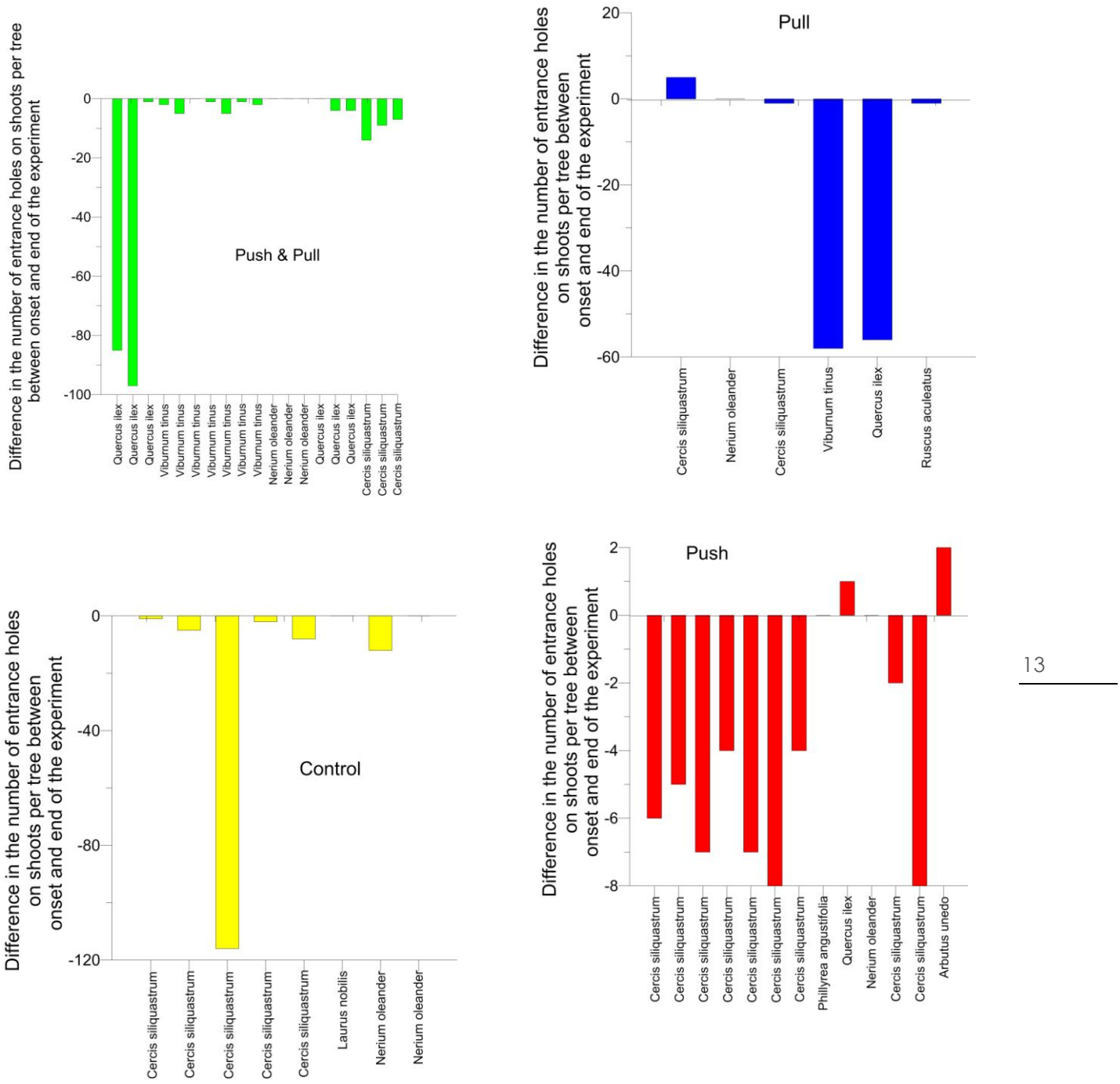

Figure 9: Differences observed in the development of shoot damage by X. compactus on trees and shrubs at Ile Sainte Marguerite between the onset and the end of the experiment according to the 4 treatment modalities

3.3. Transect trappings for defining optimal trap density for capturing *Xylosandrus* beetles

Two parallel, linear transects of 5 traps baited with the 4 attractive compounds shown in 1. were placed at increasing distances from each other in the core area of Antibes-Bois de la Garoupe. Traps were thus placed at 0m, 10m, 20m, 30m and 50m (Figure 10). The optimal distance between two traps could be inferred from the observation of reduced capture when the range of attraction of two adjacent traps begins to overlap. A formula allows the calculation of the optimal distance from the relative capture of the traps and the distance between the paired traps.

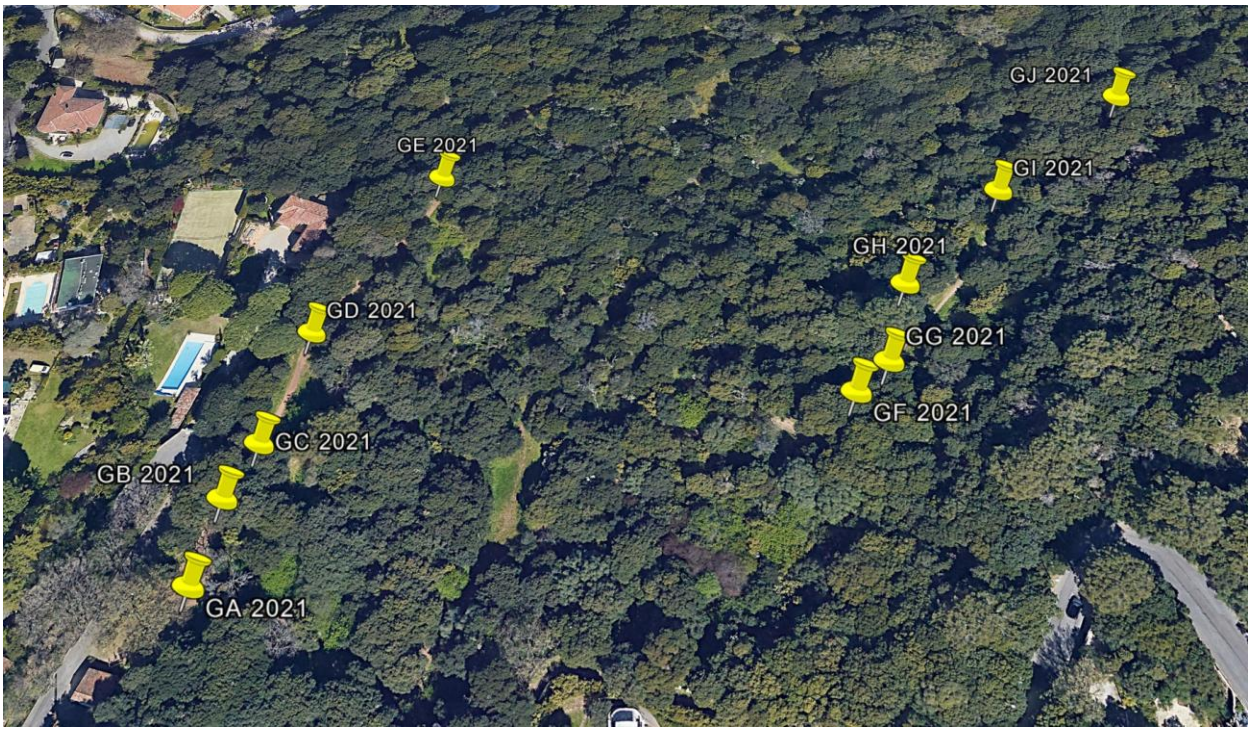

Figure 10: Trap transects of increasing distances deployed at Bois de la Garoupe in 2021

As for the other experiments, only a few beetles were trapped whilst observed damage appeared very limited at Bois de la Garoupe (> 10 shoots with entrance holes along each transects, and no damage on trunks). In total, 7 *X. compactus* were captured for 6 *X. crassiusculus* but 23 *X. germanus*. Raw data on captures per trap are presented at Figure 11.

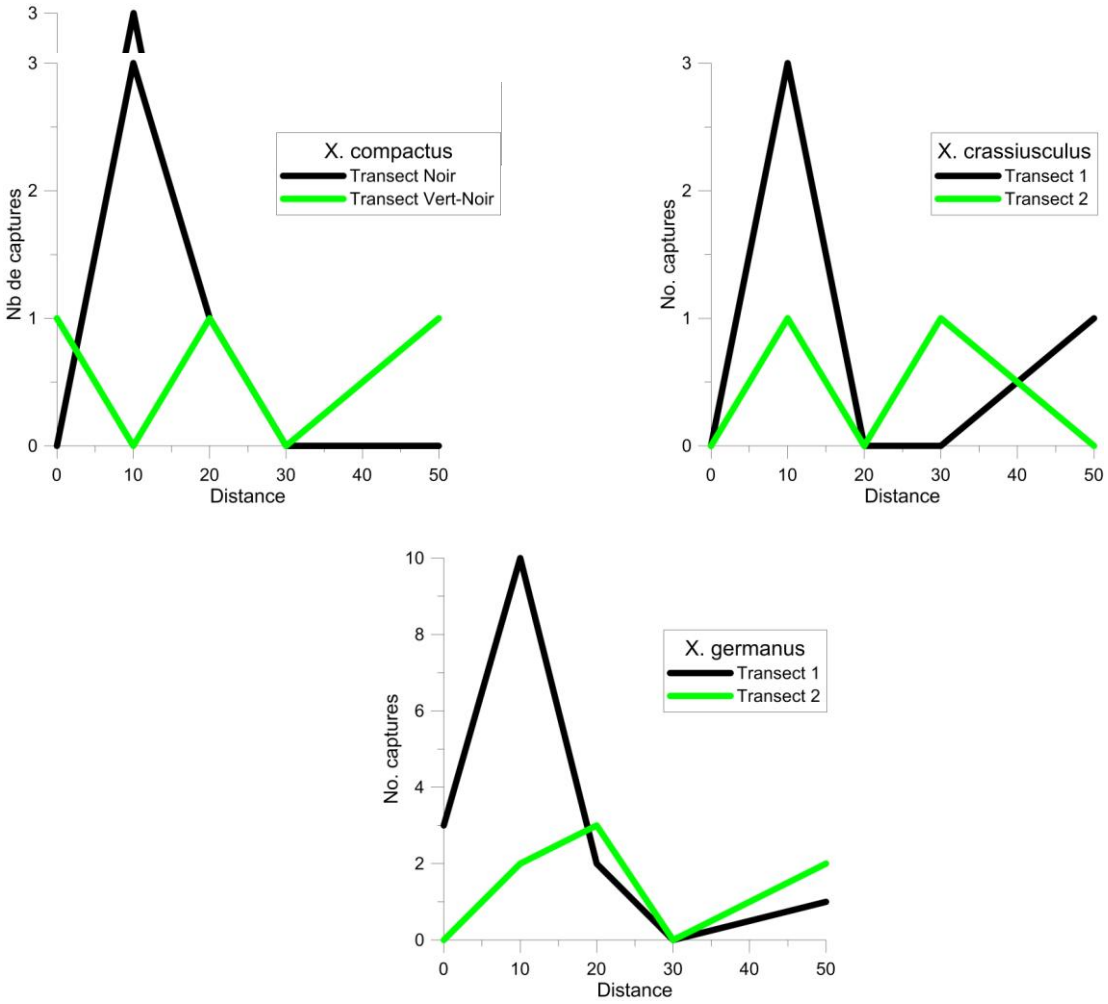

Figure 11: Total captures of the 3 Xylosandrus species along the two transects at Bois de la Garoupe in 2021

The calculations are ongoing but given the limited captures, we will need to gather data from the other transect experiments done by SAMFIX partners in order to be capable of realizing the expected statistics for estimating the optimal distances between traps.

4. Protocols to be implemented in 2022

Most of the stakeholders having attended the meeting held in February at Antibes have shown their interest in continuing the survey of *Xylosandrus* beetles in 2022 although no specific funding will be available. INRAE has agreed to supply them with the necessary lures. The trapping protocol will be the same as in 2021 (cf 1.). One black multifunnel trap baited with the 4 attractive compounds (Ethanol/ [-] a-pinene/ + quercivorol/ + a-copaene) will be deployed per site. A total of 19 sites will be surveyed. Five traps will be deployed within the Corniches de la Riviera, and 5 on its surroundings. At Antibes 3 traps will be deployed in the core area and 2 in the surroundings. Two traps will be installed at Ile Sainte Marguerite with 2 additional traps on the close seashore. All trappings are expected to start on April 15 at the latest and to continue until October with a collection of the trapped beetles every 3 weeks.

5. Conclusions

Both species are spreading in the core areas but at rather low density for the moment. It is important to continue the survey of their expansion. An important result of the SAMFIX project is that most of the involved stakeholders agreed to go on developing beetle surveys by themselves given that INRAE could provide lures.

The results of the push and pull experiments as well as those of the mass trapping experiments were rather disappointing but it could be attributed to the low density of the beetles which prevented any definitive conclusions since beetle damage even decreased in the untreated control plots. It would be important to develop again such tests in situations with high densities of the *Xylosandrus* species.