Deliverable:

Final evaluation on the effectiveness of lures

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SUMMARY

Based on literature data, the attractiveness of 9 different lure combinations for *Xylosandrus compactus* and *X. crassiusculus* was compared in trials carried out in the French Antibes area and in the Circeo Park in Italy during 2019. A combination of 4 compounds, i.e. quercivorol, a-copaene, Ethanol and a-pinene, captured significantly more *X. compactus* per trap than the other lures; but no difference was observed in the trapping of *X. crassiusculus* among the 9 lure combinations. However, in Spain this 4-compound combination showed a larger attraction for *X. crassiusculus* than a simple combination of Ethanol and a-pinene. Thus, a good compromise to attract both *Xylosandrus* species seemed the use of this combination of 4 compounds. Since crossvane and multifunnel traps did not show significant differences in *Xylosandrus* captures when baited with same lure, the use of multifunnel traps was suggested because of their easier handling.

Trap colour also appeared to play a role in attraction, black traps and traps combining a green top and a black basis being the significantly most attractive ones. Finally, it was decided to use black multifunnel traps baited with the 4-component combination lure for monitoring and early detection of *Xylosandrus spp*. since 2020 on. These further trappings effectively allowed a satisfactory early detection for the two targeted *Xylosandrus* species. However, the relationships between the number of captures per trap and the population density as well the resulting damage was more complex to be established, especially because of the low beetle populations present on the sites.

Because the 4 compounds combined in the lure are not specific of *X. compactus* or *X. crassiusculus*, large numbers of a congeneric invader *X. germanus* were also captured as well as many other scolytids. Although these side captures did not seem to limit the lure effectiveness for the two target species, they could delay the identification of the collected beetles and the early application of possible measures when the species are detected.

1. Introduction

When the SAMFIX project started, only very limited, and sometimes confusing data existed in the literature about the lures susceptible to be used for the detection of the invasive ambrosia beetles *Xylosandrus crassiusculus* and *X. compactus*. Ethanol, which is emitted by stressed trees, was already known to attract a number of ambrosia beetles (Kelsey and Joseph, 2017; Ranger et al., 2015) among which *X. crassiusculus* (Reding et al., 2011) and *X. compactus* (Burbano et al., 2012). Actually, artisanal bottles baited with ethanol effectively captured *Xylosandrus crassiusculus* in the invaded Mont Boron in southern France but no *X. compactus* although it was present there (DSF, 1998). The addition of a-pinene to ethanol then allowed to trap a few *X. compactus* in this area (Roques, unpublished data). In the USA, large numbers of *X. crassiusculus* were also trapped using a mixture of quercivorol and a-copaene (Owen et al., 2017; Kendra et al., 2017), whilst also capturing a few specimens of *X. compactus* (Owen et al., 2017; Kendra et al., 2017). In the same country, (*E*)-(±)-conophthorin, a compound used by cone beetles, apparently enhanced attraction of *X. compactus* to ethanol (Van der Laan & Ginzel, 2013). However, the genetic composition, and thus the behaviour, may largely differ among populations having invaded different continents, depending on the origin of the source populations.

Thus, experimental tests had to be performed in order to compare the attractiveness of the different compounds identified in the world as potentially attractive for *Xylosandrus crassiusculus* and *X. compactus*. Given the results in the USA, such tests had also to consider these compounds both alone and in combination.

2. Tests of potentially- attractive lures and of optimal trap design for both Xylosandrus species

2.1 Definition of optimal lures for *Xylosandrus* spp.

<u>2.1.1 Selection of the compounds to be tested</u>

Five compounds were tested, including Ethanol, (-) a-pinene, quercivorol [(1S,4R)-4-Isopropyl-1-methyl-2-cyclohexen-1-ol], a-copaene [(1S,4R)-4-Isopropyl-1-methyl-2-cyclohexen-1-ol], and *E*)-(±)-conophthorin [(E)-7-Methyl-1,6-dioxaspiro[4.5]decane]. Figure 1 shows the chemical structure of the 3 last compounds.

1a *1b* *1c*

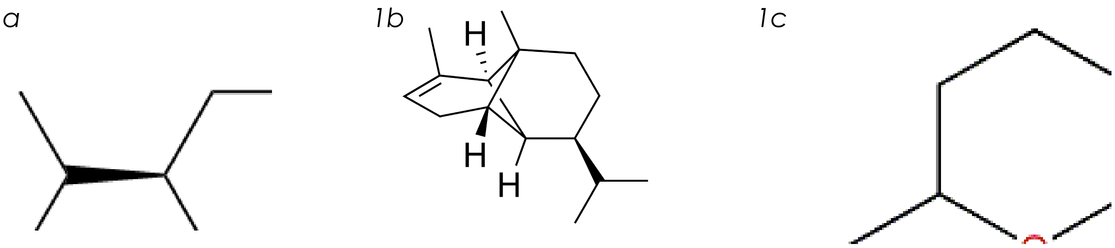


Figure 1: *Chemical structure of 1a- quercivorol; 1b- a-copaene; and 1c- E)-(±)-conophthorin.*

The tests consisted in 9 different lure combinations, each compound being used alone or in combination with others, with or without a-pinene and ethanol. The modalities were the following: 1- quercivorol (1ml); 2- a-copaene (2 ml); 3- quercivorol (1ml) + a-copaene (2 ml); 4- quercivorol (1ml) + a-copaene (2 ml)+ Ethanol + (-) a-pinene; 5- quercivorol (1ml) + Ethanol + (-) a-pinene; 6- a-copaene (2 ml) + Ethanol + (-) a-pinene; 7- (*E*)-(±)-conophthorin) + Ethanol ; 8- Ethanol + (-) a-pinene; 9- Cerambycid blend (1ml) + Ethanol + (-) a-pinene. The last one was used as a control since it was the one used for previous trappings (generic blend for cerambycids defined in Fan et al., 2019, implemented with Ethanol and a-pinene).

Quercivorol and a-copaene were obtained as bubble cups (Figure 2a) from Synergy Semiochemicals Corp. (Burnaby, BC, Canada) whereas Ethanol (100ml with 96 % purity; release rate 2 g/day at 20°C; Figure 2b) and (-) a-pinene (25 ml with 98 % purity; release rate 0.3 g/day at 20°C; Figure 2c) were obtained as diffuser packs from Econex (Spain). These compounds are expected to last 60 days. The cerambycid blend was made at INRAE and stored in tubes of 1 ml to be inverted on a dental cotton stick placed into a mini zip bag (Figure 2d).

The lures were hung on either multifunnel or crossvane traps (Figure 3). On multifunnel traps, the bubbles of quercivorol, a-copaene and E)-(±)-conophthorin were placed tied to the middle of the trap as well as the pack of ethanol and the cerambycid blend but the pack of a-pinene was tied to the 2nd funnel from the bottom. On crossvane traps, the bubbles of quercivorol, a-copaene and *E*)-(±)-conophthorin, the pack of ethanol and the ceramabycid blend were tied to different holes of the top of the central window whereas the pack of alpha-pinene was tied to a hole on the basis of the central window.

2a *2b* *2c* *2d*

Figure 2: *from left to right: bubble of Quercivorol, pack of Ethanol, pack of a-pinene and dental cotton impregnated with cerambycid blend in a minizip bag.*

Figure 3*: Examples of multifunnel (left) and crossvane (right) traps.*

2.1.2 Designs of the experimental tests

The experimental trappings including the 9 modalities were carried out in 2019 in France and Italy in places invaded by both *Xylosandrus* spp. Additional tests were carried out in 2020 in the El Tello area of Spain, where only *X. crassiusculus* was present, but these tests only compared two modalities: 2 compounds (Ethanol + [-] a-pinene) vs. 4 compounds (Ethanol + [-] a-pinene + quercivorol + a-copaene).

In France, 4 sets of 9 traps were deployed, including 2 at Villa Thuret and 2 at Bois de la Garoupe. At each site, one set used multifunnel traps and the other crossvane traps. In Italy, 4 trapping sets were deployed at different places within the Circeo Park. The trappings were carried out from 26 February to 20 November 2019 in France and from 2 May to 17 October in Italy. The traps were collected and rotated clockwise every 3 weeks. Trap rotation allowed us to consider the number of collection dates at each site as replicates, i.e. 54 replicated in France and 32 replicates in Italy. Data from France and Italy were thus combined for the statistical analyses. However, replicates from a given date that contained no *Xylosandrus* in any of the traps, for example due to inclement weather or insect phenology, were dropped from the analyses. Because data violated normality, differences between lure captures were tested using the nonparametric Friedman's Q test (Statistica 9 ®, Tibco Software Inc., Palo Alto, CA, USA). Assuming a significant overall Friedman's test, pairs of treatment means were compared with the nonparametric Dunn–Nemenyi multiple comparison test.

In Spain, experiments were performed in ten randomized complete blocks of two treatments (FourC= trap baited with Four components; TwoC = trap baited with Two components). The distance between traps was at least 100 m and the blocks were at least 700 m apart. Number of *X. crassiusculus* were log10(x + 1)-transformed to meet the assumptions of normality and homocedasticity, and subjected to ANOVA [general linear models (GLM)] for randomized complete blocks with the R software package (R Development Core Team 2020).

2.1.3 Optimal lure for *Xylosandrus compactus*

A total of 516 specimens were trapped in 2019 in tests performed in France and Italy, but with large differences according to the site (Circeo A: 174; Circeo B: 6; Circeo C: 33; Circeo D: 4; Garoupe crossvanes: 190; Garoupe multifunnels: 40; Thuret crossvanes: 35; Thuret multifunnels: 34).

Over the 86 3-week collections from France and Italy, only 40 contained at least one specimen of *X. compactus* caught by one of the baits, the number of specimens ranging up to 129 at Bois de la Garoupe on June 3 in a trap baited with a combination of quercivorol, a-copaene, Ethanol and a-pinene. However, other positive captures only ranged from 1 to 15 per trap.

Highly significant differences in the mean number of trapped specimens were noted between blends (Friedmann Q8,39 = 24.354; P = 0.002). The combination of the 4 compounds 'quercivorol, a-copaene, Ethanol and a-pinene', caught the largest number of beetles with an average of 4.00±2.22 individuals trapped per collection period (Figure 4). However, this value did not differ significantly from those obtained by traps baited with a combination of quercivorol, Ethanol and a-pinene (1.90±0.85), and by traps baited with Ethanol and a-pinene (1.83±0,63). The other blends were significantly less attractive, even conophthorin implemented with Ethanol.

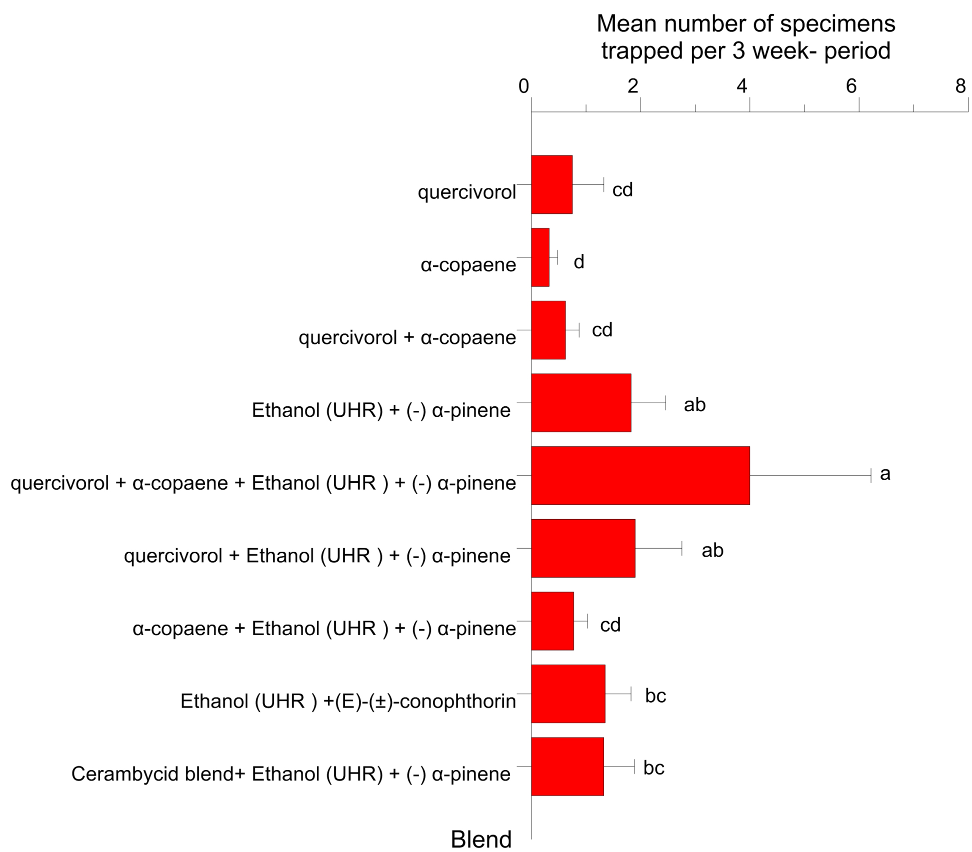


Figure 4: *Comparison of the attractiveness of the 9 blend combinations for trapping Xylosandrus compactus. Pooled data from the 2019 trials of Villa Thuret, Bois de la Garoupe and Circeo Park (40 replicates)*

2.1.4 Optimal lure for Xylosandrus crassiusculus

In France and Italy, only 239 specimens were trapped in tests performed in 2019, with large differences according to the site (Circeo A: 0; Circeo B: 21; Circeo C: 2; Circeo D: 3; Garoupe crossvanes: 9; Garoupe multifunnels: 6; Thuret crossvanes: 108; Thuret multifunnels: 90). Over the 86 3-week collections from France and Italy, only 34 contained at least one specimen of *X. crassiusculus*. Captures ranged from 1 to 17 individuals per positive trap (17 at Villa Thuret on May 14 in a trap baited with a-copaene, Ethanol and a-pinene). Unlike *X. compactus*, no significant differences in attractiveness was observed among blends (Friedmann Q8,33 = 12.959; P = 0.113). On the average, the combinations of 'quercivorol, Ethanol and a-pinene' and 'quercivorol, a-copaene, Ethanol and a-pinene', caught a few more specimens per trap than the others (1.21±0.58 and 1.15±0.42, respectively; Figure 5).

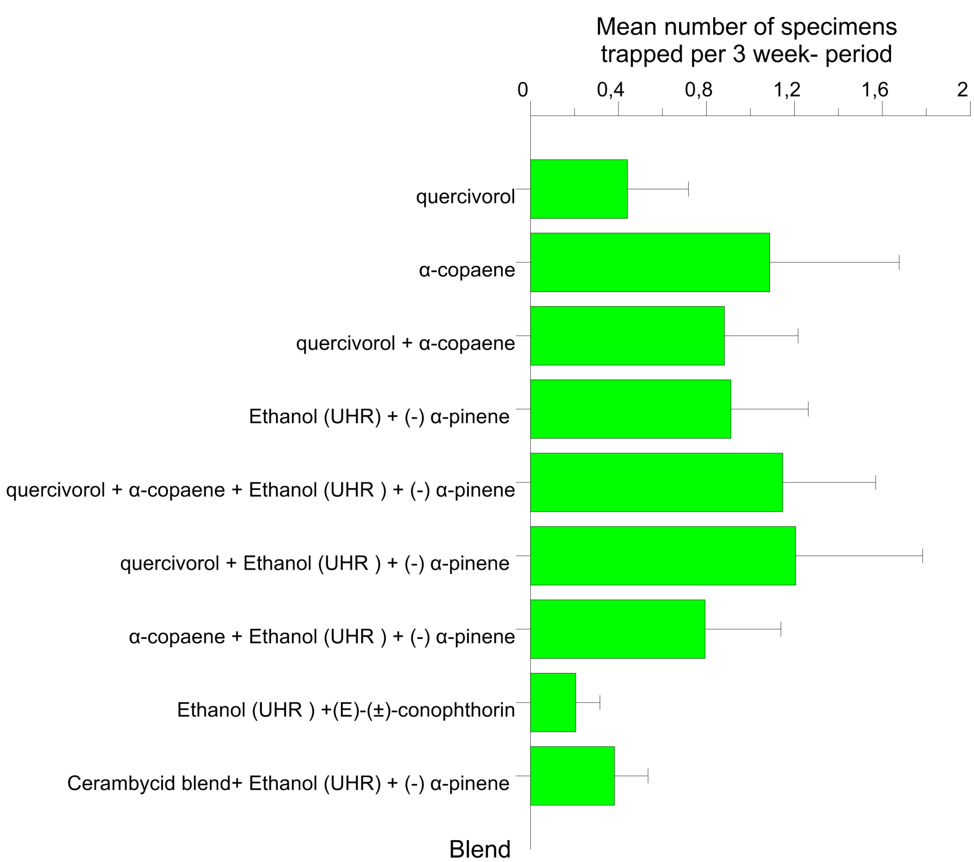
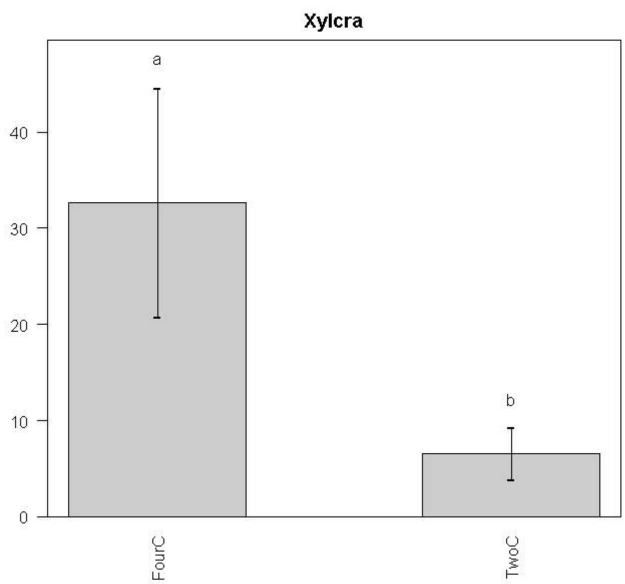


Figure 5: *Comparison of the attractiveness of the 9 blend combinations for trapping Xylosandrus crassiusculus. Pooled data from the 2019 trials of Villa Thuret, Bois de la Garoupe and Circeo Park (34 replicates)*

The 2020 trials in Spain gave somewhat different results, in the context of an apparently larger population of *X. crassiusculus*. The combination of 4 compounds (Ethanol + [-] a-pinene + quercivorol + a-copaene) appeared significantly more attractive that Ethanol combined to [-] a-pinene) (gl=1, F=5.827, Pvalue=0.039) (Figure 6a). This was verified during each collection period (Figure 6b).

6a



6b

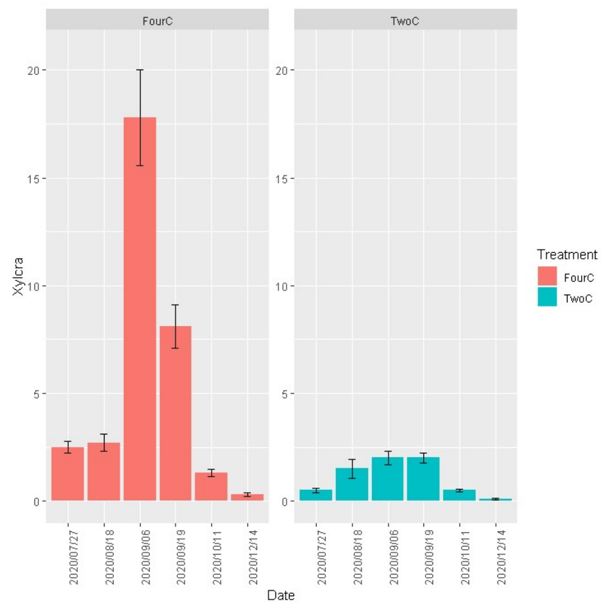


Figure 6: *Comparison of the attractiveness of the blend combining 4 compounds (Ethanol + [-] a-pinene + quercivorol + a-copaene) with the one of the blend combining 2 compounds (Ethanol + [-] a-pinene) at El Tello, Spain in 2020; A (left)- mean (± se) captures per collection; B (right)- mean captures (± se) per collection dates*

2.1.5 A surprising side effect: Optimal lure for *Xylosandrus germanus*

Another *Xylosandrus* species, *X. germanus*, also invasive from Asia but established in Europe since the 1980s, was surprisingly trapped in numbers at most locations of France and Italy during the 2019 tests. A total of 1776 specimens were thus trapped although large differences were observed according to the plot (Circeo A: 18; Circeo B: 52; Circeo C: 15; Circeo D: 152; Garoupe crossvanes: 125; Garoupe multifunnels: 960; Thuret crossvanes: 282; Thuret multifunnels: 172). One specimen at least was captured in 39 of the collections, sometimes in large numbers especially at Bois de la Garoupe where more than 100 individuals per trap were trapped 3 times.

Highly significant differences in the mean number of trapped specimens were noted between lures (Friedmann $Q_{8,38}$ =138,61; P = 0.000). The combination of the 'Ethanol and a-pinene', caught the largest number of beetles with an average of 12.18±7.41 individuals trapped per collection period (Figure 7). However, this value did not differ significantly from those obtained by traps baited with a-copaene alone (9.67±5.74), and by traps baited with the cerambycid blend plus Ethanol and a-pinene (6.05±3,61). The other blends were significaly less attractive.

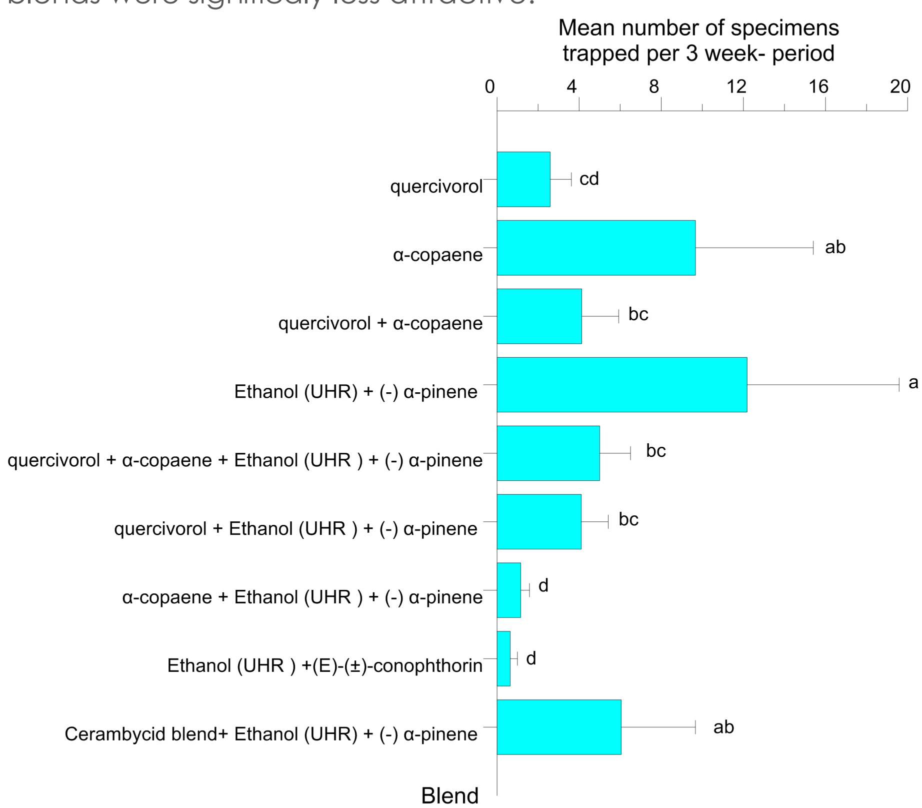


Figure 7: *Comparison of the attractiveness of the 9 blend combinations for trapping Xylosandrus germanus in the 2019 trials of Villa Thuret, Bois de la Garoupe and Circeo Park (39 replicates)*

2.1.6 Conclusion: Which lure for an optimal detection of *Xylosandrus* spp.?

A good compromise appeared to be the use of a combination of 4 products, i.e. quercivorol, a-copaene, Ethanol and a-pinene, because it appeared more attractive for *X. compactus* in France and Italy but also for *X. crassiusculus* in Spain.

2.2. Selection of the optimal trap shape

The experiments carried out in 2019 at Villa Thuret and Bois de la Garoupe in 2019 allowed to compare the captures obtained by black multifunnel traps and by black cross-vanes traps for each lure modality.

The two types of traps did not significantly differ in captures of *Xylosandrus compactus* (Figure 8), as well as of *X. crassiusculus* (Figure 9).

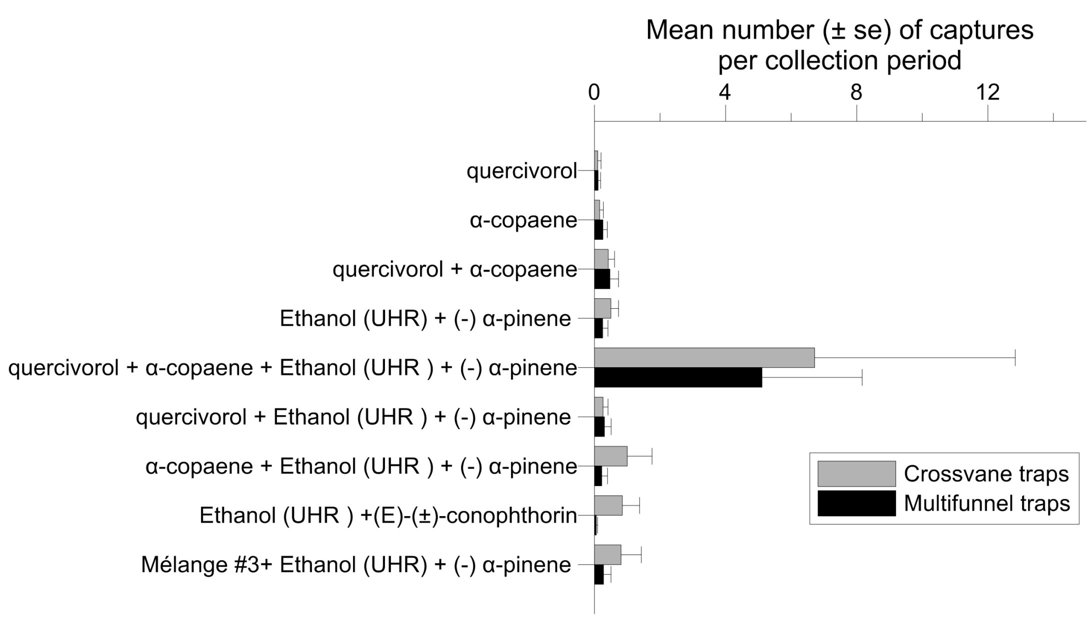


Figure 8: *Comparative captures of X. compactus by crossvane and multifunnel traps according to the 9 lure modalities in 2019 (data from Villa Thuret and Bois de la Garoupe pooled)*

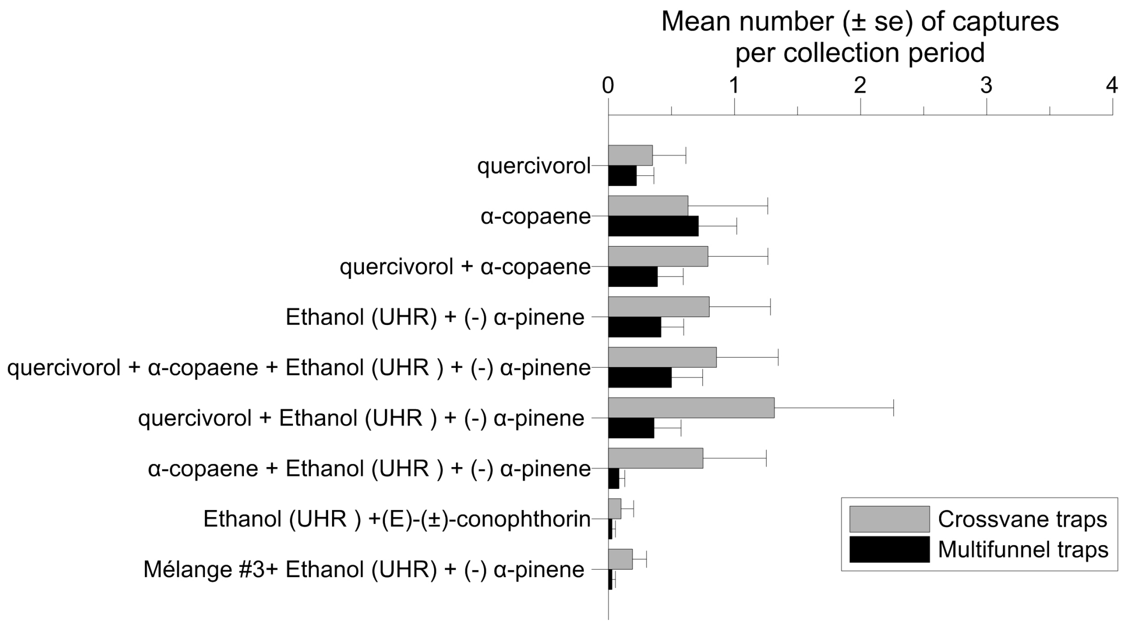


***Figure 9**: Comparative captures of X. crassiusculus by crossvane and multifunnel traps according to the 9 lure modalities in 2019 (data from Villa Thuret and Bois de la Garoupe pooled)*

Thus, because the multifunnel traps are more easy to manipulate, transport and store, it has been suggested to use this type of trap.

2.3. Selection of the optimal trap color

Experiments carried out in 2020 at Villa Thuret aimed at comparing the captures by multifunnel traps of different colors baited with the same combination of 4 compounds considered as the optimal lure. For both *X. compactus* and *X. crassiusculus*, black traps and traps consisting of a green top (6 funnels mimicking canopy) and a black basis (6 funnels mimicking trunk) were the most attractive ones but did not differ in beetle captures. Purple traps were also as attractive than the two previous ones for X. compactus but not for *X. crassisuculus* (Figure 10).

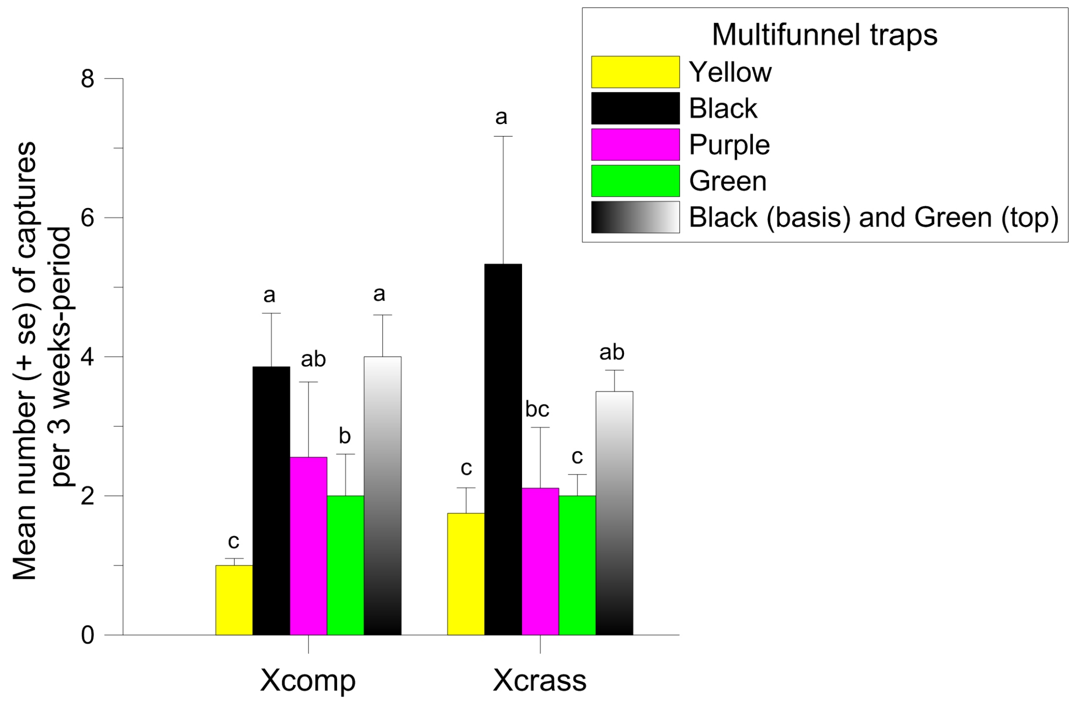


Figure 10: *Comparative attractiveness of traps of different colours baited with the same combination of 4 compounds for X. compactus* and *X. crassiusculus at Villa Thuret in 2020.*

Because entirely black traps are more easy to be designed, it was thus suggested to use black multifunnel traps for *Xylosandrus* detection and monitoring rather than green/black ones.

3. Effectiveness of the 4-component lure combination for the detection and monitoring of *Xylosandrus* spp in 2020 and 2021

Based on the results detailed above, the trapping networks deployed in 2020 and 2021 generally used black multifunnel traps baited with a combination of 4 compounds - quercivorol, a-copaene, Ethanol and a-pinene. However, because of the COVID epidemics, the 2020 trappings started very late in most sites of France and Italy, and thus usually missed the dispersing flight of the 1st generation of beetles. Therefore, the 2020 trapping data were not considered as very reliable except for Spain.

The 2021 trappings effectively started by mid-March to early April and could be considered as representative of the effectiveness of the lures. Table I is presenting the total captures and the mean captures per trap.

3.1. Lure effectiveness for monitoring and early detection of *X. compactus*

The mean number of *X. compactus* captured per trap in 2021 was very low everywhere in France and Italy except in the French core areas of Corniches de la Riviera and Ile Sainte Marguerite where more than 3 beetles were captured per trap. Compared to the 2019 test trappings using the same lure combination at Antibes (Villa Thuret and Bois de la Garoupe), the mean number of beetles per trap decreased from 35 (140 for 4 traps) in 2019 to 1.7 in 2021. At Circeo Park, the captures with the same lure combination also dropped from 5.0 (20 on 4 traps) in 2019 to 0.7 per trap in 2021. These limited captures obtained in 2021 may thus reflect a decreased density of insects at these trapping sites. The survey of damage by *X. compactus* at Villa Thuret in 2021 tended to confirm this decrease with an average of only 1.2 entrance holes per tree (10 shoots surveyed per tree on 19 susceptible trees and shrubs). In Italy, there were no statistically significant differences between theses because the current percentage of infestation of the plants due to *Xylosandrus* species appears to be, for all theses, less than 1%.

However, the effectiveness of the lure for the early detection of the beetle presence appeared satisfactory. All the traps placed at new sites where the presence of *X. compactus* was suspected from simple damage observation effectively captured individuals of the species and thus confirmed the presence, e.g., in downtown public squares at Antibes and in recycling centers at Bormes- Manjastre. The lures also allowed to detect the arrival of *X. compactus* in Corsica in 2020 on the west coast of the island before any damage was observed, and confirmed its presence on the East side. In Italy, the already extensive presence of *Xylosandrus* did not allow, in comparison with what was verified in France, to identify early detection signs of infestation.

Year	Country	Site	nb traps	*X.compactus*	*X.crassiusculus*	*X.germanus*	Other scolytids	X.compactus/trap	X.crassiusculus/trap	X. germanus/trap	Other scolytids/trap
2021	Italy	Circeo transects	45	32	4	606	1671	0,7	0,1	13,5	37,1
2021	Italy	Circeo PushPull	30	14	1	35	818	0,5	0,0	1,2	27,3
2021	Italy	Viv For	6	0	0	46	1146	0,0	0,0	7,7	191,0
2021	Italy	Monti Auraunci	6	0	4	20	736	0,0	0,7	3,3	122,7
2021	Italy	Monti Ausoni- Camposoriano	6	0	0	1	2	0,0	0,0	0,2	0,3
2021	Italy	Monti Ausoni- Villa Placitelli	2	1	86	129	39	0,5	43,0	64,5	19,5
2021	Italy	Castelli Romani	6	3	1	1144	960	0,5	0,2	190,7	160,0
2021	Italy	Tor Caldara	6	2	0	9	448	0,3	0,0	1,5	74,7
2021	Italy	Riviera di Ulysse	6	0	5	4	207	0,0	0,8	0,7	34,5
2021	Italy	Ventotene	6	0	0	0	5	0,0	0,0	0,0	0,8
2021	Italy	Castel Porziano	6	0	0	3	148	0,0	0,0	0,5	24,7
2021	Italy	Ninfa	10	13	6	98	2372	1,3	0,6	9,8	237,2
2021	Italy	Fogliano	2	0	10	3	803	0,0	5,0	1,5	401,5
2021	France	Antibes	23	39	63	324	4297	1,7	2,7	14,1	186,8
2021	France	Corniches de la Riviera	5	3	20	5	1495	0,6	4,0	1,0	299,0
2021	France	Corniches- surroundings	6	18	73	174	3711	3,0	12,2	29,0	618,5
2021	France	Ile Ste Marguerite	6	20	52	993	7643	3,3	8,7	165,5	1273,8
2021	France	Ile Ste Marguerite- surroundings	7	10	15	427	2109	1,4	2,1	61,0	301,3
2021	France	Port Cros	3	0	0	0	79	0,0	0,0	0,0	26,3
2021	France	Port Cros-surroundings	8	2	1	21	2415	0,3	0,1	2,6	301,9
2021	France	Corsica	14	13	0	108	2621	0,9	0,0	7,7	187,2
2020	Spain	El Tello	40	-	144				3,6		
2020	Spain	Naquera	2	-	1863				931,5		
2021	Spain	El Tello	40	-	135				3,4		
2021	Spain	Naquera	8	-	2091				261,4		

Table I: *Captures of Xylosandrus spp. with traps baited with the 4-component lure combination in the areas surveyed in France and Italy during 2021 and in Spain during 2020 and 2021.*

3.2. Lure effectiveness for monitoring and early detection of *X. crassiusculus*

The mean number of *X. crassiusculus* captured per trap in 2021 was frequently higher than the mean captures of *X. compactus* in France, especially at Corniches de la Riviera and Ile Sainte Marguerite, and in Italy, especially at Villa Placitelli of Monti Ausoni. Compared to the 2019 test trappings using the same lure combination at Antibes (Villa Thuret and Bois de la Garoupe), the mean number of beetles per trap also decreased, but a bit less than for *X. compactus*, from 4,5 (18 for 4 traps) in 2019 to 2.7 in 2021. This low density of beetles was confirmed at Villa Thuret where quite no damage was observed in 2021: only 1 tree showing a few entry holes on its trunk, over the 19 surveyed trees and shrubs. At Circeo Park, the captures with the same lure combination were also very low (< 1.0 per trap) in both 2019 and 2021. However, the effectiveness of the lure combination was largely shown in Spain where several hundreds of beetles were captured per trap at Naquera (Table 1) although a decrease was observed between 2020 and 2021.

The effectiveness of the lure for an early detection of *X. crassiusculus* also appeared satisfactory, with the trapping of beetles in areas where no damage was previously observed, e.g. in several sites of Corniches de la Riviera. In Italy, the already extensive presence of *Xylosandrus* did not allow, in comparison with what was verified in France, to identify early detection signs of infestation.

3.3. Lure effectiveness for monitoring and early detection of X. germanus

The 4-component lure combination appeared highly effective in trapping this congeneric species, several hundreds of captures per trap being recorded at several sites where its presence was not known, e.g. Castelli Romani in Italy and Ile Sainte Marguerite in France (Table 1). Actually, *X. germanus* was trapped quite everywhere the trappings were deployed in southeastern France and Italy. It showed both that the species is largely established all over these regions but also the detection power of the lure.

3.4. Another side effect of the lure combination: the massive trapping of other ambrosia and bark beetles

As shown in Table I above in quite all the target sites the capture ratio between the two target *Xylosandrus* is highly unbalanced, being up to 1 *Xylosandrus* for 805 other scolytids in the surroundings of Port-Cros. Although these side captures did not seem to prevent those of the target species (cf. the large captures of *X. crassiusculus* in Spain), and thus the lure effectiveness, they could have the effect to delay the identification of the collected beetles and the early application of possible measures when the species are detected.

4. General conclusions

The combination of the four compounds on black multifunnel traps effectively allowed a satisfactory early detection for the two targeted *Xylosandrus* species. However, the relationships between the number of captures per trap and the population density as well as the resulting damage is more complex to be established. The side captures of large numbers of *X. germanus* but also of many other scolytids may create problems as regards species identification and early detection of the invaders. However, it did not appear possible to define fully specific lures.

5. Literature cited

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