Action D1
Interim Evaluation on the effectiveness of lures

Deliverable:

Interim Evaluation on the effectiveness of lures

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Table of content

Summary 3

1. Previous state of the art about lures effective to monitor *Xylosandrus compactus* and *X.crassiusculus* 4

2. Samfix experiments aimed at testing the effectiveness of lures for both *Xylosandrus* species 5

2.1 Monitoring using traps baited with a-pinene and ethanol 5

2.2. Definitions of the blends to be tested in 2019 for improving lure attractiveness and specificity for both for both *Xylosandrus* species 6

2.3. Experimental sites and design of the experiments 8

3. Results 10

3.1 Effectiveness of the monitoring of *Xylosandrus compactus* and *X.crassiusculus* with traps baited with a-pinene and ethanol 10

3.2 Definition of an optimal lure to monitor *Xylosandrus compactus* 11

3.3 Definition of an optimal lure to monitor *X.crassiusculus* 13

3.4. Additional monitoring of an interesting non-native ambrosia beetle: *Xylosandrus germanus* 14

3.5. Trapping of xylophagous species which can perturbate captures and sorting of *Xylosandrus* specimens 16

4. Conclusions : which optimal lure to be used 17

5. Literature cited 17

SUMMARY

Following the results of the 2018 experiments, which identified a first attractive blend, consisting in a combination of a-pinene and ethanol, for the monitoring of *Xylosandrus compactus* and *X. crassiusculus*, traps deployed in 2019 in southeastern France allowed to detect two new invaded sites for each of these species. However, such a blend being not specific, tests had to be carried out in order to define optimal lures for monitoring both species. The attractiveness of 9 different blends was compared in 8 different trials carried out in the French Antibes area and in the Circeo Park in Italy.

A combination of 4 compounds, i.e. quercivorol, a-copaene, Ethanol and a-pinene, caught the largest number of *X. compactus* per trap but no difference was observed in the trapping of *X. crassiusculus* among the 9 blends. No significant difference in *Xylosandrus* captures was also observed between crossvane and multifunnel traps for a same blend.

The captures of additional xylophagous species, especially longhorn beetles, which can delay sorting or limit attraction of ambrosia beetles, appeared limited in traps baited by 8 of the lure combinations.

Finally, it was suggested to use for further monitoring of both *Xylosandrus* species any black trap design baited with quercivorol, a-copaene, Ethanol and a-pinene.

1. Previous state of the art about lures effective to monitor *Xylosandrus compactus* and *X.crassiusculus*

The 2018 experiments resulted in the identification a first attractive blend, consisting in a combination of a-pinene and ethanol, that was thus suggested to serve for the monitoring of both species of *Xylosandrus* in 2019 (deliverable A1, supplied in April 2019).

However, Deliverable A1 also pointed out that the combination of a-pinene and ethanol could not be considered as representing an optimal lure, the more as it is attracting a large number of species other than *Xylosandrus*, e.g. cerambycids and other bark and ambrosia beetles.

Thus, in order to define such an optimal lure, we searched in the literature which chemical compounds have previously been identified as possible attractants in other continents where these ambrosia beetle species are invasive. The following compounds were identified: p-menth-2-en-1-ol also called "quercivorol", a-copaene and (*E*)-(±)-conophthorin.

Actually, large numbers of *X. crassiusculus* were trapped in the USA using a mixture of quercivorol and a-copaene (Owen et al., 2017; Kendra et al., 2017) whereas (*E*)-(±)-conophthorin apparently enhanced attraction of the same species to alcohol (Van der Laan & Ginzel, 2013). A blend combining quercivorol and a-copaene also trapped a few specimens of *X. compactus* in the USA (Owen et al. 2017 as well as Kendra et al. (2017).

The 2019 tests were aimed at testing the attractiveness of these compounds, alone or in combination, compared ta the one of the combination "a-pinene and ethanol".

2. Samfix experiments aimed at testing the effectiveness of lures for both *Xylosandrus* species

2.1 Monitoring using traps baited with a-pinene and ethanol

Trappings using this combination were carried out in 30 locations of southeastern France in order to survey the spread of *Xylosandrus compactus* and *X.crassiusculus* and detect new infestations. The location of the trapping sites is shown in Figure 1.

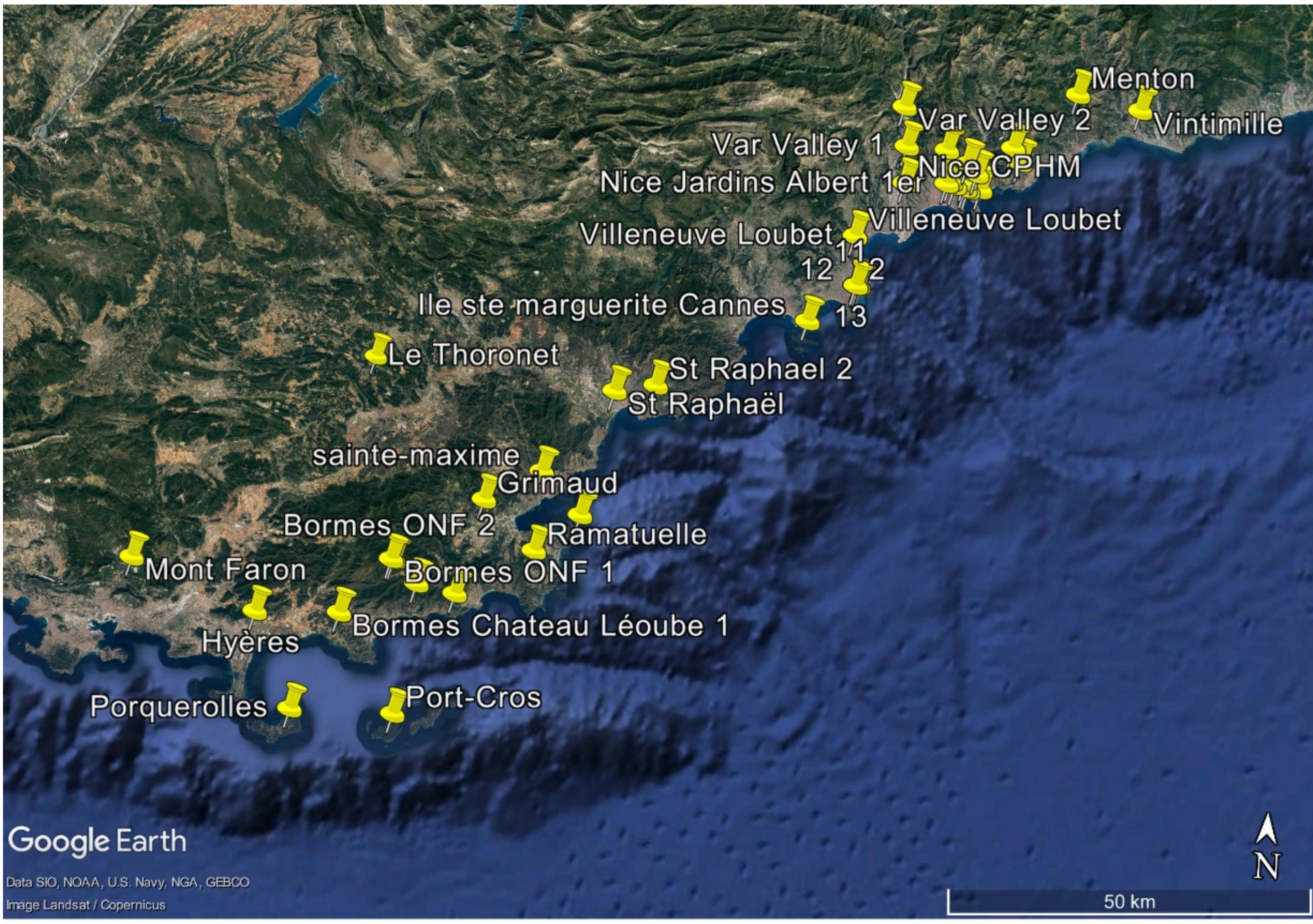
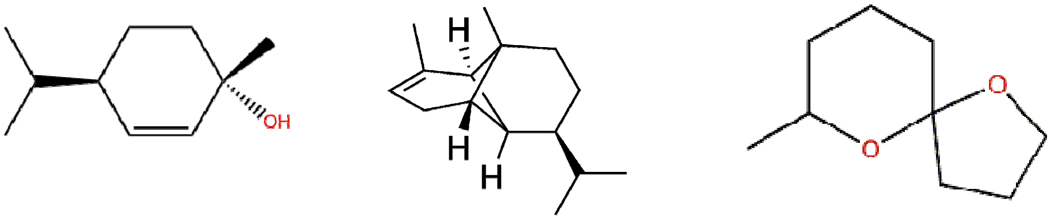


Figure 1. Location of the plots used to monitor the spread of both Xylosandrus species using traps baited with a-pinene and ethanol

2.2. Definitions of the blends to be tested in 2019 for improving lure attractiveness and specificity for both for both *Xylosandrus* species

The attractive potential of quercivorol [(1S,4R)-4-Isopropyl-1-methyl-2-cyclohexen-1-ol; Figure 2a], a-copaene [(1S,4R)-4-Isopropyl-1-methyl-2-cyclohexen-1-ol; Figure 2b], and *E*)-(±)-conophthorin [(E)-7-Methyl-1,6-dioxaspiro[4.5]decane; Figure 2c], was tested, these compounds being used alone or in combination between them, with or without a-pinene and ethanol.



2a 2b 2c

Figure 2: Chemical structure of 2a- quercivorol; 2b- a-copaene; and 2c- E)-(±)-conophthorin.

Thus, the tests consisted in 9 different lure combinations, the last one (9-) being used as a control since it was the one we mainly used for trappings in 2018 (generic blend for cerambycids defined in Fan et al., 2019, implemented with Ethanol and a-pinene)

- quercivorol (1ml);
- a-copaene (2 ml);
- quercivorol (1ml) + a-copaene (2 ml);
- quercivorol (1ml) + a-copaene (2 ml)+ Ethanol + (-) a-pinene;
- quercivorol (1ml) + Ethanol + (-) a-pinene;
- a-copaene (2 ml) + Ethanol + (-) a-pinene;
- (*E*)-(±)-conophthorin) + Ethanol
- Ethanol + (-) a-pinene;
- Cerambycid blend (1ml) + Ethanol + (-) a-pinene;

Quercivorol and a-copaene were obtained as bubble cups (Figure 3a) from Synergy Semiochemicals Corp. (Burnaby, BC, Canada) whereas Ethanol (100ml with 96 % purity; release rate 2 g/day at 20°C; Figure 3b) and (-) a-pinene (25 ml with 98 % purity; release rate 0.3 g/day at 20°C; Figure 3c) were obtained as diffuser packs from Econex (Spain). These compounds are expected to last 60 days. The cerambycid blend was made at INRA and stored in tubes of 1 ml to be inverted on a dental cotton placed into a mini zip bag (Figure 3d).

The lures were hung on either multifunnel or crossvane traps (Figure 4). 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.

Figure 3: 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 4: Examples of multifunnel (left) and crossvane (right) traps settled at Villa Thuret, Antibes.

2.3. Experimental sites and design of the experiments

The tests were carried out in France and Italy. In each country, 4 sets of 9 traps were deployed. In order to expect trapping numbers of specimens allowing statistical comparisons, we selected places where both *Xylosandrus* spp. previously showed consistent populations: the Antibes area in France and the Circeo Park in Italy.

In France, they consisted in two sets of traps at Villa Thuret, one using multifunnel traps and one crossvane traps, and 2 sets of traps at Bois de la Garoupe with a similar trap type design (Figure 5). In Italy, the 4 trapping sets were deployed in different places within the Circeo Park (Figure 6).

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, ie. 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.

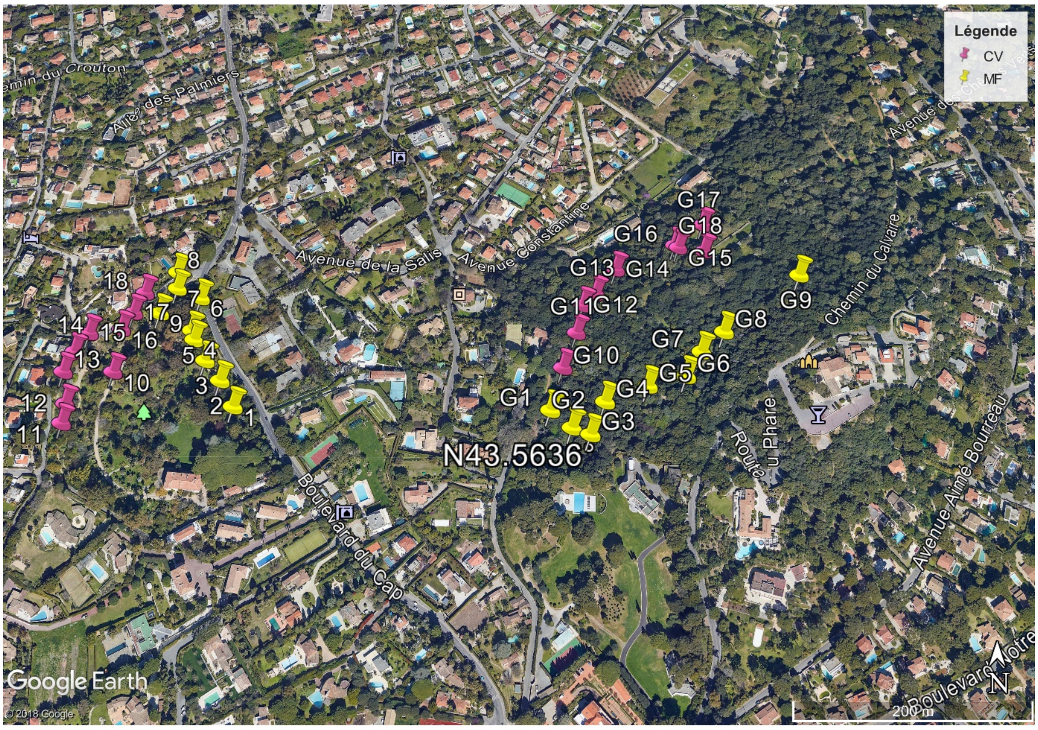


Figure 5: Experimental design at Villa Thuret (left) and Bois de la Garoupe (right- G numbers), Antibes, France. Yellow marks correspond to multifunnel traps, purple ones to crossvane traps.

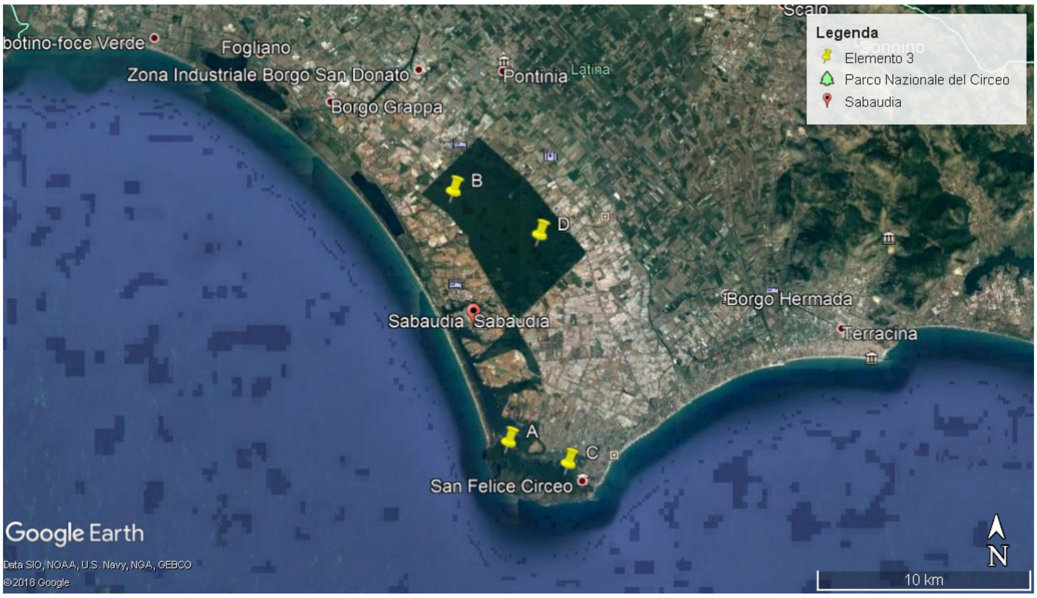


Figure 6: Experimental design at Circeo Park, Italy. A, B, C and D correspond to the location of each set of 9 traps.

3. Results

3.1 Effectiveness of the monitoring of *Xylosandrus compactus* and *X.crassiusculus* with traps baited with a-pinene and ethanol

The combination "a-pinene and ethanol" proved to be effective in detecting new populations of both species as shown in Table 1. *X. compactus* was trapped at 8 locations (including Villa Thuret and Bois de la Garoupe) over the 30 ones where such trappings were deployed. The two new sites detected at Ste Marguerite Island and at Saint Raphaël revealed an expansion to the west.

Table I: Locations where Xylosandrus spp. were detected in 2019 using traps baited with a-pinene and ethanol. New records figured in bold

Site	*Xylosandrus compactus*	*Xylosandrus crassiusculus*	*Xylosandrus germanus*	*Amasa* nr. *resecta*
Cannes - Ste Marguerite Isl.	**13**	163	565	**3**
La Turbie	0	2	0	0
Nice - Albert 1er Garden	0	2	3	0
Nice - Mount Boron	28	1769	1	0
Nice - Nursery	0	**1**	1	0
Nice - Var Valley	0	**6**	3	0
Villefranche Cap Ferrat	31	329	77	0
Bormes - Château Léoube	4	0	4	0
Saint Raphael	**1**	0	1	0
Saint Tropez	4	0	413	0
Total	81	2272	1068	3

X.crassiusculus has been detected at 9 locations (including Villa Thuret and Bois de la Garoupe), of which 2 new sites in the upper valley of Nice, revealing an expansion upwards.

The monitoring also allowed to detect the extended presence of another Asian *Xylosandrus*, *X. germanus,* which has been trapped at 11 locations (including Villa Thuret and Bois de la Garoupe), often in large numbers.

Traps baited with a-pinene and ethanol also allowed to detect the expansion of an additional ambrosia beetle of the genus *Amasa* in the Natura 2000 site of Sainte Marguerite island. This species has been detected for the first time in Europe at Villa Thuret in 2018.

3.2 Definition of an optimal lure to monitor *Xylosandrus compactus*

A total of 516 specimens were trapped but with large differences according to the plot (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 $Q_{8,39}$ = 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 7). 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 significaly less attractive, even conophthorin implemented with Ethanol.

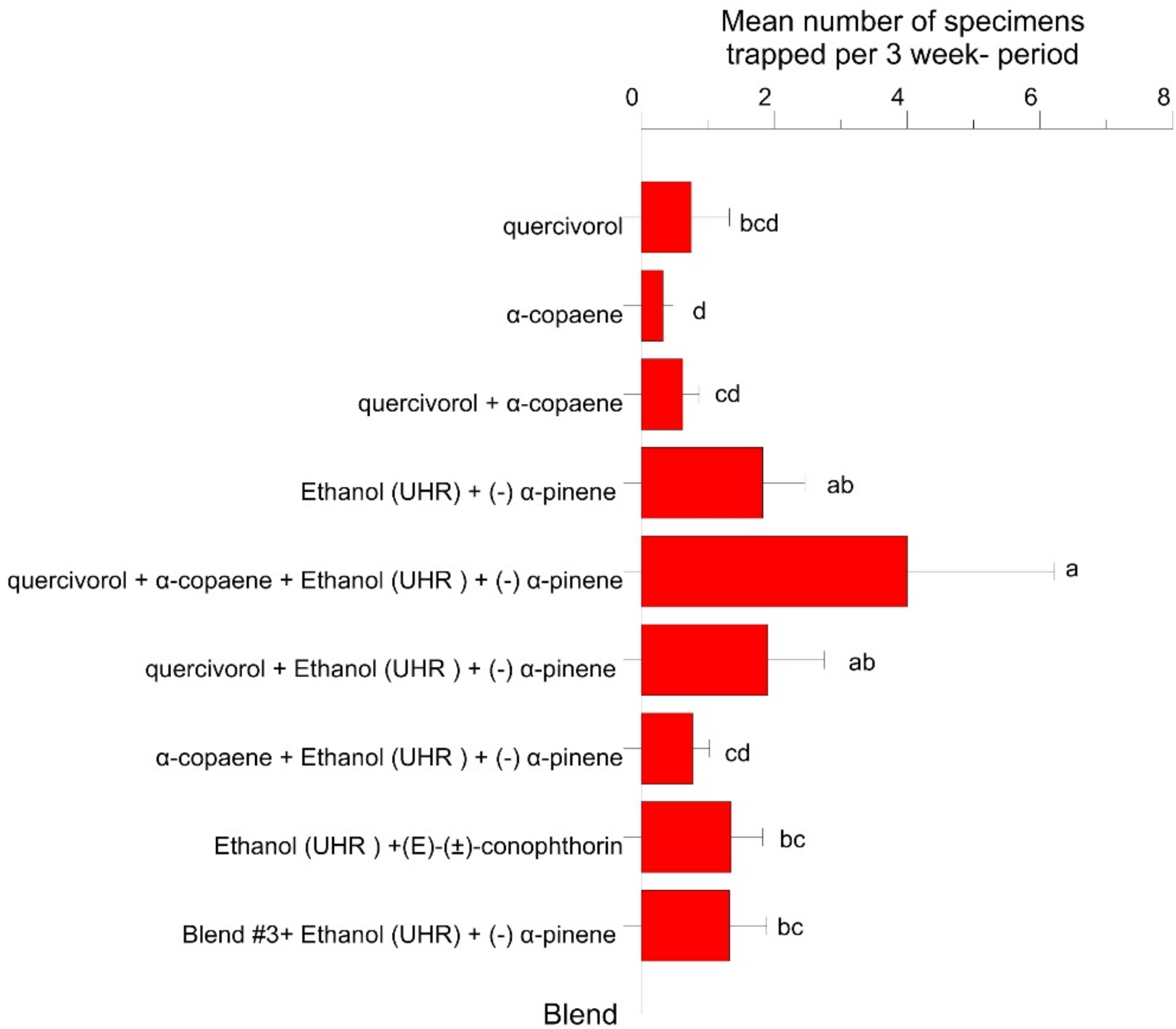


Figure 7: Comparison of the attractiveness of the 9 blends for trapping Xylosandrus compactus in the 2019 trials of Villa Thuret, Bois de la Garoupe and Circeo Park (40 replicates)

No significant difference in captures by a given blend was noted between the two trap types.

3.3 Definition of an optimal lure to monitor *X.crassiusculus*

Only 239 specimens were trapped, with large differences according to the plot (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 collections from France and Italy, only 34 contained at least one specimen of *X.crassiusculus*. Captures ranged 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).

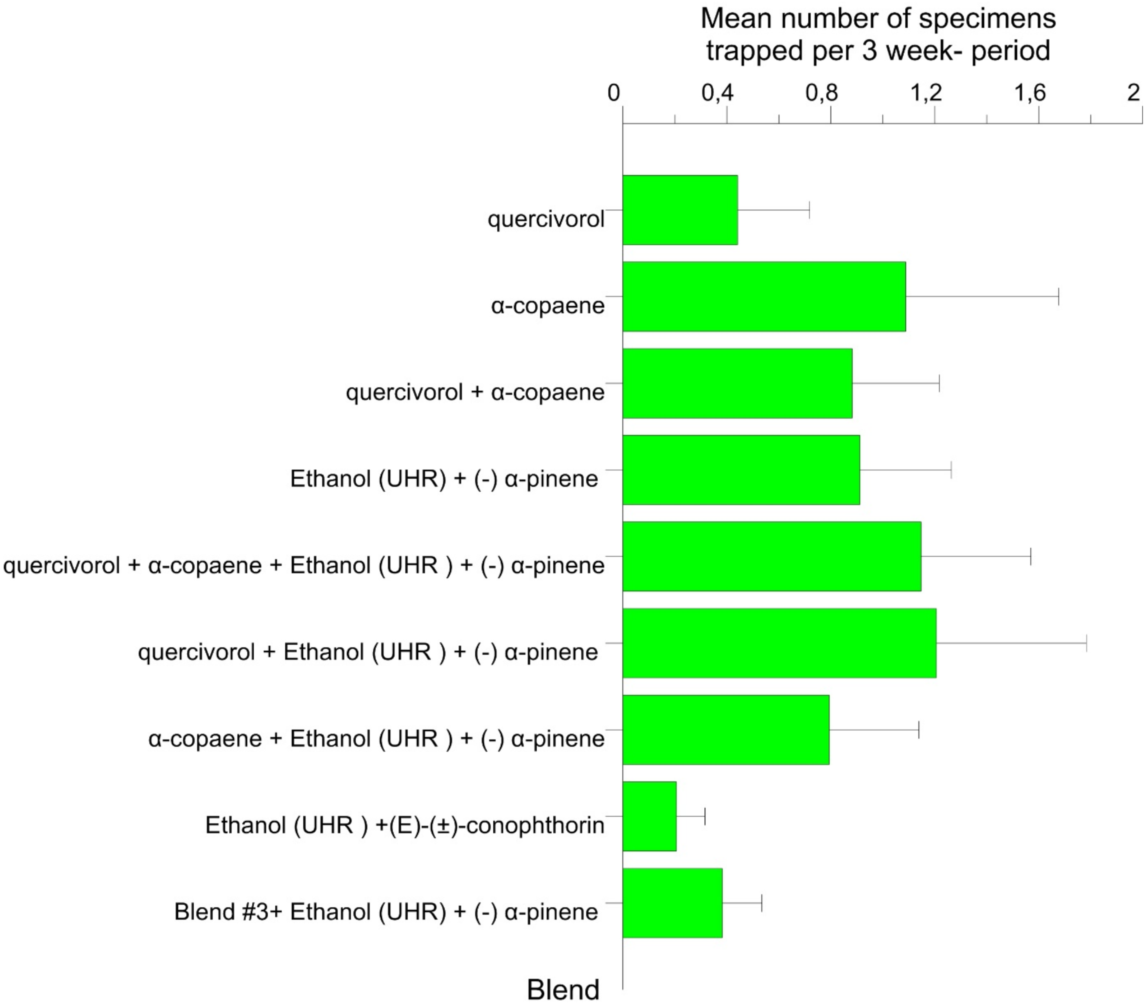


Figure 8: Comparison of the attractiveness of the 9 blends for trapping Xylosandrus crassiusculus in the 2019 trials of Villa Thuret, Bois de la Garoupe and Circeo Park (34 replicates)

Unlike *X. compactus*, no significant differences in attractiveness was observed among blends (Friedmann $Q_{8,33}$ = 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 8).

No significant differences in captures by a given blend were noted between the two trap types.

3.4. Additional monitoring of an interesting non-native ambrosia beetle: *Xylosandrus germanus*

This Asian species was largely present at all the sites used for the tests, with a total of 1776 specimens 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 blends (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 9). 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.

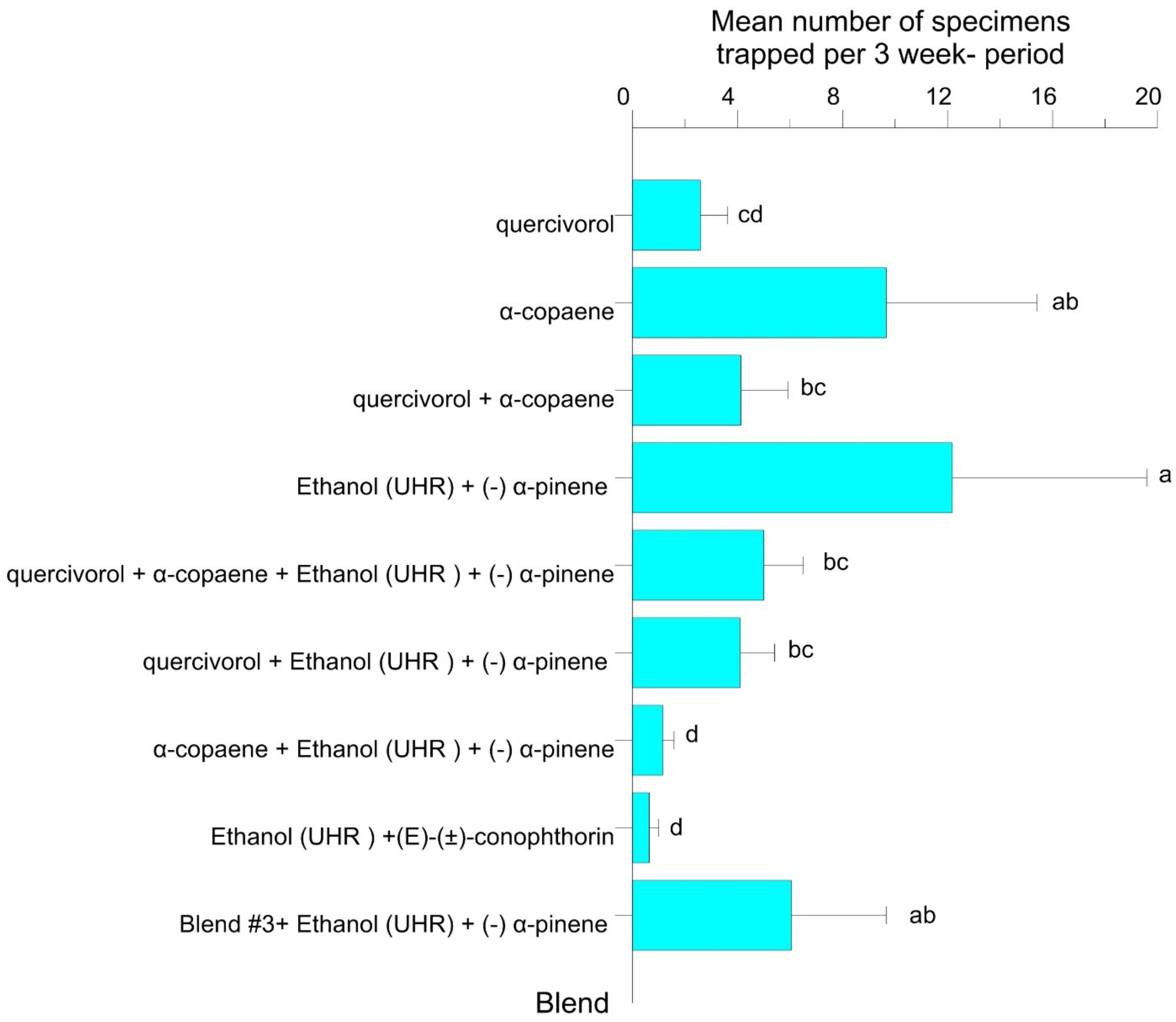


Figure 9: Comparison of the attractiveness of the 9 blends for trapping Xylosandrus germanus in the 2019 trials of Villa Thuret, Bois de la Garoupe and Circeo Park (39 replicates)

3.5. Trapping of xylophagous species which can perturbate captures and sorting of *Xylosandrus* specimens

In 2018, the collectors from the traps baited with the generic cerambycid blend implemented with a-pinene and ethanol were full of large longhorn beetles and other xylophagous species. This largely delayed the sorting of the specimens of Xylosandrus sp, and it may be hypothesized that the presence of such species could affect the trapping of ambrosia beetles.

We thus assessed if the different blends tested in 2019 resulted in limiting the presence of such additional species. The number of trapped cerambycid beetles was significantly lower when using the 8 blends other than the generic blend for longhorn beetles (#3) plus a-pinene and ethanol (Figure 10). The mean number of captures per trap thus dropped from 9.74±1.55 to less than 0.6 when traps were baited by the other blends. No significant differences were observed between these last blends.

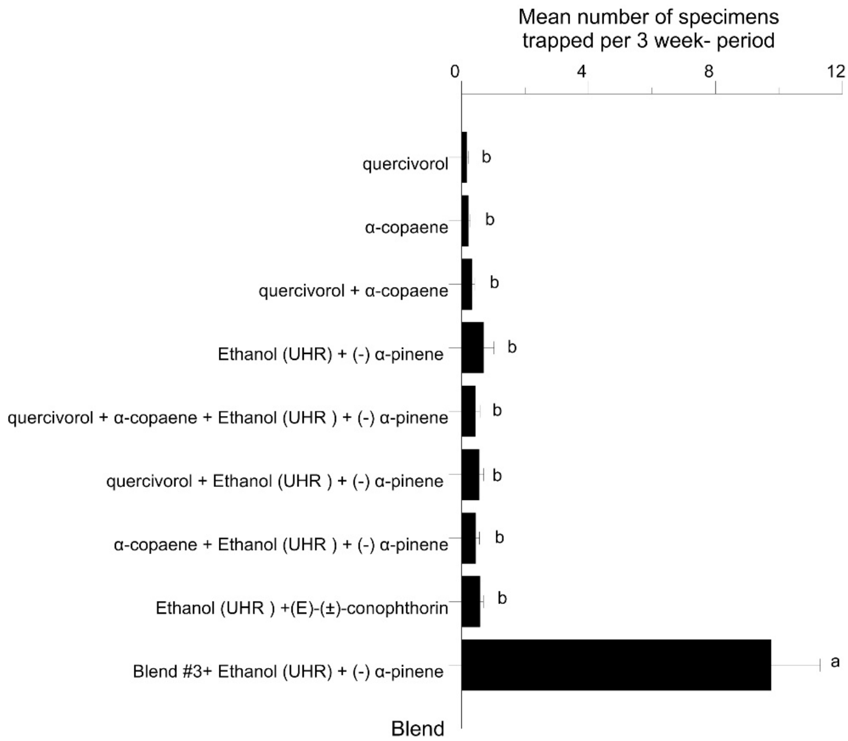


Figure 10: Comparison of the attractiveness of the 9 blends for trapping longhorn beetles in France in the 2019. All data from France pooled.

4. Conclusions : which optimal lure to be used

If traps deployed in 2019 in southeastern France for the monitoring of *Xylosandrus compactus* and *X. crassiusculus* using a combination of a-pinene and ethanol allowed to detect two new invaded sites for each of these two species, they still could not be considered as optimal lures. The comparison in France and Italy of the attractiveness of 9 different blends identified as potential attractants gave powerful results due to the number of replicates.

The results suggested that a combination of 4 compounds, i.e. quercivorol, a-copaene, Ethanol and a-pinene, could be used as a rather optimal attractant for both species although *X. crassiusculus* did not show a clear preference for it. The very limited occurence of longhorn beetles in traps baited by this combination is likely to allow and easier sorting of the specimens. Since no significant difference in captures was also observed between crossvane and multifunnel traps for this blend, **it is finally suggested to use for further monitoring of both *Xylosandrus* species any black trap design baited with quercivorol, a-copaene, Ethanol and a-pinene.**

5. Literature cited

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