Deliverable:

Trap density and distribution final evaluation

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Summary

Due to the COVID-19 pandemic, before 2021 it has not been possible to carry out the experiments designed to test the optimization of trap density to catch invasive *Xylosandrus* species. In 2021, two trials based on the same protocol were developed in parallel in the core areas of France (Bois de la Garoupe) and Italy (Circeo Park). Multifunnel traps, baited with a combination of 4 components identified as being attractive for *Xylosandrus* species, were installed along linear transects at increasing distances from each other (0, 10, 20, 30, 50m). Although only a few individuals of both targeted species were trapped, in relation to the limited damage observed in 2021 in these core areas, these transects made it possible to infer a mean trap attraction radius of 19m for *X. compactus* and 27m for *X. germanus*; unfortunately data were too limited and heterogeneous to give any reliable estimation for *X. crassiusculus*. Finally, these experiments suggested that early detection of invasive *Xylosandrus* species in susceptible areas should rely on linear transects of attractive traps spaced at 20m from each other.

1. Introduction

Monitoring insect populations is often based on trap networks baited with volatile attractants such as pheromones and/or compounds emitted by host plants. Thus, the knowledge of the attraction range of such traps is essential to optimize the design and density of the trap networks. The attraction range depends on both insect dispersal behaviour and the shape of the volatile plume, which is resulting from complex interactions between air movements, compound volatility, dispenser release pattern, as well as tree density around the traps. However, because air flows are assumed to randomly change in direction, it is often recognized that volatile molecules end up occupying the volume of a sphere over time (Byers, 2009). This leads to the definition of the 'attraction range' or 'attraction radius' (*AR*) of volatile-baited traps as the maximum distance over which insects can be shown to direct their movement to the source. Thus, a rather easy way of estimating the attraction range of traps baited with volatiles is to study interference between traps, considering that a competition for insect trapping would occur if two neighbouring traps are sufficiently close to have overlapping attraction ranges (i.e. are at a distance shorter than twice their attraction range) (Schlyter, 1992).

Such an approach has been recently used in forests to identify the attraction range of traps baited with pheromones of the pine sawyer beetle, *Monochamus galloprovincialis*, a vector of the invasive pine wood nematode (Jactel et al., 2018). The authors installed traps at increasing distances from each other, hypothesizing that the plume diffusion followed a two-dimensional Gaussian distribution shape, with a symmetrical probability distribution on both sides of the trap, and that the probability of capture follows a logistic curve. Trap competition for catching insects was considered to begin when the attraction space of two neighbouring pheromone traps started overlapping (Byers, 1987). As a result, there was no competition between two paired traps when the distance D between traps was greater than twice their attraction range ($D>2AR$) and competition occurred when $D \leq 2AR$. A related assumption was that an insect flying within this overlapping area had an equal probability of being caught in any of the two neighbouring traps, the probability of capture likely follows a logistic curve as well. The probability of capture, P, in a given trap was thus assumed to be maximal [$max(P)$] when $D>2AR$, then to decrease in the form of a logistic curve when $D \leq 2AR$ up to a minimum value [$min(P)$] when the two traps were at the same location (i.e. $D=0$). Because insects had an equal probability of being trapped in the two adjacent traps, $min(P)$ was expected to equal $max(P)/2$ [or $max(P)/min(P)=2$] (Fig. 1).

Finally, the abscissa of the point at which the logistic curve approached the asymptote [*max*(*P*)] was used to estimate *D*, and thus *AR*=*D*/2

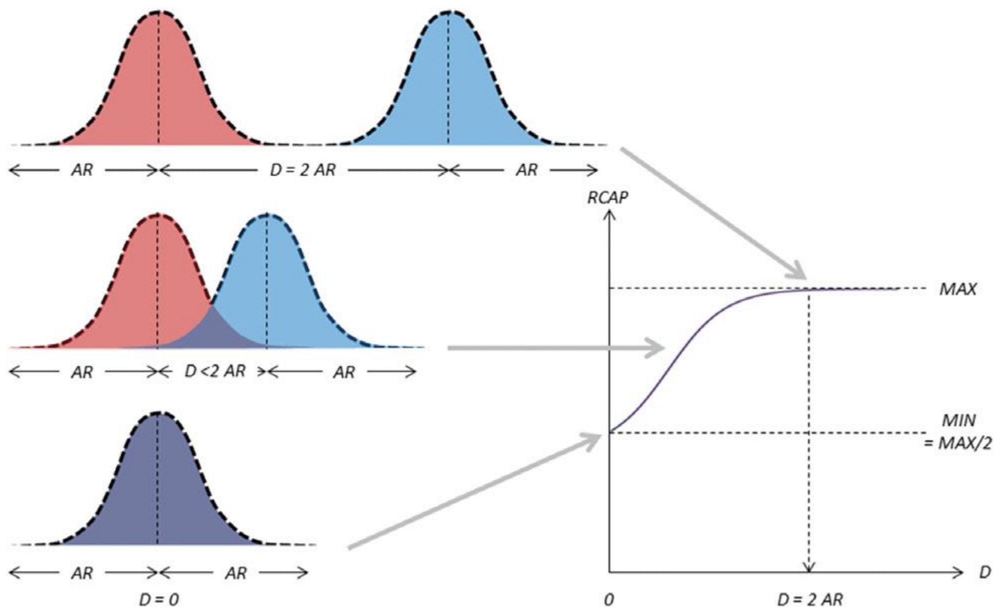

Figure 1: *Relationships between the relative percentage of insect capture (RCAP) in a trap baited with volatiles and the distance (D) to the adjacent trap. The two-dimensional shape of the volatile plume of the trap is assimilated to a Gaussian distribution, giving a Gaussian attraction surface (in red and blue for the two adjacent traps, respectively). RCAP is set to be proportional to the area below the dashed line. RCAP is expected to follow a logistic curve. When D≥2AR, the adjacent traps are assumed to be independent, leading to a maximum [max(RCAP)] of capture in any of the two paired traps. Trap competition for catching insects begins when the attraction space of two neighbouring pheromone traps starts overlapping (i.e. when D<2AR). When D=0, insects have an equal probability of being trapped in the two adjacent traps.*

2. Transect trapping experiments for defining optimal trap density for capturing *Xylosandrus* beetles

2.1 Design and timing of the experiments

Because of the COVID-19 pandemic, it has not been possible to carry out the suggested experiments before 2021 as in 2020 installment and revision of traps was only possible since early summer .

In 2021, two trials based on the same protocol were developed in parallel in France and in Italy. We used black multifunnel traps (except one transect with green top and black basis multifunnels at Garoupe) baited with the combination of 4 components, identified as being attractive for Xylosandrus species; i.e. Ethanol, (-) a-pinene, quercivorol, and a-copaene. The traps were installed at increasing distances from each other along linear transects. In each transect, the traps were thus placed at 0m, 10m, 20m, 30m and 50m (Figure 2 and 3). The trapped insects were collected every 3 weeks since the start of the experiments and the lures changed every 3 weeks.

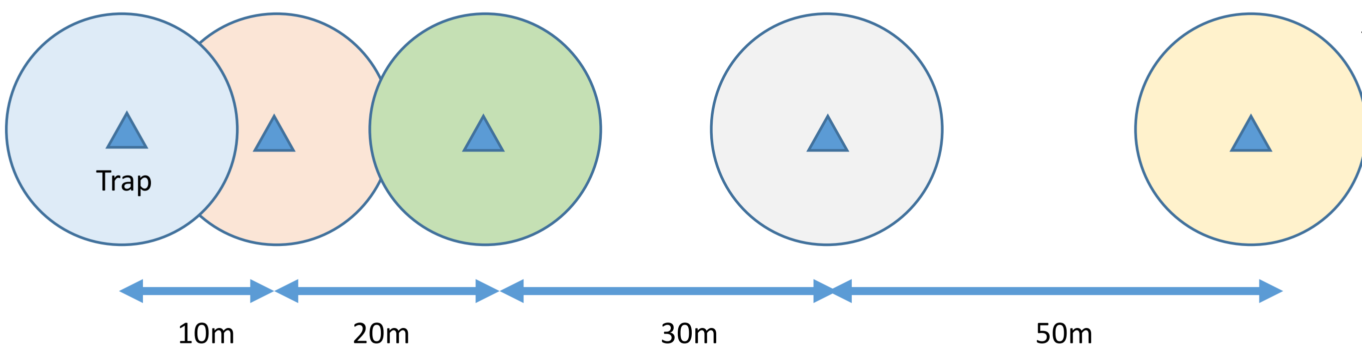

Figure 2: Trapping design of each transect. The circles around each trap represent the putative attraction range.

The trial conducted in France included 2 transects in the core area of Bois de la Garoupe, one with black multifunnels, one with green (top)/ black (basis) multifunnels (Figure 3). The traps were set up on 15 March, with a first collection on 6 April, and the trappings ended on 12 October, i.e. providing 10 collections.

The trial conducted in Italy included 9 transects in the core area of Circeo Park. The traps were set up at different times starting from 15 March. The first collection occurred on 2 April on Transect 1, 6 April on Transect 7, 7 April on Transect 3, 9 April on Transects 4 and 5, 16 April on Transect 6, 22 April on Transect, 6 May on Transect 8 and 14 May on Transect 9. Depending on the transect, the trappings ended from 9 to 23 October, i.e. providing 10 collections except in Transects 8 and 9 where only 9 collections were obtained.

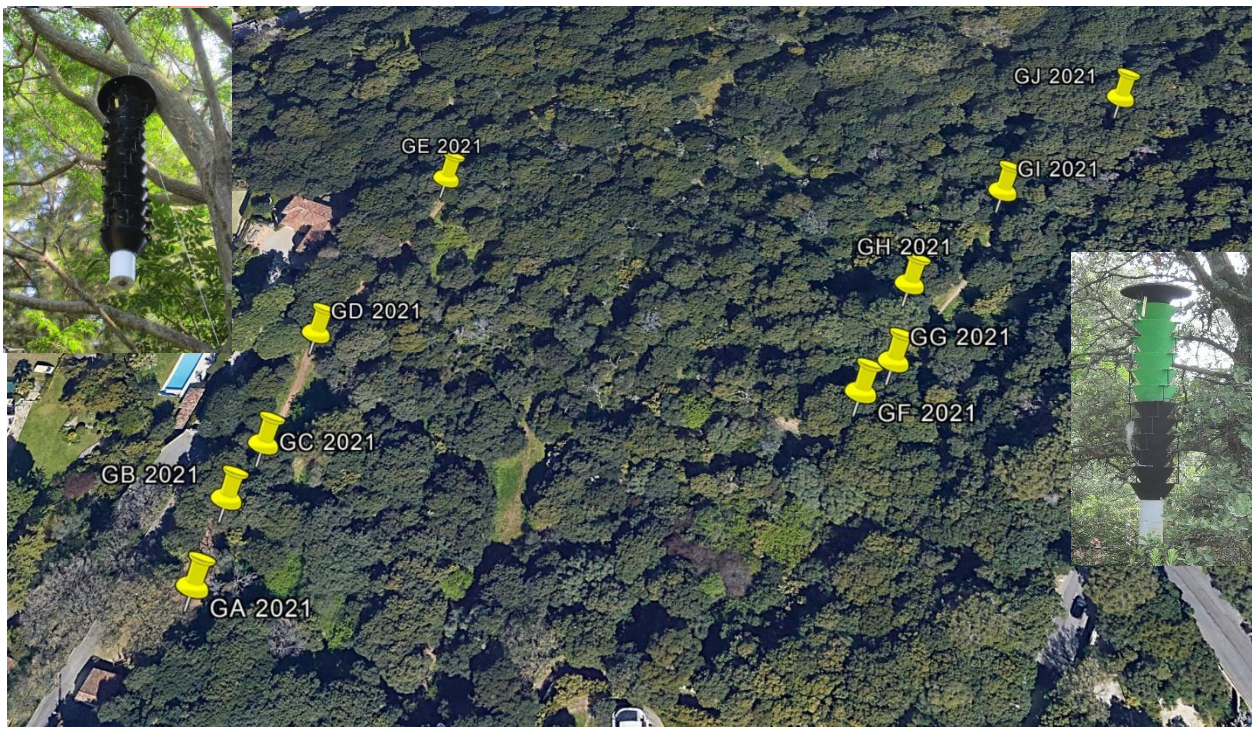

Figure 3: *An example of trap transects of increasing distances deployed at Bois de la Garoupe in 2021 (GA to GE: black multifunnels; GF to GJ : green/black multifunnels)*

2.2. Trappings results

Unfortunately, a very limited number of *X. compactus* and *X. crassiusculus* were captured in all transects of both countries, with for *X. compactus* a total of 7 individuals at Bois de la Garoupe and 32 at Circeo Park, and for *X. crassiusculus* a total of 6 individuals at Bois de la Garoupe and 4 at Circeo Park (Tables 1 and 2).

These limited captures are coherent with the very low damage observed in 2021 at Bois de la Garoupe, with less than 10 shoots with entrance holes (*X. compactus*) noticed on trees along each transect, and no damage on trunks (*X. crassiusculus*). The situation was similar at Circeo Park. However, much more individuals of the congeneric species *X. germanus* were trapped, especially in Italy (606 specimens vs. 32 in France).

Table 1: *Description of the transects deployed during 2021 at Bois de la Garoupe and corresponding total captures of Xylosandrus spp. per trap*

Transect	ID Traps	Distance (m) from the previous trap	Latitude (N)	Longitude (E)	*X. compactus*	*X. crassiusculus*	*X. germanus*
1	A	0	43,56375	7,12872	0	0	3
1	B	10	43,56393	7,12875	3	3	10
1	C	20	43,564049	7,12882	1	0	2
1	D	30	43,564317	7,12890	0	0	0
1	E	50	43,56475	7,12922	0	1	1
2	F	0	43,564066	7,13036	1	0	0
2	G	10	43,564133	7,13045	0	1	2
2	H	20	43,564331	7,13053	1	0	3
2	I	30	43,56455	7,13083	0	1	0
2	J	50	43,564783	7,13122	1	0	2

Table 2: *Description of the transects deployed during 2021 at Circeo Park and corresponding total captures of Xylosandrus spp. per trap*

Transect	ID Traps	Latitude (N)	Longitude (E)	Distance (m) from the previous trap	*X. compactus*	*X. crassiusculus*	*X. germanus*
1	1	41°20'34.79"	13° 0'54.19"	0	0	0	4
1	2	41°20'34.61"	13° 0'54.75"	10	0	0	7
1	3	41°20'34.06"	13° 0'55.27"	20	0	0	2
1	4	41°20'33.44"	13° 0'56.03"	30	0	0	1
1	5	41°20'32.34"	13° 0'56.96"	50	0	0	2
2	1	41°17'36.79"	13° 3'14.96"	0	1	0	1
2	2	41°17'37.04"	13° 3'14.76"	10	0	0	2
2	3	41°17'37.52"	13° 3'14.32"	20	0	0	1
2	4	41°17'38.30"	13° 3'13.37"	30	0	0	1

2	5	41°17'39.76"	13° 3'12.60"	50	0	0	1
3	1	41°17'33.01"	13° 3'2.88"	0	0	0	6
3	2	41°17'33.48"	13° 3'2.67"	10	0	0	6
3	3	41°17'34.08"	13° 3'1.87"	20	0	0	5
3	4	41°17'35.12"	13° 3'1.53"	30	1	0	7
3	5	41°17'36.69"	13° 2'59.93"	50	0	0	6
4	1	41°14'46.08"	13° 2'26.44"	0	4	0	3
4	2	41°14'46.03"	13° 2'26.76"	10	0	0	3
4	3	41°14'46.19"	13° 2'27.97"	20	3	0	2
4	4	41°14'46.53"	13° 2'29.18"	30	0	0	3
4	5	41°14'46.89"	13° 2'31.28"	50	1	0	3
5	1	41°13'50.31"	13° 4'16.13"	0	1	0	0
5	2	41°13'50.75"	13° 4'16.00"	10	0	0	0
5	3	41°13'50.71"	13° 4'14.97"	20	0	0	0
5	4	41°13'50.96"	13° 4'13.95"	30	0	0	0
5	5	41°13'51.38"	13° 4'11.91"	50	2	0	0
6	1	41°21'48.52"	13° 0'47.89"	0	4	1	66
6	2	41°21'48.28"	13° 0'47.40"	10	1	0	37
6	3	41°21'47.58"	13° 0'46.92"	20	1	0	81
6	4	41°21'47.17"	13° 0'45.92"	30	0	2	68
6	5	41°21'46.30"	13° 0'44.47"	50	3	0	87
7	1	41°22'2.42"	13° 2'39.78"	0	0	0	0
7	2	41°22'2.63"	13° 2'39.52"	10	0	0	0
7	3	41°22'3.19"	13° 2'38.68"	20	0	0	0
7	4	41°22'3.69"	13° 2'37.53"	30	0	0	0
7	5	41°22'5.11"	13° 2'35.88"	50	0	0	0
8	1	41°14'4.93"	13° 4'54.35"	0	0	0	6
8	2	41°14'4.64"	13° 4'53.77"	10	1	0	11
8	3	41°14'4.50"	13° 4'52.83"	20	0	0	16
8	4	41°14'4.77"	13° 4'52.12"	30	3	0	9
8	5	41°14'4.33"	13° 4'49.93"	50	2	0	4
9	1	41°20'8.15"	13° 1'18.55"	0	1	1	7
9	2	41°20'8.07"	13° 1'17.74"	10	2	0	24
9	3	41°20'7.88"	13° 1'16.94"	20	0	0	22
9	4	41°20'7.99"	13° 1'15.49"	30	1	0	38
9	5	41°20'8.36"	13° 1'13.33"	50	0	0	55

For each *Xylosandrus* species, we did not consider in the analyses the collection period of a transect when no beetle was captured in any of the 5 traps. Thus, we finally retained a total of 17 collection periods for *X. compactus*, 8 for *X. crassiusculus* and 37 for *X. germanus* (Table 3). The relative percentage of species' capture per period and trap (RCAP) was calculated by dividing the number of individuals captured by the trap by the total number of individuals captured in the transect during the period.

Table 3: *Number of collection periods per transect with at least one specimen trapped.*

Site	Transect	*X. compactus*	*X. crassiusculus*	*X. germanus*
Circeo	1	0	0	2
Circeo	2	0	0	4
Circeo	3	1	0	3
Circeo	4	3	0	3
Circeo	5	1	0	0
Circeo	6	4	2	8
Circeo	7	0	0	0
Circeo	8	1	0	3
Circeo	9	1	1	6
Garoupe	1	3	3	4
Garoupe	2	3	2	4
Total		*17*	*8*	*37*

2.3. Estimation of attraction radius for the *Xylosandrus* species

2.3.1 Estimation of attraction radius for *X. compactus*

Based on the 17 available collection periods (Table 3), the trap attraction radius resulting from the transect experiments for *X. compactus* was estimated at 19m (95% CI= 16- 26m) (Figure 4).

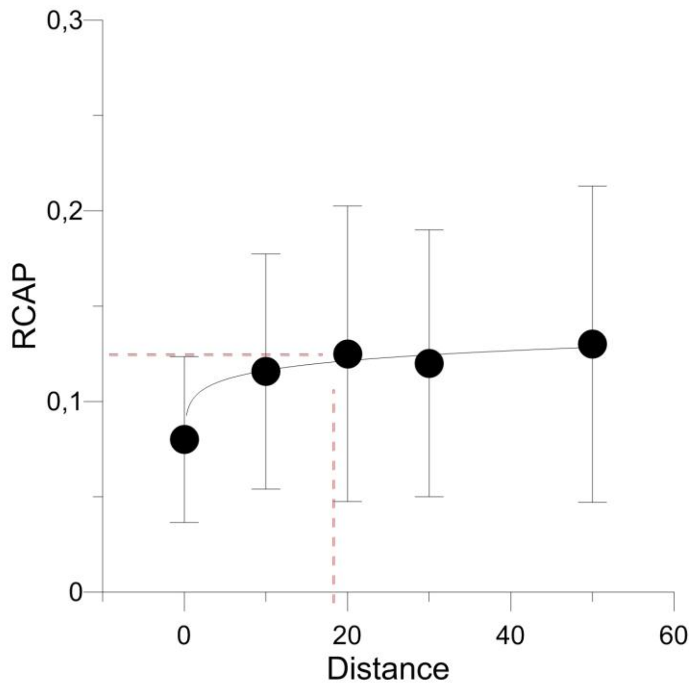

Figure 4: *Relationships between the relative capture of Xylosandrus compactus per trap and per 3-week period in each transect (RCAP) and the distance between two adjacent traps in the transect. Circles represent the mean values of RCAP (±se) per distance between adjacent traps. Data from Circeo Parka and Bois de la Garoupe pooled. The vertical red dashed line represents the estimated attraction radius.*

2.3.2 Estimation of attraction radius for *X. crassiusculus*

By contrast, the too limited number of collection periods available for *X. crassiusculus* (8 collection periods), and their large heterogeneity did not allow for any calculation of the attraction radius for this species (Figure 5). However, the absence of captures at 20m may indicate an attraction radius rather similar to the one for *X. compactus*.

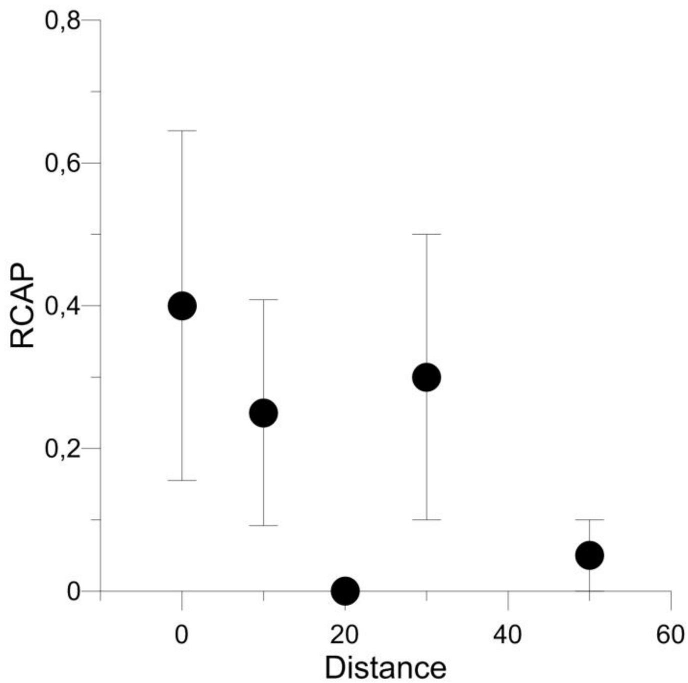

Figure 5: *Relationships between the relative capture of Xylosandrus crassiusculus per trap and per 3-week period in each transect (RCAP) and the distance between two adjacent traps in the transect. Circles represent the mean values of RCAP (±se) per distance between adjacent traps. Data from Circeo Parka and Bois de la Garoupe pooled.*

2.3.3 Estimation of attraction radius for *X. germanus*

Conversely to the two other species, a large number of collection periods (37) with at least one individual captured per transect were available for *X. germanus*. Thus, the estimated distances for the trap attraction radius for this species are more reliable. This attraction was estimated at 27m (95% CI= 23- 36m) (Figure 6).

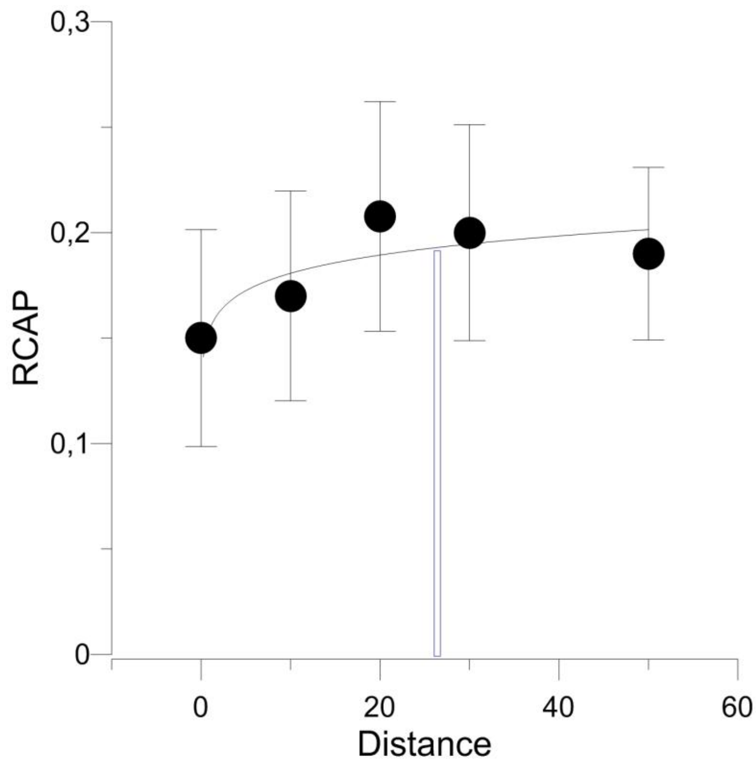

Figure 6: *Relationships between the relative capture of Xylosandrus germanus per trap and per 3-week period in each transect (RCAP) and the distance between two adjacent traps in the transect. Circles represent the mean values of RCAP (±se) per distance between adjacent traps. Data from Circeo Parka and Bois de la Garoupe pooled. The blue vertical line represents the estimated attraction radius.*

3 . General conclusions about optimisation of trap density

The low density of the targeted *Xylosandrus* species in the tested core areas largely hindered a precise measurement of the trap attraction radius. However, convergent analyses for *X. compactus* and *X. germanus* tended to indicate that traps positioned at a distance between 20 and 30m from each other could be the most effective to detect these ambrosia beetles.

Although it would be necessary to carry out other experiments, especially in areas with high densities of *X. crassiusculus* in order to get reliable data about this species, we finally suggest that, as a compromise, early detection of invasive *Xylosandrus* species in susceptible areas should rely on linear transects of attractive traps spaced at 20m from each other.

4. Literature cited

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