

# Testing trapping protocols for detecting the Citrus Longhorn Beetle, *Anoplophora chinensis* (Coleoptera: Cerambycidae)

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## Abstract

Citrus Longhorn Beetle (CLB), *Anoplophora chinensis* (Coleoptera: Cerambycidae) is a highly polyphagous species native to eastern and south-eastern Asia. In 2000, an outbreak of *A. chinensis* was detected in Lombardy Region (Italy). In 2017, an extensive trapping experiment was conducted at three infested sites in order to evaluate the effectiveness of three variables: trap model (Econex soft cross-vane trap, Witasek cross-vane trap and Witasek multi-funnel trap), trap position (on a wooden pole in an open space or in the canopy of a host tree) and type of lure (ChemTica, Synergy and Glabriwit pheromones). Each combination of variables was replicated five times at each site, giving a total of 270 traps. At the end of the study, 162 adults had been caught, with catches gradually increasing during the month of June to peak in early July. The two cross-vane traps outperformed the multi-funnel traps and the Econex traps captured more females than the Witasek traps, probably due to the structure of the collecting funnel. The three lures had similar catch performance, although the best combination was the Econex trap with Synergy blend, due to the remarkable variability in catches observed with Glabriwit blend. Finally, traps set in the tree canopy outperformed traps set on wooden poles. In conclusion, the best protocol was the use of Econex cross-vane traps baited with Synergy blend and deployed on the canopy of the host trees.

## KEYWORDS

CLB, invasive alien species, monitoring, survey, wood-boring beetles

## 1 | INTRODUCTION

The Citrus Longhorn Beetle (CLB), *Anoplophora chinensis* (Coleoptera: Cerambycidae), is a pest native to eastern and south-eastern Asia, widely distributed in China, Korea, Japan, and locally present in Indonesia, Malaysia, Myanmar, Taiwan, Vietnam and the Philippines (Haack et al., 2010; Hoppe et al., 2019; Lingafelter & Hoebeke, 2002).

The large CLB adults ( $\leq 4$  cm long) lay eggs under the bark of apparently healthy trees making characteristic T-shaped oviposition wounds in the bark of the trunk near ground level or even in the more superficial roots (Van Der Gaag et al., 2010). Larvae develop in the host at ground level or below, where they bore long galleries initially in the phloem and then deeper into the wood. The whole life cycle takes about 1 or 2 years, or even more, according to the

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latitude (Haack et al., 2010; Van Der Gaag et al., 2010). Consecutive generations of beetles may affect the same plant for several years.

*Anoplophora chinensis* is a highly polyphagous species reported from a wide range of broadleaf host-trees belonging to 26 families (Lingafelter & Hoebeke, 2002), but occasionally recorded also from conifers of the genera *Cryptomeria* and *Pinus* (Hoppe et al., 2019). In Asia, however, CLB is considered a major pest on *Citrus* spp. (Adachi, 1988,1989,1990,1994; Adachi & Korenaga, 1989; Mitomi et al., 1990) although causing serious damage also to many other deciduous trees, mainly in the genera *Acer* and *Malus*, followed by *Populus* and *Platanus* (Sjöman et al., 2014).

Due to market globalization and the speed of international trade, in the last 40 years, CLB has been intercepted several times in USA (EPPO, 1999; Haack et al., 2010). Four outbreaks have been successfully eradicated in the USA, but to date, the pest is absent from the rest of the American continent (EPPO, 2020a; Hoppe et al., 2019). In the EPPO region, CLB was first reported in 1980 in the Netherlands (Haack et al., 2010). Since 2000, several outbreaks have been reported and successfully eradicated in the Netherlands (2003, eradicated in 2010), Germany (2008, eradicated in 2017), Denmark (2011, eradicated in 2015) and Switzerland (2014, eradicated in 2019) (EPPO, 2010,2015,2020a; Hoppe et al., 2019); whereas the pest is considered transient under eradication in France (2003 and 2008), Croatia (2007) and Turkey (2015) (EPPO 2020a). Finally, in Italy, CLB is present with restricted distributions in Lombardy Region (2001, 2007, 2008, 2016 eradicated in 2021) and in Tuscany Region (2014 eradicated in 2018, 2017, 2019), whereas in Lazio Region (2008), the pest was successfully eradicated in 2019 (EPPO, 2019,2020a). CLB is considered one of the major pests of urban forests, gardens and parks infesting mainly *Acer*, *Platanus*, *Betula*, *Carpinus*, *Fagus*, *Corylus*, *Lagerstroemia*, *Malus* and *Pyrus* (EPPO, 2020a; Hoppe et al., 2019).

In addition to control measures carried out according to EU legislation, Lombardy Region implemented a specific survey of the regional territory based on the use of traps baited with lures attractive to CLB. Nevertheless, very little information is available about the best trap model and lure for CLB detection. Similarly, no data are known concerning the best trap position—that is, height from ground, type of trap support and distance from possible host trees—for CLB interception. Moreover, there is no commercially available pheromone blend specific for CLB, only those applied against the Asian Longhorn Beetle (ALB), *Anoplophora glabripennis*, which, apparently, shares one of its male-produced volatile pheromones 4-(n-heptyloxy) butanal (Hansen et al., 2015).

Improving trapping protocols available for the interception of invasive species is the first step for their detection and one of the most crucial factors facilitating their eradication. In this context, this study aims at evaluating the effectiveness of various trap models, different lure blends and trap positions for trapping CLB adults. Based on a large field experiment conducted in northern Italy, this study is focused on the identification of the best protocol (i.e., trap model, lure, trap position and their combinations) for CLB detection.

## 2 | MATERIALS AND METHODS

### 2.1 | Experimental sites and periods

The study was conducted on three CLB populations occurring in northern Italy (Figure 1), used as replicates:

- 'Milano' site, including the infestation in Milan city and in South Milan Agricultural Park;
- 'Altomilanese' site, including the infestation in Nerviano (Milan province) and neighbouring municipalities;
- 'Brescia' site, including the infestation in the municipality of Gussago (Brescia province).

All the experimental sites were within CLB infested areas ensuring the presence of the species and, therefore, suitable to evaluate the effectiveness of the tested protocols. Table 1 provides information about the infestation rates (i.e., number of monitored trees, number of infested trees, number of exit holes and percentage of infested trees) in the three sites.

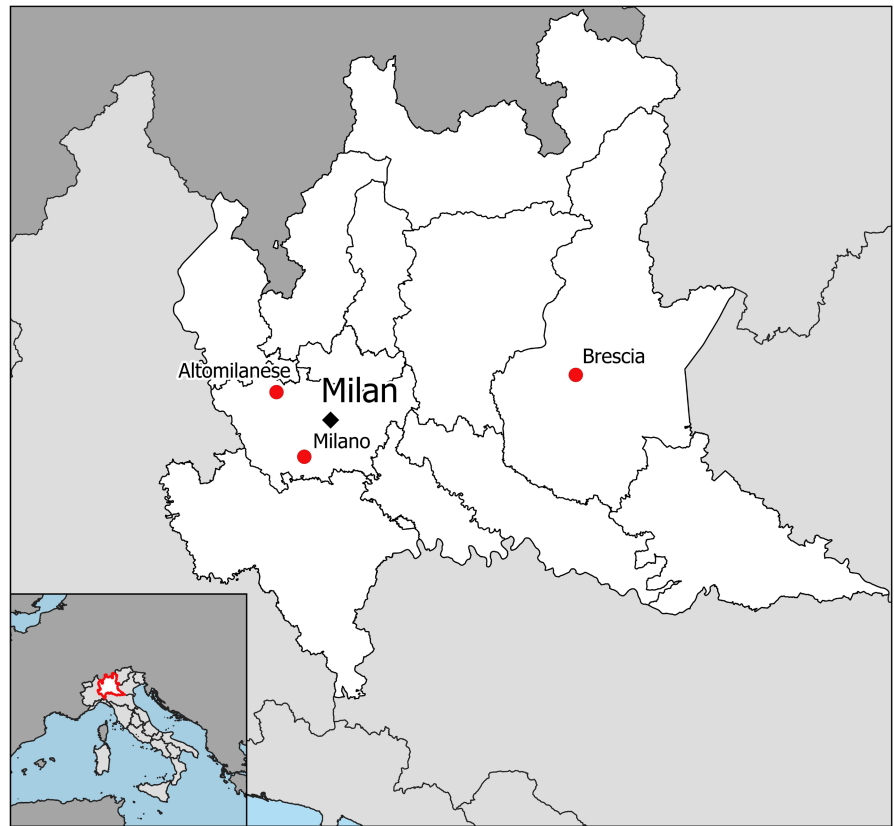
For the same reasons, the study was conducted during the period of maximum CLB flight activity of the emerging beetles which in northern Italy is generally concentrated in June and July. Traps were, therefore, set up and baited with lures between 31 May and 7 June 2017, and withdrawn between 31 July and 3 August 2017, covering 2 months. All the traps were checked and emptied every second week, that is, four times at mid and the end of June and July, recording the number of CLB males and females collected in each date and trap. The specimens were sexed on the basis of morphological characters (Lingafelter & Hoebeke, 2002) and the mating status of females was not determined. Lures were replaced after 4 weeks (in the middle of monitoring) to maintain the recommended emission rate.

### 2.2 | Trap setting

At each of the three experimental sites, traps were set up in five different locations each containing 18 traps covering all combinations of trap model (3), lure composition (3) and trap position (2) (see experimental design below). The locations were chosen as far as possible from one another to avoid overlapping effects, and never closer than 500–600 metres.

At each location, half the traps (9) were installed individually in open spaces away from trees (parks, gardens, parking lots, avenues, etc.), and fixed on wooden poles at a height of about 4–5 m from the ground. The other half of the traps (9) were set up individually at a similar height from the ground but on the canopies of suitable host plants growing in tree rows, parks and gardens, edges of urban wooded areas. The ground clearance of 4–5 m was chosen to avoid theft or trap damage. The traps were placed randomly without a precise spatial design but according to the opportunities offered by the local conditions of each individual experimental location. Each trap was provided with a wooden tag bearing a unique identification

**FIGURE 1** Map of the three CLB infestations where the study was conducted [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]



**TABLE 1** Number of exit holes, trees with exit holes and trees with other symptoms recorded in the three sites of the study in 2017

Site	No. of monitored trees	No. of trees with exit holes	No. of exit holes	No. of trees with other symptoms	Infestation rate (%)
Milano (MI)	136,185	137	208	172	0.23
Altomilanese (MI)	69,241	132	167	312	0.64
Brescia (BS)	106,882	39	47	95	0.13



**FIGURE 2** The traps used in this study. From left to right: Soft cross-vane (Econex), standard cross-vane (Witasek) and multi-funnels (Witasek) on the wooden support poles [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

code. Information boards were erected to inform citizens about the traps and the experiment.

### 2.3 | Experimental design

The study was based on an experimental design aimed at identifying the best combination of different trap models, lures and trap position in the territory in order to increase the probability of catching CLB adults.

In this regard, three different models of traps were tested (Figure 2):

- Black soft cross-vane traps produced by the Spanish company Econex (cross-vane Econex): a new model of panel-traps consisting of two soft crossed panels, longer than the classic cross-vane traps (approximately 120 cm in height compared to 80 cm for the Witasek model), mounted on a collector funnel communicating with a collector glass;

- Black cross-vane traps produced by the Austrian company Witasek (cross-vane Witasek): panel-traps with rigid crossed panels mounted on a collector funnel communicating with a collector cup;
- Black multi-funnel traps produced by the Austrian company Witasek (multi-funnel): traps made up of 12 overlapping funnels communicating with a collector cup.

Each trap model was individually activated with three different blends of aggregation pheromones and host volatiles produced and sold by the companies Synergy Semiochemicals (Canada), ChemTica (Costa Rica) and Witasek (Glabriwit, Austria) for trapping the Asian Longhorn Beetle, *Anoplophora glabripennis*, as no specific pheromone is commercially available for CLB. Formulations of the three blends are provided in Table 2.

Finally, each trap and pheromone combination was tested either on wooden poles placed in open areas or directly on the canopy of suitable host plants, for a total of 18 different combinations tested in each location (Table 3). Based on this experimental design, 90 traps for each trap model and lure were set up in each of the three monitored infested sites giving a total of 270 traps. At each site, traps were placed at least 50 m away from each other to avoid interference.

## 2.4 | Statistical analysis

Data of mean CLB captures per trap were subjected to analysis of variance (ANOVA) by the general linear model for randomized block designs (Zar, 1999) to test differences between trap model, lures, trap position and their interactions, using the STATISTICA for WINDOWS software. Homogeneity of variance was tested using Cochran's test, and when necessary, data were log-transformed [ $X = \log(x + 1)$ ] to obtain homogeneous variances. Where significant differences among variables occurred, Tukey's honestly significant difference (HSD) multiple comparison test was applied for mean separation (Zar, 1999). Differences at 0.05 level of confidence were

considered significant. Means were calculated for all the same traps across all the sites and locations: in fact, sites and locations were considered replicates with homogeneous characteristics.

## 3 | RESULTS

### 3.1 | Total captures and flight period

A total of 162 CLB adults were captured in June–July 2017, 84 males and 78 females, without significant differences between sexes (ANOVA,  $df = 1$ ; 268,  $F = 2.13$ ,  $p > 0.05$ ). Catches gradually increased during the month of June to peak in early July and then gradually drop in the following month (Figure 3). There were fewer catches in Brescia (the site with a lower level of infestation) than the two sites in Milan.

### 3.2 | Captures of different trap models

CLB catches showed significant differences between the three trap models tested (ANOVA,  $df = 2$ ; 267,  $F = 1.39$ ,  $p < 0.05$ ). In particular, Econex cross-vane traps showed the best performance with average catches of 0.86 adults per trap. A 22% lower value, although not statistically different, was provided by Witasek cross-vane traps, with average catches of 0.67 adults per trap. Multi-funnel traps showed the worst catching performance with only 0.48 insects per trap, that is, about half (–44%) of the captures obtained with the Econex cross-vane traps from which they differ significantly.

Males and females of CLB respond differently to the various traps (ANOVA,  $df = 2$ ; 267,  $F = 1.43$ ,  $p < 0.05$ ). While Econex cross-vane and multi-funnel traps did not show significant differences in capture's sex ratio, Witasek cross-vane traps caught significantly fewer females than males (Figure 4). Witasek cross-vane traps, in fact, had male capture levels similar to those observed in the Econex cross-vane traps, whereas the female catches were low and similar to those found in the multi-funnel traps.

Lure	Formulation
ChemTica	4-( <i>n</i> -heptyloxy) butanal (0.13 g) 4-( <i>n</i> -heptyloxy) butan-1-ol (0.10 g) (-)-linalool (1.30 g) + trans-caryophyllene (1.30 g) + (Z)-3-hexen-1-ol (0.16 g)
Synergy Semiochemicals	4-( <i>n</i> -heptyloxy) butanal (0.135 g) 4-( <i>n</i> -heptyloxy) butan-1-ol (0.12 g) (Z)-3-hexen-1-ol (0.84 g) + beta-caryophyllene (3.36 g) + (-)-linalool (3.8 g)
Glabriwit <sup>a</sup>	4-( <i>n</i> -heptyloxy) butanal 4-( <i>n</i> -heptyloxy) butan-1-ol (Z)-3-hexen-1-ol + (-)-linalool + caryophyllene

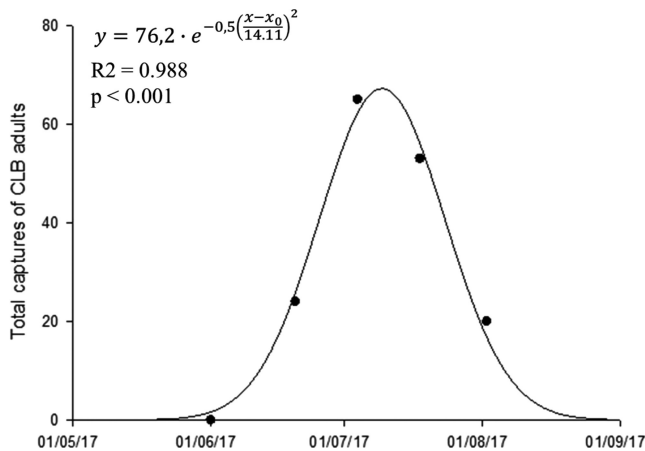
TABLE 2 List of the constituents of the three tested blends: ChemTica, Synergy and Glabriwit

<sup>a</sup>The company has not agreed to provide the exact formulation of the blend.

**TABLE 3** Experimental design adopted in each of the three monitored sites, testing the 18 possible combinations of trap model (3), lure composition (3) and trap position (2)

Trap model	Lure	Trap position	Trap number
Standard cross-vane (Witasek)	Sinergy	pole	5
		crown	5
	ChemTica	pole	5
		crown	5
	Witasek	pole	5
		crown	5
Multi-funnel (Witasek)	Sinergy	pole	5
		crown	5
	ChemTica	pole	5
		crown	5
	Witasek	pole	5
		crown	5
Soft cross-vane (Econex)	Sinergy	pole	5
		crown	5
	ChemTica	crown	5
		pole	5
	Witasek	pole	5
		crown	5
Total traps per site			90

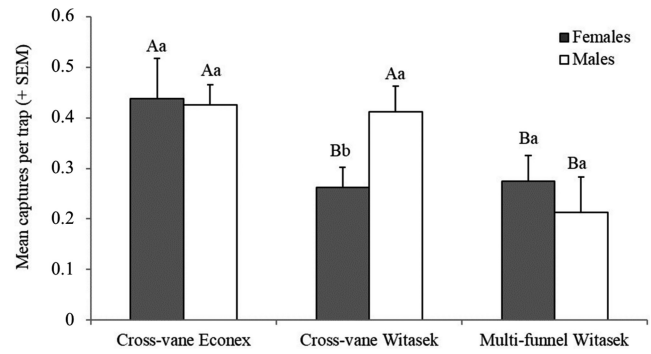
Note: In each site, all combinations were replicated five times in five different locations for a total of 90 traps.



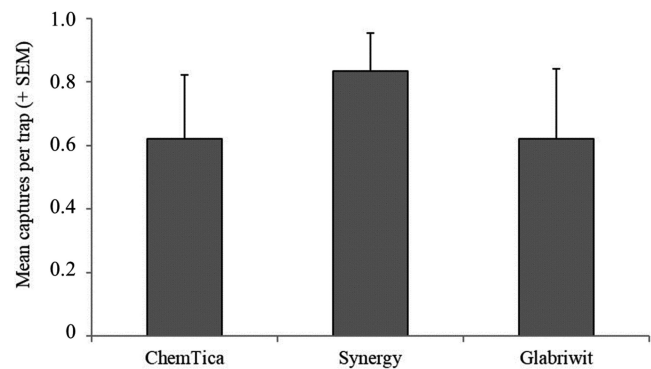
**FIGURE 3** Temporal trend of the CLB adults trapped in Lombardy (N Italy) in June–July 2017, and descriptive model of the flight curve of the species. Traps were checked and emptied every second week

### 3.3 | Attractiveness of different lures

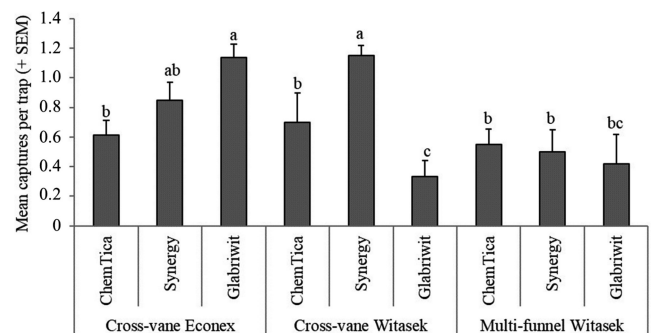
The three different tested lures did not show significant differences in the mean number of trapped CLB (ANOVA,  $df = 2; 267, F = 0.49, p = 0.61$ ), although the Canadian Synergy pheromone—with



**FIGURE 4** Mean CLB captures per trap (+SEM) in relation to trap model and insect sex. Different letters correspond to significant differences at the analysis of variance (Tukey test,  $p < 0.05$ ). Capital letters indicate differences among trap models, lower case letters indicate differences between genders within the same traps



**FIGURE 5** Mean captures of CLB per trap (+SEM) in relation to pheromone blend



**FIGURE 6** Mean CLB captures (+SEM) according to trap model and pheromone blend. Different letters correspond to significant differences at the analysis of variance (Tukey test,  $p < 0.05$ )

0.83 adults per trap—caught approximately 25% more insects than Chemtica and Glabriwit lures (both with average catches of 0.62 insects per trap) (Figure 5).

Although there were no significant differences in the total number of males (ANOVA,  $df = 2; 267, F = 1.14, p = 0.32$ ) or females (ANOVA,  $df = 2; 267, F = 0.02, p = 0.97$ ) captured with the various lures, traps with the Synergy blend caught 50% more males (0.5 per trap) than females (0.33 per trap). On the other hand, catches

of males and females with the ChemTica and Glabriwit blends were lower and almost identical to one another (about 0.3 per trap).

### 3.4 | Trap-lure interactions

Mean catches of CLB adults were significantly affected by the interactions between trap model and lure blend used to bait the trap (ANOVA,  $df = 4$ ; 264,  $F = 1.29$ ,  $p < 0.02$ ). In particular, Econex cross-vane traps baited with Synergy or Glabriwit pheromones and the Witasek cross-vane traps triggered with Synergy pheromone were the combinations providing significantly higher captures than all the others (Figure 6). The lowest trapping values were recorded with Witasek cross-vane traps baited with the pheromone Glabriwit. In general, multi-funnel traps showed relatively low catches regardless of the lure tested.

### 3.5 | Effect of the trap position

Cross-vane traps installed on tree crowns of the host plants showed significantly higher average catch levels—and almost double—the traps installed on wooden poles placed far from the trees (ANOVA,  $df = 1$ ; 268,  $F = 5.68$ ,  $p < 0.01$ ). Canopy effect was, however, non-significant in multi-funnel traps, which had mean catches similar between canopies and poles (ANOVA,  $df = 2$ ; 267,  $F = 2.59$ ,  $p < 0.05$ ) (Figure 7).

## 4 | DISCUSSION

The results of the CLB trapping protocols tested in this study indicate cross-vane traps as the best trap model to be used to increase the probability of catching CLB. This result is in agreement with previous reports for similar species: cross-vane traps are more effective than multi-funnel traps in catching many families of bark and wood-boring beetles, including long-horn beetles (Allison & Redak, 2017). Moreover, even in ALB monitoring and eradication protocols, mainly cross-vane traps were used (Eyre & Barbrook, 2021; Nehme

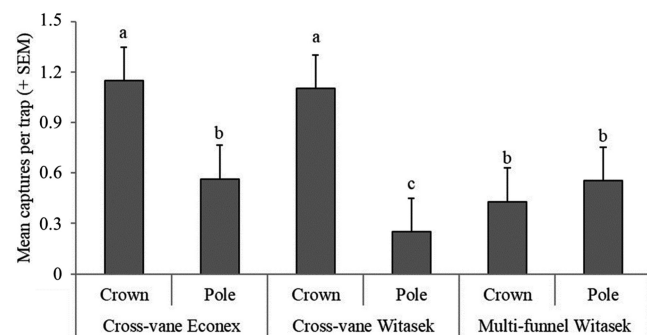


FIGURE 7 Mean CLB captures per trap (+SEM) according to trap model and trap position. Different letters correspond to significant differences at the analysis of variance (Tukey test,  $p < 0.05$ )

et al., 2014). Although non-significant differences occurred between the catches of Econex and Witasek cross-vane traps, the former showed higher mean number of captures. Actually, Econex cross-vane traps are structurally different from standard cross-vane traps as they consist of two longer panels made of soft and very slippery rubber, with a greater interception surface (approximately 4,270 cm<sup>2</sup> compared to 3,960 cm<sup>2</sup> of the Witasek model). The differences in mean adult catches observed between Econex and Witasek cross-vane traps are essentially based on the strongly reduced number of CLB females captured by the Witasek model, which could have been due to a greater ability of females to escape from the Witasek traps. This phenomenon led to an overall reduction of captures recorded in the Witasek cross-vane traps compared to Econex ones. On average, CLB females are significantly larger than males (37 vs. 21 mm) (Hoppe et al., 2019), increasing the possibility for females to escape when the funnel at the base of the cross panels has a hole towards the collector jar which is too small and easily clogged up with leaves and debris. In this respect, the diameter of the funnel hole of the Witasek cross-vane trap is a bit larger (4–7 cm compared to the 2.5–4.5 cm of those of the Econex), but the structure of the Witasek trap is different. In fact, the two panels penetrate the funnel at the base causing a narrowing, and could facilitate the escape of the insects, especially large females. Another possibility could be related to CLB's flight technique. As with the congeneric *Anoplophora glabripennis*, during flight adults hold their legs to the side and back toward their dorsum (Keena, 2018). In this way, the ventral part hits the surface of the trap first and the insect can bounce off, especially larger individuals (like females). The soft panels of the Econex cross-vane trap can reduce this phenomenon, compared to the hard ones of the Witasek model. Despite having a greater insect interception surface, Econex cross-vane traps also allow a considerable space-saving for winter storage as the panels can be folded up. Overall, the Econex soft cross-vane trap appears to be a more efficient model for CLB trapping.

Among lures tested in the study, the Synergy ones provided the best results with high mean capture levels, although they did not differ statistically from the other two blends. Looking at the formulations of the blends, it can be seen that they are similar in their components, but differ significantly in kairomone quantities; in particular, the Synergy one has the highest quantity of them. However, more kairomones does not correspond to a significant increase in catches. Considering the trap-lure interaction, the best combination for catching CLB adults are the Econex cross-vane trap baited with Synergy or Glabriwit blends. However, it is interesting to underline the remarkable variation in adult catches showed by the Glabriwit blend when tested in different trap models, even when used in the same trap type (cross-vane), suggesting its variable performance.

On average, traps hooked on tree crowns had greater catches than traps hung on the wooden poles. This result can be explained by the visual and chemical attractiveness induced in CLB adults by the canopy silhouette and host-tree volatiles, which integrate and enhance the action of the aggregation pheromones used to bait the

traps. A similar result was also observed for ALB, which was caught in higher numbers by traps hanging from trees than from bamboo poles (Nehme et al., 2010). Other researchers have found traps placed under the canopy or in the forest edges to be more effective in catching long-horned beetles than traps set up in clearings and open fields (Dodds, 2011; Sweeney et al., 2020). According to the results of our study, the protocol that provides the highest levels of CLB captures is therefore the installation of cross-vane traps in the crowns of CLB host trees. In this context, however, there is a risk of an over-spilling effect, that is, insects attracted by the pheromone to a host plant and then not caught by the trap but directly infesting the tree. In this respect, healthy plants used to install traps must be carefully surveyed to avoid initiating new local infestations.

In relation to the insect phenology recorded by this study, the best season to conduct a survey of CLB populations by pheromone traps in northern Italy—or to verify the presence of this species in a new territory—falls between mid-June and mid-July. The main flight activity of CLB adults occurs in this period and, therefore, the highest probability of insect interception.

In conclusion, the best protocol is the use of Synergy blend to bait E-conex cross-vane traps, which have a higher, though not significant, catch rate than the Witasek model and allows easier winter storage due to the soft panels. Finally, the best position for traps is on host trees, although it is necessary to plan periodic checks in order to avoid the over-spilling effect.

Finally, although the mean catches per trap recorded in this study are in general particularly low, they are in line with ALB catches reported in other papers (Nehme et al., 2014). Moreover, the use of pheromone traps against CLB allows species detection and a spatial and temporal survey of its populations, giving crucial information to set up survey programme and to assess the effectiveness of control measures applied.

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## CONFLICT OF INTEREST

We have no conflicts of interest to declare.

## AUTHOR CONTRIBUTIONS

Bianchi A, Ciampitti M and Faccoli M conceived research. Bianchi A conducted experiment. Faccoli M analysed data and conducted statistical analyses. All authors, in equal parts, wrote the manuscript. Ciampitti M secured funding. All authors read and approved the manuscript.

## DATA AVAILABILITY STATEMENT

Data used for this work are available in a public repository (Research Data Unipd), identified with <http://researchdata.cab.unipd.it/id/eprint/540> (Marchioro et al., 2021).

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