



**Research Article** 

## Testing a trapping protocol for generic surveillance of woodboring beetles in heterogeneous landscapes

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#### Abstract

Baited traps are a basic component of both specific and generic surveillance programs targeting wood-boring beetles at risk of introduction to new habitats because of global trade. Among the numerous protocols developed over the years for generic surveillance of longhorn beetles, jewel beetles, and bark and ambrosia beetles is the simultaneous use of black multi-funnel traps set up in the understory and green multi-funnel traps set up in the canopy of forested areas surrounding ports and other entry points. These traps are commonly baited with multi-lure blends of pheromones and host volatiles. In this study, we tested this trapping protocol in areas surrounding eight entry points located in Europe and North America to determine: i) the relative performance of black-understory traps and green-canopy traps among the targeted taxa; and ii) whether the dissimilarity among communities of beetles collected by the understory vs. canopy traps was affected by taxon and amount of forest cover in the traps' surroundings. A total of 96,963 individuals belonging to 358 species of wood-boring beetles were collected, including 21 non-native species. Black-understory multi-funnel traps were generally more efficient than green-canopy multi-funnel traps for detecting longhorn beetles and bark and ambrosia beetles, whereas the opposite trend was observed for jewel beetles. Differences between beetle communities caught in black-understory and green-canopy traps were mainly attributed to differences in species richness in jewel beetles, while both differences in species richness and species turnover contributed to the dissimilarity between communities of longhorn beetles and bark and ambrosia beetle. The difference in the number of jewel beetle species caught by the two trapping methodologies decreased with increasing forest cover, whereas species turnover increased when moving from an urban-dominated to a forest-dominated landscape. Overall, these results suggest that the simultaneous use of both black-understory and green-canopy multi-funnel traps can be considered a very efficient approach for generic surveillance of longhorn beetles, jewel beetles and bark and ambrosia beetles in both urban-dominated and forest-dominated areas surrounding entry points.

Key words: Buprestidae, Cerambycidae, early-detection, exotic species, monitoring, Scolytinae

## Introduction

The continuous increase in global trade in recent decades, combined with deliberate plant introductions in the past, has resulted in increasing number of non-native insects moved outside their native ranges (Seebens et al. 2017; Isitt et al. 2024). This trend is evident for wood-boring insects, especially bark and ambrosia beetles (Coleoptera: Scolytinae), longhorn beetles (Cerambycidae) and jewel beetles (Buprestidae) (Rassati et al. 2016; Ruzzier et al. 2023a) which can be accidentally transported within wood packaging materials, logs, processed wood, and live nursery stock (Meurisse et al. 2019; Fenn-Moltu et al. 2023). After introduction, wood-boring beetles may become invasive pests, with high economic, ecological, and social impacts in urban and natural forests (Aukema et al. 2011). The jewel beetle Agrilus planipennis and the longhorn beetle Anoplophora glabripennis, for example, are listed among the top 10 of all invasive species for post-invasion management costs and are estimated to be among the costliest non-native insects worldwide (Cuthbert et al. 2022). For these reasons, substantial investments globally have focused on mitigating the arrival and establishment rate of non-native wood-boring beetles through more efficient biosecurity measures (Nahrung et al. 2023), including tools and strategies for early-interception of non-native species at and around entry points.

Among the numerous tools developed for surveillance of wood-boring beetles (Poland and Rassati 2019), traps baited with attractive lures are part of the biosecurity systems of several countries around the world (Rassati et al. 2015a, 2015b; Carnegie et al. 2018, 2022; Rabaglia et al. 2019; Hoch et al. 2020; Allison et al. 2021; Holusa et al. 2023; Mas et al. 2023; Wardhaugh and Pawson 2023). The knowledge acquired over the years on the key factors influencing trap efficacy (Allison and Redak 2017; Dodds et al. 2024) and on the chemical ecology of hundreds wood-boring beetle species (e.g., Byers 2007; Millar and Hanks 2017; Ranger et al. 2021) has led to the definition of a set of trapping protocols tailored to the target taxa or objective of the surveillance program. In the case of generic surveillance aimed at intercepting as many non-native species as possible, the simultaneous use of black multi-funnel or intercept panel traps set up in the understory and green multi-funnel traps set up in the canopy, all baited with multi-lure blends, in areas surrounding entry points (e.g., Wong et al. 2012; Hanks et al. 2018; Fan et al. 2019; Rice et al. 2020; Roques et al. 2023), might be considered as a potentially efficient approach. Baited black traps set up in the understory are known to attract longhorn beetles and bark and ambrosia beetles commonly living in the lower forest strata (De Groot and Nott 2001; McIntosh et al. 2001; Dodds et al. 2010; Flaherty et al. 2019; Ulyshen and Sheehan 2019), while baited green multi-funnel traps set up in the canopy are known to catch jewel beetles well (Francese et al. 2011, 2013; Petrice and Haack 2015; Skvarla and Dowling 2017; Santoiemma et al. 2024) but also longhorn beetles and certain bark and ambrosia beetles living in mid and upper forest strata (Rassati et al. 2019; Marchioro et al. 2020). Nonetheless, this trapping protocol remains to be tested in surveillance programs targeting areas surrounding high-risk sites, such as international ports and airports or warehouses, which can be characterized by heterogeneous landscapes ranging from urban-dominated areas to forest-dominated areas.

In addition to testing overall efficacy, there is an urgent need to better understand whether the simultaneous use of baited black multi-funnel traps placed in the understory and baited green multi-funnel traps placed in the canopy is always necessary, irrespective of the characteristics of the landscape. Previous studies showed that the efficacy of a trapping methodology can be context-dependent (e.g., Bouget et al. 2009; Marchioro et al. 2020; Rassati et al. 2021) as it can be affected by a number of environmental variables (Dodds et al. 2024), including the amount of tree cover occurring around the trap (Schroeder 2013; Nunes et al. 2021). In areas surrounding high-risk sites, traps can be set up either on isolated trees that are present along streets or private gardens (i.e., in urban-dominated areas) or on trees that are present in urban parks or forest patches and that are surrounded by many potential hosts (i.e., in forest dominated landscapes or where urban areas are intermixed with forest areas) (Bashford 2008; Rassati et al. 2015a; Mas et al. 2023). Thus, understanding whether the proposed trapping protocol can be simplified depending on the amount of tree cover occurring in the trap surroundings is crucial to optimize efforts and reduce overall costs of the surveillance program (Epanchin-Niell et al. 2014; Nguyen et al. 2024).

In this study, we conducted a trapping experiment in areas surrounding eight entry points located in Europe and North America using black multi-funnel traps set up in the understory and green multi-funnel traps set up in the canopy, all baited with the same multi-component blend of longhorn beetle pheromones complemented with plant volatiles. We first compared the relative efficacy of black-understory traps and green-canopy traps for detecting different target taxa, i.e., longhorn beetles, jewel beetles and bark and ambrosia beetles. Second, we calculated dissimilarity indices to compare the communities of longhorn beetles, jewel beetles and bark and ambrosia beetles collected by black-understory *vs.* green-canopy traps, and then we tested the effect of the amount of forest cover in the trap surroundings on the dissimilarity indices. These analyses allowed us to investigate whether the simultaneous use of black-understory traps and green-canopy traps is required irrespective of the taxon and the landscape in which this protocol is used, or whether a simpler protocol (e.g., using only black-understory traps) may detect as many species of a particular taxon, depending on the surrounding landscape.

## Methods

#### Study sites, trap types and experimental scheme

The study was conducted at eight sites in five different countries in the temperate zone of Europe and North America: France, Italy, Switzerland, Canada (Nova Scotia) and USA (Ohio) (Suppl. material 1: fig. S1, table S1). Selected sites were characterized by the presence of mixed forest and urban patches, and were located in the proximity of entry points or high-risk areas such as ports, airports, warehouses and high-use recreation areas (Suppl. material 1: table S1).

At each site we used sixteen black and sixteen green multi-funnel traps (Suppl. material 1: fig. S1), supplied by Synergy Semiochemical Corporation (Delta, BC, Canada) and ChemTica Internacional (Santo Domingo, Costa Rica). Both trap types were pre-treated with a 50% solution of Fluon (active ingredient polytetrafluoroethylene) as a trap coating because of its ability to increase trap catches of wood-boring beetles (Graham and Poland 2012; Allison et al. 2016). Trap-collecting cups were filled with 150–200 ml of a 50% propylene glycol solution mixed with either water or as a pre-mixed marine/RV antifreeze solution. Propylene glycol solutions were replaced at each trap check.

Traps were set up using a  $2 \text{ km} \times 2 \text{ km}$  grid as reference (Suppl. material 1: fig. S1), with one black and one green multi-funnel trap set up in each of 16 grid cells of  $0.5 \text{ km} \times 0.5 \text{ km}$ . The only exceptions were the two sites in France, where traps were coupled along a horizontal transect due to limits in space within the selected sites and local restrictions. Black multi-funnel traps were set up with the top of the trap about 1.5–2 m above the ground on lower tree branches, whereas green multi-funnel traps were set up in the upper one-third of the tree canopy at heights ranging from 7 m to 15 m. We selected this approach even though it confounded the effects of trap height and trap color on catch of targeted taxa because the latter was already addressed in several previous studies (reviewed in Dodds et al. 2024). Canopy traps were set up following the methods of Hughes et al. (2014). The two traps within the same grid cell were suspended on different trees, separated by 50-100 m. Trees were selected based on position and suitability to hold the weight of the traps, irrespective of the species. Traps were set up in mid-May 2019 and emptied every 2-3 weeks until the end of August 2019. All trapped longhorn beetles, jewel beetles, and bark and ambrosia beetles were identified to species level using morphological features and taxonomic keys. Voucher specimens were deposited in the insect collection of each institution.

#### Lures

All traps were baited with a lure containing a blend of eight cerambycid pheromones attractive to a wide range of longhorn beetle species (Fan et al. 2019; Roques et al. 2023), plus UHR (ultra-high release rate) ethanol and alpha-pinene lures. The pheromone blend included racemic fuscumol (volume amount: 50 mg), racemic fuscumol acetate (50 mg), geranyl acetone (25 mg), racemic 3-hydroxyhexan-2-one (50 mg), prionic acid (1 mg), 2-methylbutan-1-ol (50 mg), anti-2.3-hexanediol (50 mg), and monochamol (50 mg), all dissolved in isopropanol as a carrier to a total volume of 1 ml per lure (see Fan et al. 2019 for release rate). All pheromones were purchased from ChemTica Internacional (Santo Domingo, Costa Rica) except prionic acid (Alpha Scents Inc., West Linn, Oregon, USA); the ethanol and alpha-pinene lures were provided by Econex (Spain). One-milliliter aliquots of the pheromone blend were filled in glass vials with screw caps and stored at 4 °C until used. At the beginning of the trial and during each trap check, the 1-ml aliquots were poured into a clear polyethylene sachet containing a cotton cylinder, which was hung on the trap using a string. The addition of the two host volatile lures increases both the attractiveness of the pheromones to many species of longhorn beetles (e.g., Collignon et al. 2016; Miller et al. 2017) as well as the likelihood of trapping certain species of jewel beetles (Miller et al. 2015) and bark and ambrosia beetles (Miller and Rabaglia 2009; Marchioro et al. 2020).

## Analysis of dissimilarity indices to investigate wood-boring beetle communities

To investigate differences in the communities of wood-boring beetles collected in black-understory multi-funnel traps and green-canopy multi-funnel traps, we used the  $\beta$ -diversity approach outlined in Carvalho et al. (2012) (see also Podani and Schmera 2011; Legendre 2014). The general term " $\beta$ -diversity" refers to the total

compositional change between two communities (in our case study, the community of wood-boring beetles collected using the two trapping methodologies), and can be partitioned into two components: species richness difference and species replacement (Carvalho et al. 2012). "Species richness difference" refers to the relative difference in the number of species between two communities, whereas "species replacement" refers to the substitution of species by others when two communities are compared. The two components are additive, and their sum provides the  $\beta$ -diversity index.

Given "a" = number of species exclusive to the first community, "b" = number of species exclusive to the second community, and "c" = number of species common to both communities, the  $\beta$ -diversity is given by the Jaccard dissimilarity index:

$$\beta_{cc} = (a + b)/(a + b + c)$$

with values ranging from 0 (perfect similarity) to 1 (total dissimilarity). The species richness difference component is given by:

$$\beta_{\rm rich} = |a + b|/(a + b + c)$$

with values ranging from 0 (no richness difference) to 1 (maximum richness difference). The species replacement component is given by:

$$\beta_{-3} = 2 \min(a, b)/(a + b + c)$$

with values ranging from 0 (no replacement) to 1 (maximum replacement). To calculate these indices, data collected across the entire sampling season from black-understory and green-canopy traps (i.e., total catch per trap over the entire trapping season) of each grid cell at each site were paired, creating  $n \times m$  presence/absence matrices for each taxon, whereby n = 2 (one row for each trapping method) and m = number of species. Then, for each matrix, the three indices were computed using the "vegan" package (Oksanen et al. 2022) in R software (R Core Team 2021).

#### Quantification of tree cover in the area surrounding the traps

Forest patches around each trap were manually digitized by visual inspection of high-resolution satellite images in Google Earth Pro (Google Inc.© 2023) within a buffer of 250 m radius. This spatial scale was selected based on the results of previous studies testing the attraction range of baited traps towards wood-boring beetles (e.g., Dodds and Ross 2002; Jactel et al. 2019). For each buffer, the total forest cover (%) was then quantified in GIS (Quantum GIS 3.22, QGIS Development Team 2021) after importing the digitized patches from Google Earth Pro (Google Inc.© 2023). Forest cover within the buffers ranged from 0% (where only urban patches were present) to 100% (where only forest was present), with a mean value ( $\pm$  standard deviation) of 51.4  $\pm$  39.3%.

#### Statistical analyses

We used generalized linear mixed models for all the analyses. Data collected from all sites were analyzed together to increase both the statistical power of the models and the gradient of forest cover around the traps. First, we tested the effect of the taxon and the trapping methodology on species richness and abundance. Species richness (i.e., total number of species) and abundance (i.e., total number of individuals) for each trap and pooled over the sampling rounds were considered as response variables. The taxon (categorical variable: longhorn beetles, jewel beetles, and bark and ambrosia beetles), the trapping methodology (categorical variable: black-understory and green-canopy multi-funnel traps) and their interaction were considered as explanatory variables. The site identity, the identity of each grid cell within each site and the identity of each trap within each grid cell were included in the models as nested random factors.

Second, we tested the effect of the taxon and the forest cover on beta-diversity indices. The three beta-diversity indices  $\beta_{cc}$ ,  $\beta_{rich}$  and  $\beta_{-3}$  calculated for each pair of traps (within a cell) were considered as response variables. The taxon (categorical variable: longhorn beetles, jewel beetles, and bark and ambrosia beetles), the forest cover (continuous variable: mean % of forest cover in the buffers of 250 m radius around the pair of traps present in the same cell) and their interaction were considered as explanatory variables. The site identity and the identity of each cell of the grid within each site were included in the models as nested random factors.

Models were fitted with a Poisson distribution (log link function) for species richness, negative binomial distribution (log link function) for abundance, and Gaussian distribution for the beta-diversity indices. Pairwise comparisons between each taxon and between the two trapping methodologies within each taxon were run using Tukey correction of *p*-values. All the analyses were carried out in R software (R Core Team 2021). Models were fitted using the 'glmmTMB' (Brooks et al. 2022) and 'nlme' (Pinheiro et al. 2021) packages, and validated using the 'DHARMa' (Hartig 2022) and 'car' (Fox and Weisberg 2019) packages. There was no evidence of spatial autocorrelation of models' residuals, checked with Moran's I test for distance-based autocorrelation using the 'DHARMa' package (Hartig 2022). Pairwise comparisons and slope estimates were calculated using the 'emmeans' package (Lenth 2021).

## Results

#### **General results**

A total of 96,963 individuals belonging to 358 species of wood-boring beetles were collected (Suppl. material 1: table S2). The most species-rich taxon was represented by longhorn beetles (169 species), followed by bark and ambrosia beetles (123) and jewel beetles (66). Longhorn beetles and bark and ambrosia beetles represented the most trapped species at all sites (Fig. 1). For abundance, bark and ambrosia beetles had the highest number of trapped specimens (73,109), followed by longhorn beetles (17,970) and jewel beetles (5,884) (Suppl. material 1: table S2). *Xyleborinus saxesenii* (48,042 individuals) and *Orthotomicus erosus* (4,180) were the most abundant ambrosia and bark beetle species collected, respectively, whereas *Phymatodes amoenus* (2,954) and *Agrilus olivicolor* (2,213) were the most of the trapped species were native, 21 species were non-native for 14,772 specimens. These non-native species included four longhorn beetle species (i.e., *Phymatodes testaceus* in North America, *Neoclytus acuminatus*, *Xylotrechus chinensis* and

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**Figure 1.** World map describing the communities of longhorn beetles, jewel beetles, and bark and ambrosia beetles collected at each experimental site. Circle size indicates the number of trapped species ranging from 55 (smallest circle) to 100 (biggest circle). The different colors within each circle indicate the relative percentage of species attributed to each taxon: red = longhorn beetles; green = jewel beetles; blue = bark and ambrosia beetles. Numbers in yellow circles represent the different study sites according to Suppl. material 1: table S1 column ID number.

*Xylotrechus stebbingi* in Europe), two jewel beetle species (i.e., *Agrilus cyanescens* and *Agrilus planipennis* in North America), and 15 bark and ambrosia beetle species (nine in North America, two in Europe and four in both continents) (Suppl. material 1: table S2).

## Effect of taxon and trapping methodology on trap captures

Species richness was significantly affected by taxon and by the interaction between taxon and trapping methodology (Table 1). The mean number of species ( $\pm$  standard error) was significantly higher for longhorn beetles ( $8.34 \pm 0.26$ ) than bark and ambrosia beetles ( $7.42 \pm 0.26$ ) and jewel beetles ( $1.79 \pm 0.15$ ). In addition, the effect of trapping methodology differed depending on the taxon (Fig. 2A–C). For longhorn beetles, the mean number of species caught in black-understory traps was not significantly different from the number of species caught in green-canopy traps (Fig. 2A). For jewel beetles, green-canopy traps caught significantly more species than black-understory traps (Fig. 2B), whereas the opposite trend was observed for bark and ambrosia beetles (Fig. 2C).

Abundance was significantly affected by all tested variables (Table 1). The mean number of individuals ( $\pm$  standard error) was significantly higher for bark and ambrosia beetles (286.70  $\pm$  25.47) than for both longhorn beetles (70.47  $\pm$  4.03) and jewel beetles (23.08  $\pm$  5.43). In addition, black-understory traps generally caught more individuals than green-canopy traps, even though the trapping performance

changed depending on the taxon (Fig. 2D–F). Black-understory traps outperformed green-canopy traps for both longhorn beetles (Fig. 2D) and bark and ambrosia beetles (Fig. 2F), whereas the opposite trend was observed for jewel beetles (Fig. 2E).

**Table 1.** Analysis of deviance table from the generalized linear mixed models testing the effects of taxon (longhorn beetles, jewel beetles, and bark and ambrosia beetles), trapping methodology (black-understory multi-funnel traps and green-canopy multi-funnel traps) and their interaction on species richness (Poisson distribution; log link function) and abundance (negative binomial distribution; log link function). Nested random structure used for both models: ~1|Site/Cell/Trap. Type II Wald chi-square tests ( $\chi^2$ ), degrees of freedom (df), *p*-values, and lognormal marginal (mR<sup>2</sup>) and conditional (cR<sup>2</sup>) pseudo R-squared are provided for both models.

	χ²	df	<i>p</i> -value
Species richness			
Taxon	659.517	2	< 0.001
Methodology	0.455	1	0.500
Taxon × Methodology	161.154	2	< 0.001
			$mR^2 = 0.73, cR^2 = 0.87$
Abundance			
Taxon	1040.747	2	< 0.001
Methodology	20.737	1	< 0.001
Taxon × Methodology	274.916	2	< 0.001
· · · · · · · · · · · · · · · · · · ·	· · · ·		$mR^2 = 0.73$ , $cR^2 = 0.86$



**Figure 2.** Mean ( $\pm$  standard error) species richness and abundance of longhorn beetles (**A**, **D**), jewel beetles (**B**, **E**), and bark and ambrosia beetles (**C**, **F**) for each trapping methodology. Asterisks within the plots indicate the statistical significance level from pairwise comparisons between the two trapping methodologies within each taxon from the generalized linear mixed models. Asterisks under the plots indicate the statistical significance level from pairwise comparisons among the three taxa from the generalized linear mixed models. *P*-values: \* = 0.01 - 0.05; \*\* = 0.001 - 0.01; \*\*\* = < 0.001; ns = not significant (> 0.05). *P*-values were adjusted by Tukey correction. Model details are provided in Table 1.

# Effect of taxon and forest cover in the trap surroundings on dissimilarity indices

 $\beta_{cc}$  and  $\beta_{rich}$  were significantly affected by taxon and forest cover but not by their interaction, while  $\beta_{.3}$  was significantly affected by taxon and by the interaction between taxon and forest cover (Table 2). Irrespective of the forest cover effect, jewel beetles showed higher  $\beta_{cc}$  and  $\beta_{rich}$  values, and lower  $\beta_{.3}$  values compared to longhorn beetles and bark and ambrosia beetles (Fig. 3; Suppl. material 1: table S3). For all taxa,  $\beta_{cc}$  slightly decreased with increasing amount of forest cover,



**Figure 3.** Effect of forest cover in a buffer of 250 m radius around the traps on the dissimilarity among wood-boring beetle communities found in black understory multi-funnel traps and green canopy multi-funnel traps, considering the total beta-diversity  $\beta_{cc}$  (A–C) and its components species richness difference  $\beta_{rich}$  (D–F) and species replacement  $\beta_{-3}$  (G–I). Plots include model estimate (colored line) and 95% confidence intervals (colored shading). Model details are provided in Table 2. Pairwise comparisons among taxa are provided in Suppl. material 1: table S3. Slope estimates, along with their corresponding 95% confidence intervals, are provided in Suppl. material 1: table S4.

**Table 2.** Analysis of deviance table from the generalized linear mixed models testing the effects of taxon (longhorn beetles, jewel beetles, and bark and ambrosia beetles), forest cover (mean % in buffers of 250 m radius around the pair of traps) and their interaction on  $\beta$ -diversity ( $\beta_{cc}$ ), species richness difference ( $\beta_{rich}$ ) and species replacement ( $\beta_{.3}$ ) indices (Gaussian distribution used for all models). Nested random structure used for all models: ~1|Site/Cell. Type II Wald chi-square tests ( $\chi^2$ ), degrees of freedom (df), *p*-values, and delta marginal (mR<sup>2</sup>) and conditional (cR<sup>2</sup>) pseudo R-squared are provided for all models. Pairwise comparisons among taxa are provided in Suppl. material 1: table S3. Slope estimates, representing the relationship between each index and forest cover, along with their corresponding 95% confidence intervals, are provided in Suppl. material 1: table S4.

	χ <sup>2</sup>	df	<i>p</i> -value	
$\beta_{cc}$				
Taxon	391.167	2	< 0.001	
Forest	4.282	1	0.039	
Taxon × Forest	0.899	2	0.638	
$mR^2 = 0.53$ , $cR^2 = 0.58$				
$\beta_{\rm rich}$				
Taxon	262.048	2	< 0.001	
Forest	4.617	1	0.032	
Taxon × Forest	5.073	2	0.079	
$mR^2 = 0.45, cR^2 = 0.47$				
β.3				
Taxon	17.470	2	< 0.001	
Forest	0.426	1	0.514	
Taxon × Forest	7.250	2	0.027	
$mR^2 = 0.07$ , $cR^2 = 0.10$				

although the trend was not significant (Fig. 3A–C; Suppl. material 1: table S4). For jewel beetles,  $\beta_{rich}$  decreased with increasing amount of forest cover (Fig. 3E; Suppl. material 1: table S4), whereas the opposite trend was found for  $\beta_{.3}$  (Fig. 3H; Suppl. material 1: table S4). For longhorn beetles and bark and ambrosia beetles, both  $\beta_{rich}$  and  $\beta_{.3}$  showed no relationship with forest cover (Fig. 3D, F, G, I, Suppl. material 1: table S4).

## Discussion

Our study confirmed that the use of baited traps around high-risk sites represents an efficient approach for generic surveillance of wood-boring beetles (Brockerhoff et al. 2006; Rassati et al. 2015a, 2015b; Fan et al. 2019; Rabaglia et al. 2019; Mas et al. 2023). The simultaneous use of black-understory multi-funnel traps and green-canopy multi-funnel traps baited with a multi-lure blend of pheromones and host volatiles allowed us to catch 21 non-native species from all the three targeted families (i.e., longhorn beetles, bark and ambrosia beetles and jewel beetles), as well as more than 300 native species. In addition to the importance of records of newly introduced or expanding non-native species, knowledge of distribution and abundance data for native species in areas near entry/export sites represents a second key benefit of the application of this or similar trapping protocols (Rassati et al. 2015a, 2018; Mas et al. 2023). In fact, these data can be crucial not only to increase our ability to predict which species are at most risk from being introduced in other countries via exported commodities (Mas et al. 2023) but also to monitor range expansion or shifts of native species, which could become invasive within their native distributional range (Rassati et al. 2018; Ruzzier et al. 2023b).

Comparing the efficacy of the two trapping methodologies, we found that black multi-funnel traps baited with the multi-lure blend and set up in the understory caught significantly more bark and ambrosia beetle species and individuals than green multi-funnel traps baited with the same blend and set up in the canopy, but significantly less jewel beetle species and individuals. For longhorn beetles, a difference between the two trapping methodologies was found in the total number of individuals (more in black-understory traps), but not in the number of species. The trends observed in our study are likely explained by the combined effect of trap height and trap color (Dodds et al. 2024), two variables that are well known to affect trap efficacy towards the targeted taxa. Several studies, for example, showed an increasing abundance and richness of longhorn beetles and jewel beetles with increasing trap height (Ulyshen and Hanula 2007; Maguire et al. 2014; Flaherty et al. 2019; Rassati et al. 2019; Sheehan et al. 2019; Sweeney et al. 2020) and/ or the opposite trend for bark and ambrosia beetles (Ulyshen and Hanula 2007; Hanula et al. 2011; Dodds 2014; Hardersen et al. 2014; Flaherty et al. 2019). Other studies showed that green colored traps are more efficient than black or dark colored traps in catching jewel beetles, especially Agrilus spp. (e.g., Crook et al. 2009, 2014; Francese et al. 2010a, b, 2011; Petrice and Haack 2015; Skvarla and Dowling 2017; Rassati et al. 2019; Tobin et al. 2021), and/or that black or dark colored traps are generally more or similar efficient than green colored traps in attracting bark and ambrosia beetles (Cavaletto et al. 2020; Marchioro et al. 2020) and longhorn beetles (Kerr et al. 2017; Rassati et al. 2019; Cavaletto et al. 2021). Although our study does not allow us to disentangle the individual contribution of trap color and trap height on beetle catches, it shows that the simultaneous use of black-understory and green-canopy multi-funnel traps in generic trapping programs targeting these three taxa at the same time is essential.

Analyzing dissimilarity indices, we also found that differences between beetle communities caught in black-understory traps and green-canopy traps were more evident for jewel beetles than for both longhorn beetles and bark and ambrosia beetles. For jewel beetles, these differences were mainly attributed to differences in species richness, while both differences in species richness and species turnover contributed to explain the dissimilarity of communities of longhorn beetles and bark and ambrosia beetles between trapping methodologies. These results are especially useful when planning surveillance activities targeting only one of the three taxa. For longhorn beetles and bark and ambrosia beetles, the simultaneous use of black-understory and green-canopy multi-funnel traps is always recommended, as different species with different flight patterns can be caught by these two trapping methodologies. Previous studies testing different trap types, environmental gradients, and/or lures (Skvarla and Dowling 2017; Flaherty et al. 2019; Rassati et al. 2019) confirmed that the diversification of trapping methodologies is always advantageous when targeting longhorn beetles and bark and ambrosia beetles (Dodds et al. 2024). For jewel beetles, especially Agrilus spp., green-canopy multi-funnel traps should be prioritized over black-understory traps in some, but not all, landscapes. In fact, jewel beetles were the only taxon for which dissimilarity indices were affected by the amount of forest cover in the trap surroundings. In particular, the difference in the number of jewel beetle species caught in black-understory and

green-canopy multi-funnel traps decreased with increasing amount of forest cover, whereas the species turnover increased. In other words, jewel beetle species collected in black-understory traps were a subset of the species collected in green-canopy traps in urban-dominated landscapes, whereas the number of jewel beetle species exclusive to either black-understory or green-canopy traps increased in forest-dominated landscapes. Thus, the use of black-understory along with green-canopy multi-funnel traps is recommended only in forest-dominated landscapes.

## Conclusions

Baited traps are an essential component of both specific and generic surveillance programs around the world, making the development of efficient trapping protocols a research priority. Here we showed that the simultaneous use of black-understory and green-canopy multi-funnel traps baited with a multi-lure blend of longhorn beetle pheromones and host volatiles can be considered a very efficient approach for generic surveillance of longhorn beetles, jewel beetles and bark and ambrosia beetles in both urban-dominated and forest-dominated areas surrounding entry points. The only case in which this protocol can be simplified using only green-canopy multi-funnel traps is when targeting jewel beetles in urban-dominated landscapes. Despite the general efficiency of the trapping protocol we tested, it is very likely that not all longhorn beetle, bark and ambrosia beetle and jewel beetle fauna present in the sampled area was represented by trap catches. Fan et al. (2019), for example, using the same multi-lure blend, collected 48% of the 238 longhorn beetle species native to France, percentage that would be likely lower for bark and ambrosia beetles and jewel beetles. Overall, these results highlight that further improvements to the trapping protocol are possible. For example, the use of traps of different colors (e.g., yellow, blue) (Cavaletto et al. 2020, 2021) or traps integrated with more complex visual stimuli (Masaguè et al. 2024) is very likely to increase the diversity of species that can be collected, especially within those taxa strongly relying on color vision at the adult stage (e.g., Lepturinae). Similarly, advances in the knowledge of the chemical ecology of still understudied taxa (i.e., jewel beetles) will further improve trap attractiveness with direct benefits for national and international surveillance.

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## **Additional information**

## **Conflict of interest**

The authors have declared that no competing interests exist.

#### **Ethical statement**

No ethical statement was reported.

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#### Author contributions

GS: data curation, formal analysis, writing – original draft, writing – review & editing; AB: funding acquisition, writing – review & editing; CC: lure preparation, methodology; GC: beetle identification; MF: beetle identification, writing – review & editing; NF: investigation; JAF: funding acquisition, investigation, writing – review & editing; EKLF: beetle identification, investigation; FG: beetle identification; MMG: funding acquisition, investigation, writing – review & editing; CK: beetle identification, investigation; MM: conceptualization, investigation; DN: conceptualization, data curation, formal analysis; AMR: funding acquisition, investigation, writing – review & editing; JS: funding acquisition, investigation, writing – review & editing; KVR: beetle identification, investigation, writing – review & editing; AR: investigation, investigation; DR: conceptualization, data curation, project administration, validation, visualization, writing – original draft, writing – review & editing. All authors approved the text.

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#### Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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## **Supplementary material 1**

## Testing a trapping protocol for generic surveillance of wood-boring beetles in heterogeneous landscapes

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