



Efficacy of trapping protocols for *Agrilus* jewel beetles: a multi-country assessment

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Abstract

The genus *Agrilus* is one of the most diverse insect genera worldwide. The larval feeding activity causes extensive damage in both forests and orchards. In addition, more than 30 species have been introduced outside their native range so far, including the emerald ash borer *Agrilus planipennis* Fairmaire. Thus, the availability of efficient trapping protocols for early detection of *Agrilus* species at entry points is of utmost importance. In this study we tested whether trapping protocols developed for surveillance of *A. planipennis* in North America were also effective for other *Agrilus* species. In particular, through a multi-country assessment we compared the efficacy of detecting *Agrilus* species on: (i) green glue-coated prism traps vs. green Fluon-coated multi-funnel traps when baited with the green leaf volatile (Z)-3-hexenol or left unbaited; and (ii) green multi-panel traps vs. green multi-panel traps baited with dead adult *Agrilus* beetles (decoys). A total of 23,481 individuals from 45 *Agrilus* species were caught. Trap design significantly affected both species richness and abundance of *Agrilus* species in several of the countries where the trapping experiments were carried out, and green prism traps outperformed green multi-funnel traps in most cases. On the contrary, the addition of a (Z)-3-hexenol lure or dead adult beetle decoys on to traps did not improve trap catches. Our study highlights that reliable trap models to survey *Agrilus* species are already available, but also that there is the clear need to further investigate chemical ecology of *Agrilus* species to develop semiochemical lures that can improve detection efficacy.

Keywords Biosecurity · Early detection · Forest pests · Lures · Traps · Visual stimuli · *Agrilus*

Key message

- The genus *Agrilus* has > 3,300 species and includes a number of potentially invasive species
- Efficient trapping protocols are needed for surveillance and early detection of *Agrilus* species
- Green glue-coated prism traps generally performed better than green multi-funnel traps

- The addition of a (Z)-3-hexenol lure or adult beetle decoys to traps did not increase trap catches

Introduction

The genus *Agrilus* (Coleoptera; Buprestidae) is one of the most diverse insect genera with over 3,300 species described worldwide (Jendek 2016; Kelnarova et al. 2019; Jendek and Grebennikov 2023). Adults mainly feed on leaves of host trees while larvae develop in living subcortical tissues (Kelnarova et al. 2019). The larval feeding activity can be sufficient to kill a host, especially when it has already been weakened by other abiotic (e.g., drought) or biotic (e.g., defoliation) factors (Muzika et al. 2000; Brück-Dyckhoff

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et al. 2019), causing extensive damage in both forests and orchards (Brown et al. 2015; Zhang et al. 2021; Perish et al. 2023). In addition, the absence of visible evidence of feeding within wood products has likely facilitated invasion by *Agrilus* species (Ruzzier et al. 2023). More than 30 species have been introduced outside their native range (Ruzzier et al. 2023), including the emerald ash borer *A. planipennis* Fairmaire, which has caused massive ecological and economic damage in North America (Kovacs et al. 2010; Klooster et al. 2018). Given that further introductions can be expected due to increasing trade and climate change (Brockenhoff and Liebhold 2017; Meurisse et al. 2019; Pureswaran et al. 2022), the availability of efficient trapping protocols for the early detection of introduced *Agrilus* species at entry points is of utmost importance.

Among the numerous trap types developed for *Agrilus* monitoring programs around the world (e.g., Poland et al. 2019; Imrei et al. 2020a), glue-coated prism traps and Fluon-coated multi-funnel traps are the most commonly adopted and recommended types (European Food Safety Authority et al. 2020; Evans et al. 2020; Silk et al. 2020a). Indeed, both were found to efficiently catch high numbers of *Agrilus* species and detect (i.e., the ability to catch a single beetle) them at low density (Francese et al. 2008, 2010a, 2011, 2013; Marshall et al. 2010; Petrice et al. 2013; Crook et al. 2014; Brown et al. 2017; Rhainds et al. 2017). Other trap types and trapping strategies for *Agrilus* species have been developed over the years, including double-decker traps (McCullough and Poland 2017), branch-traps (Domingue et al. 2013), modified multi-funnel traps (Imrei et al. 2020b), fan-traps (Grégoire et al. 2022), and girdled or stressed trap trees (McCullough et al. 2009a, 2009b; Mercader et al. 2013). Some of these other trap types have proven effective at detecting *A. planipennis* at sites with low population densities (e.g., double-decker traps, girdled trap trees), and for capturing a range of other *Agrilus* species (e.g., branch traps, modified multi-funnel traps), but with the exception of the modified multi-funnel traps (Imrei et al. 2020b), none are commercially available.

Irrespective of the trap type, visual stimuli present on the trap can strongly affect its efficacy. *Agrilus* species strongly rely on color vision when searching for mates, suitable brood hosts, and food (Lelito et al. 2007; Domingue et al. 2011). Electroretinogram assays demonstrated that both male and female *A. planipennis* adults are sensitive to green, blue, red and ultraviolet specific wavelengths (Crook et al. 2009). Subsequent field studies showed that green and purple traps are efficient to catch *A. planipennis* and other *Agrilus* species (Crook et al. 2009; Francese et al. 2010b, 2011, 2013; Petrice et al. 2013; Poland and McCullough 2014; Kim et al. 2016; Rhainds et al. 2017; Cavaletto et al. 2020). In particular, specific shades of green (i.e., wavelength range 525–540 nm, and

reflectance in the 49–67% range) are very attractive for male *A. planipennis* (Francese et al. 2010b; Domingue et al. 2012; Poland et al. 2019; Parker et al. 2020) presumably because it resembles foliage where they can find both mates and food (Rodriguez-Soana et al. 2007); thus, green is currently the most commonly adopted trap color for trapping *Agrilus* species (Rassati et al. 2019; Evans et al. 2020; Sallé et al. 2020; Wittman et al. 2021). Another key visual cue used by males to locate females seems to result from the light scattering effect produced by the microstructures present on elytra of adult beetles (Lelito et al. 2008; Domingue et al. 2014). In fact, dead adult beetles or bioreplicated decoys attached on leaves or plastic sticky cards were found to readily evoke the typical descending flights of males occurring when spotting a mate (Lelito et al. 2008; Domingue et al. 2014, 2015). Nonetheless, whether the presence of dead adult beetles or bioreplicated decoys on traps can increase trap efficacy is still unclear.

Besides visual stimuli, *Agrilus* species rely on olfactory stimuli when searching for mates, suitable host trees, and food (Pureswaran and Poland 2009; Silk et al. 2019; Imrei et al. 2020a). Many species breed in stressed trees and are particularly attracted to volatiles emitted from their hosts, suggesting that decisions regarding host suitability are made prior to alighting on the tree. For example, significantly more *A. planipennis* (McCullough et al. 2009a, 2009b), *A. bilineatus* (Weber) (Dunn et al. 1986), and *A. anxius* Gory (Silk et al. 2020b) or oak-associated *Agrilus* species (Sallé et al. 2020) were captured on traps placed on, or adjacent to, stressed (i.e., girdled) host trees compared to healthy host trees. Positive responses to extracts of host foliage and/or bark, as well as synthetic blends of compounds identified in host volatiles, have been observed in *A. planipennis* (Crook et al. 2008; Grant et al. 2010, 2011; Silk et al. 2011), other *Agrilus* species (Domingue et al. 2013; Vuts et al. 2016), and other species in the subfamily Agrilinae, namely *Coraebus fasciatus* (Herbst) (Fürstenau et al. 2012) and *Coraebus undatus* Fabricius (Fürstenau et al. 2015). Among these volatiles, one of the most adopted is the commercially available green leaf volatile, (Z)-3-hexenol. This compound was found to efficiently attract *A. planipennis* adult beetles both alone and in combination with other lures (De Groot et al. 2008; Grant et al. 2010, 2011; Silk et al. 2011; Ryall et al. 2012; Crook et al. 2014) and is commonly used as the main attractant in surveillance programs targeting the latter species.

In this study we tested whether trapping protocols most commonly adopted for surveillance of *A. planipennis* were also effective for other *Agrilus* species. In particular, we carried out a multi-country assessment comparing the efficacy of detecting *Agrilus* species on: (i) green glue-coated prism traps vs. green Fluon-coated multi-funnel traps when baited with the green leaf volatile (Z)-3-hexenol or left unbaited;

and (ii) green multi-panel traps vs. green multi-panel traps baited with dead adult *Agrilus* beetles (decoys).

Materials and methods

Study sites and general experimental methods

The three trapping trials were carried out in 2021 and 2022 in nine different countries in Europe and North America: Austria, Canada, France, Germany, Italy, Poland, Slovenia, United Kingdom (UK), and United States of America (USA) (Table S1). In USA, three states were involved (Massachusetts, Ohio and Kentucky). Not all countries carried out all three trapping trials (Table S1).

Irrespective of the trapping trial, some general criteria were followed. Traps were always set up in a complete randomized block design within an oak-dominated forest, with 20–40 m spacing between treatments and about 60 m among blocks. Using a big-shot catapult (Hughes et al. 2014) or a carbon-fibre telescopic pole, traps were always suspended in the mid to upper canopy, 10–30 m above the ground, preferably in open and sunny spots and avoiding complete shade under branches. Mid-upper canopy was preferred over ground level because several studies showed that species richness and abundance of *Agrilus* species are generally higher at the canopy level than close to the ground (e.g., Francese et al. 2008; Crook et al. 2008, 2009; Ryall et al. 2012; Rassati et al. 2019; Sallé et al. 2020). Similarly, open sunny spots were preferred over shaded ones because traps more exposed to sunlight tend to catch more adults than those in shaded locations (e.g., Francese et al. 2008; Lyons et al. 2009). Traps were established in mid to late May, and beetles were collected every 2–4 weeks until mid to late August to cover the main flight period of *Agrilus* species (i.e., 4–8 collections).

Trial 1: Effect of trap design (green prism traps vs. green multi-funnel traps) on *Agrilus* detection on unbaited traps

Field trials were conducted in 2021 in Canada, France, Germany, Italy, Slovenia, UK and USA (Massachusetts and Ohio) (Table S1). Green (540 nm, 49% reflectance; see Wittman et al. 2021), glue-coated prism traps were purchased from Andermatt Group (Grossdietwil, Switzerland and Fredericton, New Brunswick, Canada) (Fig. S1A), whereas green (530 nm, 49% reflectance; described by Francese et al. 2011) multi-funnel traps were purchased from ChemTica Internacional (Santo Domingo, Costa Rica) (Fig. S1B). Multi-funnel traps were pre-treated with a 50% solution of Fluon as a trap coating because of its ability to increase trap catch of woodboring beetles (Graham and Poland 2012; Allison et al. 2016) including

A. planipennis (Lyons et al. 2012; Francese et al. 2013). Each trap type was replicated 10 times in all but two of the countries (i.e., Slovenia and France) (Table S1). The collecting cup of the multi-funnel traps was filled with approximately 200 ml of 50% propylene glycol solution either in water or in a solution as a pre-mixed marine / RV antifreeze in most of the countries. However, in a few cases either a saturated salt solution (table salt in water) or ventilated cups for dry trapping supplied with an insecticide net were used. The contents of the collecting cups from the multi-funnel traps were transferred to labelled containers and transported back to the laboratory for further analysis (the trap collection cup was reset with fresh solution following each collection). The green prism traps were examined at each collection date and any *Agrilus* beetles captured on the glue surface of the trap were picked off with forceps and transferred to a labelled collection vial or bag.

Trial 2: Effect of trap design (green prism traps vs. green multi-funnel traps) and (Z)-3-hexenol lure (present vs. absent) on *Agrilus* detection

Field trials were conducted in 2022 in the same sites and countries as trial 1 in 2021 (Table S1), except for the addition of a site in each of Austria and Sweden and the replacement of the Ohio site with a site in Kentucky in USA. Green prism traps and green multi-funnel traps were obtained from the same companies as in 2021 (i.e., Andermatt and ChemTica Internacional). (Z)-3-hexenol lure pouches were purchased from Synergy Semiochemicals Corporation (Delta, BC, Canada) or purchased together with the traps at Andermatt Group (i.e., Germany), and had a release rate of 50 mg/day at 25°C. Each treatment was replicated 5–10 times depending on the country (Table S1). Traps were checked as explained above for trial 1.

Trial 3: Effect of decoys on *Agrilus* detection in green multi-panel traps

Field trials were conducted in 2022 in Canada, France, Italy, Poland, UK, and USA (Massachusetts and Ohio) (Table S1). Green multi-panel traps (i.e., Synergy Semiochemicals Corporation (Delta, BC, Canada). Three individual dead *Agrilus* adult specimens were glued onto each panel surface of the multi-panel trap (i.e., 18 individuals/trap) to serve as decoys (Fig. S1C, D). Each country used as decoys the preserved cadavers of the most common *Agrilus* beetle species captured in the 2021 field trial, namely *A. olivicolor* Kiesenwetter in Italy, *A. laticornis* (Illiger) in France and UK, *A. biguttatus* (Fabricius) in Poland, *A. crinicornis* Horn in Canada and *A. bilineatus* in USA. We decided to bait traps with dead adults of a single species and test the effect on multiple species because previous studies showed

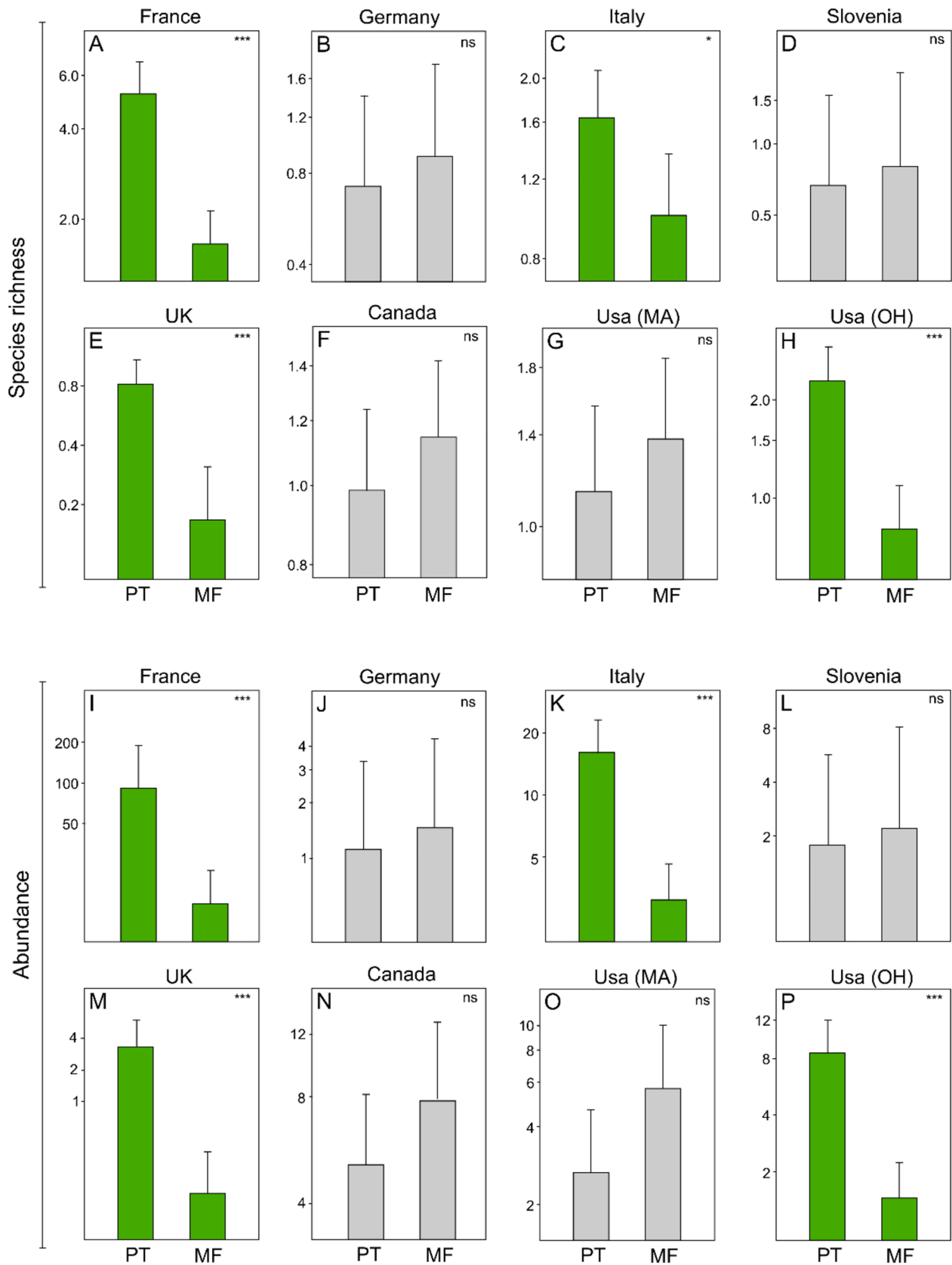


Fig. 1 Means from the generalized linear mixed models (Poisson or negative binomial distribution with a log link-function) testing the effect of trap type on the mean number of species (i.e., species richness) and mean number of individuals (i.e., abundance) of *Agrilus* species collected per trap per sampling period in each of the eight different countries where the trial was carried out in 2021 (trial 1). Trap type: PT=green prism traps; MF=green multi-funnel traps. P-values: *=0.05–0.01; **=0.01–0.001; ***=<0.001; ns=not significant (>0.05). Bars are green for countries where a significant effect of trap type was found and grey for countries where the effect was not significant. Error bars indicate the 95% positive confidence interval. Note the nonlinearity of the y-axis (due to log-link function in the models)

that the light-scattering effect produced by physical microstructures present on *Agrilus* species body is not species-specific (Lelito et al. 2008; Domingue et al. 2011, 2014). Each treatment was replicated 10 times in all countries except France (Table S1). The collecting cup of the multi-panel traps was filled with approximately 200 ml of 50% propylene glycol solution either in water or in a solution as a pre-mixed marine / RV antifreeze in most of the countries, except Canada where a saturated salt solution was used.

Statistical analyses

The effects of trap design (trial 1), trap design, lure and their interaction (trial 2), and decoy presence (trial 3), were tested using generalized linear mixed models (GLMMs). Models were fitted with a Poisson distribution, or negative binomial distribution when data were over dispersed, using a log link-function. We opted for GLMMs over classic ANOVA given the non-normality and the correlated and longitudinal structure of the data. For each country and trial, species richness (i.e., total number of *Agrilus* species), total abundance (i.e., total number of *Agrilus* individuals), and the abundance of each *Agrilus* species (when represented by at least 10 individuals) were considered as response variables. In all cases, we treated the data collected from each trap and sampling period as a distinct statistical unit. This approach ensures more accurate parameter estimates and better model fit compared to pooling data from different sampling rounds. The block identity and the trap identity within each block were included in the models as random factors to account for the temporal pseudoreplication (repeated measures on each trap) and for the nested design of the trials. For trial 2, pairwise multiple comparisons were run using post-hoc tests with Holm correction of *p*-values. All the analyses were carried out in R (R Core Team 2021). Models were fitted using the ‘glmmTMB’ package (Brooks et al. 2022). Pairwise comparisons were run using the ‘emmeans’ package (Lenth et al. 2022). Models were checked for overdispersion, residual distribution, and residual temporal and spatial autocorrelation using the ‘DHARMa’ package (Hartig 2022).

Results

General results

A total of 23,481 individuals from 45 *Agrilus* species were caught (Table S2). Six species were represented by more than 1,000 individuals; among them, *A. laticornis* was the most abundant species (6,665 individuals), followed by *A. olivicolor* (4,136), *A. angustulus* (Illiger) (3,158), *A. obscuricollis* Kiesenwetter (2,069), *A. hastulifer* Ratzeburg (1,296), and *A. crinicornis* Horn (1,240). By contrast, 21 species were represented by less than 10 individuals (Table S2). Three countries were represented by more than 2,000 individuals, from 10 or more different species, i.e., France (8,048), Austria (5,521) and Italy (2,449). Only Poland and Sweden, in which only one trapping experiment was conducted, were represented by less than 200 individuals (Table S2). In general, species richness at the sampling sites tended to decrease with increasing latitude.

Trial 1: Effect of trap design (green prism traps vs. green multi-funnel traps) on *Agrilus* detection on unbaited traps

Trap type significantly affected both species richness and abundance of *Agrilus* species in France (Fig. 1A, I), Italy (Fig. 1C, K), UK (Fig. 1E, M) and Ohio (Fig. 1H, P), where green prism traps always outperformed green multi-funnel traps. On the contrary, no significant effect of trap type was observed in Germany (Fig. 1B, J), Slovenia (Fig. 1D, L), Canada (Fig. 1F, N), or Massachusetts (Fig. 1G, O) neither for species richness nor abundance.

At the species level, trap type significantly affected abundance of 14 of the 33 *Agrilus* species collected in 2021, even though the effect was not evident in all countries where the species were collected (Table 1). In countries where the effect of trap type was significant, *A. angustulus*, *A. arcuatus* (Say), *A. celti* Knull, *A. convexicollis* Redtenbacher, *A. curtulus* Mulsant & Rey, *A. hastulifer*, *A. laticornis*, *A. masculinus* Horn, *A. obscuricollis*, *A. olivicolor*, *A. putillus* Say, and *A. viridis* L. were caught significantly more in green prism traps than green multi-funnel traps, while the opposite trend was observed only for *A. bilineatus* (Table 1). The only species for which the effect of trap type was not consistent between countries was *A. sulcicollis* Lacordaire (i.e., better performance of green prism traps in France and green multi-funnel traps in Slovenia) (Table 1).

For species composition, most *Agrilus* species were collected by both trap types (i.e., 23), seven exclusively by green prism traps and three exclusively by green multi-funnel traps (Fig. S2A). When singletons and doubletons were excluded, no species was found exclusively in green

Table 1 Output of the generalized linear mixed models testing the effect of trap type (trial 1) on abundance of single *Agrilus* species. In case of significant effect, the most efficient trap model is shown in parenthesis. PT=green prism traps; MF=green multi-funnel traps.

P-values: ns= not significant (> 0.05); * = 0.05–0.01; ** = 0.01–0.001; *** = < 0.001 . nt = species represented by less than 10 individuals which was not statistically analyzed. – = species not collected in the country

	Europe					North America		
	France	Germany	Italy	Slovenia	UK	Canada	USA (MA)	USA (OH)
<i>A. angustulus</i>	*** (PT)	ns	nt	nt	–	–	–	–
<i>A. anxius</i>	–	–	–	–	–	ns	–	–
<i>A. arcuatus</i>	–	–	–	–	–	nt	ns	*** (PT)
<i>A. atricornis</i>	–	–	–	–	–	–	–	nt
<i>A. benjamini</i>	–	–	–	–	–	–	–	nt
<i>A. biguttatus</i>	nt	nt	–	nt	–	–	–	–
<i>A. bilineatus</i>	–	–	–	–	–	ns	** (MF)	–
<i>A. celti</i>	–	–	–	–	–	–	–	*** (PT)
<i>A. cephalicus</i>	–	–	–	–	–	–	–	nt
<i>A. convexicollis</i>	nt	nt	*** (PT)	nt	–	–	–	–
<i>A. crinicornis</i>	–	–	–	–	–	ns	–	nt
<i>A. curtulus</i>	* (PT)	–	–	–	–	–	–	–
<i>A. derasofasciatus</i>	nt	–	–	–	–	–	–	–
<i>A. ferrisi</i>	–	–	–	–	–	–	–	nt
<i>A. frosti</i>	–	–	–	–	–	–	nt	–
<i>A. geminatus</i>	–	–	–	–	–	–	–	nt
<i>A. graecus</i>	nt	–	–	–	–	–	–	–
<i>A. graminis</i>	ns	–	ns	–	–	–	–	–
<i>A. hastulifer</i>	* (PT)	–	nt	nt	–	–	–	–
<i>A. juglandis</i>	–	–	–	–	–	–	nt	–
<i>A. laticornis</i>	*** (PT)	ns	ns	ns	*** (PT)	–	–	–
<i>A. lecontei</i>	–	–	–	–	–	–	–	ns
<i>A. litura</i>	–	–	nt	–	–	–	–	–
<i>A. masculinus</i>	–	–	–	–	–	–	–	* (PT)
<i>A. obscuricollis</i>	*** (PT)	nt	nt	ns	–	–	–	–
<i>A. obsoletoguttatus</i>	–	–	–	–	–	nt	nt	nt
<i>A. olivicolor</i>	*** (PT)	ns	*** (PT)	ns	–	–	–	–
<i>A. otiosus</i>	–	–	–	–	–	–	–	nt
<i>A. planipennis</i>	–	–	–	–	–	–	nt	–
<i>A. politus</i>	–	–	–	–	–	nt	–	–
<i>A. putillus</i>	–	–	–	–	–	–	–	** (PT)
<i>A. sulcicollis</i>	* (PT)	ns	–	* (MF)	nt	–	–	–
<i>A. viridis</i>	*** (PT)	ns	nt	–	–	–	–	–

multi-funnel traps, two exclusively in green prism traps, and 21 in both trap types (Fig. S2A).

Trial 2: Effect of trap design (green prism traps vs. green multi-funnel traps) and (Z)-3-hexenol lure (present vs. absent) on *Agrilus* detection

Both species richness and abundance of *Agrilus* species were affected by trap type in Austria (Figs. 2A, 3A), France (Figs. 2B, 3B), Italy (Figs. 2D, 3D), Slovenia (Figs. 2E, 3E), Sweden (Figs. 2F, 3F), UK (Figs. 2G, 3G), Kentucky

(Figs. 2I, 3I) and Massachusetts (Figs. 2J, 3J), where green prism traps outperformed green multi-funnel traps in all sites but Massachusetts, where the opposite trend was observed. On the contrary, there were no significant effects of trap type on species richness or abundance in Canada (Figs. 2H, 3H) and Germany (Figs. 2C, 3C). The (Z)-3-hexenol lure and the interaction between trap type and lure never affected species richness or abundance.

Trap type also significantly affected abundance of 15 out of the 36 *Agrilus* species collected in trial 2, but the effect was not evident in all countries where the species were

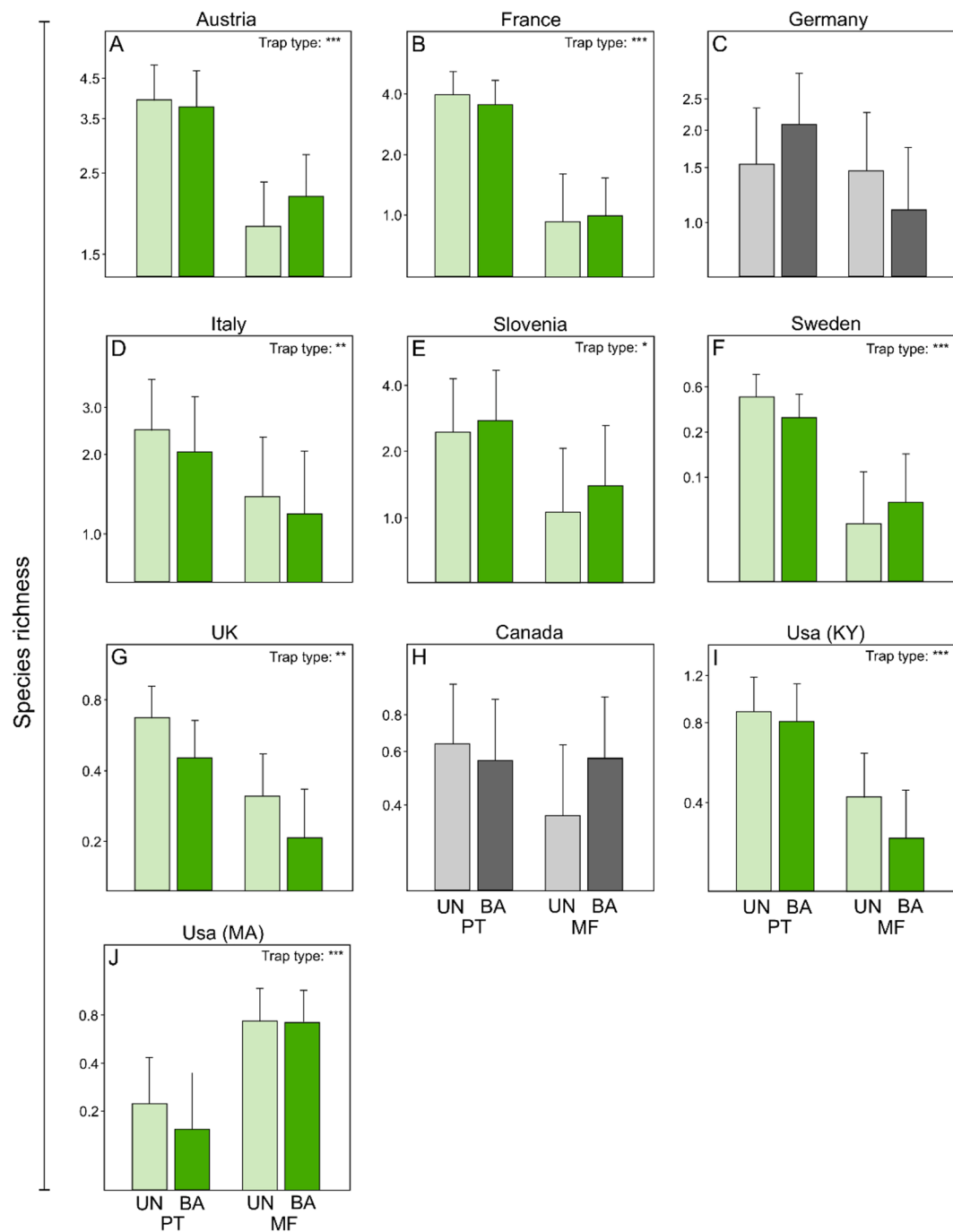


Fig. 2 Means from the generalized linear mixed models (Poisson or negative binomial distribution with a log link-function) testing the effect of trap type, (Z)-3-hexenol lure and their interaction on the mean number of species (i.e., species richness) of *Agrilus* species collected per trap per sampling period in each of the ten different countries where the trial was carried out in 2022 (trial 2). (Z)-3-hexenol lure: UN=unbaited; BA=baited. Trap type: PT=green prism traps;

MF=green multi-funnel traps. *P*-values: *=0.05–0.01; **=0.01–0.001; ***= <0.001. Bars are green for countries where a significant effect of either trap type, lure or their interaction was found and grey for countries where the effect of the latter variables were not significant. Error bars indicate the 95% positive confidence interval. Note the nonlinearity of the y-axis (due to log-link function in the models)

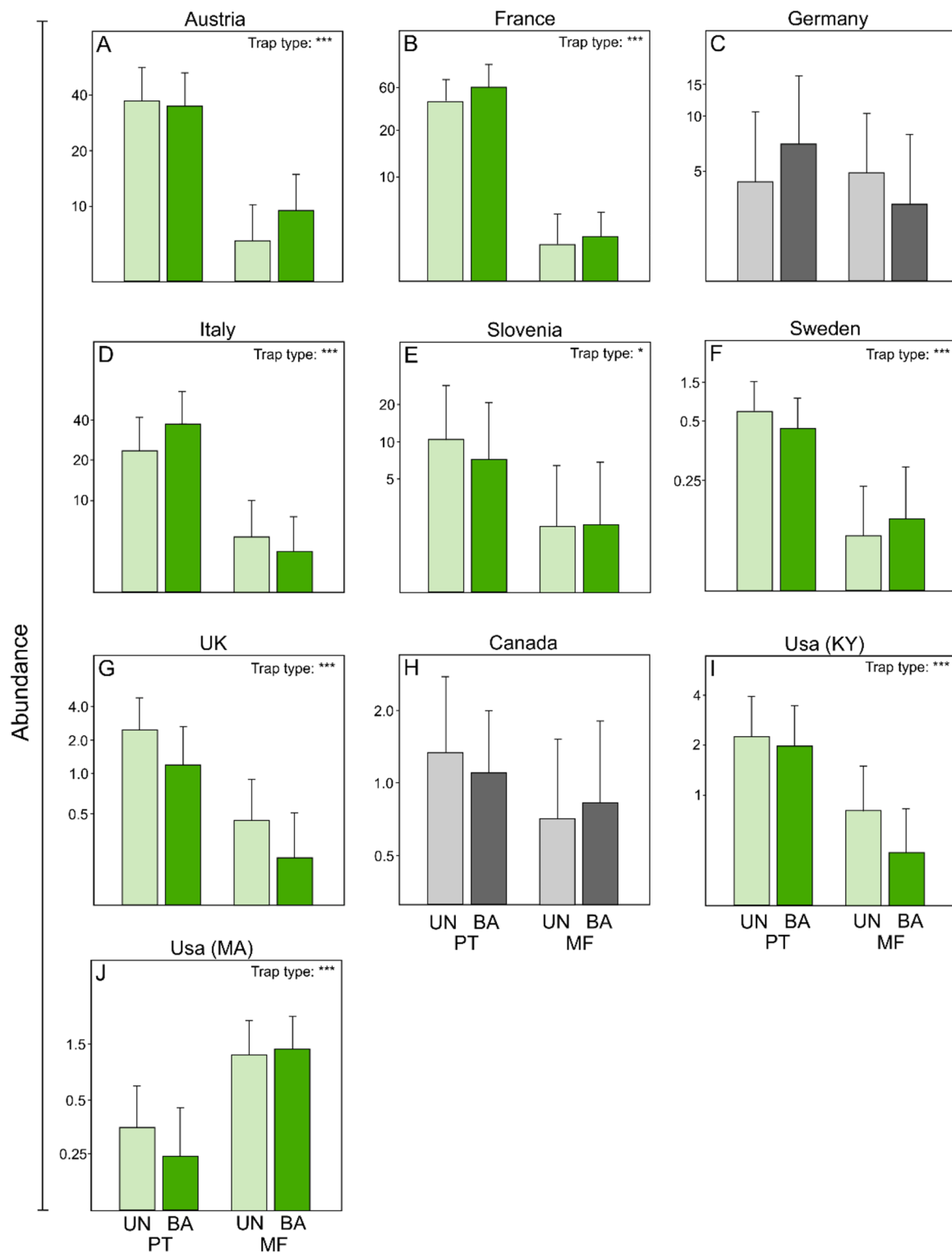


Fig. 3 Means from the generalized linear mixed models (Poisson or negative binomial distribution with a log link-function) testing the effect of trap type, (Z)-3-hexenol lure and their interaction on the mean number of individuals (i.e., abundance) of *Agrilus* species collected per trap per sampling period in each of the ten different countries where the trial was carried out in 2022 (trial 2). (Z)-3-hexenol lure: UN=unbaited; BA=baited. Trap type: PT=green prism traps;

MF=green multi-funnel traps. *P*-values: *=0.05–0.01; **=0.01–0.001; ***= <0.001. Bars are green for countries where a significant effect of either trap type, lure or their interaction was found and grey for countries where the effect of the latter variables were not significant. Error bars indicate the 95% positive confidence interval. Note the nonlinearity of the y-axis (due to log-link function in the models)

Table 2 Output of the generalized linear mixed models testing the effect of trap type and (Z)-3-hexenol lure (trial 2) on species richness, total abundance, and abundance of single *Agrilus* species. In case of significant effect, the most efficient trap model is shown in parenthesis. PT=green prism traps; MF=green multi-funnel traps. *P*-values:

ns = not significant (> 0.05); * = 0.05–0.01; ** = 0.01–0.001; *** = < 0.001 . nt = species represented by less than 10 individuals which was not statistically analyzed. The effects of (Z)-3-hexenol and the interaction between trap type and lure are not reported since they were never significant. – = species not collected in the country

	Europe							North America		
	Austria	France	Germany	Italy	Slovenia	Sweden	UK	Canada	USA (KY)	USA (MA)
<i>A. anxius</i>	–	–	–	–	–	–	–	ns	–	–
<i>A. arcuatus</i>	–	–	–	–	–	–	–	nt	ns	*** (MF)
<i>A. auricollis</i>	–	–	–	–	nt	–	–	–	–	–
<i>A. angustulus</i>	*** (PT)	*** (PT)	ns	nt	ns	–	–	–	–	–
<i>A. atricornis</i>	–	–	–	–	–	–	–	–	nt	–
<i>A. bilineatus</i>	–	–	–	–	–	–	–	ns	nt	*** (MF)
<i>A. biguttatus</i>	nt	nt	ns	–	nt	–	nt	–	–	–
<i>A. celti</i>	–	–	–	–	–	–	–	–	*** (PT)	–
<i>A. convexicollis</i>	nt	–	ns	*** (PT)	nt	nt	–	–	–	–
<i>A. crinicornis</i>	–	–	–	–	–	–	–	ns	nt	–
<i>A. croaticus</i>	–	–	–	–	nt	–	–	–	–	–
<i>A. curtulus</i>	–	ns	–	–	nt	–	–	–	–	–
<i>A. cyanescens</i>	–	–	–	–	nt	–	–	–	–	–
<i>A. defectus</i>	–	–	–	–	–	–	–	–	–	nt
<i>A. egeniformis</i>	–	–	–	–	–	–	–	–	nt	–
<i>A. egenus</i>	–	–	–	–	–	–	–	–	nt	–
<i>A. fallax</i>	–	–	–	–	–	–	–	–	nt	–
<i>A. ferrisi</i>	–	–	–	–	–	–	–	–	nt	–
<i>A. geminatus</i>	–	–	–	–	–	–	–	–	*** (PT)	–
<i>A. graminis</i>	*** (PT)	*** (PT)	–	nt	ns	–	–	–	–	–
<i>A. hastulifer</i>	*** (PT)	*** (PT)	–	ns	–	–	–	–	–	–
<i>A. laticornis</i>	*** (PT)	*** (PT)	ns	*** (PT)	* (PT)	*** (PT)	*** (PT)	–	–	–
<i>A. lecontei</i>	–	–	–	–	–	–	–	–	** (PT)	nt
<i>A. litura</i>	*** (PT)	–	–	nt	nt	–	–	–	–	–
<i>A. masculinus</i>	–	–	–	–	–	–	–	nt	ns	nt
<i>A. obscuricollis</i>	*** (PT)	*** (PT)	nt	nt	ns	–	–	–	–	–
<i>A. obsoletoguttatus</i>	–	–	–	–	–	–	–	ns	nt	nt
<i>A. olivicolor</i>	*** (PT)	*** (PT)	ns	*** (PT)	** (PT)	–	–	–	–	–
<i>A. otiosus</i>	–	–	–	–	–	–	–	–	ns	–
<i>A. pensus</i>	–	–	–	–	–	–	–	nt	–	–
<i>A. planipennis</i>	–	–	–	–	–	–	–	–	nt	–
<i>A. putillus</i>	–	–	–	–	–	–	–	–	* (PT)	–
<i>A. subrobustus</i>	–	–	–	–	–	–	–	–	nt	–
<i>A. sulcicollis</i>	*** (PT)	*** (PT)	** (PT)	nt	* (PT)	nt	–	–	–	–
<i>A. suvorovi</i>	–	–	–	–	nt	–	–	–	–	–
<i>A. viridis</i>	nt	*** (PT)	nt	nt	–	–	–	–	–	–

collected (Table 2). In countries where the effect of trap type was significant, *A. angustulus*, *A. celti*, *A. convexicollis*, *A. geminatus* (Say), *A. graminis* Kiesenwetter, *A. hastulifer*, *A. laticornis*, *A. lecontei* Saunders, *A. obscuricollis*, *A. olivicolor*, *A. putillus*, *A. sulcicollis*, and *A. viridis* were caught significantly more in green prism traps than green

multi-funnel traps, while the opposite trend was observed only for *A. arcuatus* and *A. biguttatus* (Table 2).

For species composition, most *Agrilus* species were collected by all treatments irrespective whether singletons and doubletons were included or excluded (i.e., 21) (Fig. S2B). Only a few species were instead found to be exclusive to

Table 3 Output of the generalized linear mixed models testing the effect of *Agrilus* decoy presence (trial 3) on species richness, total abundance, and abundance of single *Agrilus* species. The species used as decoy is indicated right under the country name. In case of significant effect, the most efficient trap model is shown in parenthe-

ses. Y = decoy presence; N = decoy absence. *P*-values: ns = not significant (> 0.05); * = $0.05 - 0.01$; ** = $0.01 - 0.001$; *** = < 0.001 . nt = species represented by less than 10 individuals which was not statistically analyzed. – = species not collected in the country

	Europe				North America		
	France <i>A. laticornis</i>	Italy <i>A. olivicolor</i>	Poland <i>A. biguttatus</i>	UK <i>A. laticornis</i>	Canada <i>A. crinicornis</i>	USA (MA) <i>A. bilineatus</i>	USA (OH) <i>A. bilineatus</i>
Species richness	ns	ns	ns	ns	ns	ns	ns
Total abundance	ns	ns	ns	ns	ns	ns	ns
<i>A. angustulus</i>	ns	nt	nt	–	–	–	–
<i>A. arcuatus</i>	–	–	–	–	nt	ns	nt
<i>A. betuleti</i>	nt	–	–	–	–	–	–
<i>A. biguttatus</i>	nt	–	ns	nt	–	–	–
<i>A. bilineatus</i>	–	–	–	–	ns	nt	nt
<i>A. celti</i>	–	–	–	–	–	–	ns
<i>A. convexicollis</i>	–	ns	nt	–	–	–	–
<i>A. crinicornis</i>	–	–	–	–	ns	–	–
<i>A. curtulus</i>	nt	–	–	–	–	–	–
<i>A. defectus</i>	–	–	–	–	–	–	nt
<i>A. geminatus</i>	–	–	–	–	–	–	* (Y)
<i>A. graminis</i>	ns	nt	nt	–	–	–	–
<i>A. hastulifer</i>	* (N)	ns	nt	–	–	–	–
<i>A. laticornis</i>	ns	nt	ns	ns	–	–	–
<i>A. lecontei</i>	–	–	–	–	–	–	nt
<i>A. masculinus</i>	–	–	–	–	–	–	ns
<i>A. obscuricollis</i>	ns	nt	nt	–	–	–	–
<i>A. obsoletoguttatus</i>	–	–	–	–	nt	–	nt
<i>A. olivicolor</i>	ns	ns	–	–	–	–	–
<i>A. osburni</i>	–	–	–	–	nt	–	–
<i>A. otiosus</i>	–	–	–	–	–	–	nt
<i>A. planipennis</i>	–	–	–	–	–	–	nt
<i>A. putillus</i>	–	–	–	–	–	–	ns
<i>A. sulcicollis</i>	nt	–	** (N)	nt	–	–	–
<i>A. viridis</i>	nt	nt	–	–	–	–	–

certain treatments, and this value was even lower when singletons and doubletons were excluded (Fig. S2B).

Trial 3: Effect of decoys on *Agrilus* detection in green multi-panel traps

The presence of adult *Agrilus* decoys on green multi-panel traps did not affect species richness and abundance of *Agrilus* species in any country (Table 3). At the species level, a significant effect of the *Agrilus* decoys was observed only on 3 of the 25 *Agrilus* species collected, i.e., *A. hastulifer* in France, *A. sulcicollis* in Poland, and *A. bilineatus* in Ohio (Table 3). Traps baited with decoys of *A. laticornis* collected

fewer *A. hastulifer* than did unbaited traps. Similarly, traps baited with decoys of *A. sulcicollis* collected fewer *A. sulcicollis* than did unbaited traps. Conversely, traps baited with *A. bilineatus* decoys collected more *A. geminatus* than did unbaited traps (Table 3).

For species composition, most *Agrilus* species were collected by both unbaited and *Agrilus* decoy-baited multi-panel traps (i.e., 21), two exclusively by unbaited multi-panel traps and two exclusively by decoy-baited multi-panel traps (Fig. S2C). When singletons and doubletons were excluded, no species was found exclusively in unbaited multi-panel traps, one exclusively in decoy-baited multi-panel traps, and 21 in both trap types (Fig. S2C).

Discussion

More than 30 *Agrilus* species have been introduced outside their native range, including European species introduced into North American forests (e.g., *A. sulcicollis*) and North American species introduced into European forests (e.g., *A. bilineatus*) (Hızal and Arslangündoğdu 2018). For this reason, the availability of efficient trapping protocols that can be integrated in surveillance programs is of utmost importance (Poland and Rassati 2019; Nahrung et al. 2023). Through extensive trapping trials in Europe and North America, we showed that trap types commonly used for early detection and monitoring of *A. planipennis* (i.e., green prism traps and multi-funnel traps) are reliable also for detection of other *Agrilus* species, even though depending on the scope of the surveillance programs one trap design might be more suitable than the other. In addition, our trials indicated that baiting traps with (Z)-3-hexenol or adult beetle decoys does not seem to increase general trap efficacy.

Trap design significantly affected both species richness and abundance of *Agrilus* species in several of the countries where the trapping experiments were carried out. In particular, when a significant effect was found, green prism traps outperformed green multi-funnel traps in all cases but one. A very similar pattern was observed also at the species level. The efficacy of colored prism traps in attracting *Agrilus* species is well known (Francese et al. 2008; Petrice et al. 2013; Poland et al. 2019), and this trap design has been used for monitoring the spread of the emerald ash borer in USA and Canada. Nonetheless, the higher efficacy of prism traps compared to multi-funnel traps that we found was rarely observed in previous studies (e.g., Francese et al. 2005), which often highlighted a similar efficacy of the two trap designs or even a higher efficacy of multi-funnel traps (Francese et al. 2011, 2016; Crook et al. 2014; Poland et al. 2019). We suggest three possible reasons why green prism traps generally performed better than green multi-funnel traps in our study. First, previous *Agrilus* trapping studies have primarily focused on *A. planipennis*, while our study assessed the responses of other *Agrilus* species which may display different behaviors and preferences. Secondly, compared to multi-funnel traps, the glue-coated prism traps may have retained a greater proportion of beetles that alighted on the trap surface. Although Fluon-coating increases the capture rate of beetles in multi-funnel and intercept panel traps (Allison et al. 2011; Graham and Poland 2012), studies suggest that a proportion of beetles that contact the surface of multi-funnel traps do not fall into the collecting cup (Allison et al. 2014). Thirdly, adult *Agrilus* individuals

landing on prism traps remain attached to the panel and could act as positive visual cues for other *Agrilus* species (Domingue et al. 2014, 2015) whereas this does not occur in multi-funnel traps.

Besides overall efficacy, other factors to consider when deciding to use prism traps or multi-funnel traps for *Agrilus* surveys include the need to reuse traps for multiple years, ease of deployment, and the overall goal of the trapping program. Prism traps are discarded after one field season while multi-funnel traps can be reusable for many years if properly maintained (Francese et al. 2011). Additionally, at each trap check prism traps must be thoroughly examined in the field, which is time consuming particularly in bad weather, while multi-funnel traps are simply emptied by pouring the content of the collector cup in a plastic container (Francese et al. 2011). Prism traps may also be at a disadvantage in areas with high dust and pollen counts. This debris can coat the surface of the trap and render the glue inefficient. Glue-coated surfaces in hot, sunny locations can also slough off of the trap surface taking samples with it (Francese et al. 2011). Furthermore, removal of beetles from prism traps and subsequent treatments to remove the glue from their body can damage them, negatively affecting morphological identification. Several studies showed that multi-funnel traps also efficiently catch longhorn beetles (Rassati et al. 2019), bark and ambrosia beetles (Marchioro et al. 2020; Thurston et al. 2022), and leaf-dwelling beetles (Sallé et al. 2020), while whether this is true also for sticky prism traps is still unclear (e.g., Skvarla and Holland 2011). Thus, despite our results suggest that for generic surveillance programs targeting *Agrilus* species green prism traps might be preferred over green multi-funnel traps, the latter trap design might be preferred when targeting *Agrilus* species that do not show preferences for a specific trap design as found for more than half of the species caught in this study.

We also found that the addition of the green leaf volatile (Z)-3-hexenol did not affect species richness, total abundance, or abundance of any *Agrilus* species in any of the countries where the trapping trials were carried out. This result is in contrast to the results of previous studies showing that (Z)-3-hexenol significantly increased trap catches of *A. planipennis* (De Groot et al. 2008; Grant et al. 2010, 2011; Silk et al. 2011), *A. auroguttatus* (Coleman et al. 2014), and *A. sulcicollis* (Domingue et al. 2014). However, lack of positive response to (Z)-3-hexenol has previously been observed for *A. anxius* (Silk et al. 2019), *A. angustulus*, *A. graminis*, *A. laticornis*, and *A. obscuricollis* (Domingue et al. 2013). Because so many of the same foliar and cortical volatile compounds are shared among different genera of trees, it follows that *Agrilus* species and other beetles that use olfactory

cues during host selection, likely respond most positively to particular blends and relative ratios of volatiles emitted from suitable hosts, more so than any individual compound. Indeed, van Wijk et al. (2011) suggested that the blend of compounds emitted from a suitable host is perceived as a distinct odor, different from its individual components.

Our field trials also indicated that the inclusion of adult beetle decoys on trap panels did not affect species richness and total abundance of most *Agrilus* species; however there did appear to be an effect for three individual species. Two species, *A. laticornis* and *A. sulcicollis*, were seemingly repelled by the presence of adult beetles of a different or the same species, respectively, while *A. geminatus* seemed to be attracted by the presence of adult beetles of *A. bilineatus*. The general lack of an effect might be due to the small number of *Agrilus* individuals (i.e., three) that we glued on each trap panel. It is possible that any attractive effects of the decoys were obviated by stronger combined effects of the green color and the large size of the multi-panel traps, contrary to what was observed when smaller traps (i.e., branch-traps) were used (Lelito et al. 2008; Domingue et al. 2014). The few significant repellent or attractive effects observed at the species level might be due to greater sensitivity of certain species to light scattering effects produced by adult beetles. In a previous study, only individuals of *A. sulcicollis* were significantly more attracted to traps baited with decoys than unbaited traps, while no effect was observed for *A. angustulus* and *A. laticornis* (Domingue et al. 2013). In addition, different species might display different mating behavior (Lelito et al. 2011), leading to heterogeneous responses to decoy-baited traps.

Trapping programs are commonly carried out at entry points or other high-risk sites (e.g., Rassati et al. 2015a,b; Rabaglia et al. 2019; Mas et al. 2023), but their efficacy strongly depends on the availability of efficient trapping protocols for the target group of species. Our study highlighted that both green prism traps and green multi-funnel traps can be considered reliable trap models to monitor *Agrilus* species, but also that the commonly used (Z)-3-hexenol is not universally attractive to all *Agrilus* species. Given that host volatiles and pheromones, especially when combined, can be used to increase trap attractiveness (Parker et al. 2020), more efforts should be done to discover new species-specific and generic lures to be used in surveillance programs for *Agrilus* species. A direct comparison of the three trap designs tested in this study would also be useful to understand whether green multi-panel traps can be eventually more efficient than green prism-traps and/or green multi-funnel traps. Despite the lack of an effect of adult beetle decoys glued on traps, future studies should further investigate whether

it is possible to reproduce the light scattering effect on trap panels, for example using more adult beetles as decoys or exploiting technological advances to reproduce the microstructures present on beetle elytra on surfaces that can be then attached to trap panels. Traps of other colour wavelengths (and designs) should be also further evaluated (Imrei et al. 2020a). Finally, additional multi-country experiments involving more continents should be conducted. As shown in this and other studies (e.g., Roques et al. 2023), sharing information from trapping experiments conducted on different continents provides us with direct information on the efficacy of traps, lures, and other factors for detecting target species potentially at risk of introduction to our respective continents.

Author's contributions

DW, JAF, JS, and DR conceived the study; DR and GS wrote the first draft of the manuscript; BH, GS, GCa, EB, SD, EKLF, EGB, SMD, DLP, EF, CH, JMG, RP, KS, EG, TH, MdG, AS, DW, AE, GH, WH, JC conducted field experiments; GCu, EF, MG, CK, JMG, KVR, EG, AS, DW, AE, JC, WH identified beetle specimens; GS, DR and MdG analyzed the data; BH, DW, DR, MdG, JS, JAF, AS, GH, AMR acquired funds. All authors contributed to and approved the manuscript.

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Data availability The datasets generated and/or analysed during the current study are available from the corresponding authors on reasonable request.

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Declarations

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