



Euphresco

Final Report

Project title

Developing and assessing surveillance methodologies for *Agrilus* beetles

Project duration:

Start date:	2021-04-01
End date:	2023-03-31



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2. Project overview

The jewel beetle genus *Agrilus* (Family Buprestidae) has over 3000 species (Kelnarova et al., 2019), all of which are strictly phytophagous, with adults feeding on leaves, and their larvae feeding on the living subcortical tissues of trees and shrubs. Larval feeding can be sufficient to kill a host, especially when it has already been weakened by other abiotic (e.g. drought), and/or biotic (e.g. defoliation) factors (Kelnarova et al., 2019 and references therein). Furthermore, *Agrilus* species have a proven invasive potential facilitated by their relatively long-lived larvae that are readily transported within nursery plants and wood products (firewood, wood packaging material etc.), whilst adult beetles have good dispersal ability through active flight periods (Kelnarova et al., 2019). As a consequence, some *Agrilus* species have become important invasive pests after being accidentally introduced into new geographic areas leading to wide ranging environmental, economic and social impacts. Hence, *Agrilus* beetles constitute a high-risk group of invasive pests, comparable to both longhorn (Cerambycidae) and bark beetles (Curculionidae: Scolytinae and Platypodinae) and should be considered a priority group when developing early detection and surveillance programmes.

With the exception of emerald ash borer (*Agrilus planipennis*), there is relatively little information published within the scientific literature on surveillance and monitoring protocols for the wood-boring beetles of the *Agrilus* genus. However, across Europe and North America there have been scattered trials and research projects undertaken in the past decade, along with anecdotal evidence of current ongoing research programmes that have started to investigate methodologies for capturing and assessing *Agrilus* species in a variety of contexts. This Euphresco project aimed to consolidate the European/North American studies that have been conducted, and with collaboration from North American researchers start to develop monitoring tools for either specific *Agrilus* species (e.g. *A. anxius*, *A. bilineatus*, *A. biguttatus*, *A. auroguttatus*), and/or develop a more generic trapping technique for this group of wood-boring insects. As well as gathering together the current knowledge on available trapping/monitoring techniques employed for *Agrilus* species, we encouraged collaborators to evaluate trap designs with and without volatile lures in a variety of forest/woodland settings to assess the efficiency and species diversity of captures.



The main objectives of the project were:

- Collate and report evidence from previous European and North American *Agrilus* species surveillance and monitoring studies.
- Consolidate information on current protocols implemented in national surveillance and monitoring programmes for *Agrilus* beetles.
- Contribute to designing and evaluating species-specific and generic *Agrilus* trapping techniques.
- Validate detection methods to determine specific *Agrilus* species presence; potential lures and traps will be deployed and assessed to effectively trap native and invasive *Agrilus* species and allow early detection by deployment at high-risk sites.

There is mounting evidence of introductions of *Agrilus* beetles into new geographic areas, hence there is a real need to develop early detection and monitoring approaches for intercepting this group of wood-boring beetles. In North America there have been at least 12 non-native *Agrilus* species that have been accidentally introduced and which have subsequently established (Digirolomo et al., 2019), with emerald ash borer (*Agrilus planipennis*) being the most infamous. Similarly, emerald ash borer has also invaded and established in Europe, in both Russia (Baranchikov et al., 2008) and Ukraine (Drogvalenko et al., 2019). A North American species of *Agrilus*, the two-lined chestnut borer (*Agrilus bilineatus*), has also been introduced, and likely established, in Turkey (Hizal & Arslangündoğdu, 2018; EPPO 2020). The North American bronze birch borer (*Agrilus anxius*) is – like emerald ash borer – regulated as a priority pest in the EU (Commission Delegated Regulation (EU) 2019/1702). As the *Agrilus* genus has over 3000 known species there is somewhat of an inevitability that in response to the ever-expanding global trade in resources and commodities, and changing climate patterns, there will be an increase in the frequency with which *Agrilus* spp. will be intercepted in new locations around the world. Hence, understanding what trapping approaches could be utilised for detecting these wood-boring insects and monitoring their spread is a vital first step in establishing national invasive insect monitoring programmes.



Both the USA and Canada, along with several European countries already have ongoing research and monitoring activities underway concerning several species of buprestids with an emphasis on *Agrilus* beetles, so the current project is an opportunity to consolidate and assess the variety of approaches that may be used to detect and monitor for this large family of wood-boring beetles. With a significant emphasis on conducting fieldwork, the results of the field trials, conducted over the two years of the project, should lead to research outputs that contribute to developing best practice guidelines for early detection methodologies, and surveillance and monitoring strategies for the buprestids as a whole, and for specific *Agrilus* species.

References

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3. Work-packages (WP)

WP1 - Project management and co-ordination

WP2 - Review of *Agrilus* trapping protocols and methodologies (both European - WP2.1 and North America – WP2.2) excluding emerald ash borer

WP3 - European trapping trials – evaluating effectiveness of traps and lures in capturing *Agrilus* species

WP4 - North America trapping trials - evaluating effectiveness of traps and lures in capturing *Agrilus* species

3.1. WP1 - Project management and co-ordination

- Full project proposal drafted and agreed with participants (March 2021); Co-operation agreement (memorandum of understanding) drafted and passed to all collaborators following review by Euphresco co-ordinator at EPPO (April/May 2021). However, subsequent discussions with the Euphresco co-ordinator and Defra suggest that this is no longer a formal requirement for Euphresco projects.
- Meetings arranged as appropriate. End of field season meetings were held online through MS Teams on 8th November 2021 and 29th September 2022, end of year one meeting was held on 8th March 2022, and final project meeting held in person (Vienna) 20th March 2023. Communication was maintained throughout the duration of the project with many of the project collaborators, through regular updates and exchanging of relevant information, primarily undertaken via email and phone/MS Teams/Zoom discussions.
- Reporting of results, literature reviews and collation of information was undertaken throughout the duration of the project. Summaries and update reports were provided to Euphresco coordinator Dr Baldissera Giovani at 6 month intervals – which was subsequently used in the meeting of the EPPO Panel on Inspection, and in a speech on 'Euphresco and its role in surveillance' within the framework of the EU Presidency (March



2022). Meetings with Defra colleagues were undertaken quarterly, end of year one report for Defra submitted in March/April 2022, and end of project report is due April/May 2023. A presentation on project progress was given to the EPPO entomology diagnostics panel on 15th September 2022, a final project overview was given at the Forest Protection Colloquium meeting in Vienna, Austria (21-22nd March 2023), and collaborators have presented results at various meetings, workshops and conferences throughout the duration of the project.

3.2. WP2 – Review of *Agrilus* trapping protocols and methodologies (both European and North American)

Review of literature on factors affecting efficacy of trapping surveys for detection of *Agrilus* species

The goal of this review is to summarize the research on factors affecting foraging behavior of *Agrilus* species and their detection in traps to help us determine ways of improving surveillance and early detection of non-native *Agrilus* species in Europe, North America, and Asia. Much of what we have learned about the chemical ecology and foraging behavior of *Agrilus* species has come from research on the emerald ash borer, *Agrilus planipennis* (e.g., Crook and Mastro, 2008; Silk et al., 2015a, 2019a) and much of its behaviour appears common to other *Agrilus* species. Like other wood-boring beetles, Buprestidae use both visual and olfactory stimuli when searching for food, mates and brood hosts (Lelito et al., 2007; Domingue and Baker, 2012; Silk et al., 2019a), so trap colour, trap position and semiochemical lures have significant effects on diversity and abundance of *Agrilus* species in traps. Trap design and trap coatings also affect detection efficacy and interactions among these factors are common. We have therefore organized this literature review into two main sections: 1) Factors affecting *Agrilus* foraging and detection in traps, including olfactory stimuli or semiochemicals (plant volatiles, pheromones), visual stimuli (trap color, shape), trap position (sun exposure, height, edge effects), and trap type (sticky vs. non-sticky); and 2) European perspectives on *Agrilus* detection in traps. Because our knowledge of buprestid chemical ecology is so limited, we also include relevant genera of jewel beetles other than *Agrilus*.



1. Factors affecting *Agrilus* detection in traps

Olfactory stimuli – Semiochemicals

A number of studies have shown that *Agrilus* species respond to olfactory stimuli when foraging for food, suitable brood hosts, and mates. Many *Agrilus* species breed in stressed hosts and are particularly attracted to volatiles emitted from those hosts, suggesting that decisions regarding host suitability are made prior to alighting on the host. For example, significantly more *Agrilus bilineatus* (Dunn et al., 1986a), *A. planipennis* (McCullough et al., 2009a, b) and *Agrilus anxius* (Silk et al., 2019b) were captured on traps placed on or adjacent to stressed (i.e., girdled) host trees compared to healthy trees. In laboratory bioassays, female *A. planipennis* responded positively to *Fraxinus mandshurica* seedlings that had been fed upon by conspecifics (Rodriguez-Saona et al., 2006). Other studies have shown positive responses to extracts of host foliage and/or bark, as well as synthetic blends of compounds identified in host volatiles, and individual compounds, such as the green leaf volatile, Z-3-hexan-1-ol (Z-3-hexenol) (Table 1). Fewer studies have investigated the role of pheromones in *Agrilus* chemical ecology (Silk et al., 2011, 2015a, b, 2019; Ryall et al., 2012).

Crook et al. (2008) found a number of volatile sesquiterpene compounds, namely α -cubebene, α -copaene, 7-epi-sesquithujene, trans- β -caryophellene, and α -humulene, that were emitted in greater amounts from girdled vs. healthy ash; these compounds elicited antennal response in *A. planipennis* and were hypothesized to be used as olfactory cues to assess host suitability. Peterson et al. (2020) noted that the monoterpenes, Z- β -ocimene and linalool are both emitted in larger amounts from stressed as opposed to healthy ash and birch trees (Rodriguez-Saona et al., 2006; Vuorinen et al., 2007) and would be good candidates to test for effects on trap catch but to date this has not been done. They also suggested sabinene may be an attractant of *A. planipennis* because it is emitted from the highly attractive black ash (*Fraxinus nigra*) in volumes 6.5X those emitted from the much less attractive Manchurian ash (*Fraxinus mandshurica*). Pureswaran and Poland (2009) suggested the preference of *A. planipennis* for green vs. Manchurian ash in olfactometer bioassays was due to the former's lower emissions of volatiles.



Behavioral responses to olfactory stimuli have been measured in laboratory bioassays (e.g. y-tube olfactometer) and field trapping bioassays, and both methods are useful. However, results from laboratory bioassays are not always supported by data from trapping bioassays (e.g. Silk et al. 2010) and we consider evidence from trapping bioassays that have demonstrated significant positive effects of a compound or compound blend on trap catch more conclusive when developing a trap lure for operational surveillance. Therefore, we have categorized the evidence of *Agrilus* species response to semiochemicals separately for laboratory bioassays and trapping bioassays (Table 1).

Host volatiles

Laboratory bioassays - Studies usually consider that insects have made a positive or negative response to a test compound in a y-tube olfactometer when the proportion of individuals that choose the arm containing the test stimulus vs. the control arm differs from a 1:1 ratio, using a chi-square test, or when the insects spend more time in one arm of the y-tube than the other. *Agrilus biguttatus* responded positively to 1) foliage of *Quercus robur*; 2) bark of *Q. robur*; and 3) synthetic blends of antennally-active volatiles from oak foliage and oak bark (Vuts et al., 2016) (Table 1). Female (but not male) *Coroebus florentinus* (Buprestidae) (also known as *Coroebus fasciatus*) were attracted to volatiles collected from freshly cut branches of *Quercus suber*, extracts from *Q. suber* leaves, and several synthetic green leaf volatiles presented either individually or in a blend (Fürstenau et al., 2012) (Table 1). Silk et al. (2011) observed attraction of male and female *A. planipennis* to Z-3-hexenol, and both positive (at low dose) and negative response (at high dose) of *A. planipennis* males to Phoebe oil. In y-tube olfactometer assays, Bari et al., (2019) reported attraction of *Capnodis tenebrionis* (Buprestidae) females to Z-3-hexenol and 3-methyl butanol, and male attraction to both 3-methyl butanol and 1-pentanol whereas females were repelled by benzaldehyde and 2-hexanone and males were repelled by S-limonene, (Z)-3-hexen-1-ol and 2-hexanone.

Field trapping bioassays - Sesquiterpenes are difficult to synthesize but all those that Crook et al. (2008) found emitted in greater quantities from girdled vs. healthy ash with the exception of 7-epi-sesquithujene are contained in commercially available Manuka oil. Baiting sticky traps with



Manuka oil and later Phoebe oil (which contained all five compounds) significantly increased mean catch of *A. planipennis* compared to unbaited traps (Crook et al., 2008). The purple prism trap baited with Manuka oil was adopted as the standard operational method for EAB survey by USDA APHIS. Subsequently, Coleman et al. (2014) showed that the gold-spotted oak borer, *Agrilus auroguttatus*, also preferred traps baited with Manuka oil or Phoebe oil compared to unbaited traps. Mercador et al. (2013) reported that girdled ash trees wrapped with sticky bands outperformed purple sticky prism traps baited with Manuka oil for detection of *A. planipennis* in low density populations. Dunn et al. (1986b) showed that *A. bilineatus* were attracted to a steam distillate of phloem from stressed *Q. alba* but unlike Cerambycidae and Scolytinae, were not attracted to ethanol.

The common green leaf volatile Z-3-hexenol significantly increased trap catches of *A. planipennis* (De Groot et al., 2008), the bronze birch borer, *A. anxius* (Silk et al., 2019b) and *A. auroguttatus* (Coleman et al., 2014). Another green leaf volatile, Z-3-hexenyl acetate is antennally active but emitted in relatively large amounts by healthy Manchurian ash which are avoided by female *A. planipennis*; for this reason, Peterson et al. (2020) suggested it may be a deterrent to females. With the exception of Z-3-hexenol, most synthetic host volatiles have not proven attractive to *Agrilus* species compared to host extracts or blends of volatiles like Phoebe oil (Table 1). van Wijk et al. (2011) suggested that the blend of compounds, e.g., emitted from a suitable host, is perceived as a distinct odor, different from its individual components.

Fürstenau et al. (2015) observed significantly greater trap catch of the cork oak borer, *Coroebus undatus*, on purple sticky prism traps baited with a 5-component blend of green leaf volatiles that approximated the volatile profile from freshly cut branches of cork oak, dissolved in ethanol and released at a rate of 0.3–2.6 mg/day (see Table 1), compared to ethanol alone. The traps were placed 1.5-2m above the ground – only female *C. undatus* were captured.

Pheromones

Behavioral bioassays have suggested that females of some *Agrilus* species emit sex pheromones that attract conspecific males. Dunn and Potter (1988) captured significantly more male *A. bilineatus* on field cages that contained live females and oak logs vs. cages with oak logs only



and hypothesized attraction was due either to olfactory or auditory stimuli produced by the females. In short range lab bioassays, Pureswaran and Poland (2008) showed that *A. planipennis* males that had been blinded with model paint found females as quickly as normal males, but males with compromised (painted) antennae took significantly longer than normal males to find females. Bartelt et al. (2007) identified a macrocyclic lactone (3Z)-12-dodecenolide [(3Z)-lactone] emitted predominantly by female *A. planipennis* but reported no behavioral activity. Field trials testing attraction of the lactone to *A. planipennis* have had mixed results which suggest that visual cues (e.g., trap color) and trap position (e.g., canopy vs. understory) interact with olfactory cues and affect trap catch. Adding a 3Z-lactone lure to green traps co-baited with Z-3-hexenol and placed in the upper canopy significantly increased mean catches of *A. planipennis* (Silk et al., 2011; Ryall et al., 2012) as well as the proportion of traps that detected at least one *A. planipennis* (Ryall et al., 2013). Similarly, adding the lactone to green sticky “branch traps” (Domingue et al., 2015) co-baited with Z-3-hexenol significantly increased catch of both male and female *A. planipennis*, and male catch was further increased by the presence of dead female conspecific decoys (Domingue et al., 2016). It is likely that the effect of the lactone is relatively short range and dependent on context, e.g., it must be combined with the green leaf volatile on a trap placed in the upper canopy of the host tree. Silk et al., (2019a) hypothesized that the lactone may act more as a “flight arrestant” rather than an elicitor of upwind anemotaxis. 12-Dodecanolide, the saturated analog of 3Z-lactone, also increased catch of *A. planipennis* in green traps co-baited with Z-3-hexenol and is less expensive to synthesize than Z-3-lactone (Silk et al., 2015b).

In y-tube olfactometer bioassays, virgin males of the black-banded oak borer, *Coroebus florentinus* were attracted to abdominal extracts of live females; conversely, virgin females were not attracted to volatiles or abdominal extracts of either sex (Fürstenau et al., 2012). However, both males and females responded positively to a synthetic blend of three compounds prominent in the abdominal extracts: nonanal, decanal, and geranylacetone. When these compounds were tested individually in the olfactometer, decanal attracted males, geranylacetone attracted females, and nonanal attracted neither sex. In a study by Lopez et al (2021), both sexes of *C. undatus* released the spiroacetal 1,7-dioxaspiro[5.5]undecane (olean), but females were found to be more responsive than males to high amounts of this compound. In double-choice assays, adults older



than seven days were significantly attracted to clean, whereas this attraction was not detected in insects aged less than seven days. Indeed, a repellent effect was observed in young females.

Trap position

Trap height - Several studies reported greater activity (Yu, 1992) and greater trap catches of male buprestids in sunny vs. shaded areas, i.e., as found in the upper canopy of host trees, open grown trees or along the edge of stands (Fraser et al., 2006; Francese et al., 2008; Lance et al., 2007; Bonsignore and Jones, 2013). Observations of *A. planipennis* mating behavior by Lelito et al. (2007) indicated that males fly near the canopy of ash trees and rapidly descend on females perched on ash leaves. Both sexes of *A. planipennis* were positively phototactic in lab trials, i.e., they moved towards light (Chen and Poland, 2009), and Lelito et al. (2007) observed males searching for and copulating with females perched on ash leaves in the upper canopy. Canopy traps have also collected greater species richness and abundance of buprestids than understory traps (Wermelinger et al., 2007; Rassati et al., 2019; Sallé et al., 2020). Ulyshen and Sheehan (2017) found greater species richness and abundance of phloem feeding beetles (Buprestidae, Cerambycidae) in canopy traps vs. understory traps whereas the converse was true for Scolytinae. However, this trend has not always been observed (Ulyshen and Hanula, 2007). *Agrilus* species use a complex of chemical and visual cues when foraging for hosts and mates and interactions between these stimuli are likely very common (Domingue and Baker, 2012; Silk et al., 2015a, 2019a). For example, Hardersen et al. (2014) observed no apparent effect of trap height on detection of any buprestid species in an Italian forest when they used Malaise traps. However, others (e.g. Rassati et al., 2019) have found significantly greater species richness and abundance of *Agrilus* species in the canopy vs. the understory in green or purple Fluon-coated Lindgren multifunnel traps. Similarly, the combination of 3Z-lactone and Z-3-hexenol did not increase trap catches of male *A. planipennis* on purple sticky prism traps which were also baited with either Phoebe oil or green leaf volatiles, however, they do increase trap catches of male *A. planipennis* when used to bait green sticky prism traps placed in the canopy of ash trees. Conversely to male-biased catches in the canopy, catches of *A. planipennis* in the understory have been significantly female-biased (Lyons et al., 2009).



Edge effects - Purple prism traps captured significantly more *A. planipennis* when placed in an open field 15 m from a woodlot or along the woodlot edge, compared to 15 m inside the woodlot (Francese et al., 2008). Lyons et al., (2009) captured more *A. planipennis* on sticky bands placed on ash trees along the edges of woodlots than on trees within the woodlots. Other studies have also found greater species richness and abundance of buprestids in open fields or along the edge of forest stands compared to the forest interior (Wermelinger et al., 2007).

Visual stimuli – Trap design and colour

Much work on the effects of visual stimuli on *Agrilus* species has been done with the emerald ash borer, *A. planipennis*, by Francese et al., (2010a,b, 2011, 2013a,b) and Crook et al., (2009) who have shown through field assays and the use of electroretinograms that female *A. planipennis* are sensitive to wavelengths in the purple part of the spectrum and that males are sensitive to green and yellow wavelengths. They have also shown that green traps of wavelengths from 525 – 540 nm tend to catch more adult *A. planipennis* than traps of other colour wavelengths (Francese et al., 2010), especially when placed in the upper canopy of ash trees. Female *A. planipennis*, on the other hand, tend to be captured in greatest numbers on purple traps (Francese et al., 2010b). Trap design has also been found to be important with Francese et al. (2011, 2013b) and Crook et al. (2014) showing that multifunnel traps manufactured in colors based on these attractive green wavelengths, and coated with fluon are comparable in trap catch and detection to prism traps.

There are now several review papers looking at trapping approaches for emerald ash borer in particular, that provide an overview of the influence of factors such as trap design (sticky prism vs. multiple funnel traps vs. double-decker traps), trap colour, trap positioning in the tree canopy and effects of trap coatings (e.g. Fluon) (Petrice & Haack, 2015; Poland et al., 2019; Tobin et al., 2021). These trapping approaches have now started to be tested and adapted for wider *Agrilus* surveillance and monitoring programmes. For example, in Europe Rassati et al. (2019) showed that green traps captured a greater abundance of *Agrilus convexicollis* and *A. olivicolor* than did purple traps, and this preference of *A. convexicollis* and other European *Agrilus* species for green traps over other colours has been confirmed by several studies (Cavaletto et al., 2020; Sallé et al., 2020). In North America, 30 species of *Agrilus* were caught in green multifunnel traps, and



the genus in general showed a preference for green multifunnel traps over purple prism and multifunnel traps (Francese et al., 2016).

2. European perspectives on *Agrilus* detection in traps

While a substantial amount of work has been done on trapping for certain *Agrilus* species, particularly emerald ash borer (*A. planipennis*), focusing on chemical ecology and behavior, published information from Europe is relatively sparse. With the increasing concern that other *Agrilus* species could become invasive pests in the future, interest in developing surveillance methods for this genus in Europe has increased. Most studies reported here were carried out to test traps and lures specifically for *Agrilus* species, whilst some focused on other buprestids or other xylobiontic species.

Trap type

Several field trials have evaluated the most common trap types, such as sticky prism traps or Lindgren multi-funnel traps. A study in Spanish cork oak stands compared both trap types, with purple prism traps catching more *Coroebus undatus* than purple multi-funnel or sticky single panel traps (Fürstenau et al., 2015). Multi-funnel traps and panel traps used in several different studies evaluating the effect of trap colour on wood-boring beetles successfully attracted buprestid species including *Agrilus* and *Coroebus* species (Rassati et al., 2019; Cavaletto et al., 2020; Sallé et al., 2020).

Both flight interception (window) traps and yellow pan traps were used to study xylobiontic beetles in Switzerland, and the pooled trap catches from these two trap types suggested that there were good catches of Buprestidae, with good numbers of *Agrilus viridis*, *A. olivicolor* and *A. convexicollis* captured (Wermelinger et al., 2007). However, no details of which trap design actually captured the Buprestidae are given. Similarly, flight interception traps were also used in a study of flight activity of *Agrilus viridis* in South Germany (Brück-Dyckhoff et al., 2017).

There have also been attempts to develop novel trap designs for buprestids, particularly *Agrilus* species. Green plastic branch traps (i.e. sticky panels mounted to a branch in the crown of an oak



tree) were successfully used in field experiment in oak forest in Hungary testing several other trap types, with these trap designs accounting for 75% of captured buprestid specimens (Domingue et al., 2013). In another field experiment in Hungary, a novel construction of a multi-funnel trap (differing from the widely used type of Lindgren multi-funnel trap) gave catches comparable to conventional sticky traps (Imrei et al., 2020). The authors highlight the advantage of a non-sticky trap for operation in the field as well as for morphological determination of beetles.

Trap color

Overall, green colors turn out to be most promising for catching *Agrilus* species in European field studies, but there are some indications for species-specific differences. Green multifunnel traps caught significantly more individual Buprestidae and Agrilini specimens as well as more species than purple multifunnel traps in an experiment in Italy, with the most abundant species, *A. convexicollis* and *A. olivicolor* primarily caught in green traps (Rassati et al., 2019). Similar results were observed in France, with a marked preference of several *Agrilus* and *Coroebus* species (*A. angustulus*, *A. biguttatus*, *A. hastulifer*, *A. laticornis*, *A. olivicolor*, *A. viridis*, and *C. undatus*) for green multifunnel traps over purple ones (Sallé et al., 2020; Sallé et al., unpublished data). The preference of *A. convexicollis* and other European *Agrilus* spp. (i.e. *A. angustulus*, *A. biguttatus*, *A. graminis*, *A. hastulifer*, *A. laticornis*) for green traps over other colors was confirmed by Cavaletto et al. (2020). Green color was also important for catches of *Agrilus* species in a field experiment in Hungary (Domingue et al., 2013).

Yellow sticky traps were used to trap *Agrilus* species in hazelnut orchards, with seven species being caught, and *A. olivicolor* was the most abundant species captured (Corte, 2009). Yellow pan traps were used in combination with flight traps to evaluate xylobiontic insects in Switzerland and Germany, with several species of *Agrilus* captured (Wermelinger et al., 2007). In neither of these cases, comparisons to other colors were made, and it is unclear from the Wermelinger et al. (2007) study which trap type actually captured the *Agrilus* species.

None or very few catches of *Agrilus* were made with transparent or purple traps in a study in an oak forest in Hungary (Imrei et al., 2020). Species specific preferences were reported by Rhainds et al. (2017), with the most *A. convexicollis* specimens (> 95 %) being caught on green prism



traps while significantly more *A. viridis* were caught on purple prism traps. Similarly, purple prism traps (used with green leaf volatiles) have been reported as being more effective at capturing *C. undatus* (Fürstenau et al., 2015).

Catches of *Agrilus* in black traps used for other xylobiontic beetles are generally very low (see below).

Decoys

Visual cues are important for *Agrilus* species. Besides color, male beetles searching for mating partners also orient towards the shape of beetles. The importance of visual mate location behavior was ascertained in field observation studies in Hungary for *A. biguttatus*, *A. angustulus*, and *A. sulcicollis* (Domingue et al., 2011). This study also highlighted substantial cross-attraction among the species. In other field experiments, *A. biguttatus*, *A. sulcicollis* and *A. angustulus* showed visually mediated approaches toward dead, pinned beetle models. Male mate-finding behavior depended on visual location of females on foliage or green plastic background (Imrei et al., 2020). Therefore, using decoys may be an option to increase the attractiveness of traps. Using emerald ash borer visual decoys pinned on to green traps often increased trap catches particularly for *A. biguttatus* in Hungary (Domingue et al., 2013).

Olfactory cues

Several studies have tested natural plant volatiles or synthetic blends of compounds from leaves in laboratory and field trials. Green leaf volatiles (natural blend or synthetic (E)-2-hexenol, 1-hexanol, and (Z)-3-hexenyl acetate) were attractive for *C. florentinus* in olfactometer experiments (Fürstenau et al., 2012). Similarly, a mixture of green leaf volatiles combined in a lure and used with purple prism traps increased trap catches of adult *C. undatus* in a trapping experiment in cork oak stands (Fürstenau et al., 2015). Laboratory olfactometer studies have shown attraction of *A. biguttatus* virgin females and males to odor from oak leaf material. In this study, males and females positively responded to synthetic blends of EAG-active compounds from oak foliage, and gravid females responded to odor from bark or synthetic blends of bark compounds (Vuts et al., 2016).



Small but significant effects of olfactory cues were found in a field experiment in Hungary for *A. angustulus* (catches in (Z)-9-tricosene traps were greater than on unscented traps or the other odor treatments) and *A. sulcicollis* (all tested odor lures – manuka oil, (Z)-3-hexen-1-ol, or (Z)-9-tricosene – had significantly higher captures than the control) (Domingue et al., 2013). Trials in Slovakian beech and poplar forests found that either there was no effect of using lures (cubeb oil or 3Z-hexenol) on trap catches of *Agrilus* species, or that purple prism traps baited with a cubeb oil lure increased catches of female *A. viridis* (Rhainds et al., 2017).

***Agrilus* species as bycatch in traps for other xylobiontic insects**

In general, low numbers of Buprestidae, particularly *Agrilus* species, tend to be captured in trials that focus predominantly on trapping for Scolytinae or Cerambycidae using black traps. Low numbers of buprestids were reported from bark beetle traps with the *Ips typographus* lure Pheroprax, with only 145 specimens being captured in 46 traps over an eight-year period in Slovakia, and the only *Agrilus* species caught was *A. cyanescens* (Zach, 1997). *Agrilus graminis* was the only *Agrilus* species caught in black multi-funnel traps baited with alpha-pinene, bark beetle pheromone components and/or *Monochamus* (Cerambycidae) pheromone in an experiment in mixed spruce-beech forest in Austria (Halbig, 2013; Halbig et al., 2014). Black multi-funnel and cross-vane traps baited with cerambycid pheromone blends, alpha pinene and ethanol were tested for trapping cerambycids in Austria (Hoch et al., 2020). Although no data on other families were reported in the paper, the unpublished data from this study showed that there were only low numbers of buprestids captured and no *Agrilus* specimens were trapped in either a pine forest or a mixed hardwood riparian forest (Hoch et al., unpublished data). Nonetheless, catches of *Agrilus* species can increase when blends developed for longhorn beetles are used on green traps (Rassati et al., 2019; Cavaletto et al., 2020).

Table 1. List of olfactory stimuli (individual host volatiles and blends, pheromones, and combination thereof) and behavioral responses elicited in Buprestidae in laboratory olfactometer and/or field trapping bioassays.

Plant volatiles	Context	Species/sex	Olfactometer /lab bioassays	References	Trapping bioassays	References
Ethanol	synthetic	Buprestidae			neutral	Montgomery and Wargo 1983; Dunn et al., 1986b
Ethanol		<i>A. bilineatus</i>			neutral	Dunn et al., 1986b
Z-3-hexenol	Alone or added to other green leaf volatiles on purple or green sticky prism traps	<i>A. planipennis</i> ♂♀ (male biased)	positive	Silk et al., 2011;	positive	De Groot et al., 2008; Grant et al., 2010, 2011; Ryall et al., 2012
Z-3-hexenol	1M conc. in n-hexane, 5 ul on filter paper	<i>Capnodis tenebrionis</i> ♀	positive	Bari et al., 2019		
Z-3-hexenyl acetate		<i>A. planipennis</i>			neutral	Grant et al., 2010
Z-3-hexenyl acetate	synthetic	<i>C. florentinus</i> ♀	positive	Fürstenau et al., 2012		
n-hexyl acetate	synthetic	<i>C. florentinus</i> ♀	neutral	Fürstenau et al., 2012		
1-hexanol	synthetic	<i>C. florentinus</i> ♀	positive	Fürstenau et al., 2012		
E-2-hexanal	synthetic	<i>C. florentinus</i> ♀	neutral	Fürstenau et al., 2012		
		<i>A. planipennis</i>			neutral	de Groot et al., 2008

Z-3-hexenal		<i>A. planipennis</i>			neutral	Grant et al., 2010
<i>E</i> -2-hexenol	synthetic	<i>C. florentinus</i> ♀	positive	Fürstenau et al., 2012		
<i>E</i> -2-hexenol		<i>A. planipennis</i>			neutral	De Groot et al., 2008
2-hexanone	1M conc in n-hexane, 5 ul on filter paper	<i>C. tenebrionis</i> ♂♀	negative	Bari et al., 2019		
Nonanal	synthetic	<i>C. florentinus</i> ♂ <i>C. florentinus</i> ♀	neutral neutral	Fürstenau et al., 2012		
Decanal	synthetic	<i>C. florentinus</i> ♂ <i>C. florentinus</i> ♀	positive neutral	Fürstenau et al., 2012		
geranylacetone	synthetic	<i>C. florentinus</i> ♂ <i>C. florentinus</i> ♀	neutral positive	Fürstenau et al., 2012		
conophthorin	synthetic	<i>A. anxius</i> ♂♀			neutral	Silk et al., 2019b
3-methyl butanol	synthetic	<i>C. tenebrionis</i> ♂♀	positive	Bari et al., 2019		
Benzaldehyde	1M conc in n-hexane, 5 ul on filter paper	<i>C. tenebrionis</i> ♀	negative	Bari et al., 2019		
S-limonene	1M conc in n-hexane, 5 ul on filter paper	<i>C. tenebrionis</i>	negative	Bari et al., 2019		
Blend of Z-3-hexen-1-ol and <i>E</i> -2-hexenol	synthetic	<i>A. anxius</i> ♂♀			neutral	Silk et al., 2019b
Blend of nonanal, decanal, geranylacetone (1:1:1)	synthetic	<i>C. florentinus</i> ♂♀	positive	Fürstenau et al., 2012		
Blend of nonanal, decanal, geranylacetone (1:1:1)	synthetic	<i>C. undatus</i> ♀			neutral	Fürstenau et al., 2015
Blend of 4 green leaf volatiles: Z-3-hexenol <i>E</i> -2-hexanol	synthetic	<i>A. planipennis</i> ♂♀			positive (as attractive)	Poland et al., 2007

<i>E</i> -2-hexenal hexenal					as Manuka oil)	Crook et al., 2008
Blend of 5 green leaf volatiles: <i>E</i> -2-hexenal <i>E</i> -2-hexanol 1-hexenol <i>Z</i> -3-hexenyl acetate <i>n</i> -hexyl acetate (1:1:1:1:1)	synthetic	<i>C. florentinus</i> ♂ <i>C. florentinus</i> ♀	neutral positive	Fürstenau et al., 2012		
Same 5 compounds as above in ratio found in headspace volatiles from freshly cut branches of <i>Quercus suber</i> (cork oak), i.e., 93:100:51:66:11, dissolved in 2.5 ml ethanol vs. ethanol alone. The 5GLV blend was released from a 55 cm x 0.6 cm OD semipermeable thermoplastic elastomer tubing sealed at both ends; RR by weight loss at 25C was 0.3-2.6 mg/d	synthetic	<i>C. undatus</i>			positive (dry-cup Lindgren funnels & sticky purple prism traps, 1.5-2 m off the ground)	Fürstenau et al., 2015 (also found that sticky prism purple traps captured more than panel traps which caught more than dry cup 8-funnel Lindgrens)
Manuka oil (contains sesquiterpenes α -cubebene, α -copaene, α -humulene, <i>trans</i> -caryophellene, also emitted by stressed ash)	natural extract	<i>A. planipennis</i> ♂♀			positive	Crook et al., 2008 Grant et al., 2010
Manuka oil		<i>A. anxius</i> ♂♀			neutral	Silk et al., 2019b
Phoebe oil (shares the same sesquiterpenes that Manuka oil shares with stressed ash, plus 7-epi-sesquithujene)	natural extract	<i>A. planipennis</i> ♂ <i>A. planipennis</i> ♂♀	positive (low dose) negative (high dose)	Silk et al., 2011	neutral positive positive	Grant et al., 2011 Crook et al., 2008; Silk et al., 2011

		<i>A. anxius</i> ♂♀			neutral	Silk et al., 2019b
Blend 1 of EAG-active oak leaf volatiles Z-3-hexen-1-ol Z-2-hexanal Z-3-hexenyl acetate Z-ocimene E-ocimene Linalool oxide (E)-4,8-dimethyl-1,3,7-nonatriene m-ethylacetophenone	synthetic blend at same relative ratios detected in <i>Q. robur</i> foliage	<i>A. biguttatus</i>	positive	Vuts et al., 2016		
Blend 2 of EAG-active oak leaf volatiles: Z-3-hexen-1-ol Z-2-hexanal Z-3-hexenyl acetate	synthetic blend at same relative ratios detected in <i>Q. robur</i> foliage	<i>A. biguttatus</i>	positive	Vuts et al., 2016		
Blend of EAG-active oak bark volatiles: p-cymene 1,8-cineole E-ocimene γ-terpinene, (R/S)-camphor	synthetic blend at same relative ratios detected in <i>Q. robur</i> bark	<i>A. biguttatus</i>	positive	Vuts et al., 2016		
Foliar headspace volatiles	<i>Quercus robur</i>	<i>A. biguttatus</i>	positive	Vuts et al., 2016		
Bark headspace volatiles	<i>Quercus robur</i>	<i>A. biguttatus</i>	positive	Vuts et al., 2016		
leaf extract	<i>Quercus suber</i>	<i>C. florentinus</i> ♂ <i>C. florentinus</i> ♀	neutral positive	Fürstenau et al., 2012		
volatiles from cut branches	<i>Quercus suber</i>	<i>C. florentinus</i> ♂ <i>C. florentinus</i> ♀	neutral positive	Fürstenau et al., 2012		

Live seedlings treated with methyl jasmonate or fed upon by <i>A. planipennis</i>	<i>Fraxinus mandshurica</i>	<i>A. planipennis</i> ♀ <i>A. planipennis</i> ♂	positive neutral	Rodriguez-Saona et al., 2006		
Live female <i>C. florentinus</i>		<i>C. florentinus</i> ♂ <i>C. florentinus</i> ♀	positive neutral	Fürstenau et al., 2012		
Abdominal extract of female <i>C. florentinus</i>	e.g., containing nonanal, decanal and geranylacetone	<i>C. florentinus</i> ♂ <i>C. florentinus</i> ♀	positive neutral	Fürstenau et al., 2012		
Stressed (girdled) <i>Quercus alba</i>	sticky bands around trunks	<i>A. bilineatus</i>			positive	Dunn et al., 1986a
Steam distillate from girdled <i>Q. alba</i>	sticky bands + clear cross vane traps	<i>A. bilineatus</i>			positive	Dunn et al., 1986b
Stressed (girdled) <i>Fraxinus</i> species	sticky bands around trunks	<i>A. planipennis</i>			positive	McCullough et al., 2009a,b; Mercador et al., 2013
Stressed (girdled) <i>Betula papyrifera</i>	purple sticky prism traps 2 m height on host trees	<i>A. anxius</i> ♂♀			positive	Silk et al., 2019b
Pheromones						
Live female <i>Agrilus bilineatus</i>	live females	<i>A. bilineatus</i> ♂			positive*	Dunn and Potter 1988
Live female <i>A. planipennis</i>	live females	<i>A. planipennis</i> ♂ with intact antennae found faster than males with painted antennae; blinded males were just as fast as untreated males	positive	Pureswaran and Poland (2008)		
Z-3-lactone	when combined with Z-3-hexenol on green traps placed in the host tree canopy	<i>A. planipennis</i> ♂	neutral	Silk et al., 2011	positive	Silk et al., 2011, 2015b Ryall et al., 2012, 2013

						Domingue et al., 2016
Z-3-lactone	when combined with Z-3-hexenol on green branch traps with dead female conspecifics	<i>A. planipennis</i> ♀			positive	Domingue et al., 2016
Z-3-lactone	alone	<i>A. planipennis</i> ♂	neutral	Silk et al., 2015b	neutral	
<i>E</i> -3-lactone	alone	<i>A. planipennis</i> ♂ <i>A. planipennis</i> ♀	positive neutral	Silk et al., 2011, Silk et al., 2015b		
12-dodecanolide (saturated analog of Z-3-lactone)	alone	<i>A. planipennis</i> ♂ ♀	positive	Silk et al., 2015b		
12-dodecanolide (saturated analog of Z-3-lactone)	combined with Z-3-hexenol on green traps in the host tree canopy	<i>A. planipennis</i> ♂			positive	Silk et al., 2015b

*attraction of males to live females may have been olfactory or auditory

Table 2. Effect of trap position on detection of jewel beetles (Buprestidae) in traps with particular emphasis on *Agrilus* species

Trap position	Species/sex	References
Canopy > Understory	<i>Agrilus planipennis</i> ♂♀	Francese et al., 2008 (purple sticky traps ; female biased);
	<i>Agrilus planipennis</i> ♂	Lelito et al., 2008 (green leaf sticky traps)
	<i>Agrilus viridis</i>	Wermelinger et al., 2007
	<i>Agrilus convexicollis</i>	Vodka and Cizek 2013
	Buprestidae abundance/richness	Rassati et al. (2019), Sallé et al. (2020)
	Agrilinae abundance/richness	Rassati et al. (2019), Sallé et al. (2020)
Canopy = Understory	<i>Agrilus olivicolor</i>	Wermelinger et al., 2007
	<i>Anthaxia quadripunctata</i>	Wermelinger et al., 2007
	Buprestidae abundance	Ulyshen and Hanula 2007 (clear cross-vane traps)
Understory > Canopy	<i>Anthaxia nitidula</i>	Wermelinger et al., 2007
	<i>Anthaxia helvetica</i>	Wermelinger et al., 2007
	<i>Trachys minuta</i>	Wermelinger et al., 2007
No effect of trap height		
Open field > Edge > Interior	<i>Agrilus planipennis</i>	Francese et al., 2008, McCullough et al., 2009a
	Buprestid species richness & abundance	Wermelinger et al., 2007
	<i>Anthaxia nitidula</i>	Wermelinger et al., 2007

	<i>Anthaxia quadripunctata</i>	Wermelinger et al., 2007
Interior = Edge > Open field	<i>Agrilus viridis</i>	Wermelinger et al., 2007
No edge effect		
Sun-exposed > Shaded	<i>Capnodis tenebrionis</i>	Bonsignore and Jones 2013
	<i>A. planipennis</i>	McCullough et al., 2009

Table 3. List of visual stimuli (colors) and trap designs found to be attractive to Buprestidae with an emphasis on *Agrilus* spp.

Visual Stimulus / Color	Trap Design	Species/sex	References
Red, Magenta, Purple	“wallpaper” strip	16 species of buprestids inc. <i>Acmaeodera tubulus</i> <i>Acmaeodera</i> spp. <i>Agrilus obsoletoguttatus</i> <i>Agrilus</i> spp. <i>Anthaxia quercata</i> <i>Anthaxia viridifrons</i> <i>Chrysobothris adelpha</i> <i>Chrysobothris azurea</i> <i>Chrysobothris femorata</i> complex <i>Chrysobothris pusilla</i> <i>Chrysobothris sexsignata</i>	Oliver et al., 2002
Yellow	Multz trap	<i>Agrilus</i> spp.	Imrei et al., 2020
Purple	“Box” – 4-sided corrugated plastic	<i>Agrilus planipennis</i>	Francese et al., 2005
Purple	Prism	<i>Agrilus planipennis</i>	Francese et al., 2008
Purple	Prism	<i>Agrilus auroguttatus</i>	Coleman et al., 2014

Purple	Prism	<i>Agrilus viridis</i> <i>Coroebus undatus</i>	Rhains et al., 2017 Fürstenau et al., 2015
Green	Prism		Crook et al., 2009 Francese et al., 2010a 2010b, 2013a Tobin et al., 2021
Green	Prism	<i>Agrilus convexicollis</i>	Rhains et al., 2017
Purple	Double-decker trap (two purple prism traps hung on a 3m pole)	<i>Agrilus planipennis</i>	Poland et al., 2011 Tobin et al., 2021
Green	Double-decker trap (two green prism traps)	<i>Agrilus planipennis</i>	Poland et al., 2019
Green/Purple	Double decker trap (upper green prism, lower purple prism)	<i>Agrilus planipennis</i>	Poland et al., 2019
Yellow	Sticky trap	<i>Agrilus olivicolor</i>	Corte, 2009
Green	Multifunnel	<i>Agrilus planipennis</i>	Francese et al., 2011, 2013 Crook et al., 2014 Poland et al., 2019 Tobin et al., 2021
Green	Multifunnel	<i>Agrilus cephalicus</i> <i>Agrilus lecontei</i> <i>Agrilus obsolettoguttatus</i> <i>Ptosima gibbicollis</i>	Skvarla and Dowling, 2017 Tobin et al., 2021
Green	Multifunnel	<i>Agrilus</i> spp. (includes 31 species, but excludes <i>A.</i> <i>planipennis</i>) <i>Anthaxia</i> spp. (4 species)	Francese et al., 2016
Green	Multifunnel	<i>Agrilus convexicollis</i>	Rassati et al., 2019



		<i>Agrilus olivicolor</i> <i>Agrilus angustulus</i> <i>Agrilus biguttatus</i> <i>Agrilus graminis</i> <i>Agrilus hastulifer</i> <i>Agrilus laticornis</i> <i>Agrilus olivicolor</i> <i>Agrilus viridis</i>	<p>Sallé et al., unpublished data Sallé et al., 2020</p>
Purple	Multifunnel	<i>Chrysobothris</i> spp. (as many as 5 species) <i>Dicerca</i> spp. (as many as 7 species) <i>Chrysobothris affinis</i> <i>Coroebus undatus</i>	<p>Francese et al., 2016</p> <p>Sallé et al., unpublished data (from Sallé et al., 2020)</p> <p>Fürstenau et al., 2015</p>
Purple	'Lindgren funnel' (multifunnel)	<i>Dicerca lurida</i> <i>Dicerca obscura</i> <i>Ptosima gibbicollis</i>	Skvarla and Dowling, 2017
emerald ash borer 'visual Decoy' (dead, pinned insect)		<i>Agrilus planipennis</i>	<p>Lelito et al., 2007 Domingue et al., 2013</p>
emerald ash borer 'visual decoy' (fabricated 3D model)		<i>Agrilus planipennis</i>	Domingue et al., 2014, 2015
emerald ash borer 'visual Decoy' (nanofabricated model)	Electrocution trap	<i>Agrilus planipennis</i>	Domingue et al., 2014
emerald ash borer 'visual decoy' (fabricated 3D model)	Electrocution trap	<i>Agrilus planipennis</i>	Domingue et al., 2014



Green Yellow	Corrugated plastic sticky card	<i>Agrilus egenus</i> <i>Agrilus subsinctus</i>	Petrice and Haack, 2015
Yellow	Panel trap	<i>Anthaxia thalassophila</i>	Cavaletto et al., (2020)
Purple	Panel trap	<i>Chrysobothris affinis</i>	Cavaletto et al., (2020)
Green	Panel trap	<i>Agrilus convexicollis</i> <i>Agrilus angustulus</i> <i>Agrilus biguttatus</i> <i>Agrilus graminis</i> <i>Agrilus hastulifer</i> <i>Agrilus laticornis</i> <i>Lamprodila mirifica</i>	Cavaletto et al., (2020)

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3.3. WP3 & WP4 – European & North American trapping trials – evaluating effectiveness of traps and lures in capturing *Agrilus* species

WP3.1 & WP4.1 – standardised trapping approaches in 2021 & 2022

Introduction

Since the *Agrilus* genus has over 3000 known species there is somewhat of an inevitability that in response to the ever-expanding global trade in resources and commodities, and changing climate patterns, there will be an increase in the frequency with which *Agrilus* species will be intercepted in new locations around the world. Hence, understanding what trapping approaches could be utilised for detecting these wood-boring insects and monitoring their spread is a vital first step in establishing national invasive insect monitoring programmes. The development of trapping protocols for the now infamous emerald ash borer (*Agrilus planipennis*) has focused primarily on the use of two trap designs, sticky prism traps and multifunnel traps, with traps of both green and purple colouration being predominantly used. Subsequent studies using these two trap designs for assessing other *Agrilus* species is ongoing in numerous countries, with researchers citing benefits and disadvantages to both trap designs.

Within the current Euphresco project we aimed to assess the effectiveness of both green sticky prism traps and green (Fluon coated) multifunnel trap designs (both with and without the green leaf volatile 3Z-hexenol) in catching *Agrilus* beetles within oak woodlands/forests. This was to gather baseline evidence to establish whether one trap design may be more effective at capturing *Agrilus* beetles in general, and hence contribute to NPPO surveillance and monitoring approaches for the early detection of these wood-boring insects.

The principal aims of the 2021 and 2022 trials were to establish these two trap designs in oak forests to evaluate *Agrilus* species abundance and diversity, and specifically to:

1. Evaluate the total trap catch of all *Agrilus* species captured by both trap types – which trap type catches the most *Agrilus* beetles? Does 3Z-hexenol influence trap catches?

2. Assess the *Agrilus* species diversity captured in the two trap types – does one trap type catch more *Agrilus* species than the other? Are trap catches dominated by one species? How effective are traps at capturing rarer species?

A. Material & Methods for 2021 field trial – trap design trial

This trial aimed to evaluate the effectiveness of green sticky prism traps and green fluon-treated multifunnel (12 funnels) traps at capturing *Agrilus* beetles with no green leaf volatile lures used. Woodlands/forests selected for use in the trial were oak-dominant locations, and within each oak woodland/forest site 10x prism traps and 10x multifunnel traps were deployed. Traps were deployed in the mid to upper canopy of oak trees (ideally at 10m or higher), no closer than 20-25m to one another, and preferably in an open, sunny part of the canopy, with an emphasis on trying to avoid positioning traps in extreme shade or under branches (Figure 1). In addition, it was important to avoid placing traps near areas where a lot of dust and debris could reduce the stickiness of the glue trap. The height of the trap from the ground and the dbh of each trapped tree was measured. The collecting cup of the multi-funnel traps was filled with approximately 200ml of 50% propylene glycol solution (alternatively if propylene glycol was not available a saturated salt solution was used, or ventilated cups for dry trapping supplied with an insecticide net). Traps were established in mid to late May, with collections from traps being made every two or three weeks, and the traps were run until mid to late August to cover the main flight period of *Agrilus* species (i.e. 6-8 collections). The contents of the collecting cups from the multifunnel traps were transferred to labelled pots and transported back to the laboratory for further analysis (the trap collection pot was reset with fresh solution following each collection). The sticky 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 pot. All *Agrilus* species captured within each of the twenty traps were tallied and identified to species (using morphological keys), with all data updated onto spreadsheets so that it was possible to identify what each individual trap captured, the height of the trap, and dbh of the tree in which it was placed. The tree composition of the woodland/forest used was also recorded (i.e. pure stand of oak, broadleaved forest dominated with oak and beech/birch/ash etc), and the percentage oak composition was estimated.

B. Material & Methods for 2022 field trial – trap design + green leaf volatile trial

The experimental set-up in 2022 was comparable to the 2021 trial and was focused again within oak woodlands, with traps being set-up exactly as highlighted above, but with an additional 10x prism and 10x multifunnel traps being deployed each with a 3Z-hexenol lure attached. This enabled us, to once again, evaluate trap design on *Agrilus* captures for a second year, but also allowed us to determine whether the addition of the green leaf volatile, 3Z-hexenol, had any influence on trap catches of *Agrilus* beetles. Traps were deployed in the mid to upper canopy of oak trees (ideally at 10m or higher), and ideally in an open, sunny part of the canopy, with an emphasis on trying to avoid positioning traps in extreme shade or under branches (Figure 1). The collecting cup of the multi-funnel traps was filled with approximately 200ml of 50% propylene glycol solution (or fully saturated salt solution). Traps were again set-up in mid to late May 2022 with collections from traps being made every two or three weeks, and the traps were run until mid to late August to cover the main flight period of *Agrilus* species (i.e. 6-8 collections).

Figure 1. Green sticky prism and multifunnel traps were installed in open, sun exposed parts of the canopy of oak trees.



All traps used in the above 2020 & 2021 trials were purchased from the same supplier to standardise the approach in each country, hence green fluon coated multifunnel traps (12 funnel) were purchased from ChemTica Internacional (<http://www.chemtica.com/>), and green sticky prism traps were purchased from Sylvar Technologies Inc. (<https://www.sylvar.ca/>). This company is based in Fredericton, Canada, however they are now part of the Andermatt group, so they have distributors in Europe (France, Switzerland, UK). The 3Z-hexenol lure pouches were purchased from Synergy Semiochemicals Corporation (Canada) and had a release rate of 100mg/day at 30°C (with an estimated release rate decrease of 50% with every 5°C drop in temperature, so 50mg/day at 25°C and 25mg/day at 20°C, M. Jones pers. comm.).

C. Material & Methods for 2022 field trial - *Agrilus* decoy trial

The key objective of this trial was to establish whether adult *Agrilus* individuals (decoys) glued to the surface of green panel traps could be used to increase trap catches, when compared to traps without decoys. The experimental design was again comparable to the above protocols in the sense that traps were deployed in mid-May and were set in the mid to upper canopy of oak trees. The traps used in this decoy trial were the green Multitrap panel traps supplied by Synergy Semiochemicals Corporation (<https://semiochemical.com/synergy-multitrap-platform/#tab-id-4>). Each participating collaborator in this trial glued 3 individual *Agrilus* beetles onto each panel surface of the green panel traps (so 18 *Agrilus* beetles/trap), with the most common *Agrilus* beetle captured in the 2021 field trial in each respective country used as the decoy:

Italy – decoy = *Agrilus olivicolor*

France – decoy = *Agrilus laticornis*

UK – decoy = *Agrilus laticornis*

Poland – decoy = *Agrilus biguttatus*

Canada – decoy = *Agrilus crinicornus*

USA – decoy = *Agrilus bilineatus*

Both decoy and non-decoy traps were again deployed in the canopy of oak trees, at a height of 10m or higher, again in open, sunny parts of the canopy in spring 2022. The collecting cup of the panel traps was filled with approximately 200ml of 50% propylene glycol solution (alternatively if propylene glycol was not available a saturated salt solution was used). Traps were established in mid to late May, with collections from traps being made every two or three weeks, and the traps were run until mid to late August to cover the main flight period of *Agrilus* species (i.e. 6-8 collections). The contents of the collecting cups from the panel traps were transferred to labelled pots and transported back to the laboratory for further analysis (the trap collection pot was reset with fresh solution following each collection). All *Agrilus* species captured within each trap were tallied and identified to species, with all data updated onto spreadsheets.

A. Results for 2021 trap design trial

The initial analysis from the 2021 trial suggested that both trap designs are suitable for use in detecting *Agrilus* species within oak woodland/forest settings (Table 4). No clear trend emerged, since in some countries/states multi-funnel traps outperformed prism traps in terms of total *Agrilus* captured, whilst in other countries/states the opposite was observed (Table 4). Most trap catches in any given location were dominated by 1-3 *Agrilus* species, and these were not always oak associated species. This was primarily because traps were deployed in mixed broad leaved forest locations where oak dominated but other tree species were also present.

Overall, across all locations, the prism traps caught more adult *Agrilus* beetles (n = 7114) than multifunnels (n = 3477), but the total number of species captured did not differ between the two trap types, with both trap types each capturing 22 *Agrilus* species (Table 5) (although not all *Agrilus* beetles were identified to species level).

Several issues were noted by collaborators from the year 1 (2021) trial – Firstly there does seem to be a general lack of ‘good’ morphological identification keys for *Agrilus* species. An attempt has been made therefore to collate together sources of information from Europe, North America and Asia to aid in the morphological identification of *Agrilus* species (Appendix 2). Secondly, there were considerable differences in opinion on the ‘useability’ of the two main trap types used in the 2021 and 2022 trial (sticky prism traps vs multifunnel traps). Hence, project collaborators combined their experiences and opinions on the two trap designs into a

pros and cons table (Appendix 3), to help inform future users. Although it would be fair to state that both trap designs will likely have a role to play in early detection, monitoring and surveillance programmes for *Agrilus* species.

Further analysis will be undertaken as soon as the full data set becomes available and will be incorporated into a journal paper.

Table 4. Total number of adult *Agrilus* captured in each trap design in each location from the 2021 Euphresco trial – multifunnels vs prism traps (yellow shading highlights highest trap catches in a given location).

Country ¹	Multifunnels ²	Prism traps ²	Total (total no. adults captured)
Austria (n=10)	325 (8 species)	529 (11 species)	854 – 11 species
Canada (n=10)	652 (5 species)	475 (6 species)	1127 – 6 species
France (n=9)	1409 (13 species))	3960 (12 species)	5369 - 13 species
Germany (n=10)	375 (8 species)	135 (8 species)	510 – 8 species
Italy (n=10)	123 (5 species)	662 (9 species)	785 - 9 species
Slovenia (n=5)	173 (8 species)	68 (5 species)	241 - 9 species
UK (n=10)	12 (1 species)	276 (2 species)	288 - 2 species
USA (MA) (n=10)	264 (7 species)	95 (5 species)	359 – 7 species (provisional data) ³
USA (OH) (n=10)	144 (9 species)	914 (13 species)	1058 – 14 species (provisional data) ³
Total	3477	7114	10591 – (at least 33 species)

¹ n = number of each trap design deployed in 2021

² Total number of adult *Agrilus* captured in traps and the number of *Agrilus* species captured (in brackets)

³ Not all *Agrilus* specimens were identified to species

Table 5. The *Agilus* species captured in 2021 trial – multifunnels (m) vs prism traps (p)

European Species	UK	Germany	Italy	France	Slovenia	Austria
<i>A. angustulus</i>	-	m & p	p	m & p	m	m & p
<i>A. biguttatus</i>	-	m & p	-	m & p	m	-
<i>A. convexicollis</i>	-	m & p	m & p	m & p	p	m & p
<i>A. curtulus</i>	-	-	-	m & p	-	-
<i>A. derasofasciatus</i>	-	-	-	m	-	p
<i>A. graecus</i>	-	-	-	m & p	-	-
<i>A. graminis</i>	-	-	m & p	m & p	-	m & p
<i>A. hastulifer</i>	-	-	m & p	m & p	m	m & p
<i>A. laticornis</i>	m & p	m & p	m & p	m & p	m & p	m & p
<i>A. litura</i>	-	-	p	-	-	m & p
<i>A. obscuricollis</i>	-	m & p	p	m & p	m & p	m & p
<i>A. olivicolor</i>	-	m & p	m & p	m & p	m & p	p
<i>A. sulcicollis</i>	m & p	m & p	-	m & p	m & p	m & p
<i>A. viridis</i>	-	m & p	p	m & p	-	p
Unidentified spp.	-	-	-	-	m	-

N. American species	Canada	USA (MA)	USA (OH)
<i>A. anxius</i>	m & p	-	-
<i>A. arcuatus</i>	m & p	m & p	m & p
<i>A. atricornis</i>	-	-	p
<i>A. benjamini</i>	-	-	p
<i>A. bilineatus</i>	m & p	m & p	-
<i>A. celti</i>	-	-	m & p
<i>A. cephalicus</i>	-	-	m & p
<i>A. crinicornis</i>	m & p	-	m
<i>A. ferrisi</i>	-	-	p
<i>A. frosti</i>	-	m	-
<i>A. geminatus</i>	-	-	p
<i>A. juglandis</i>	-	m	-
<i>A. lecontei</i>	-	-	p
<i>A. masculinus</i>	-	-	m & p
<i>A. obsoletoguttatus</i>	m & p	-	m & p
<i>A. otiosus</i>	-	-	m & p
<i>A. planipennis</i>	-	m & p	-
<i>A. politus</i>	p	-	-
<i>A. putillus</i>	-	-	m & p
Unidentified spp.	-	m & p	m & p

B. Results for 2022 trap design + leaf volatile trial

There were 11,392 adult *Agrilus* beetles captured in the 2022 field trial across both European and North American locations, and this was comprised of at least 32 *Agrilus* species (there may be other species within the 322 unidentified specimens). In general results from this 2022 field trial again highlighted that both trap types were suitable for use in trapping programmes for *Agrilus* beetles, however it was more apparent from the 2022 field trial that sticky prism traps were more effective at trapping *Agrilus* species both in general (Table 6), and at an individual species level (Table 7). Results from European locations in particular, really demonstrated the effectiveness of the sticky prism traps at capturing many of the *Agrilus* species, whereas the difference between the two trap types was not quite so apparent from the North American field sites. In fact, in some instances in North America some *Agrilus* species were caught more frequently in the multifunnel traps (Table 7).

The results from both Europe and North America also indicated that there was very little evidence that adding 3Z-hexenol lures to the traps improved trap catches of *Agrilus* beetles in general. Although from Europe there was perhaps a slight indication that catches of two individual species of *Agrilus* may potentially have been improved by the addition of the 3Z-hexenol to traps. For example, *A. olivicolor* and *A. hastulifer* catches were higher on prism traps when the 3Z-hexenol lures were present (although they weren't on the multifunnel traps), but this finding was primarily based on observations from the trap catches from France in 2022 (Table 7), hence further statistical analysis needs to be undertaken to evaluate this fully. Similarly, in North America *A. bilineatus* was caught slightly more frequently in multifunnel traps when the 3Z-hexenol lure was present (but not on sticky prism traps). Hence, further trapping trials would need to be conducted to confirm whether this specific green leaf volatile was effective at attracting these particular species to traps more frequently. From a wider perspective, it would seem more probable that each individual *Agrilus* species will respond to other host plants volatiles or a blend of host plant volatiles, rather than to 3Z-hexenol alone, which is somewhat of a ubiquitous green leaf volatile.

Interestingly, from the 2022 field trial established in Kentucky, North America, one of the prism traps picked up a solitary specimen of *Agrilus subrobustus*, which is an invasive species first detected in the US in Georgia in 2006 (Westcott, 2007). This particular *Agrilus* species is of East Asian origin and its only known host of mimosa (*Albizia julibrissin*) is a widely planted ornamental tree species in the southeast of the US. Whilst this *Agrilus* species is now known

from at least 5 US states (Swink *et al.*, 2015), it is nevertheless noteworthy that it was picked up within this particular field trial, where traps were predominantly deployed in oak dominant woodlands. This clearly highlights the benefits of undertaking surveillance and early detection trapping trials in a variety of woodland locations for native and invasive insect pest monitoring programmes and emphasises the effectiveness of these trap designs for *Agrilus* detection.

Table 6. Total number of *Agrilus* beetles captured (all species combined) in each trap and lure combination in a given location, from the 2022 field trial comparing multifunnel vs prism traps with and without a 3Z-hexenol lure. Yellow highlighted cells within a row highlight the highest trap catches within a given location. (MA = Massachusetts, KY = Kentucky).

Country	<u>Multifunnel traps</u>		<u>Prism traps</u>		Total	No. of <i>Agrilus</i> species
	No lure	+ lure	No lure	+ lure		
UK (n=10)	59	10	295	185	549	2
Sweden (n=7)	0	4	50	17	71	3
France (n=5)	15	29	622	921	1587	11
Italy (n=5)	98	68	420	747	1333	10
Austria (n=8)	369	540	2385	2227	5521	11+
Germany (n=5)	268	99	121	184	672	8
Slovenia (n=5)	60	104	183	123	470	14+
Canada (n=10)	41	68	79	52	240	7
USA (MA) (n=10)	77	81	18	9	185	6+
USA (KY) (n=10)	170	54	299	241	764	17+
Total	1157	1057	4472	4706	11392	

Table 7. Total number of each *Agilus* species captured in each trap/lure combination in the 2022 field trial. Data combined from UK, Sweden, France, Italy, Austria, Slovenia & Germany for European *Agilus* species (n=45 traps/treatment), and data combined for Canada and USA (Massachusetts and Kentucky) for North American *Agilus* species (n=30 traps/treatment).

<i>Agilus</i> species	Multifunnel	Multifunnel + 3Z-hexenol	Sticky prism	Sticky prism + 3Z-hexenol	Total
<u>European</u>					
<i>A. laticornis</i>	346	230	1362	954	2892
<i>A. angustulus</i>	218	307	1071	1070	2666
<i>A. olivicolor</i>	167	125	467	1245	2004
<i>A. obscuricollis</i>	34	75	587	563	1259
<i>A. sulcicollis</i>	37	47	199	129	412
<i>A. hastulifer</i>	10	7	138	224	379
<i>A. graminis</i>	15	14	83	68	180
<i>A. convexicollis</i>	21	11	48	45	125
<i>A. litura</i>	1	0	18	8	27
<i>A. viridis</i>	4	2	7	12	25
<i>A. biguttatus</i>	9	2	6	8	25
<i>A. curtulus</i>	0	6	3	3	12
<i>A. auricollis</i>	0	0	1	0	1
<i>A. cyanescens</i>	0	0	1	0	1
<i>A. croaticus</i>	0	1	0	0	1
<i>A. suvorovi</i>	0	1	0	0	1
Unidentified spp.	7	26	85	75	193
Total	869	854	4076	4404	10203
<u>North American</u>					
<i>A. geminatus</i>	70	14	130	105	319
<i>A. crinicornis</i>	32	39	58	25	154
<i>A. masculinus</i>	67	10	17	18	112
<i>A. celti</i>	3	4	46	48	101
<i>A. arcuatus</i>	28	44	16	8	96
<i>A. bilineatus</i>	18	41	14	10	83
<i>A. otiosus</i>	9	1	26	10	46
<i>A. lecontei</i>	5	7	17	16	45
<i>A. anxius</i>	0	3	6	15	24
<i>A. obsoletoguttatus</i>	8	6	4	5	23
<i>A. putillus</i>	1	1	15	5	22
<i>A. fallax</i>	1	1	1	6	9
<i>A. egeniformis</i>	0	0	2	6	8
<i>A. atricornis</i>	6	0	0	0	6
<i>A. egenus</i>	0	0	0	5	5
<i>A. planipennis</i>	1	0	1	0	2
<i>A. pensus</i>	0	0	2	0	2
<i>A. defectus</i>	1	0	0	0	1
<i>A. ferrisi</i>	0	0	1	0	1
<i>A. subrobustus</i>	0	0	1	0	1
Unidentified spp.	38	32	39	20	129
Total	288	203	396	302	1189

The field trial established in Portuguese cork-oak stands during 2022, using 20 green multifunnel traps, half of them with 3Z-hexenol lures, did not catch a single *Agrilus* specimen. The absence of catches was found to be related to the incorrect green colour of the trap, which had been purchased from an alternative European supplier (mistake of Portuguese supplier that bought from a different manufacturer than other Euphresco partners, despite precise instructions given), but also reveals the low effect of 3Z-hexenol to lure *Agrilus*. (Note: The European supplier of the alternatively coloured green multifunnel trap is in the process of redeveloping the traps and improving the green colouration to match the standard ChemTica traps).

C. Results for 2022 *Agrilus* decoy trial

In general, results from the 2022 'decoy' field trial indicated that affixing an *Agrilus* decoy beetle to the green panel traps did not improve trap catches of *Agrilus* species in general (Table 8). This was a little disappointing since previous studies that have used *Agrilus* decoys have found that in some instances they can increase trap catches (Lelito et al., 2008; Domingue et al., 2012, 2013). These previous decoy trials used emerald ash borer (EAB) (*Agrilus planipennis*) as the decoy, which is quite a large *Agrilus* species, so perhaps any subsequent trials of this nature should also focus on either using larger species of *Agrilus* as the decoy, or perhaps simply increasing the numbers of decoys used on the trap surface may also improve trap catches.

Similarly, there was very little evidence that the actual decoy *Agrilus* species glued to the trap in each location attracted greater numbers of conspecifics (Table 9). In hindsight an alternative approach could have utilised a standardised decoy used across all locations, in which case EAB decoys could have been used to attempt to replicate the Domingue et al. (2012, 2013) studies.

Table 8. Results from the 2022 trial using decoy *Agrilus* beetles (green interception traps with and without adult *Agrilus* decoys), highlighting the total number of *Agrilus* beetles captured (all species combined) at each location (MA = Massachusetts, OH = Ohio).

Country	Traps with decoy	Traps without decoy	Total	No. of <i>Agrilus</i> species
UK (n=10)	107	101	208	3
France (n=5)	504	738	1242	11
Italy (n=10)	194	137	331	8
Poland (n=10)	49	118	167	8
Canada (n=10)	41	37	78	5
USA (MA) (n=10)	20	20	50	2+
USA (OH) (n=10)	164	156	320	10+
Total	1079	1317	2396	

Table 9. Results from the 2022 trial using decoy *Agrilus* beetles (green interception traps with and without *Agrilus* decoy), and the total number of conspecifics (i.e. same species as the decoy) that were captured in each location (MA = Massachusetts, OH = Ohio).

Country	Decoy used	Decoy traps	No decoy traps	Total
UK (n=10)	<i>Agrilus laticornis</i>	107	99	206
France (n=5)	<i>Agrilus laticornis</i>	194	423	617
Italy (n=10)	<i>Agrilus olivicolor</i>	127	112	239
Poland (n=10)	<i>Agrilus biguttatus</i>	10	11	21
Canada (n=10)	<i>Agrilus crinicornis</i>	27	23	50
USA (MA) (n=10)	<i>Agrilus bilineatus</i>	2	2	4
USA (OH) (n=10)	<i>Agrilus celti</i>	17	59	76
Total		484	729	1213

Conclusions

The over-riding conclusions from the field trials conducted over the 2021-2022 seasons were that both trap designs were clearly effective tools for use as early detection, monitoring and surveillance approaches for adult *Agrilus* beetles, with perhaps the evidence suggesting that the sticky green prism traps outperform the green multifunnel traps. In addition, it also seemed that adding 3Z-hexenol, the green leaf volatile used in emerald ash borer monitoring and surveillance programmes in North America, did not improve trap catches of *Agrilus* in general trapping programmes within oak woodlands for these insects. Other plant volatiles may increase trap catches of target *Agrilus* species, but considerable effort working closely with chemical ecologists would be required to identify the key host plant volatiles that might be effective at an individual species level for invasive species in particular.

It is important to note that other trap designs are available, or are currently being developed, for trapping for *Agrilus* beetles and their effectiveness should be evaluated in fully replicated trials alongside the two trap designs evaluated within this Euphresco work package. The complete absence of *Agrilus* catches in the trial established in Portugal, where multifunnel traps of a different green colour were used, reinforces the significant importance of visual stimuli, and demonstrates the importance of fully testing new trap designs with effective ones.

There was very little evidence from the decoy trial conducted within this Euphresco project that there was a positive influence on trap catches of having *Agrilus* decoys attached to the trap surfaces. Whilst visual stimuli are important to adult *Agrilus* beetles, as has been shown for emerald ash borer (*A. planipennis*), there is still considerable effort required to identify what visual stimuli are important for other *Agrilus* beetles. For example, we cannot assume that green traps are going to work for every *Agrilus* species, so even evaluation of other trap colours and shapes (since trap profiles may be important) is likely to be vital for other emerging invasive *Agrilus* species.

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3.4. WP3.2 & WP4.2 – additional trapping trials 2021 and 2022

WP3.2 The AGRITRAP approach for Belgium

Objective

The primary aim within this project area was evaluate the response of Belgian native *Agilus* species to the visual cues provided by two types of green multi-funnel traps and green self-made traps, i.e. ‘bottle traps’ in 2021, and ‘fan traps’ in 2022, with or without decoy insects. The focus was on the most harmful indigenous species for Belgium, including *A. biguttatus* (two-spotted oak buprestid, implicated in oak decline events across Europe and strongly linked to Acute Oak Decline in the UK), *A. viridis* (flatheaded wood borer, playing a role in the mass mortality of beech in Hungary and Germany), and *A. sinuatus* (sinuate pear borer, a pest in pear orchards). Therefore, the focus on the trapping was within beech and oak forests and pear orchards.

Material & Methods for the 2021 field trial

The aim was to deploy traps at 20 different locations in Belgium. At each site, the traps were hung in 3 groups, at least 20-25m apart. The distribution of the trap groups per location was as follows:

- 1 Lindgren funnel-trap (ChemTica - <http://www.chemtica.com/site/?p=3731>) / 1 Hungarian funnel-trap (Csalomon – MULTz - <http://www.csalomontraps.com/6trapdesigns/osszerakasipdfek/multzassembling.pdf>) / 1 double bottle trap with decoy / 1 double bottle trap without decoy
- 1 Lindgren funnel-trap / 1 Hungarian funnel-trap / 1 double bottle trap with decoy / 1 double bottle trap without decoy
- 1 double bottle trap with decoy / 1 double bottle trap without decoy

(Figures 2 and 3)

All traps were green, but in different shades (bottle trap = RAL 6038) and coated with fluon. As a decoy for half of the bottle traps, a dead *A. planipennis* adult (obtained from Dr Ben Slager, Supervisory Entomologist, USDA APHIS PPQ, USA) was glued/pinned on the trap.

Approximately 250ml of a 50% propylene glycol solution was added to the collecting pots, and traps were hung at least 10m from the ground, in a sunny spot in the tree canopy. The trapping period started in May/June and ended in September, and traps were emptied every two weeks. Due to problems with the delivery of the ChemTica traps, these traps were deployed slightly later than planned. Due to the cold and wet spring in Belgium in 2021, the activity of *Agrilus* spp. was expected to start later than usual, so a few catches were missed due to the later start date of the monitoring.

Trap locations were selected in accordance with regional forest services and city administrations, with an emphasis on selecting oak and beech stands with canopy dieback; however, this was no guarantee of the presence of (large) *Agrilus* populations. To compensate for the initially disappointing catches at most locations, new locations were selected using waarnemingen.be/observations.be. The platform was searched for recent sightings of *A. viridis* and *A. biguttatus* by citizen scientists.

Figure 2. Close up of the double bottle trap (left) and the Hungarian MULTz multifunnel trap (right). Photo: Gilles San Martin.



Figure 3. Type of traps tested on site in the canopy of an oak tree in 2021. From left to right: double bottle trap with decoys, Lindgren multifunnel trap (ChemTica), Hungarian MULTz multifunnel trap and double bottle trap without decoy. Photo: Gilles San Martin



Material & Methods for the 2022 field trial

In 2022, we used a similar trial design as in 2021, but now with the following traps:

- Lindgren funnel-traps (Chemtica)
- Hungarian funnel-traps (MULTz)
- Fan-trap green
- Fan-trap green with decoys
- Fan-trap yellow

- Fan-trap yellow with decoys

At each site, the traps were hung in two groups, at least 20-25m apart. Each group contained one of each type of trap.

Note that, in 2022, the bottle traps were replaced by fan traps (Creative Commons license; Grégoire et al. 2022), painted either in fluorescent yellow (cf. MULTz) or green (cf. Lindgren). These fan traps were hung in double pairs, half with decoys (two/trap), the others without (Figures 4 and 5).

The trapping period started in early May and ended in early September, and traps were emptied every month. Except for the one successful site in Rochefort (see results below), none of the 2021 trap locations were retained in 2022. The main focus was again on oak, beech and pear, but in addition some poplar stands were also monitored. On a limited number of locations (3), the chemical lure (3Z)-hexenol was tested in combination with Lindgren traps. Lastly, 48 green and yellow Fan-traps were sent to France (C. Bouget, INRAE), decoys were added to half of them, and 24 green and yellow fan-traps of both colors were deployed in Canada (C. Hughes & J. Sweeney, NRCan).

Figure 4. Close up of the double paired yellow fan traps (left) and the double paired green fan traps (right). Photo: Gilles San Martin.



Figure 5. Type of traps tested on site in the canopy of an oak tree in 2022. From left to right: Hungarian MULTz multifunnel trap (CSALOMON), double paired green fan traps, Lindgren multifunnel trap (ChemTica) and double paired yellow fan-traps. Photo: Alexandre Kuhn

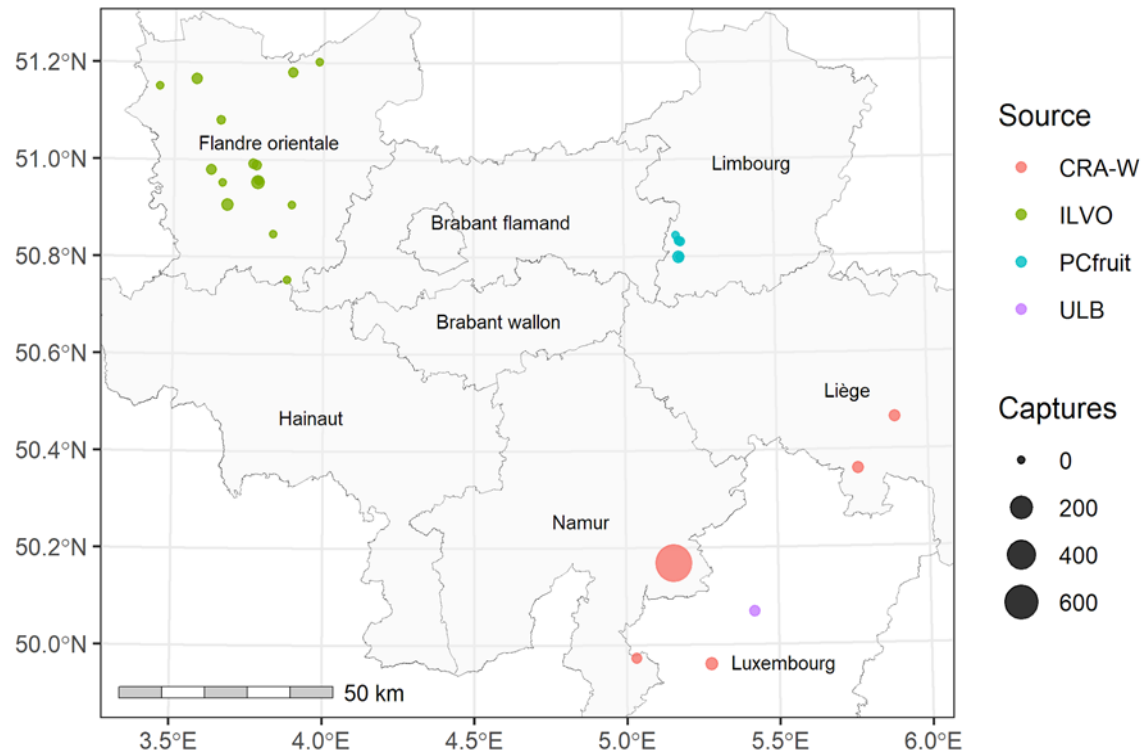


Results 2021

In total, 172 traps were deployed across 27 sites (Figure 6), with 870 buprestids (862 *Agilus*) subsequently being captured during the 2021 season in Belgium (Table 10).

Because of the high numbers of buprestids that were trapped in Rochefort compared to all other sites, and the deviating monitoring period of the ILVO traps (due to the continuous search for more suitable sites), a statistical analysis was not really suitable here and hence we decided to mainly look at the apparent trends within the data.

Figure 6. Overview map of the trapping sites and the number of catches for Belgium in 2021.



Observations

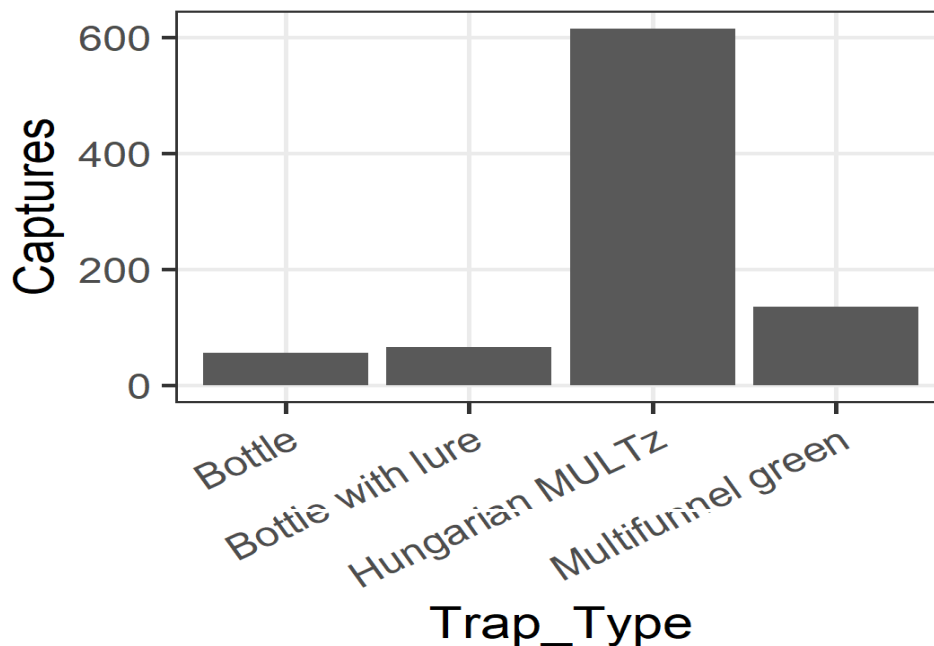
- Overall, the number of catches was rather low, except for the Rochefort site.
- Broadly speaking, the Hungarian MULTz multifunnel traps caught considerably more *Agilus* individuals - the bright green colour of these traps probably explains their success.
- The ChemTica green Lindgren multifunnel traps performed better at ILVO, but the failure of the Hungarian MULTz traps may have been due to the fact that no preservative (propylene glycol) was used (escaping adults).
- The bottle traps were not very successful probably because of their small surface area and problems with the Teflon layer. Therefore, the possible effect of the decoy could not be determined.

- In the pear orchards, the numbers of *A. sinuatus* trapped were generally quite low, even at sites where clearly active adults were observed - any differences in trap efficiency could not be detected.

Trap types

Looking at the total sum of the *Agilus* captures by trap type, it emerged that the Hungarian MULTz traps caught the largest number of buprestids (Figure 7). The results from the Rochefort site obviously determined this trend, which may be species-related (see below).

Figure 7. Total numbers of *Agilus* spp. captured in each trap design in Belgium in the 2021 trapping trial.



Species composition

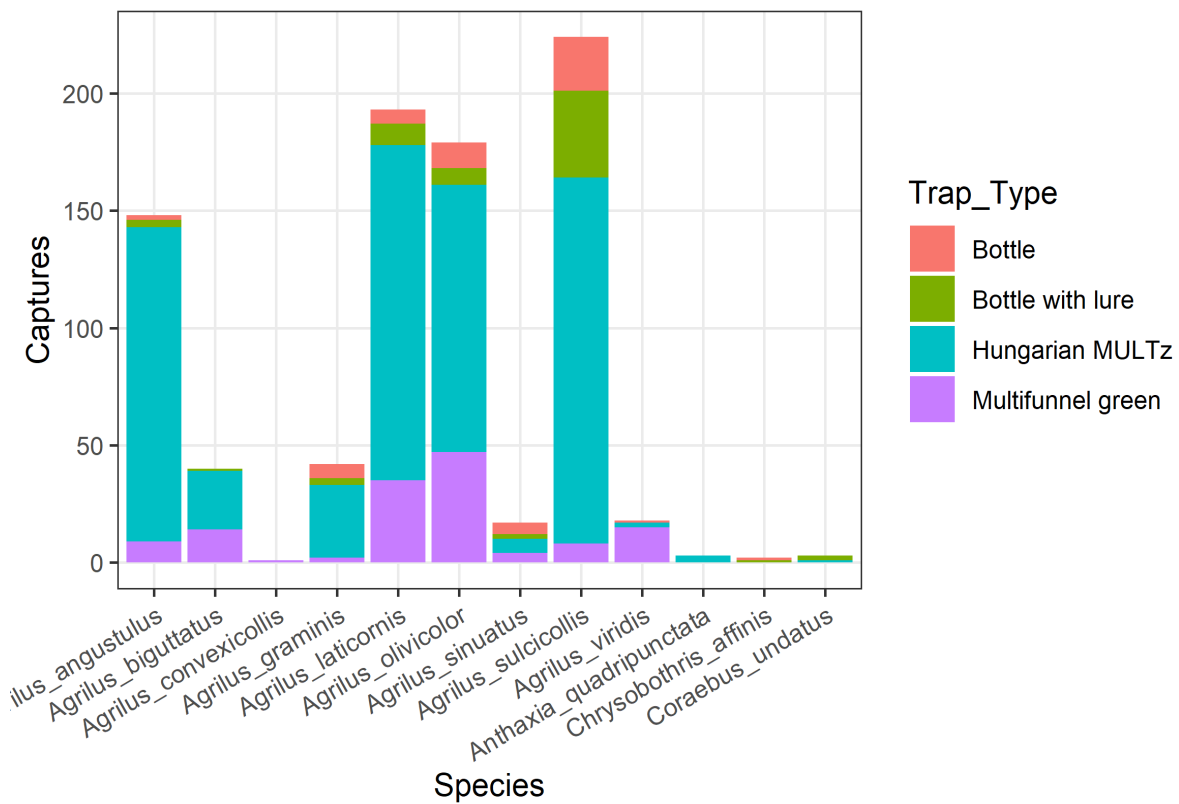
Twelve species of Buprestidae were identified in the catches, including 9 *Agilus* species (Table 10). Given the abundance in Rochefort, the most common species were all associated with oak trees.

Table 10. Total number of Buprestidae caught by species.

Species	Total numbers trapped
<i>Agrilus angustulus</i>	148
<i>Agrilus biguttatus</i>	40
<i>Agrilus convexicollis</i>	1
<i>Agrilus graminis</i>	42
<i>Agrilus laticornis</i>	193
<i>Agrilus olivicolor</i>	179
<i>Agrilus sinuatus</i>	17
<i>Agrilus sulcicollis</i>	224
<i>Agrilus viridis</i>	18
<i>Anthaxia quadripunctata</i>	3
<i>Chrysobothris affinis</i>	2
<i>Coroebus undatus</i>	3
Total	870

Looking at the species composition per trap type, the Hungarian MULTz multifunnel traps caught the largest number of specimens among the species of which more than 5 individuals were caught, with the exception of *A. viridis*. For *A. viridis* most specimens were caught with the green Lindgren multifunnel traps (Figure 8).

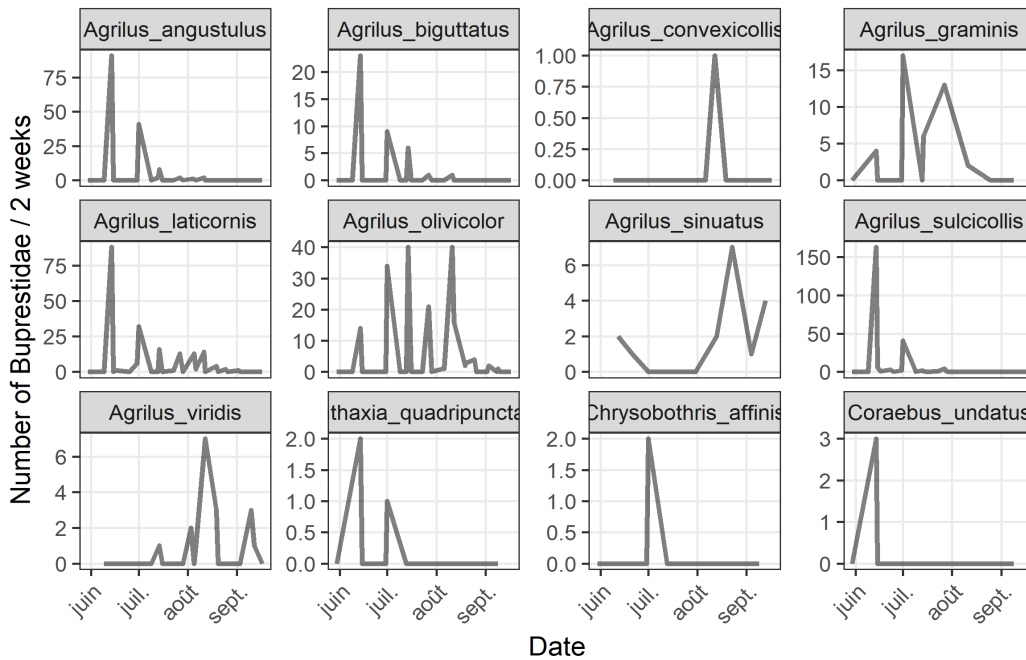
Figure 8. Species composition of the buprestids caught, per type of trap tested.



Phenology

Looking at the phenology for each buprestid species in 2021, some clear differences could be observed (Figure 9). The phenological peak seemed to coincide with the beginning of the trapping season (June) for *A. biguttatus*, *A. angustulus*, *A. sulcicollis* and *A. laticornis*. Other species though, seemed to peak later in the season, July to August for *A. olivicolor* and in August for *A. viridis* and *A. sinuatus*.

Figure 9. Species phenology of the buprestids trapped in 2021 in Belgium.



Results 2022

In total, 244 traps were deployed across 28 sites, with 3691 buprestids being captured during the 2022 season in Belgium. In France, 48 traps were set in 48 locations, with 779 buprestid individuals caught, and in Canada, 15 double fan-traps were hung in 5 different locations and 33 buprestids were captured.

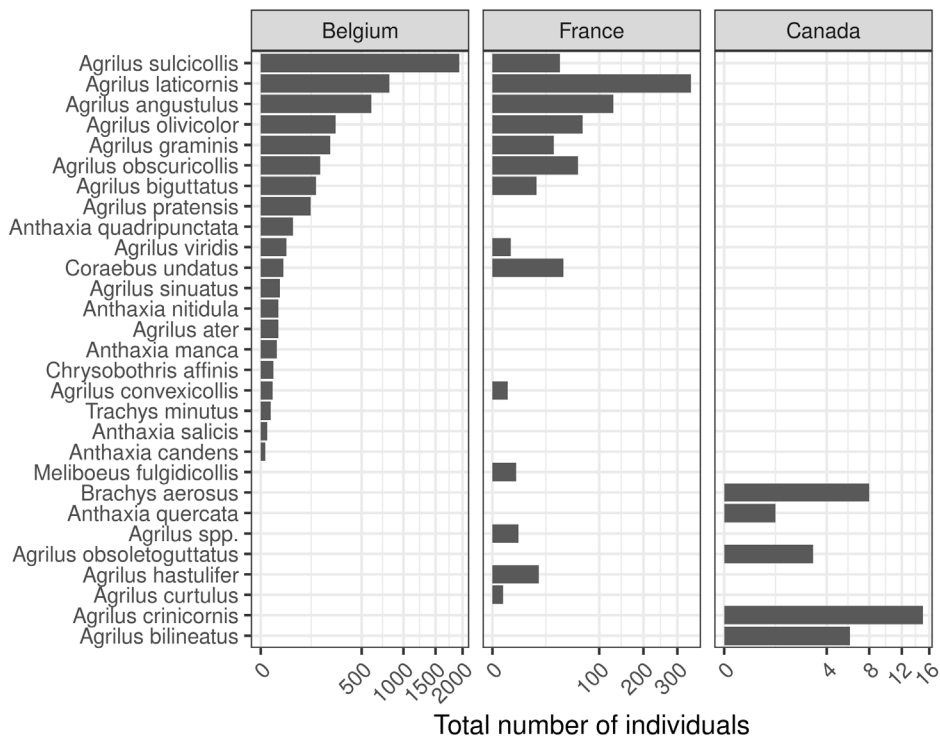
There was a very large variation between sites. At some sites we caught almost no Buprestidae, probably because there were very few at those sites. This was taken into account during the analysis (see below). For pear orchards, where the only species caught was *Agrilus sinuatus*, it is possible that this species in particular is little attracted by the traps used.

Species composition

During the 2021/2022 monitoring campaign, 12 different *Agrilus* spp. were observed in Belgium, including 2 species new to the Belgian fauna: *Agrilus graminis* and *Agrilus obscuricollis*. The most common oak-related species in the Belgian traps were *Agrilus*

sulcicollis, *Agrilus laticornis*, *Agrilus angustulus*, *Agrilus olivicolor*, *Agrilus graminis*, *Agrilus obscuricollis* and *Agrilus biguttatus* (Figure 10). Also worth mentioning are *A. viridis* (beech) and *A. pratensis* (poplar). The species composition between France and Belgium is quite similar, with different abundances (*A. sulcicollis* was more common in Belgian traps, for example) and some additional French species, such as *A. curtulus* and *A. hastulifer*. Obviously, the buprestid fauna in Canada is completely different from that in Belgium and France (Figure 10).

Figure 10. Total number of buprestid individuals per species for Belgium (2021 + 2022), France (2022) and Canada (2022).

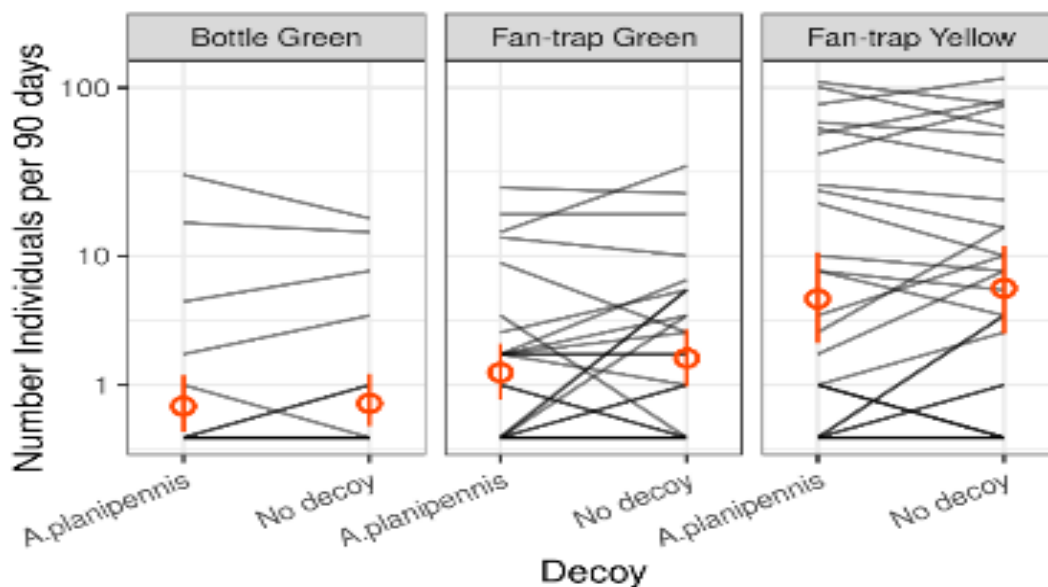


Decoy effect

Before evaluating differences in *Agrilus* attractiveness between traps, we first considered the effect of the decoys on the number of catches, to find out whether pooling of decoy/non-decoy catches was allowed. The decoy effect is shown in Figure 11 for both bottle- (2021) and fan-

(2022) traps. An Anova analysis revealed no interaction between trap type and decoy, nor a main effect of decoy, while trap type had a highly significant effect (see below).

Figure 11. Graphical summary of the decoy (i.e. dead *Agrilus planipennis*) effect on the number of *Agrilus* individuals trapped by using homemade traps. The grey lines represent the total captures of a pair of similar traps from the same tree with or without decoy. The number of individuals has been standardized to have a comparable number of trapping days (90 days). The red dots represent the average, and the bars represent the 95% confidence interval (bootstrap).



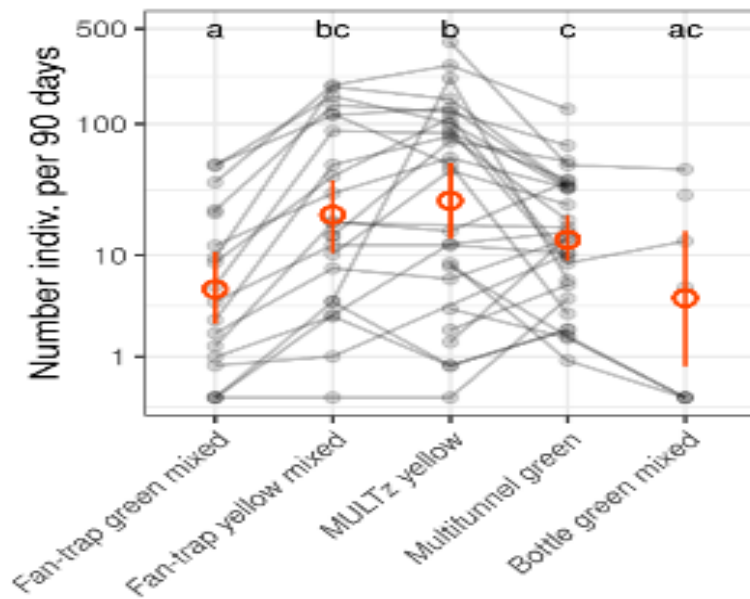
The results of the above could be masked by specific responses to the decoys: some species are attracted, and others are not, or the decoy effect could be different between males and females. However, further analysis on a subset of species for which we have sufficient data (only at Belgian and French *Quercus* sites) indicate that there is neither a species- nor sex-related decoy effect on *Agrilus* catches.

We could find no evidence of the effect of decoys on the number of catches. Therefore, we believe that we can safely group the catches of the pairs of traps with and without decoys for the rest of the analysis.

Trap types

To determine the effect of trap type on the total number of individuals caught, data on *Fagus* and *Quercus* from 2021 and 2022 were combined and transformed to equal numbers of trapping days. The Anova analysis shows a highly significant effect of trap type, and also a more marginal effect of tree species (*Fagus* vs *Quercus*), but no year effect. Figure 12 summarises the model, where we omitted catches below five individuals per tree from the analysis. The Hungarian MULTz traps have significantly more catches than the green Lindgren multifunnel ones, while the yellow fan traps occupy an intermediate position (no significant difference between yellow fan traps and green Lindgren or MULTz). When comparing fan traps with multifunnel traps, we need to consider the trapping area. As such, yellow fan traps were equally or even more attractive than the MULTz-traps. The green fan traps have significantly fewer catches than the aforementioned three types of traps. The catches of the green bottle traps are only significantly lower than those of the MULTz. It should be noted that there is no significant difference between bottle traps and fan traps, but this may be due to the fact that these two traps were not present in the same year, making it difficult to compare them. Relative to their smaller area, yellow fan traps were equally or even more attractive than the MULTz-traps.

Figure 12. Graphical summary of trap type effect on the number of *Agrilus* catches in oak and beech stands in 2021-2022. The grey lines represent traps from the same tree. In red: the mean and the 95% bootstrap confidence interval. The compact letter representation shows which pairwise comparisons are significantly different from each other (with p-value correction).



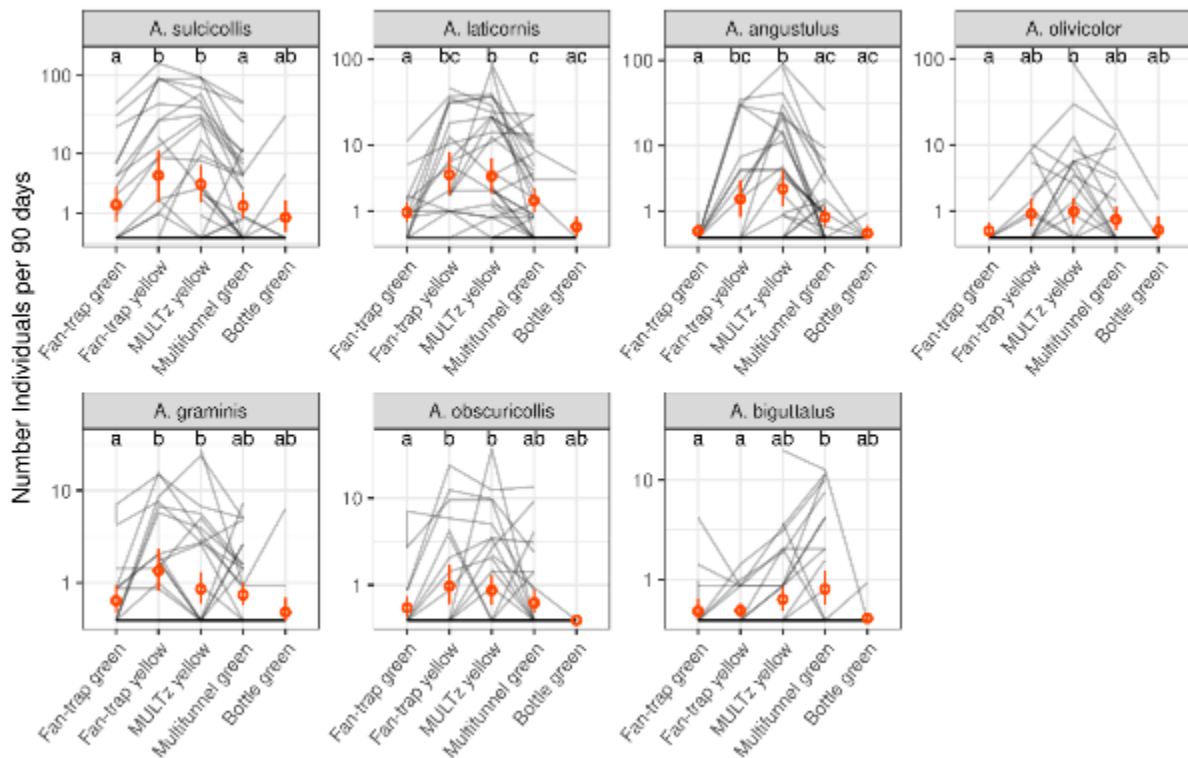
Trap type effect per species and per sex

Next, we checked whether some *Agrilus* species (on oak) are more attracted to certain trap types. The Anova analysis showed a highly significant trap type x species effect. This means that there are differences in the number of catches between trap types, but that these differences are not the same for each *Agrilus* species. Figure 13 provides a graphical summary of the model. The differences between trap types are more or less the same for the six most common species, but *A. biguttatus* shows a slightly different pattern. For these six species, catches tend to be more numerous in the two yellow traps (fan traps and MULTz) and lower in the green fan traps and to a lesser extent in the green Lindgren traps. Catches in the green bottle traps are often very low, but usually the differences with the other trap types are not significant (probably for several reasons: the number of repetitions is low, they were never placed in the same locations as the fan traps). The differences between traps become less obvious as the overall abundance of the species decreases. For *A. biguttatus*, however, the highest catches were made in the green Lindgren trap and the green and yellow fan traps

caught significantly fewer individuals, while the difference with the yellow MULTz is not significant.

Finally, we also wondered whether males or females are attracted differently to certain trap types. Based on the statistical analysis we found no evidence that one type of trap attracts more males or females.

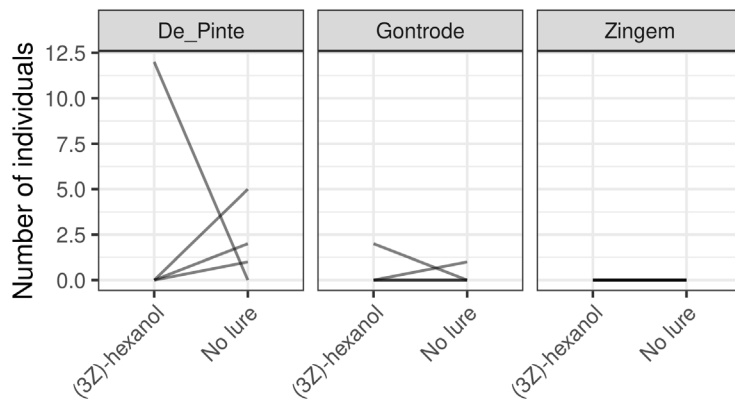
Figure 13. Graphical summary of trap type per species effect on the number of *Agrilus* catches for the *Quercus* sites (2021-2022). Grey lines represent the total captures for each species in a given trap from the same tree. The number of individuals has been standardized to have a comparable number of trapping days (90 days). Red dots represent the average, and the bars represent the 95% confidence interval (bootstrap). Trap types sharing the same letter are not significantly different.



3Z hexanol effect

We conducted a small preliminary experiment with this chemical lure (not a pheromone) that is attractive to *Agrilus planipennis*. This was a small separate experiment at different sites from the rest of the study. Three Lindgren multi-tunnel traps were lured with (3Z)-hexanol. They were placed at three different sites and paired with a non-lure Lindgren trap at the same site (but in a different tree). All six traps were collected four times. On the graphical summary of the analysis (Figure 14), there are no obvious differences. However, it is rather difficult to draw conclusions with only three traps of each. One of the sites also had zero catches.

Figure 14. Graphical summary of the effect of (3Z)-hexanol on the number of *Agrilus* catches. Each line refers to trap catches from the same location (sub-graphs) on the same date (i.e. similar catches).



Pear orchards

In pear orchards, as expected, we captured only the target species: *Agrilus sinuatus*. However, we trapped only 17 individuals in 2021 and 0 (zero) in 2022, despite the fact that their presence was demonstrated by visual observations (beating tray). Note that most catches came from only one of the 5 sites of that year and that many captures occurred quite late in the season (late August - September).

Differences between trap types seem to be limited (Figure 15). Based on the number of individuals caught, corrected by the number of trapping days and by the number of traps, the bottle traps seem less efficient, but this is based on very few real catches.

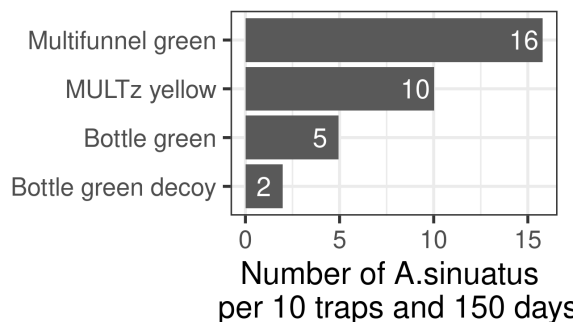
We conclude that based on these 2-year catches, the use of the tested traps for monitoring *Agrilus sinuatus* in pear orchards appears to be of limited value. According to tests in Hungary (Z. Imrei, personal communication), sticky traps would be more successful for such species. This will be verified in an organic pear orchard at the Belgian coast side in 2023.

Because this *Pyrus* dataset is very different from the rest and there are very few catches, these pear sites were not included in the rest of the analysis.

Figure 15. Graphical summary of the number of *A. sinuatus* catches in pear in 2021-2022. Note that, for comparative purposes, the actual number of individuals caught was corrected by an arbitrary number of trapping days (150) and by the number of traps (10).

Nb catches in pear orchards (2021)

NB : 0 catches in 2022



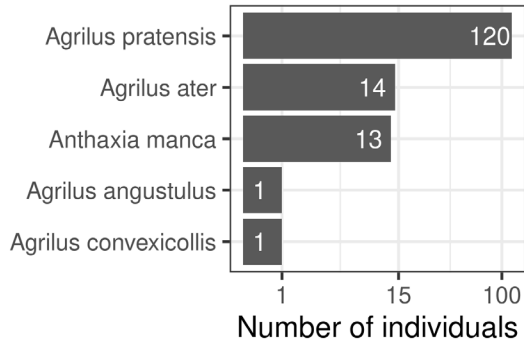
Poplars

Poplars have a very different fauna, and the monitored sites were more open, not forests. As expected, the two most common species are poplar specialists: *Agrilus pratensis* and *Agrilus ater*. *Anthaxia manca* is a rare species that lives on *Ulmus* spp. (all 10 specimens collected in the same sample); *Agrilus convexicollis* lives on *Fraxinus excelsior* (Figure 16).

Traps were set at 11 sites, but with very uneven sampling intensity. At most sites, only one or two MULTz or Lindgren traps were placed for a short period. One site in Oud-Heverlee received a full design with fan-traps with or without decoy MULTz and Lindgren traps and the vast majority of catches came from that site. *Agrilus ater* was the only species also caught at other sites (2) (8 individuals).

Figure 16. Graphical summary of the number of buprestid catches in poplar in 2022.

Nb catches on Poplars

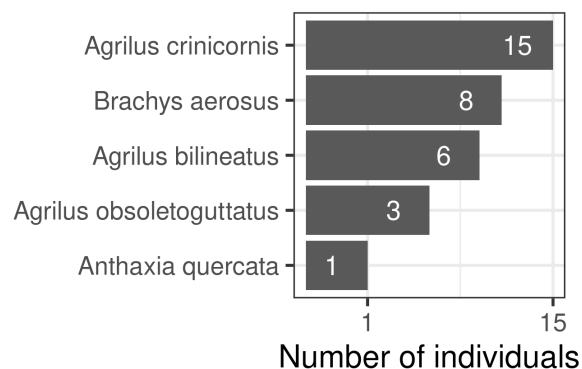


Canadian trial

As expected, the Canadian species composition is quite different from the European one (Figure 17). The number of replicates was not very high in Canada: there were only three different traps in three different trees (9 traps in total). Consequently, it was not appropriate to include the Canadian data in the wider analysis. Nevertheless, it is very interesting to see how the fan traps compare with the traditional green Lindgren traps in the case of a totally different fauna.

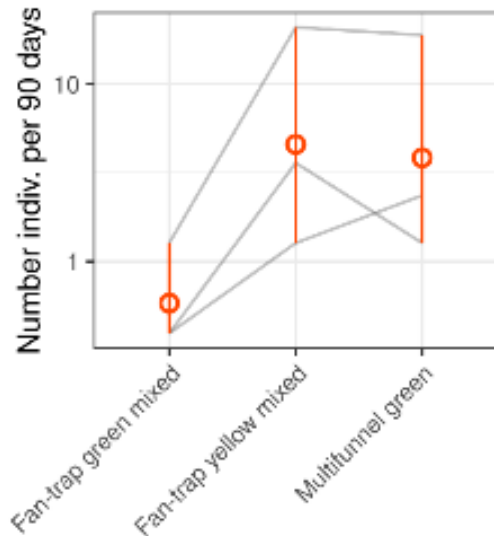
Figure 17. Graphical summary of the number of buprestid catches in Canada in 2022.

Nb catches in Canada



In terms of total catches, the results are more or less in line with what has been observed in Europe: the green fan traps catch less, while the yellow fan traps are equivalent to the green Lindgren traps (Figure 18). So, the yellow fan traps are also clearly attractive to non-European fauna.

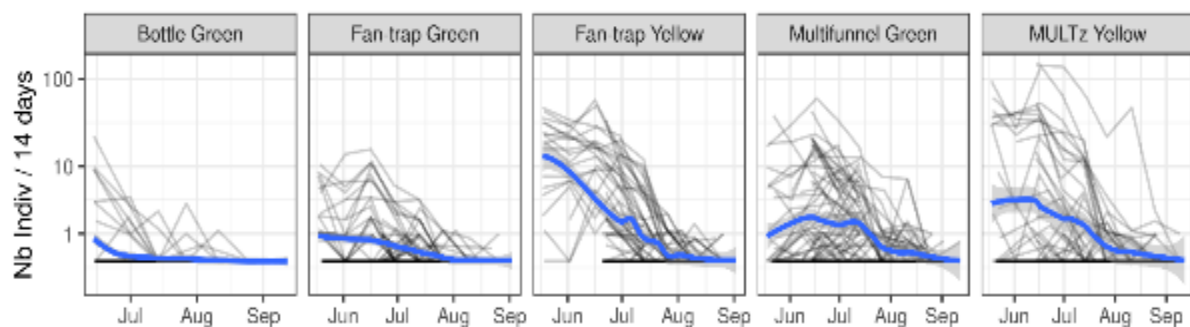
Figure 18. Graphical summary of the trap type effect on the number of *Agrilus* catches in the Canadian trials in 2022.



Phenology

As for the common phenology of the trapped *Agrilus* spp. we see that activity is in full swing in June and starts to decline from late July onwards (Figure 19). Interestingly, the yellow fan trap catches significantly more individuals in June, while later in the year there are fewer differences compared to the other traps. MULTz performs slightly better than the Lindgren trap, especially early in the season. The green bottle traps and green fan traps are clearly not suitable for monitoring *Agrilus* spp. throughout the season.

Figure 19. Number of *Agrilus* individuals caught during the 2021-2022 campaign plotted over time and per trap type. Each grey line represents the same trap. The blue line represents the average trend.



Conclusions

- There was no effect of the decoy on the number of Buprestidae trapped, so decoy/no-decoy catches could be pooled.
- A limited value of using the tested traps for monitoring *Agrilus sinuatus* in pear orchards was found. Sticky traps will be tested out in subsequent trials in 2023.
- The number of catches were host tree related (*Quercus* vs. *Fagus* vs. *Pyrus* vs. *Populus*)
- There was a clear effect of trap type on the number of *Agrilus* individuals caught.
- Yellow MULTz-traps were more successful than the commonly used green multifunnel traps.
- Yellow (fan-) traps outperformed green (fan-) traps.
- Relative to their smaller area, yellow fan-traps were equally or even more attractive than the MULTz-traps.
- During the 2021/2022 monitoring campaign, 12 different *Agrilus* spp. were observed in Belgium, including 2 species new to the Belgian fauna: *Agrilus graminis* and *Agrilus obscuricollis*.

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Grégoire, J. C., Caiti, E., Hasbroucq, S., Molenberg, J. M., & Willenz, S. (2022). When the Beetles Hit the Fan: The Fan-Trap, an Inexpensive, Light and Scalable Insect Trap under a Creative Commons License, for Monitoring and Experimental Use. *Insects*, 13(12), 1122. doi: [10.3390/insects13121122](https://doi.org/10.3390/insects13121122)

3.5. Conclusions and recommendations to policy makers

1. Including green traps (either Fluon-treated multifunnel traps, or sticky prism traps) in the upper canopy of hardwood trees in areas at risk of woodborer introduction via global trade, will increase the probability of detecting *Agrilus* spp, in annual surveillance programs for non-native woodborers. The field trials conducted within the framework of this Euphresco project highlighted that green traps even without lures can be useful tools for monitoring *Agrilus* when proper placement of traps (in sun exposed parts of host tree crowns) is observed. However, it must be ensured that experts are available for determination of the *Agrilus* specimens caught in the traps.
2. There is an ongoing need to support research focused on developing improved tools for the early detection and surveillance of *Agrilus* spp. across countries and continents. Hence, further field trials evaluating other designs of traps should be established in different geographical and forest/woodland settings.
3. *Agrilus* species are particularly difficult to identify morphologically, hence creating a database of researchers and taxonomists who can reliably identify *Agrilus* specimens, as well as developing national reference specimen collections (along with points of contact to acquire specimens) should be initiated.
4. Considering point 3 above, greater emphasis probably needs to be made on building a molecular database for identifying *Agrilus* species. Whilst work on this has been instigated (see Kelnarova et al., 2019), it is unclear how this initiative is currently progressing.
5. High risk *Agrilus* species should be identified and trapping trials should be undertaken within their existing geographic range to determine the best approaches for surveillance and monitoring – rather than waiting for them to arrive in a new location and spending an inordinate amount of time, money and effort trying to develop novel approaches to detect and monitor outbreaks.

3.6. Benefits from trans-national cooperation

1. 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 *Agilus* spp. (and other wood boring beetles) potentially at risk of introduction to our respective continents. The transatlantic cooperation within the project provided useful and important information to respective plant protection organizations on potential optimal surveillance approaches for this target group of wood-boring insects.
2. Replicating field experiments a number of times in a wide range of geographical areas with different beetle species compositions provides very robust and useful data sets.
3. The co-operation of collaborators within the project was particularly helpful in the determining/identifying of some *Agilus* species, in the sense that it helped connect experts and the exchange of pre-determined species.
4. The project facilitated new associations and collaborations that will be invaluable in developing new project areas in the future, and trapping trials for *Agilus* spp. are likely to continue after the official end of the Euphresco project.

APPENDIX

1. Project outputs

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3. Pros and cons of using multifunnel traps vs sticky prism traps for *Agrilus* trapping

Multi-funnel traps		Sticky prism traps		
	Pro's	Con's		
1.		Expensive	Relatively inexpensive	Can be as expensive as multi-funnel traps
2.	Reusable across trapping seasons (no issues with traps filling up with invertebrates) with cleaning and care			Can only be used for one trapping season (for a short time 6-8 weeks – due to becoming covered with other invertebrates)
3.	Easy and time efficient in the field to collect samples in from traps		If the assessment is for conspicuous and easily recognizable species (e.g. EAB in European ash stands – as they are bigger and easily identified) then the detection and counting of specimens would be easier and faster than in a MFT (no need to sort the specimens)	Time consuming in the field to collect captured insect specimens from the sticky surfaces of the traps (some smaller species of <i>Agrilus</i> may not be spotted)
4.	These traps can be used for assessing other woodboring insects (+ other insect families)			Would be really difficult to use these traps for assessing other wood-boring insects (or other insect families) because of the glue.



Multi-funnel traps		Sticky prism traps		
	Pro's	Con's		
			Pro's	
			Con's	
5.	No Glue! Insects captured are easily identifiable without having to use solvents/cleaning agents. However traps must be coated with fluon.			Glue – insect specimens captured are covered in the adhesive and are difficult to identify. Need to use a solvent/cleaning agent to dissolve the glue (e.g. Histo-Clear/Histo-Clear II)
6.		Can unfortunately catch bats if traps are not modified with a wire mesh in the bottom funnel		Some evidence of birds, reptiles and amphibians being caught in very low numbers on these traps
7.	If using propylene glycol/saline in the collecting pot – then insects can be used for molecular analysis		Collected insect specimens, despite being covered in glue, could be used in molecular analysis	
8.		When using a wire mesh (as outlined above in 6.) the traps can get clogged with leaves and twigs potentially affecting their efficiency		In dusty or high-pollen areas, trap surfaces can become clogged with dust and debris, affecting their efficiency
9.		Must be stored during the off-season, which can take up a lot of storage space	Disposed of at the end of the season (see 10.)	

Multi-funnel traps		Sticky prism traps		
	Pro's	Con's		
			Pro's	
			Con's	
10.				While corrugated plastic is 100% recyclable (Resin Code 5), many recycling centres will not take traps once they are coated with glue
11.	Effective across a wide temperature range			Some evidence that in very high temperatures, the glue may potentially slough off the traps, in which case insect samples are likely to be lost and traps need to be replaced
12.	They are also resizable, have replacement parts and are easy to transport		Can be covered with cling film and dismantled and transported flat at end of season	Not easy to deal with (transport) at end of field season

4. Acknowledgments

Austria: National funding for this project was provided by the Austrian Federal Ministry for Agriculture, Forestry, Regions and Water Management (Project no. 101607). We thank Urbarialgemeinde Neckenmarkt for permission to carry out the experiment in their forests. J. Connell, W. Hinterstoisser, M. Studera, D. Self, and A. Daxer contributed to field work and laboratory analysis of the samples. Gianfranco Curlietti (Carmagnola, Italy) and Wolfgang Barries (Vienna, Austria) helped with determination of *Agrilus*.

Belgium: Funding was provided by the Belgian Federal Public Service of Health, Food Chain Safety and Environment through the contract R120/A-337 Agritrap. We thank Mallory Akhamlich, Tim Belien, Emilio Caiti, Peter Dewitte, Séverine Hasbroucq, Louis Hautier, Alexandre Kuhn, Gilles San Martin, Quentin September, and Olivier Vanhoutte for their contribution to designing the project and their help in the field and in the laboratory.

Canada: Funding was provided by the Canadian Food Inspection Agency (project #02316/N-000338.001.01) and Natural Resources Canada, Canadian Forest Service under the Pest Risk management Program. We thank Cory Hughes for setting up and conducting the field trapping trials, Chantelle Kostanowicz and Mischa Giasson for sorting and identifying the specimens of *Agrilus* and other woodboring beetles captured in traps, and the New Brunswick Department of Natural Resources and Energy Development for granting us permission to conduct experiments at the Cranberry Lake protected natural area.

Germany: Funding for this project was provided by the German Federal Ministry of Food and Agriculture (BMEL). We thank Andreas Baderschneider (Lower Saxony State Forest) for the provision of experimental sites. Stephanie Feltgen, Matthias Becker, Maximilian Lorbeer, Tobias Wille and Silke Steinmöller are thanked for various help and support with fieldwork and organization of the project.

Italy: Funding was provided via the Dotazione Ordinaria Ricerca (DOR) by the University of Padova. We thank Alice Martinelli, Giacomo Santoemma and Giacomo Cavaletto for help in field activities and Gianfranco Curletti for species identification.

Slovenia: Funding was provided via the Administration of the Republic of Slovenia for Food Safety, Veterinary Sector and Plant Protection. We thank the Slovenia Forest Service for the help with the selection of the forest. Tine Hauptman, Eva Groznic and Martin Križaj contributed to the field work and laboratory analysis of the samples.

UK: Funding was provided by the Department of Environment, Food & Rural Affairs (Defra). We are grateful to Bob Thurston for permission to carry out fieldwork at the National Trust site Attingham Park. Many thanks also to Forestry England for allowing us to undertake fieldwork within Alice Holt Forest. Abi Enston and Tom Staton assisted with field work and laboratory identification of insect samples.

USA: Funding was provided by a USDA Animal and Plant Health Inspection Service Plant Protection and Quarantine Pest Detection project and through Plant Protection Act 7721. Everett Booth and Sarah Devine contributed to field work in Massachusetts. Emily Franzen and Annie Ray (Xavier University) contributed to field work in Ohio and Kentucky. Lawrence

Barringer (Pennsylvania Department of Agriculture) assisted with insect identifications. Nicole Kelleher (Massachusetts Department of Conservation and Recreation) assisted with securing permission to trap at Douglas State Forest and at Freetown/Fall River State Forest. Permission was also granted by Northern Kentucky University to use their experimental station, and Ohio Department of Natural Resources to trap in East Fork State Park.