UNIVERSITÀ DEGLI STUDI DI PADOVA

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# Improving efficiency of surveillance protocols for longhorn and jewel beetles (Coleoptera; Cerambycidae and Buprestidae)

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che nel terminare questo percorso mi tiene la mano.

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# ABSTRACT

International trade is the main driver of the increasing introduction of non-native wood-boring beetles around the world. The principal pathways of introduction are the trade of “plants for planting” and the transport of wood packaging materials. Early-detection of plant pests using baited traps at points of entry and their surrounding areas is one of the most important and used measures of prevention and control. There are several surveillance tools that can be used, but traps are easy to use and cheaper compared to the other tools. Longhorn (Cerambycidae) and jewel beetles (Buprestidae) are among the wood borers most commonly intercepted at points of entry that cause severe damages to forests and ecosystems. Green traps are the most efficient tool for jewel beetles of the genus *Agrilus*, while black or green traps baited with pheromones are commonly used for longhorn beetles. Whether green traps baited with longhorn beetle pheromones can be a good approach to catch longhorn beetles without interfering negatively with jewel beetles is still unclear. In order to answer this question, we carried out a trapping study in a lowland forest of Friuli Venezia Giulia region and we compared 4 treatments of traps in a randomized block design: i) unbaited green multi-funnel traps; ii) green multi-funnel traps baited with ethanol; iii) green multi-funnel traps baited with ethanol and longhorn beetle pheromones commonly used by species in the family Cerambycinae (i.e., D6, K6, K8); iv) green multi-funnel traps baited with ethanol and longhorn beetle pheromones commonly used by species in the family Lamiinae (i.e., EZF, EZFA). A total of 952 beetle individuals were collected, among which 63% were beetles belonging to the genus Agrilus and 37% were longhorn beetles. For longhorn beetles, traps baited with D6, K6, K8 and ethanol caught significantly more species and individuals compared to the other treatments, even though the number of species was not different to that achieved in traps baited with EZF, EZFA, and ethanol. In addition, all but one of the species caught on traps baited with EZF, EZFA, and ethanol were also caught in traps baited with D6, K6, K8 and ethanol. For jewel beetles of the genus *Agrilus*, no significant difference among the tested treatment was found. In conclusion, these results indicate that green-multi-funnel traps baited with D6, K6, K8 and ethanol can be used to survey both taxa simultaneously, reducing the number of traps needed and the overall costs of the surveillance program.

# RIASSUNTO

Il commercio internazionale è il principale responsabile dell'introduzione di specie non native di insetti xilofagi. La via principale d'introduzione di tali specie è il commercio di piante ed il trasporto di imballaggi in legno. Il rilevamento tempestivo di specie esotiche attraverso l'uso di trappole attivate con attrattivi e posizionate presso i punti d'ingresso e le aree ad essi circostanti, è una delle misure più importanti ed utilizzate per l'intercettazione precoce. I cerambicidi (Cerambycidae) ed i buprestidi (Buprestidae) possono causare danni gravi a foreste ma anche agli interi ecosistemi e sono tra gli xilofagi più comunemente intercettati presso i punti di ingresso. Trappole multi-funnel verdi sono le più efficaci per i buprestidi del genere *Agrilus*, mentre trappole nere multi-funnel innescate con feromoni possono essere un buon approccio per catturare i cerambicidi. Se le trappole verdi innescate con feromoni possano essere un buon approccio per la cattura di cerambicidi senza interferire negativamente con i buprestidi del genere *Agrilus*, ancora non è chiaro. Al fine di rispondere a questa domanda, abbiamo svolto uno studio sull'efficienza delle trappole in una foresta planiziale del Friuli Venezia Giulia, comparando 4 trattamenti in uno schema a blocchi randomizzati: I) trappole verdi multi-funnel senza attrattivi; II) trappole verdi multi-funnel innescate con etanolo; III) trappole verdi multi-funnel innescate con etanolo e feromoni di cerambicidi generalmente usati per le specie appartenenti alla sottofamiglia Cerambycinae (i.e. D6, K6, K8); IV) trappole verdi multi-funnel innescate con etanolo e ferormoni di cerambicidi generalmente usati per le specie appartenenti alla sottofamiglia Lamiinae (i.e. EZF, EZFA). Per quanto concerne i cerambicidi, in riferimento al numero di specie e di individui catturati, le trappole innescate con D6, K6, K8 ed etanolo hanno ottenuto risultati significativamente migliori se confrontate agli altri trattamenti utilizzati. Tuttavia, il numero di specie catturate mediante l'utilizzo di trappole innescate con EZF, EZFA ed etanolo non differisce in modo significativo. Le specie catturate dalle trappole innescate con EZF, EZFA ed etanolo sono state le stesse catturate nelle trappole innescate con D6, K6, K8 ed etanolo, fatta eccezione per una. Per quanto riguarda il genere *Agrilus*, non è stata riscontrata alcuna differenza significativa tra i trattamenti. Per concludere, questi risultati indicano che le trappole verdi multi-funnel innescate con D6, K6, K8 ed etanolo possono essere utilizzate per l'intercettazione precoce di entrambi i taxa simultaneamente, riducendo il numero di trappole necessarie ed i costi.

# 1.INTRODUCTION

## 1.1 Characteristics of the families Cerambycidae and Buprestidae (Coleoptera)

### 1.1.1 Cerambycidae

Beetles members of the family Cerambycidae represent one of the major families of wood-boring insects, with 35.000 estimated species. These beetles are able to cause damages to forests and their products and are responsible for wood biodeterioration both at larval stage and in the adult phase (Allison et al., 2004). These insects are commonly known as longhorned or longicorn beetles due to their distinctive long antennae (especially in males) which can reach up to twice their body length, that can vary from 2 mm to >160mm with elongate and subcylindrical shape. Diet of adult beetles is composed of different plant substrates as foliage, fruits, flowers but also roots, sap and bark; while at larval stage they mainly bore in stem and branch tissue of unhealthy or recently dead trees eating wood (Duffy et al., 1953). Female oviposition mostly occurs in cracks and cervices in bark of woody plant; in some species they cover the eggs with a jelly substance and a single female can lay up to 600 eggs in her life span (Kariyanna, 2017). Due to the incapability of the larvae to disperse, larvae develop only inside the tree host with a duration from months to even more than ten years (Allison et al., 2004). Larvae are legless (or legs are very short) usually white or yellowish with powerful mandibles capable to bore in the wood. Once the development is complete, the pupal stage is quite short and it can last from weeks to months. Adult emergence occurs through a hole excavated in the bark (Hanks, 1991).

*Figure 1 Different species of longhorn beetles. Ph: M.M. Kumawat, https://threatenedtaxa.org/index.php/JoTT/article/view/2378/3426*

Besides mechanical damages caused by larvae boring activity, longhorn beetles can also be important vectors of other organisms including nematodes (e.g., Bursaphelencus xylophillus) or fungal pathogens (e.g., Dutch elm disease) (Allison et al., 2004).

Host selection in longhorn beetles relies on chemical volatiles and pheromones. Volatiles released by stressed trees are extremely attractive for longhorn beetles: for example, floral volatiles attract on flowers where they feed on nectar and pollen; smoke-related volatiles released after forest fires often lure cerambycids which oviposit in debilitate trees; trunk and leaf volatiles, including monoterpenes (defensive compounds released from plant tissues, especially if the tissues are stressed or damaged) and ethanol (produced by anaerobic respiration in stressed, dying and dead trees) also attract longhorn beetles (Sweeney et al., 2004; Allison et al., 2004).

### 1.1.2 Buprestidae

The family Buprestidae counts more than 15.000 species mainly distributed in the warm parts of the world and it is one of the most important groups of woodborers (Ruzzier et al., 2023). They are commonly known as jewel beetles because of their metallic colours and a highly variability in coloration and shape. Adults present a short head with an evident biting mouthpart; some species feed on foliage, others are associated with flowers. Larvae are dorso-ventrally flattened and segmented, mostly bore galleries in branches, trunks and in roots as well (Bellamy et al., 2002). Most jewel beetles are considered oligophagous during all their life, that means that they are associated with a single plant family. Oviposition occurs singly or in small groups generally into cracks of the bark of dead, dying, or stressed trees; however, some species colonize healthy hosts. Generally, the larvae develop in four instars that end with the pupation and with the emergence of the adult in spring (Evans et al., 2007).

*Figure 2 Double side of a jewel beetle. Ph: Nikola Rahmé, https://memim.com/buprestidae.html*

Even though the jewel beetles have acquired a big importance in the phytosanitary context, accurate information regarding their identity, their areas of origin, their entrance pathways are complex to find in literature (Ruzzier et al., 2023).

## 1.2 Cerambycidae and Buprestidae as invasive species

International trade is greatly responsible for the introduction of alien species that can cause important damages in the country of import (Meurisse et al., 2018). The globalization and so the speed of transports and frequency of commerce generated a well-established connection worldwide, which allows alien species to overcome geographical barriers that prevented their spread in the past.

In particular, of the main pathway of introduction of non-native longhorn and jewel beetles is represented by "plants for planting" (FAO 2011) and the transport of wood packaging materials (WPMs). The definition of plants for planting refers to live plants rooted or unrooted but also bonsai and bulbs, and to a lesser extent seeds, flowers, and ornamental foliage. There are few reasons why live plants are considered the most high-risk form for moving insects and to promote their establishment: they are transported rapidly, often with soil and they are planted outdoor. Regarding WPM, it is very dangerous because it is commonly made from recently cut trees, often with bark residual, and also because it is generally made by repaired and reused wood from different countries.

Therefore, the wood, in all its form, is the principal medium to transport non-native beetles but also travellers and their baggage can play accidentally an important role: logs, wood chips, processed wood, other wood items and cones, containers, machinery and travellers with baggage and food items, these are some examples that contribute to the diffusion of non-native species (Meurisse et al., 2018).

The International Plant Protection Convention (IPPC) developed phytosanitary certificates which establish that all the requirements of the importing country are verified. Beyond that, the import inspections are another strategy of control although these differ by country and so they aren't equal and effective everywhere (Eschen et al., 2015). Unfortunately, the speed and the volume of all the commodities don't permit an accurate inspection, in fact has been estimated that in U.S. only the 2% of the international cargo is inspected (Rassati et al., 2016).

Another cause of non-native species invasions is linked to the global warming and to the increased frequency of extreme events, i.e., the climate change. The rising temperature in fact permits the range expansion of exotic species into areas with characteristics previously not favourable for them. Additionally, the extreme events can debilitate tree species and even provoke their death releasing volatile compounds (e.g., ethanol) that are extremely attractive for many wood-borers (Rassati et al., 2016).

Longhorn and jewel beetles are among the most commonly intercepted beetles at points of entry worldwide. In addition, both beetle families can have a very strong negative impact on natural ecosystems altering habitats and food supply, as well as in orchards or plantations causing severe economic losses (Ruzzier et al., 2023). Most of the introductions are accidental, only in a few cases species were introduced intentionally. This is the case of four species of Buprestidae (i.e., *Sphenoptera jugoslavica*; Agrilus hypericin; Hylaeogena jureceki; Lius Poseidon) have been intentionally introduced to act as biological control agents against some invasive plants in North America, South Africa, Australia, and Hawaii (Ruzzier et al., 2023).

The mechanisms and factors mentioned above explain why longhorn and jewel beetles are so important as non-native species despite phytosanitary certificates and inspections. Thus, actions to early-intercept incoming species are crucial to prevent their establishment, saving money and resources which would be needed for effective eradication programs (Rassati et al., 2016; Ruzzier et al., 2023).

## 1.3 Monitoring systems for exotic wood-borers beetles

Biosecurity surveillance has a key importance to intercept the initial stage of invasion combining multiple surveillance tools (Poland et al., 2018).

According to a study on the influence of landscape characteristics for the establishment of non-native species, the monitoring processes must be developed "in ports with large volumes of imports and in the surrounding broadleaf forests" because these two components are the best conditions for trapping alien wood-boring species (Rassati et al. 2015).

Biosecurity surveillance varies in the type of interventions according to the stage of invasion:

- *Pre-border* biosecurity regards the production of policies toward a safer import of commodities.
- *Border* surveillance concerns the prevention of possible establishment in the initial phase.
- *Post-border* surveillance applicable at large spatial scale when the population is established but it is still low.
- *Containment* is actualized when the established population is still low but it is focused specially around the infested areas.

Depending on the target species to which it refers, biosecurity surveillance can be divided in two other different groups: *specific*, when targets a single species or *generic* when targets a broad range of species.

Tools, strategies, and applications for the biosecurity surveillance are useful in different situations and context. Some of the most commonly used approaches are described below (Poland et al., 2019):

- Visual inspection: this method permits a good preventive analysis but it guarantees the control of only a small percentage of shipments; generally, it is used for *border* and *containment* surveillance operated by pest specialists
- Baited traps: traps are a successful form of control based on the attraction of non-native species to traps baited with lures. Beside the design, several variables, such as trap type, colours, treatments with lubricants and type of collection cup can affect trap efficacy. Traps are indicated for different types of target species and they can be useful for very large areas. Nonetheless, an important limit to consider is that they detect only adult individuals during flight activity. Baited traps are commonly used by in the contexts of *border* surveillance at points of entry, post-border, and containment surveillance. Moreover, they can be used both for *specific* surveillance using a single attractant and for *generic* surveillance using multiple attractants.
- Sentinel trees: they refer to planted trees that are close to high-risk areas or trees already planted on purpose that are stressed or baited with volatiles. Larger is the area of interest, more sentinel trees are needed and that could necessitate too many efforts to control them. Sentinel trees can be useful when pests are already established and so in *post-borders* and *containment* surveillance, and they can attract a single species or multiple species.
- Sniffer dogs and predatory insects: the use of trained dogs to intercept non-native beetles is a good solution for *specific border* surveillance and eventually for *containment* surveillance. Cons of this solution are that dogs need an adequate training and their resistance have some time limitations (i.e., in a day of work), on the other hand pros regard their competence in finding both adult and larvae stages and they may recognize the presence of the beetles even in absence of visible symptoms of infestation. The use of predatory insects is a good approach for more cryptic target species (e.g., Agrilus jewel beetles) but the surveillance is limited by the duration of the detections. This method is suited in *post-border* and *containment* surveillance.
- Genetic tools: molecular approaches are essential for the correct identification of trapped specimens and recent technological advances allow their use also in the field (e.g., LAMP-based machines). Genetic tools are commonly used in the context of *border*, *post-border*, and *containment* surveillance.

- Remote sensing and aerial survey: they provide information about the state of vegetation that can be linked to the presence of non-native species. They are useful on large areas and only if the insects cause evident damages, so for *post-border* or *containment* surveillance.
- Citizen science: participation of non-experts in the detection of alien pests. The risk is to obtain information of low quality that needs further verification from specialists. Nonetheless, it represents an opportunity to raise awareness and educate citizens by using technological tools of easy and speed access.

The integration of the tools described above and the realization of accurate and precise detection methods represent a key step for increment biosecurity of non-native species.

*Figure 3 Purple multifunnel trap. Source: https://www.chemtica.com/site/?p=3731*

*Figure 4 Predatory wasp Cerceris fumipennis preying a jewel beetle. Ph: flatpickit, 2016 https://bugguide.net/node/view/1242949*

## 1.4 The use of baited-coloured traps for longhorn and jewel beetle surveillance

Baited traps are very effective tools to intercept non-native species after their arrival and to complement visual inspections (Poland et al., 2019; Marchioro et al., 2020). Traps are usually placed at points of entry and in their surroundings and are commonly used for both longhorn and jewel beetles.

For longhorn beetles, several studies over the last years focused on the chemical ecology of longhorn beetles, and nowadays pheromones are known for more than 200 species.

Pheromones are generally species specific but in the case of longhorn beetles, they can be attractive to a wide range of species. This led to the development of multi-lure baited traps, which allow to catch simultaneously several species (Roques et al., 2023). The main advantages of this approach are the detection of a larger number of species simultaneously, the reduction of the number of traps needed, and thus a reduction of costs (Rassati et al., 2021; Marchioro et al., 2021; Poland et al., 2019). The biggest con in the use of multilure traps is that different blend components may decrease the effect of others reducing the attraction of some species.

For jewel beetles, chemical ecology is much less studied and pheromones are known for only few species. Visual stimuli, instead, represent the key factor exploited to attract them. Jewel beetles in the genus *Agrilus*, for example, are strongly attracted to green traps, colours that are used during mate searching (Rassati et al., 2018). Recent studies showed that green traps can be used not only to monitor jewel beetles but also longhorn beetles (Rassat et al., 2019) and other wood-borers (Marchioro et al. 2020).

One of the main aspects that is still unclear is whether green multi-funnel traps baited with pheromones of longhorn beetles can be used to survey also jewel beetles. In other words, this requires that pheromones of longhorn beetles are not repellent for jewel beetles.

## 2. OBJECTIVES

The aim of our study was that of determining the efficacy of green multi-funnel traps baited with longhorn beetle pheromones to survey also jewel beetles, in particular species belonging to the genus Agrilus. To this aim, we carried out a field study comparing the following four treatments:

i) unbaited green multi-funnel traps.

ii) a blend composed of the pheromones D6, K6, K8 and ethanol which is known to be attractive for longhorn beetles in the subfamily Cerambycinae

iii) a blend composed of the pheromones EXF, EZFA and ethanol which is known to be attractive for longhorn beetles in the subfamily Lamiinae.

iv) green multi-funnel traps baited only with ethanol.

This study not only can help us to understand whether green-multi funnel traps baited with longhorn beetle pheromones can be used to reliably attract also jewel beetles, but also to understand whether ethanol can be considered as a lure to attract species of the latter beetle group.

# 3. MATERIALS AND METHODS

## 3.1 Study area

The study was carried out in an oak-hophornbeam forest located in Muzzana, Friuli-Venezia Giulia region, northeastern Italy. This forest, together with other few woods in the same region and nearby Veneto region, are remnants of a larger forest that covered the Po valley after the end of the ice age, reason why they are defined as "relicts" woods. The geological characteristics of the area present many springs with groundwater that make possible the presence of some "glacial relict" species as *Populus tremula*. These forests have always been a source of income for their municipality although in the 20th Century the operations of clearance and draining of the marshes provoked a significant reduction of the woods making space for agriculture. Nowadays the area is protected by conservation laws at European, national, and regional levels, and the competent municipality promotes the active protection and the management of the woods in order to extend them and to increase their ecological, social, and historical importance; in fact, since 1995 Muzzana woods are part of the areas included in the Natura 2000 network. This area is very important for the biodiversity, having hundreds of species of plants, mosses and fungi, and its climate is recognized as "humid subtropical" by Köppen classification.

## 3.2 Monitoring and sampling

### 3.2.1 Traps, installation period and treatments

We used green multi-funnel traps (Synergy Semiochemicals, Canada), which are composed by 12-funnels and a collection cup at the bottom. This type of trap is simple and quick to set up, which makes it useful at high-risk zones as ports of entry (Rassati et al. 2018). Traps were installed on the 26, 27, 28 of May 2021 and were monitored every three weeks until mid-August. Four treatments were compared:

- Unbaited green multi-funnel traps. This trap color was selected because it was found to be attractive both for jewel beetles and several longhorn beetles (Rassati et al., 2018 and Cavalletto et al., 2020)
- Green multi-funnel traps baited only with ethanol. The ultra-high release rate (UHR) ethanol, which simulates stressed or dying tree, is an effective attractant both per se and in combination with other semiochemicals (Rassati et al., 2016)

- Green multi-funnel traps baited with racemic 3-hydroxyhexan-2-one + 3-hydroxyoctan-2-one + *syn*-2,3-hexanediols + UHR ethanol. These longhorn beetle pheromones constitute a blend attractive for longhorn beetles, particularly for the subfamily Cerambycinae (Rassati et al., 2018)
- Green multi-funnel traps baited with *E/Z*-fuscumol + *E/Z*-fuscumol acetate + UHR ethanol. Fuscumol is an important component of male-produced aggregation attractants, and with fuscumol acetate forms a blend known to be attractive for longhorn beetles, especially in the subfamily Lamiinae (Mitchell et al., 2001)

*Figure 5 Green multifunnel trap baited with pheromones. Source: https://semiochemical.com/synergy-multitrap-platform/*

To simplify and to make more rapid the comprehension in the text, the pheromones compounds used in these experiments were identified with an alphanumeric code: **D6** represents sy-2,3-hexanediol; **K6** represents racemic 3-hydroxyhexan-2-one; **K8** refers to racemic 3-hydroxyoctan-2-one; **EZF** is (E/Z-fuscumol) and **EZFA** is (E/Z)-fuscumol acetate.

Semiochemicals were released from two types of dispensers: D6, K6, and K8 were released through a cellulose sponge sealed in a polyethylene pouch, whereas EZF and EZFA from a rubber septa device.

### 3.2.2 Experimental design and installation of traps

We used a complete randomized block design, with treatments replicated 6 times in 6 blocks. A distance of about 50 m was kept among blocks. A total of twenty-four traps were installed. In order to set up traps in the tree canopy we selected high trees with solid branches, irrespective of the species. The height at which the traps were set up was on average 10.5 meters. The collection cup was half-filled with a solution having 50% of water and 50% of ethylene glycol which helps to kill the caught insects and to maintain them in a good conservation status.

The installation of the traps was carried out thanks to the use of a slingshot which permitted to launch a guide rope towards the selected tree in order to anchor the rope around a stable branch.

*Figure 6 Use of the slingshot for the installation of the traps*

Once the rope was anchored, the trap was raised up along the course of the guide rope until the height of the canopy was reached. The guide rope was pulled down and the trap was fixed with its own rope thanks to the use of a nail in the trunk of the nearest tree.

*Figure 7 Raising up a multifunnel trap to reach the desired height*

### 3.2.3 Traps control

Trap checks were carried out every three weeks starting from the installation date.

Traps were checked and emptied lowering them using the rope already fixed in the trunk of the closest tree. The first phase of control consisted in a visual inspection of the tools (traps, collection cups and ropes) in order to assess each time their efficacy and to check the presence of any damage. Traps were then cleaned up by using a solution of water and ethanol to remove the insects stuck in the funnels and to permit their eventual fall in the collection cup.

In the middle of control period, on the 5th of July, the dispensers of D6, K6, K8 and EZF, EZFA were replaced to ensure their efficacy until the end of the study.

The collection cups were emptied into plastic containers of 50mL with a screw top; then each container was labelled with a code to identify the block, the day of control, and the respective treatment used in the trap just emptied. In this way, the content in each container was correctly stored and reported for the following analysis. For each block, all the collection cups were refilled using a funnel and a tank containing the solution to replace based on water and ethylene glycol.

## 3.3 Laboratory analysis

After each day of sampling the work continued in the laboratory of entomology of the University of Padua, DAFNAE department.

One by one the plastic containers were emptied transferring all the contained material into a fine-mesh sieve to eliminate the liquids in excess without losing the insects. The drained compound was moved into a Petri dish where an accurate cleaning was effectuated in order to eliminate unnecessary residuals as pieces of leaves and other kind of organisms. Once the material was clear enough, a first observation of the insects was made to obtain an initial classification of them. The insects were sorted and separated in Buprestidae and Cerambycidae.

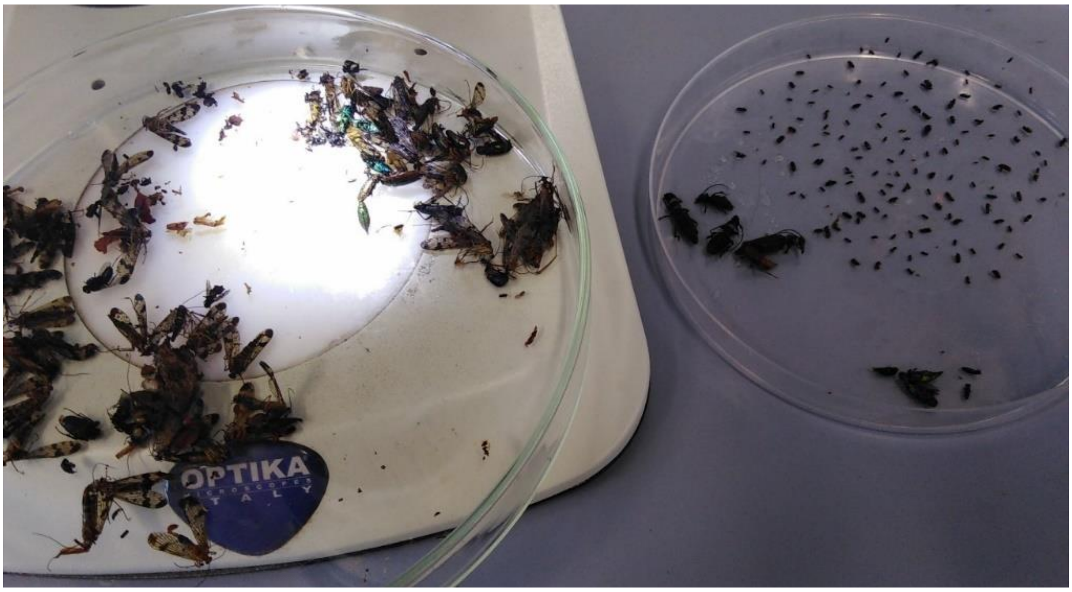

*Figure 6 Separation of the caught beetles in Buprestidae and Cerambycidae. Photo made in DAFNAE laboratory, personal source*

*Figure 7 Two jewel beetles before their identification. Photo made in DAFNAE laboratory, personal source*

Individuals were then identified to species level by Gianfranco Curletti (Buprestidae) and Filippo Giannone (Cerambycidae).

### 3.4 Statistical analysis

The effect of trap lure was tested using generalized linear mixed models. Four models were fitted with a Poisson distribution, using a log link-function. Species richness (*i.e.*, total number of Cerambycidae species and *Agrilus* species) and total abundance (*i.e.*, total number of Cerambycidae individuals and *Agrilus* individuals) were considered as response variables. Data collected from each trap and pooled over the sampling rounds were treated as a distinct statistical unit. The block identity was included in the models as a random factor. Pairwise multiple comparisons were run using post-hoc tests with Tukey correction of p-values. All the analyses were carried out in R software (R Core Team, 2021).

# 4. RESULTS

## 4.1 General results

A total of 952 beetle individuals were collected, among which 596 (63%) were jewel beetles belonging to the genus *Agrilus* and 356 (37%) were instead longhorn beetles among them, 182 individuals belonging to the subfamily Lamiinae and 160 to the subfamily Cerambycinae.

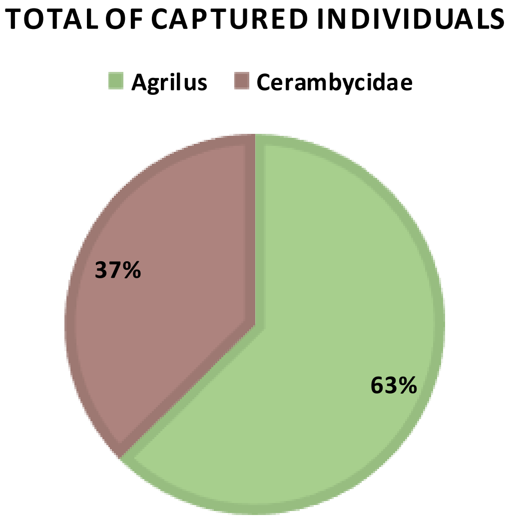

*Figure 8 Percentage of the total individuals captured*

A total of 32 beetle species were collected, 24 Cerambycidae and 8 *Agrilus* spp (Table 1). 30 species were native and only 2 were exotic, both longhorn beetles (i.e., *Neoclytus acuminatus* and *Xylotrechus stebbingi*).

| | | Treatment | | | | |
|---|---|---|---|---|---|---|
| | **Status** | **EMPTY** | **ETH** | **EZF_EZFA_ETH** | **D6_K6_K8_ETH** | **Total** |
| **Buprestidae** | | **163** | **153** | **151** | **129** | **596** |
| *Agrilus angustulus* | Native | | | 2 | 1 | 3 |
| *Agrilus convexicollis* | Native | 7 | 15 | 11 | 15 | 48 |
| *Agrilus graminis* | Native | | 1 | | | 1 |
| *Agrilus hastulifer* | Native | 1 | 7 | | 1 | 9 |
| *Agrilus laticornis* | Native | 9 | 9 | 2 | 10 | 30 |
| *Agrilus obscuricollis* | Native | 1 | 2 | | | 3 |
| *Agrilus olivicolor* | Native | 145 | 112 | 136 | 102 | 495 |
| *Agrilus sulcicollis* | Native | | 7 | | | 7 |
| **Cerambycidae** | | **24** | **50** | **104** | **178** | **356** |
| *Aegomorphus clavipes* | Native | | | 21 | | 21 |
| *Anaesthetis testacea* | Native | | 1 | 1 | 18 | 20 |
| *Anaglyptus gibbosus* | Native | | | | 1 | 1 |
| *Anoplodera sexguttata* | Native | 1 | 1 | | | 2 |
| *Clytus arietis* | Native | 1 | 1 | | 5 | 7 |
| *Deroplia genei* | Native | | | | 1 | 1 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Exocentrus adspersus* | Native | 3 | 1 | 1 | 1 | 6 |
| *Exocentrus punctipennis* | Native | 3 | 25 | 13 | 1 | 42 |
| *Gracilia minuta* | Native | | | | 7 | 7 |
| *Grammoptera ruficornis* | Native | 3 | 6 | | 1 | 10 |
| *Leiopus nebulosus* | Native | 4 | 1 | 32 | 3 | 40 |
| *Mesosa nebulosa* | Native | | | | 1 | 1 |
| *Neoclytus acuminatus* | Exotic | | 1 | | 20 | 21 |
| *Oberea linearis* | Native | 2 | 2 | 2 | 2 | 8 |
| *Phymatodes testaceus* | Native | | | | 7 | 7 |
| *Plagionotus arcuatus* | Native | | | | 1 | 1 |
| *Plagionotus detritus* | Native | | | | 12 | 12 |
| *Rutpela maculata* | Native | | 2 | | | 2 |
| *Saperda punctata* | Native | 3 | 3 | 24 | 4 | 34 |
| *Tetrops praeustus* | Native | 1 | 1 | 4 | 1 | 7 |
| *Tetrops starkii* | Native | 2 | | | | 2 |
| *Xylotrechus antilope* | Native | | 2 | 1 | 14 | 17 |
| *Xylotrechus stebbingi* | Exotic | 1 | 3 | 5 | 78 | 87 |
| **Total** | | **187** | **203** | **255** | **307** | **952** |

*Table 1 List of all the captured species*

In general, traps baited with D6-K6-K8 and ethanol allowed to catch the highest number of individuals (i.e., 307, 32%) followed by traps baited with EZF-EZFA and ethanol (255 individuals, 27%), traps baited with ethanol (203, 21%) and empty traps (187, 20%).

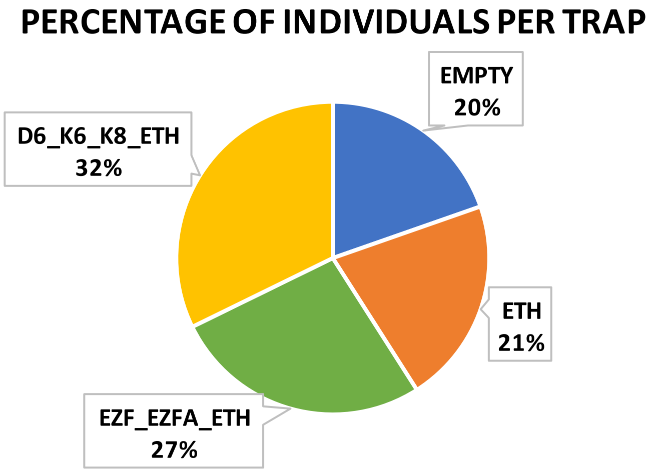

*Figure 9 Percentage of total individuals captured per traps*

### 4.2 Effects of trap treatment on richness and abundance of longhorn beetles

The mean number of longhorn beetle species was significantly higher in traps baited with D6-K6-K8 and ethanol than in traps baited with ethanol and in empty traps ($\chi^2$ = 19,755; df=3; p-value=0.0002) but not different from traps baited with EZF-EZFA and ethanol (Fig. 10). For longhorn beetle abundance, traps baited with D6-K6-K8 and ethanol caught significantly more individuals than all the other treatments ($\chi^2 = 128,23$; df=3; p-value=0.0001). In addition, traps baited with EZF-EZFA and ethanol caught significantly more beetles than empty and ethanol-baited traps and ethanol baited traps more beetles than empty traps (Fig. 11).

The empties and only with ethanol baited traps have not a significant difference, whereas the traps baited with EZFA and EZFA show a little significant difference but the D6, K6, K8 traps have a strong significant difference respect to the others. For the EMPTY traps the mean of Cerambycidae richness is 3 with standard deviation (SD) equal to 2,7; in ETH baited traps the mean is 3,8 and the SD is 1,8; in EZF_EZFA_ETH baited traps the mean is 4,8 and the SD is 0,8; in D6_K6_K8_ETH baited traps the mean is 8,5 and the SD is 2,1. For what concerns the Cerambycidae abundance, the mean of EMPTY traps is equal to 4 with SD of 4,2; in ETH baited traps the mean is 8,3 and the SD is 8; in EZF_EZFA_ETH baited traps the mean is 17,3 and the SD 9,9; in D6_K6_K8_ETH baited traps the mean is 29,7 and the SD 12,5.

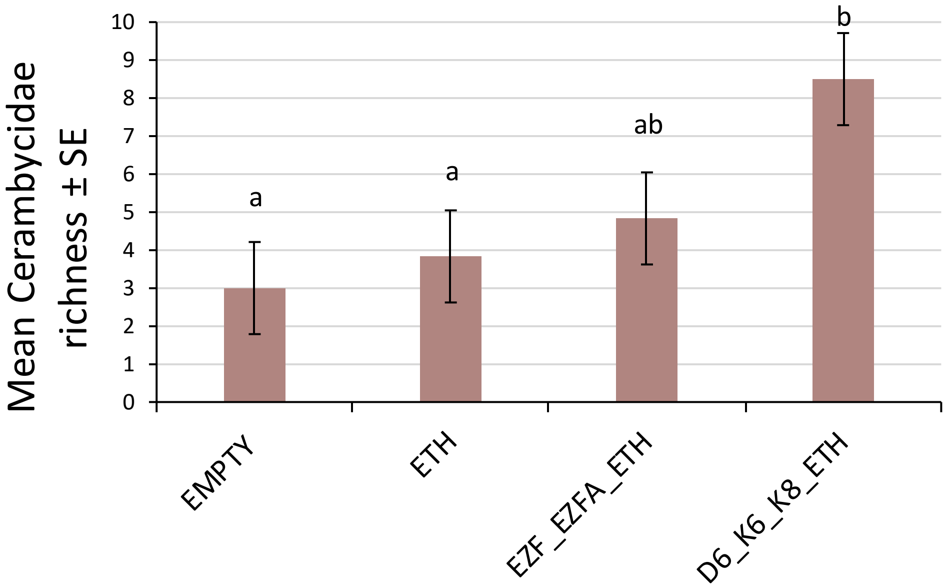

*Figure 10 Differences of the traps respect the mean Cerambycidae richness ± SE*

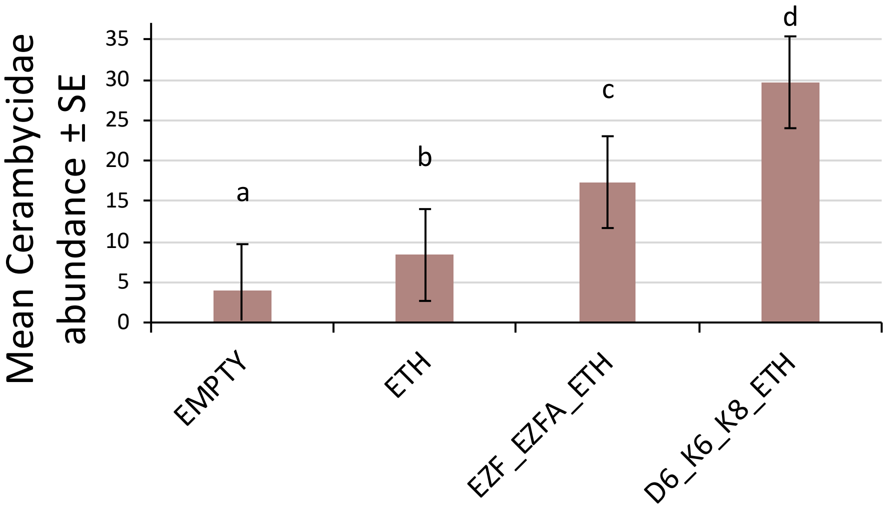

*Figure 11 Differences of the traps respect the mean Cerambycidae abundance ± SE*

## 4.3 Effects of trap treatment on richness and abundance of Agrilus spp.

The richness of Agrilus spp. does not present significant differences between the different treatments ($\chi^2$ = 0,1427; df = 3; p-value = 0,9863) (Fig. 12). In the same way, for Agrilus spp. abundance none of the traps used show significant differences ($\chi^2$ = 4,12; df = 3; p-value = 0,2487) (Fig. 13).

For the EMPTY traps the mean of Agrilus richness is equal to 2,3 with SD of 1,5; in ETH baited traps the mean is 2,5 and the SD is 2,3; in EZF_EZFA_ETH baited traps the mean is 2,2 and the SD is 1,3; in D6_K6_K8_ETH baited traps the mean is 2,3 and the SD is 1,5. For what concerns Agrilus spp. abundance, the mean of EMPTY traps is equal to 27,2 with SD of 28,7; in ETH baited traps the mean is 25,5 and the SD is 16,7; in EZF_EZFA_ETH baited traps the mean is 25,2 and the SD is 23,5; in D6_K6_K8_ETH baited traps the mean is 21,5 and the SD is 14,3.

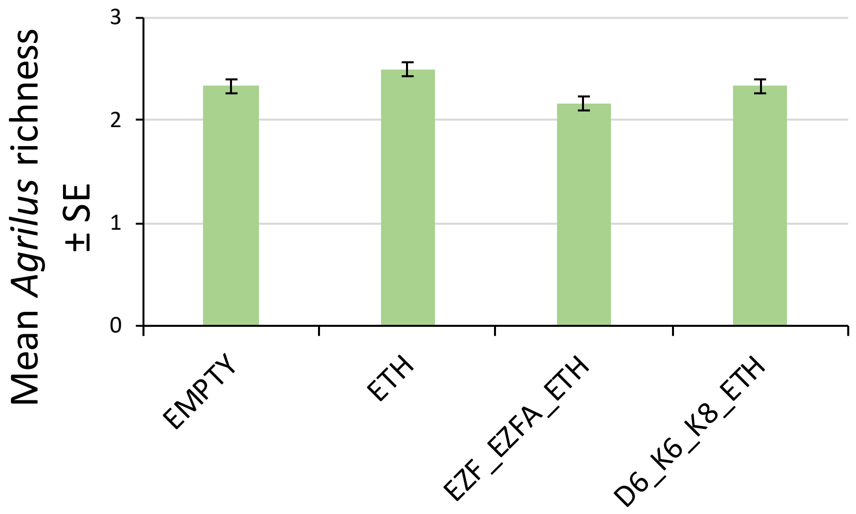

*Figure 12 Differences of the traps respect the mean Agrilus richness ± SE*

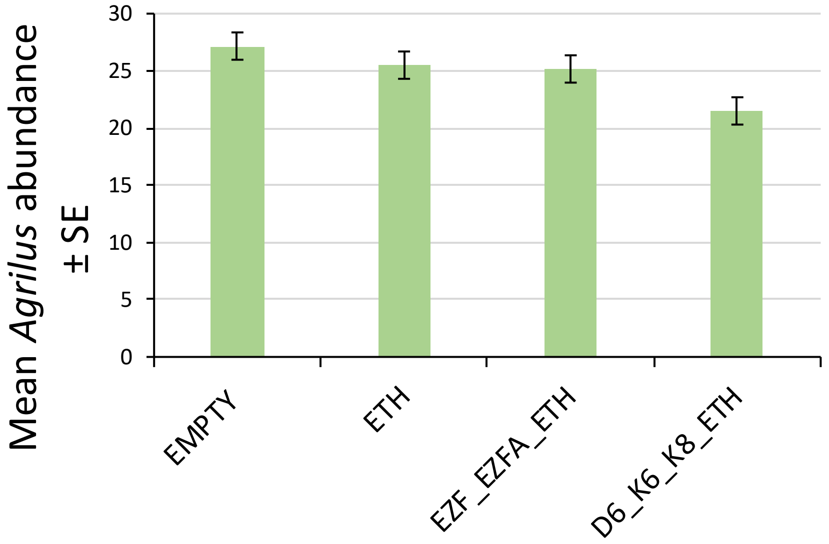

*Figure 13 Differences of the traps respect the mean Agrilus abundance ± SE*

### 4.4 Difference in longhorn beetle communities in traps baited with the different pheromone blends

The Venn diagram is widely used to show the logical relations between sets, in this study it has been used to highlight the relation between the two baited traps which caught the highest number of species of longhorn beetle species, that are D6_K6_K8_ETH and EZF_EZFA_ETH. Traps baited with D6_K6_K8_ and ethanol caught 7 exclusive species (i.e., *Anaglyptus gibbosus, Deroplia genei, Gracilia minuta, Mesosa nebulosa, Phymatodes testaceus, Plagionotus arcuatus, Plagionotus detritus* ), traps baited with EZF_EZFA and ethanol caught only 1 exclusive species (i.e., *Aegomorphus clavipes*), while 9 species were in common (*Anaesthetis testacea; Exocentrus adspersus; Exocentrus punctipennis; Leiopus nebulosus; Oberea linearis; Saperda punctata; Tetrops praeustus; Xylotrechus antilope; Xylotrechus stebbingi*).

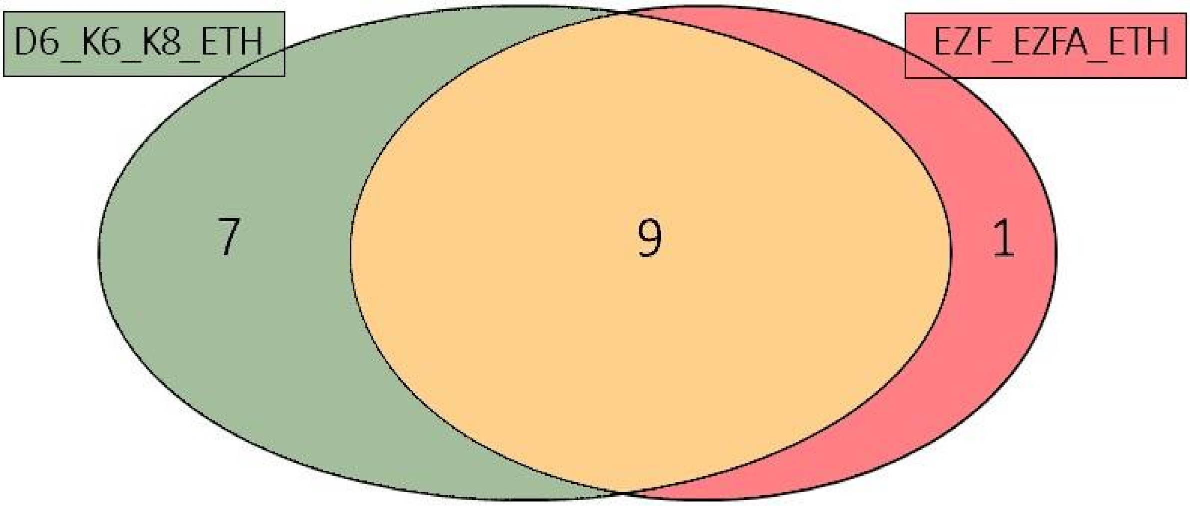

*Figure 14 Relation between baited traps with D6_K6_K8_ETH and baited traps with EZF_EZFA_ETH, in the capture of Cerambycidae species*

# 5. DISCUSSION

Early-detection of plant pests using baited traps at points of entry and their surrounding areas is one of the most important and used measures of prevention and control (Poland and Rassati 2019). There are several surveillance tools that can be used, but traps are easy to use and cheaper compared to the other tools.

Beetles within the families Cerambycidae and Buprestidae, especially the genus *Agrilus*, are wood-borers that can provoke economic and ecological damages in natural and anthropized ecosystems (Ruzzier et al., 2023). Coloured traps baited with attractive lures are commonly used also for early-detection of these beetle groups. In particular, green traps are the most efficient for jewel beetles of the genus *Agrilus*, while black or green traps baited with pheromones are commonly used for longhorn beetles. Whether green traps baited with longhorn beetle pheromones can be a good approach to catch longhorn beetles without interfering negatively with jewel beetles is still unclear. This combination would permit the capture of both taxa, reducing time and costs associated to the use of different trap models.

For longhorn beetles, green baited-multi-funnel traps allowed us to catch a high number of species of longhorn beetles, belonging to three different subfamilies, i.e., Lamiinae, Cerambycidae and Lepturinae. In particular, traps baited with D6, K6, K8 and ethanol caught significantly more species and individuals compared to the other treatments, even though the number of species was not different to that achieved in traps baited with EZF, EZFA, and ethanol. On the other hand, traps baited only with ethanol or left unbaited did not show an attractive effect to the sampled longhorn beetles These results are in line with previous studies (e.g., Rassati et al., 2018 and Hanks et al., 2012) for which the use of multi-lure and so more than one pheromone allows the attraction and the capture of different species and individuals of longhorn beetles simultaneously. According to Sweeney et al., (2014) and Mitchell et al., (2011), the addition in the same trap of the ethanol lure can increase the effectiveness of the pheromones used. It is also interesting to notice that all but one of the species caught on traps baited with EZF, EZFA, and ethanol were also caught in traps baited with D6, K6, K8 and ethanol, clearly indicating that the latter blend is the best choice to bait green traps in surveillance programs.

For jewel beetles of the genus *Agrilus*, no significant difference among the tested treatment was found in our study. Despite this is a predictable result because the jewel beetles based their movements on visual stimuli at all their stages of life for this reason they are attracted by the colour of traps, in particular green (Rassati et al., 2018), it is important because indicates that longhorn beetle pheromones did not have a repellent effect against jewel beetles. Thus, green multi-funnel traps baited with longhorn beetle pheromones, especially D6, K6, K8, and ethanol can be considered a good tool for simultaneous early-detection of both longhorn beetles and jewel beetles. It is also interesting to notice that ethanol did not improve *Agrilus* spp. catches compared to unbaited traps, indicating that ethanol is not a primary cue used by these jewel beetle species. Surveillance programs targeting only jewel beetles can thus be carried out simply using unbaited green multi-funnel traps.

In conclusion, results presented in this thesis represent an important improvement of the existing trapping protocols for longhorn beetles and jewel beetles. Our indication to use green-multi-funnel traps baited with D6, K6, K8 and ethanol to survey both taxa simultaneously can allow to reduce the number of traps needed and the overall costs of the surveillance program. This represents a very important aspect as budgets for this kind of activity are often limited, so it is the available personnel. Our study also indicates that a better understanding of jewel beetle chemical ecology might be a key aspect to achieve as no lure is available at present.

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