UNIVERSITÀ
DEGLI STUDI
DI PADOVA

Head Office: Università degli Studi di Padova

Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE)

Ph.D. COURSE IN: CROP SCIENCE

SERIES XXXIV

Development of protocols for surveillance and monitoring of alien pests in points-of-entry and surrounding forests

Coordinator: Chiar.mo Prof. Claudio Bonghi

Supervisor: Chiar.mo Prof. Massimo Faccoli

Ph.D. student: Matteo Marchioro

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Padua, 29 September 2021 Matteo Marchioro

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Summary

Increased international trade has led to an increased risk of introducing new alien species. Insects are one of the most succesfull groups of invaders and in the last decades the number of alien insect species invading Europe increased exponentially. Several regulations, protocols and tools were developed in order to prevent new introductions or mitigate their effects and they can be classified depending on the targeted invasion stage. *Border surveillance* is applied at the initial stage of the invasion process, at points-of-entry (*i.e.*, ports, airports) and it aims to prevent the establishment of an alien species just arrived. *Post-border surveillance*, instead, is applied at the first establishment stage and it aims to detect alien species that eluded border surveillance when the population level is still low. Lastly, when an invasive species is established, *eradication* or *containment* protocols can be applied to reduce economic and environmental impacts. The general aim of this thesis is to provide new tools and protocols useful for contrasting the problem of the invasive alien species in Europe. The thesis is divided into three sections, one for each stage of the biosecurity surveillance. For the border surveillance section, the effectiveness of a new light-sticky trap for the use inside containers during shipment was investigated. Results are positive for all the tested species: the use of white, ultraviolet or red light is the best solution against Diptera and Lepitoptera, whereas red light is the most attractive for Coleoptera, but the use of stronger entomological glue is required to ensure their capture. For the post-border surveillance section, trapping protocols for longhorn and jewel beetles were improved in order to maximizes catches also of ambrosia and bark beetles. Results showed that dark (black or purple) traps set in the understory and green traps set in the canopy, all of them baited with a blend composed by longhorn beetle pheromones and host volatiles, are the best combination. Then, an effective trapping protocol was explored against *Anoplophora chinensis* Thomson (Coleoptera: Cerambycidae), an extremely polyphagous Asian beetle introduced in Europe. Different trap models, lures and trap positions were tested and the best combination is the use of a soft cross-vane trap baited with the Synergy blend and set in the crown of trees. For the containment and eradication protocols section, two studies on two important

invasive wood-boring beetles were conducted. In the first research the dispersal capacity and factors affecting dispersal of *Pityophthorus juglandis* Blackman (Coleoptera: Curculionidae, Scolytinae), an important pest of walnut orchards, were investigated. An annual mean distance was estimated in about 9 km, with peaks of over 40 km; factors affecting the risk of infestation are the distance between an healty orchard and an infested one, the size of the orchard and the walnut species. Results highlighted the inadequacy of the containment measures adopted so far. Lastly, the successful eradication program undertaken in Cornuda municipality (Treviso province, Italy) against *Anoplophora glabripennis* Motschulsky (Coleoptera: Cerambycidae) was presented. All the actions applied during the protocol (suitable trees survey, felling and destruction of infested trees, trapping protocol, mitigation plan and communication with citizens) were accurately described in order to provide a usefull guide for future eradications.

Riassunto

L'aumento del commercio internazionale ha portato ad un aumento del rischio di introdurre nuove specie aliene. Gli insetti sono una delle classi di maggior successo e negli ultimi decenni il numero di specie di insetti aliene invasive in Europa è aumentato esponenzialmente. Diversi regolamenti, protocolli e strumenti sono stati sviluppati al fine di prevenire nuove introduzioni e possono essere classificati a seconda della fase dell'invasione su cui agiscono. La *border surveillance* viene applicata nella fase iniziale del processo di invasione, nei punti di ingresso (es. porti, aeroporti) ed è finalizzata a prevenire l'insediamento di una specie aliena appena arrivata. La *post-border surveillance*, invece, viene applicata al primo stadio di insediamento e mira a individuare le specie aliene che sono sfuggite alla sorveglianza di confine quando il livello di popolazione è ancora basso. Infine, quando una specie invasiva si è stabilita, si possono applicare protocolli di *eradicazione* o di *contenimento* per ridurre gli impatti economici e ambientali. L'obiettivo generale di questa tesi è quello di fornire nuovi strumenti e protocolli utili per contrastare il problema delle specie aliene invasive in Europa. Questa tesi è suddivisa in tre sezioni, una per ogni stadio della sorveglianza fitosanitaria. Per la sezione sulla border surveillance, è stata studiata l'efficacia di una nuova trappola luminosa adesiva da utilizzare all'interno dei container durante la spedizione. I risultati sono stati positivi con tutte le specie testate: l'uso di luce bianca, ultravioletta o rossa è la soluzione migliore contro Ditteri e Lepitotteri, mentre la luce rossa è la più efficacie per i Coleotteri, ma è necessario l'uso di un vischio entomologico più forte per garantirne la cattura. Per la sezione sulla post-border surveillance, è stato studiato come migliorare i protocolli di cattura per cerambicidi e buprestidi al fine di massimizzare le catture anche di scolitidi. I risultati hanno mostrato che le trappole scure (nere o viola) posizionate al livello del sottobosco e le trappole verdi posizionate nella chioma, tutte attivate con una miscela composta da feromoni di cerambicidi e sostanze volatili delle piante ospiti, sono la migliore combinazione. Inoltre è stato studiato un protocollo di cattura efficace contro *Anoplophora chinensis* Thomson (Coleoptera: Cerambycidae), un coleottero asiatico estremamente polifago introdotto in Europa. Sono stati analizzati diversi

modelli di trappole, attrattivi e posizioni delle trappole e la migliore combinazione è stata l'utilizzo di trappole cross-vane morbide con la miscela di attrattivi Synergy e posizionate nella chioma degli alberi. Per la sezione sui protocolli di contenimento ed eradicazione, sono stati condotti due studi su due importanti coleotteri xilofagi invasivi. Nella prima ricerca sono state studiate la capacità di dispersione e i fattori che influenzano la dispersione del *Pityophthorus juglandis* Blackman (Coleoptera: Curculionidae, Scolytinae), un importante parassita dei noceti. Una dispersione media annua di circa 9 km è stata stimata, con picchi di oltre 40 km; i fattori che influenzano il rischio di infestazione sono la distanza tra un frutteto sano e uno infestato, la dimensione del frutteto e la specie di noce. I risultati hanno evidenziato l'inadeguatezza delle misure di contenimento adottate finora. Infine, è stato presentato il caso del programma di eradicazione intrapreso nel comune di Cornuda (provincia di Treviso, Italia) contro *Anoplophora glabripennis* Motschulsky (Coleoptera: Cerambycidae). Tutte le azioni applicate durante il protocollo (la sorveglianza degli alberi suscettibili, l’abbattimento e la distruzione degli alberi infestati, i protocolli di monitoraggio con trappole, il piano di mitigazione e la comunicazione con i cittadini) sono state accuratamente descritte al fine di fornire una guida utile per future eradicazioni.

Chapter 1

Introduction

Alien insects in Europe

Insects represent the second most numerous alien invaders Class in Europe, after Magnoliopsida (Plantae kingdom), with 2,960 alien species recorded from the discovery of the Americas (about 21% of the total amount of the recorded alien species) (Roques et al. 2009; European Commission, 2021). Moreover, the number of alien species introduced each year is constantly increasing and is expected to continue to increase in the future years (Hulme 2009, Roques 2010, Seebens et al. 2017). In fact, in 2010 the number of alien insects recorded in Europe amounted to 1,390 (Roques et al. 2009, Roques 2010), and in just ten years this number has more than doubled (European Commission, 2021). Considering the number of alien insects introduced per year, numbers are even more impressive: it is estimated that between 1950 and 1974 an average of 10.9 species were introduced in Europe, whereas between 2000 and 2008 the average rose to 19.6 and, in the last decade, has increased more than eightfold reachen a mean of 157 per year (Roques, 2010; European Commission, 2021). Despite the fact that insects constitute such large proportions, they have received less attention in past decades than other groups, such as plants, vertebrates and aquatic organisms (Kenis et al. 2009).

The main pathways by which a species can overcome boundaries of its natural distribution ranges are: the deliberate release (which represents the only "intentional" pathway), the escape from a contained environment, the import of contaminated commodities and goods, the arrival as hitchhikers with vector vehicles and cargo, the use of human corridors that link regions previously unconnected, and the natural spread from a neighbouring region where the species maybe itself alien or native (the natural extension of the distribution range of native species caused by global warming is, in fact, a rapidly growing phenomenon) (Hulme et al. 2008, Walther et al. 2009). However, the introduction and establishment of new alien species is mainly driven by expansion of world-wide trade, globalisation of economies and climate change. It is well-known that the taxonomy and geographical pattern of biological invasors are closely linked to human trade and activities (Perrings et al. 2005, Meyerson and Mooney 2007).

An important pathway for invasions of new alien pests are shipping containers used in international trade (Meurisse et al. 2019). It is estimated that about 90% of global trade are carried with containers on ships (IMO, 2012). Although world maritime trade has slowed down in recent years in terms of percentage growth of total volumes, and the current global pandemic has further accentuated this contraction, in 2019 volumes of commodities were estimated to have reached 11.08 billion tonnes and are expected to recover again in the coming years (UNCTAD, 2019, 2020). The main orders of alien insects introduced via containers are Coleoptera, Diptera and Lepidoptera, followed by Hemiptera, typically as hitchhiking adults (Meurisse et al. 2019). Even a low rate of cargo infestation can represent a large number of potential invasions, given the huge volume of containers that move globally every day.

The first actions of governments related to alien species risk occurred in late 1800s and concerned live-stock, traded goods (Eiswerth et al. 2018) and, subsequently, trade in live plants (Liebhold and Griffin 2016). Then, in 1995, Sanitary and Phytosanitary Multinational Agreement regulates quarantine measures, which must be justified and based on an established risk to the importing country, and requires adherence to international Sanitary and Phytosanitary standards (Epanchin-Niell et al. 2021). Today, other international agreements have been signed, like the World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures (SPS), the International Plant Protection Convention (IPPC) of the Food and Agricultural Organization of the United Nations and the Convention for Biological Diversity (CBD). In Europe, only plant pest insect species are regulated (Regulation 2016/2031 of the European Parliament and of the Council on protective measures against pests of plants) and listed in the EPPO’s central communication database. Using information contained in this database, phytosanitary inspectors at Europe’s borders must check all goods and cargos that could contain black-listed insects. However, due to the increasing of international trade and the consequent enormous volumes of cargo that every day arrive at points-of-entry, inspectors are able to sample only a small percentage of total imports (Everett 2000, Surkov et al. 2008) and consequently the risk of new species crossing borders increases.

The impacts caused by establishment and spread of alien species can be different and, with a few exceptions like exotic biological control agents successfully used for pest control, they are usually considered to be negative (Kenis and Branco 2010). All the consequence of interactions between an alien species and economically valuable indigenous species (usually plants) were considered socio-economic impacts (Williamson 1996). Direct socio-economic impacts, that can be easily expressed in monetary values, occur when alien species causes yield losses or increasing production costs (Pimentel et al. 2002a,b). On the other hand, indirect socio-economic impacts include restriction on trade flow, changes in market values and consumer demand, changes in land use and landscape structure, costs associated with research, reduction of tourism, and they are often difficult to evaluate (Born et al. 2005, Xu et al. 2006). Other impacts caused by alien species may concern environment (affecting populations of native species and disturbing natural ecosystem processes), human and animal health, and human well-being (Kenis and Branco 2010, Vaes-Petignat and Nentwig 2014). This second group of environmental and ecological impacts may be assessed, instead, with more difficulty.

Wood-boring alien beetles

A significant number of invasive alien species belong to the group of wood-boring beetles (in particular Buprestidae, Cerambycidae and Scolytinae) (Cocquempot and Lindelöw 2010, Kirkendall and Faccoli 2010, Rassati et al. 2016, Eyre and Haack 2017), that include some of the most important tree pests (Kirkendall and Faccoli 2010, Haack 2017). Currently, 63 long-horn beetles and 229 curculionids (of which Scolytinae are a large percentage) alien species are recorded in Europe (Cocquempot and Lindelöw 2010, Sauvard et al. 2010). These insects, hidden inside the wood, can overcome the adverse conditions of the journey and escape phytosanitary controls (Humble 2010).

The main pathways for wood-boring beetle invasions are considered to be the international movement of wood packaging materials (WPM) and live plants, cutting, logs and processed woods (Eschen et al. 2015, Eyre et al. 2018). WPM are an ideal vector for wood-boring beetles as they are made from wood coming from different parts of the world and are therefore subject to different phytosanitary treatments (Clarke et al. 2001). It has already been observed that several alien wood-boring beetle species have been introduced via WPM, at all the life stages under residual bark or at egg, larva or pupa stages within the wood (Meurisse et al. 2019). Some examples are *Anoplophora glabripennis* Motschulsky (Coleoptera: Cerambycidae) in the USA (Haack et al. 2010) and *Ips grandicollis* Eichhoff (Coleoptera: Curculionidae, Scolytinae) in Australia (Morgan 1967). Beside WPM, live plants may also carry alien wood-boring beetles, which have been recorded (typically at egg, larva or pupa stages) in woody stems (Meurisse *et al.*, 2019), e.g. *Callidiellum rufipenne* (Motschulsky) (Coleoptera: Cerambycidae) (Cocquempot 2007) and *Xylosandrus morigerus* (Blandford) (Coleoptera: Curculionidae, Scolytinae) (Kirkendall and Faccoli 2010). Logs, as well firewood, are considered high-risk pathways for forest pests and wood borers, as they are often transported with the bark and are often untreated (Morrell 1995, Tkacz 2002, Solano et al. 2021). For example, the movement of logs and firewood is considered one of the main causes of the spread of *Pityophthorus juglandis* Blackman (Coleoptera: Curculionidae, Scolytinae) in the United States and its arrival in Europe (Newton and Fowler 2009, EPPO 2015).

Biosecurity surveillance

When a non-native species population establishes and expands into a new area, the management becomes more difficult and expensive (Liebhold and Tobin 2008), as demonstrated, for examples, by the enormous efforts and costs incurred in eradicating *Anoplophora glabripennis* from North-East Italy and England (Faccoli and Gatto 2016, Eyre and Barbrook 2021). For this reason, detecting an alien species at early stages of its invasion allows immediate mitigation measures to be taken, reducing damage and costs, and increasing the possibilities of its eradication (Poland and Rassati 2019).

Hulme (2014) classified the biosecurity surveillance actions depending on the invasion stage. *Pre-border surveillance* includes all the policies and the international agreements that allow to import commodities safely, reducing trade restrictions (Poland and Rassati 2019). Through international cooperation, information about emerging pests can be shared and risks associated with the import of new goods can be considered (Hulme 2014). *Border surveillance* covers all actions taken at point-of-entry, at the early stages of the invasion, to prevent the alien species establish and spread. Phytosanitary inspectors are the key to this stage: operating at points-of-entry they can check incoming loads and decide whether to reject or destroy infested goods. *Post-border surveillance* aims to intercept all alien species that have escaped border controls, at the early establishment stages, when population level is still low (Liebhold and Tobin 2008, Poland and Rassati 2019). Post-border surveillance operates at a spatial scale larger than border surveillance, typically in natural, urban and industrial areas surrounding points-of-entry. If an alien species successfully establishes itself in the new ecosystem, eradication or containment measures can be taken. *Eradication* is the reduction of a population in a specific geographic area, aimed at preventing its reproduction and, therefore, bringing it to the local extinction (Myers et al. 1998, Liebhold and Tobin 2008). *Containment*, on the other hand, includes all the strategies aimed at slowing or stopping the invading species spread, in order to contain the infestation in a given area (Liebhold and Kean 2019). Once invasion occurred, eradication is always preferable than containment, but not always possible (and very often difficult, costly and uncertain in any case). Certain conditions (*i.e.*, early detection of the pest, ability to detect and identify the invader and availability of effective tools of pest monitor and control) and the biological characteristics of the target species (low rate of reproduction and dispersal and limited host range) may increase the probability of success of an eradication programme (Brockerhoff et al. 2010, Tobin et al. 2014).

Traps are widely used in active detection of exotic species, due to their effectiveness, cheapness and versatility of use (Augustin et al. 2012). Insect traps are, in fact, available in several models and can be used with a wide range of stimuli as lures: lights, colours, semiochemicals, sounds (Walker 1988, Vrdoljak and Samways 2012, Rassati et al. 2019, Neupane et al. 2020, Ruchin et al. 2020). Traps can be used both in *border surveillance* context, for the interception of alien insects at their arrive at points-of-entry (Rassati et al. 2015, Fan et al. 2019), and in *post-border surveillance* context, for monitoring neighbouring areas (Tobin et al. 2007, Tobin and Blackburn 2007, Rabaglia et al. 2008). Moreover, traps can be used against a specific target species (*i.e.*, quarantine species) using specific pheromones or lures, or for generic broad-spectrum surveillance (Poland and Rassati 2019). Lastly, traps can be used also for *eradication* and *containment* programs, by mass-trapping protocols (Sanchez-Husillos et al. 2015, Bali et al. 2021).

Research objectives and thesis structure

Studies presented in this thesis deal with different stages of biosecurity surveillance, exploring issues that have not yet been addressed in the past. For each stage, a new topic was presented and investigated, contributing to the new knowledge to expand tools and protocols useful for contrasting the problem of the invasive alien species in Europe. The thesis is composed by 3 different sections, each reporting chapters focused on different topics of interception and management of model alien species, as follows:

Section 1: Border surveillance

Chapter 2 presents results regarding a new type of light-sticky trap set up inside containers used during international shipments. The light-sticky trap, which aims to intercept alien insects travelling with commodities, was tested against four model species, in empty and loaded containers. The optimal trap density was also investigated.

Chapter 3 deepens the study presented in the previous chapter. Specifically, the effect of different light colours (*i.e.*, wavelength) of light-sticky traps and the addition of entomological glue and insecticide were investigated on the catching performance.

Section 2: Post-border surveillance

Chapter 4 describes the possible application of trapping protocols commonly used to survey native and exotic longhorn and jewel beetles (Coleoptera: Buprestidae and Cerambycidae) for detecting also bark and ambrosia beetles (Coleoptera: Scolytinae). Three key variables (*i.e.*, trap colour, trap position, and attractive blend) were investigated on the species richness and abundance of bark and ambrosia beetles found in the traps.

Chapter 5 presents results of a detection protocol carried out in Lombardy against the Citrus Longhorn Beetle, *Anoplophora chinensis* (Coleoptera: Cerambycidae), testing three trap models, three lure blends and two trap position. The study presents result in terms of catches of the 18 possible combinations of the three variables, in order to identify the best protocol for the monitoring of the species.

Section 3: Containment and eradication programs

Chapter 6 presents results of an 8-year survey carried out on the alien Walnut Twig Beetle (WTB), *Pityophthorus juglandis* (Coleoptera: Curculionidae, Scolytinae), in North Eastern Italy since 2013. The aim of the study is to analyse the effective dispersal capacity of WTB, the factors affecting dispersal, and the colonization-risk of healthy walnut plantations.

Chapter 7 describes all the actions and procedures carried out during an 11-year eradication program conducted in order to eradicate the Asian Longhorn Beetle (ALB) *Anoplophora glabripennis* (Coleoptera: Cerambycidae) from North-eastern Italy, providing a useful example for current and future ALB eradication programs in Europe.

References

Augustin, S., N. Boonham, W. J. De Kogel, P. Donner, M. Faccoli, D. C. Lees, L. Marini, N. Mori, E. Petrucco Toffolo, S. Quilici, A. Roques, A. Yart, and A. Battisti. **2012**. A review of pest surveillance techniques for detecting quarantine pests in Europe. EPPO Bull. 42: 515–551.

Bali, E.-M. D., C. A. Moraiti, C. S. Ioannou, V. Mavraganis, and N. T. Papadopoulos. **2021**. Evaluation of Mass Trapping Devices for Early Seasonal Management of Ceratitis Capitata (Diptera: Tephritidae) Populations. Agronomy. 11: 1101.

Born, W., F. Rauschmayer, and I. Bräuer. **2005**. Economic evaluation of biological invasions—a survey. Ecol. Econ. 55: 321–336.

Brockerhoff, E. G., A. M. Liebhold, B. Richardson, and D. M. Suckling. **2010**. Eradication of invasive forest insects: Concepts, methods, costs and benefits. New Zeal. J. For. Sci. 40: 117–135.

Clarke, J. W., M. S. White, and P. A. Araman. **2001**. Performance of pallet parts recovered from used wood pallets. For. Prod. J. 51: 55–62.

Cocquempot, C. 2007. Alien longhorned beetles (Coleoptera Cerambycidae): original interceptions and introductions in Europe, mainly in France, and notes about recently imported species. Redia. 89: 35–50.

Cocquempot, C., and Å. Lindelöw. **2010**. Longhorn beetles (Coleoptera, Cerambycidae). Chapter 8.1. BioRisk. 4: 193–218.

Eiswerth, M., C. Lawley, and M. H. Taylor. **2018**. Economics of Invasive Species. *In* Oxford Res. Encycl. Environ. Sci. Oxford University Press.

Epanchin-Niell, R., C. McAusland, A. Liebhold, P. Mwebaze, and M. R. Springborn. **2021**. Biological Invasions and International Trade: Managing a Moving Target. Rev. Environ. Econ. Policy. 15: 180–190.

EPPO. **2015**. Pest Risk Analysis for Thousand cankers disease (Geosmithia morbida and Pityophthorus juglandis). Paris.

Eschen, R., J.-C. Grégoire, G. M. Hengeveld, B. M. de Hoop, L. Rigaux, and R. P. J. Potting. **2015**. Trade patterns of the tree nursery industry in Europe and changes following findings of citrus longhorn beetle, Anoplophora chinensis Forster. NeoBiota. 26: 1–20.

European Commission. 2021. Joint Research Centre - European Alien Species Information Network (EASIN). https://easin.jrc.ec.europa.eu/

Everett, R. A. 2000. Patterns and pathways of biological invasions. Trends Ecol. Evol. 15: 177–178.

Eyre, D., and J. Barbrook. 2021. The eradication of Asian longhorned beetle at Paddock Wood, UK. CABI Agric. Biosci. 2: 12.

Eyre, D., and R. A. Haack. **2017**. Invasive Cerambycid Pests and Biosecurity Measures, pp. 563–618. *In* Wang, Q. (ed.), Cerambycidae World Biol. Pest Manag. CRC Press, Boca Raton, FL.

Eyre, D., R. Macarthur, R. A. Haack, Y. Lu, and H. Krehan. **2018**. Variation in Inspection Efficacy by Member States of Wood Packaging Material Entering the European Union. J. Econ. Entomol. 111: 707–715.

Faccoli, M., and P. Gatto. **2016**. Analysis of costs and benefits of Asian longhorned beetle eradication in Italy. Forestry. 89: 301–309.

Fan, J. ting, O. Denux, C. Courtin, A. Bernard, M. Javal, J. G. Millar, L. M. Hanks, and A. Roques. **2019**. Multi-component blends for trapping native and exotic longhorn beetles at potential points-of-entry and in forests. J. Pest Sci. (2004). 92: 281–297.

Haack, R. A. 2017. Cerambycid pests in forests and urban trees, pp. 352–384. *In* Wang, Q. (ed.), Cerambycidae World Biol. Pest Manag. CRC Press, Boca Raton, FL.

Haack, R. A., F. Hérard, J. Sun, and J. J. Turgeon. **2010**. Managing Invasive Populations of Asian Longhorned Beetle and Citrus Longhorned Beetle: A Worldwide Perspective. Annu. Rev. Entomol. 55: 521–546.

Hulme, P. E. 2009. Trade, transport and trouble: managing invasive species pathways in an era of globalization. J. Appl. Ecol. 46: 10–18.

Hulme, P. E. 2014. An Introduction to Plant Biosecurity: Past, Present and Future, pp. 1–25. *In* Handb. Plant Biosecurity. Springer Netherlands, Dordrecht.

Hulme, P. E., S. Bacher, M. Kenis, S. Klotz, I. Kühn, D. Minchin, W. Nentwig, S. Olenin, V. Panov, J. Pergl, P. Pyšek, A. Roques, D. Sol, W. Solarz, and M. Vilà. **2008**. Grasping at the routes of biological invasions: a framework for integrating pathways into policy. J. Appl. Ecol. 45: 403–414.

Humble, L. 2010. Pest risk analysis and invasion pathways–insects and wood packing revisited:

what have we learned? NZ J. For. Sci. 40: S57–S72.

International Maritime Organization. 2012. International shipping facts and figures information resources on trade, safety, security, environment. IMO, London, UK. https://www.imo.org/en/KnowledgeCentre/Pages/MaritimeFactsFigures-Default.aspx

Kenis, M., M.-A. Auger-Rozenberg, A. Roques, L. Timms, C. Péré, M. J. W. Cock, J. Settele, S. Augustin, and C. Lopez-Vaamonde. **2009**. Ecological effects of invasive alien insects. Biol. Invasions. 11: 21–45.

Kenis, M., and M. Branco. **2010**. Impact of alien terrestrial arthropods in Europe. Chapter 5. BioRisk. 4: 51–71.

Kirkendall, L., and M. Faccoli. **2010**. Bark beetles and pinhole borers (Curculionidae, Scolytinae, Platypodinae) alien to Europe. Zookeys. 56: 227–251.

Liebhold, A. M., and J. M. Kean. **2019**. Eradication and containment of non-native forest insects: successes and failures. J. Pest Sci. (2004). 92: 83–91.

Liebhold, A. M., and R. L. Griffin. **2016**. The Legacy of Charles Marlatt and Efforts to Limit Plant Pest Invasions. Am. Entomol. 62: 218–227.

Liebhold, A. M., and P. C. Tobin. **2008**. Population Ecology of Insect Invasions and Their Management. Annu. Rev. Entomol. 53: 387–408.

Meurisse, N., D. Rassati, B. P. Hurley, E. G. Brockerhoff, and R. A. Haack. **2019**. Common pathways by which non-native forest insects move internationally and domestically. J. Pest Sci. (2004). 92: 13–27.

Meyerson, L. A., and H. A. Mooney. **2007**. Invasive alien species in an era of globalization. Front. Ecol. Environ. 5: 199–208.

Morgan, F. D. 1967. Ips grandicollis in South Australia. Aust. For. 31: 137–155.

Morrell, J. J. 1995. Importation of unprocessed logs into North America: A review of pest mitigation procedures and their efficacy. For. Prod. J. 45: 41.

Myers, J. H., A. Savoie, and E. van Randen. **1998**. Eradication and pest management. Annu. Rev. Entomol. 43: 471–491.

Neupane, B., S. Aryal, and J. Shrestha. **2020**. Assessing diversity of insect pests of grain legumes using light traps. Fundam. Appl. Agric. 6: 1.

Newton, L., and G. Fowler. **2009**. Pathway Assessment: Geosmithia sp. and Pityophthorus juglandis Blackman movement from the western into the eastern United States. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Washington, D.C.

Perrings, C., K. Dehnen-Schmutz, J. Touza, and M. Williamson. **2005**. How to manage biological invasions under globalization. Trends Ecol. Evol. 20: 212–215.

Pimentel, D., L. Lach, R. Zuniga, and D. Morrison. **2002a**. Environmental and economic costs associated with non-indigenous species in the United States., pp. 208–306. *In* Pimentel, D. (ed.), Biol. Invasions. Econ. Environ. Costs Alien Plants, Anim. Microbe Species. CRC Press, Boca Raton, USA.

Pimentel, D., S. McNair, J. Janecka, J. Wightman, C. Simmonds, C. O'Connell, E. Wong, L. Russel, J. Zern, T. Aquino, and T. Tsomondo. **2002b**. Economic and environmental threats of alien plant, animal and microbe invasions., pp. 307–330. *In* Pimentel, D. (ed.), Biol. Invasions. Econ. Environ. Costs Alien Plants, Anim. Microbe Species. CRC Press, Boca Raton, USA.

Poland, T. M., and D. Rassati. **2019**. Improved biosecurity surveillance of non-native forest insects: a review of current methods. J. Pest Sci. (2004). 92: 37–49.

Rabaglia, R., D. Duerr, R. Acciavatti, and I. Ragenovich. **2008**. Early Detection and Rapid Response for Non-Native Bark and Ambrosia Beetles. US Dep. Agric. For. Serv. For. Heal. Prot. 1–12.

Rassati, D., M. Faccoli, E. Petrucco Toffolo, A. Battisti, and L. Marini. **2015**. Improving the early detection of alien wood-boring beetles in ports and surrounding forests. J. Appl. Ecol. 52: 50–58.

Rassati, D., F. Lieutier, and M. Faccoli. **2016**. Alien Wood-Boring Beetles in Mediterranean Regions, pp. 293–327. *In* Insects Dis. Mediterr. For. Syst. Springer International Publishing, Cham.

Rassati, D., L. Marini, M. Marchioro, P. Rapuzzi, G. Magnani, R. Poloni, F. Di Giovanni, P. Mayo, and J. Sweeney. **2019**. Developing trapping protocols for wood-boring beetles associated with broadleaf trees. J. Pest Sci. (2004). 92: 267–279.

Roques, A. 2010. Taxonomy, time and geographic patterns. Chapter 2. BioRisk. 4: 11–26.

Roques, A., W. Rabitsch, J. Rasplus, C. Lopez-vaamonde, W. Nentwig, and M. Kenis. **2009**.

Alien Terrestrial Invertebrates of Europe, pp. 63–79. *In* DAISIE (ed.), Handb. Alien Species Eur. Springer Netherlands, Dordrecht.

Ruchin, A. B., L. V. Egorov, A. A. Khapugin, N. E. Vikhrev, and M. N. Esin. **2020**. The use of simple crown traps for the insects collection. Nat. Conserv. Res. 5: 87–108.

Sanchez-Husillos, E., I. Etxebeste, and J. Pajares. **2015**. Effectiveness of mass trapping in the reduction of Monochamus galloprovincialis Olivier (Col.: Cerambycidae) populations. J. Appl. Entomol. 139: 747–758.

Sauvard, D., M. Branco, M. Branco, F. Lakatos, M. Faccoli, M. Faccoli, L. Kirkendall, and L. Kirkendall. **2010**. Weevils and Bark Beetles (Coleoptera, Curculionoidea). Chapter 8.2. BioRisk. 4: 219–266.

Seebens, H., T. M. Blackburn, E. E. Dyer, P. Genovesi, P. E. Hulme, J. M. Jeschke, S. Pagad, P. Pyšek, M. Winter, M. Arianoutsou, S. Bacher, B. Blasius, G. Brundu, C. Capinha, L. Celesti-Grapow, W. Dawson, S. Dullinger, N. Fuentes, H. Jäger, J. Kartesz, M. Kenis, H. Kreft, I. Kühn, B. Lenzner, A. Liebhold, A. Mosena, D. Moser, M. Nishino, D. Pearman, J. Pergl, W. Rabitsch, J. Rojas-Sandoval, A. Roques, S. Rorke, S. Rossinelli, H. E. Roy, R. Scalera, S. Schindler, K. Štajerová, B. Tokarska-Guzik, M. van Kleunen, K. Walker, P. Weigelt, T. Yamanaka, and F. Essl. **2017**. No saturation in the accumulation of alien species worldwide. Nat. Commun. 8: 14435.

Solano, A., S. L. Rodriguez, L. Greenwood, K. J. Dodds, and D. R. Coyle. **2021**. Firewood Transport as a Vector of Forest Pest Dispersal in North America: A Scoping Review. J. Econ. Entomol. 114: 14–23.

Surkov, I. V., A. G. J. M. Oude Lansink, O. van Kooten, and W. van der Werf. **2008**. A model of optimal import phytosanitary inspection under capacity constraint. Agric. Econ. 38: 363–373.

Tkacz, B. M. 2002. Pest risks associated with importing wood to the United States. Can. J. Plant Pathol. 24: 111–116.

Tobin, P. C., J. M. Kean, D. M. Suckling, D. G. McCullough, D. A. Herms, and L. D. Stringer. **2014**. Determinants of successful arthropod eradication programs. Biol. Invasions. 16: 401–414.

Tobin, P. C., and L. M. Blackburn. 2007. Slow the spread: a national program to manage the gypsy moth. USDA Forest Service General Technical Report NRS-6, Newtown Square, PA.

Tobin, P. C., A. M. Liebhold, and E. A. Roberts. **2007**. Comparison of methods for estimating the spread of a non-indigenous species. J. Biogeogr. 34: 305–312.

United Nations Conference on Trade and Development. 2019. Review of maritime transport 2019. UNCTAD, New York, NY.

United Nations Conference on Trade and Development. 2020. Review of maritime transport 2020. UNCTAD, New York, NY.

Vaes-Petignat, S., and W. Nentwig. **2014**. Environmental and economic impact of alien terrestrial arthropods in Europe. NeoBiota. 22: 23–42.

Vrdoljak, S. M., and M. J. Samways. **2012**. Optimising coloured pan traps to survey flower visiting insects. J. Insect Conserv. 16: 345–354.

Walker, T. J. 1988. Acoustic traps for agriculturally important insects. Florida Entomol. 71: 484–492.

Walther, G.-R., A. Roques, P. E. Hulme, M. T. Sykes, P. Pyšek, I. Kühn, M. Zobel, S. Bacher, Z. Botta-Dukát, and H. Bugmann. **2009**. Alien species in a warmer world: risks and opportunities. Trends Ecol. Evol. 24: 686–693.

Williamson, M. 1996. Biological Invasions. Population and Community Biology series. V. 15. London: Chapman and Hall. 244 p.

Xu, H., H. Ding, M. Li, S. Qiang, J. Guo, Z. Han, Z. Huang, H. Sun, S. He, H. Wu, and F. Wan. **2006**. The distribution and economic losses of alien species invasion to China. Biol. Invasions. 8: 1495–1500.

SECTION 1

BORDER SURVEILLANCE

Chapter 2

Light-traps in shipping containers: a new tool for the early-detection of insect alien species

Paper published as: Marchioro M., Battisti A. & Faccoli M. (2020). Light-traps in shipping containers: a new tool for the early-detection of insect alien species. Journal of Economic Entomology, 113: 1718-1724.

JOURNAL OF ECONOMIC ENTOMOLOGY

Light Traps in Shipping Containers: A New Tool for the Early Detection of Insect Alien Species

Matteo Marchioro ✉, Andrea Battisti, Massimo Faccoli

Journal of Economic Entomology, Volume 113, Issue 4, August 2020, Pages 1718–1724,
https://doi.org/10.1093/jee/toaa098
Published: 15 May 2020

Abstract

Insects are one of the most successful groups of invasive species, and the number of new introductions has been increasing in the last decades. Insect invasions are affected mainly by the increase in international trade, as most of them travel across the world inside shipping containers. The effectiveness of sticky light traps was tested for the interception of alien pests inside the containers. The tested hypotheses were that light traps have a valuable broad-spectrum attraction and their trapping performance differs between empty or loaded containers. The optimal trap density in a container was also investigated. Trapping tests were conducted on four model species: *Cadra cautella* Walker (Lepidoptera: Pyralidae), *Drosophila melanogaster* Meigen (Diptera: Drosophilidae), *Sitophilus zeamais* Motschulsky and *Ips typographus* L. (Coleoptera: Curculionidae). Insects were released within a standard shipping container, in either empty or loaded conditions, where sticky light traps were deployed for 15 h. Traps were tested with light on (activated) or off (control). Activated traps captured more Lepidoptera and Diptera than control ones, with no differences between empty and loaded container. Instead, Coleoptera were rarely caught, probably because of their ability to escape from traps. Results show that higher trap density in the container (from 1 to 8) increases the probability of insect capture. In conclusion, positive results on *C. cautella* and *D. melanogaster* suggest a possible application of sticky light traps against some small Lepidoptera and Diptera species flying in containers and infesting seeds, grains and fruits, while traps need improvement for application against beetles.

Introduction

Arthropods are one of the most successful groups of invasive species in the world and the number of new introductions is increasing worldwide (Seebens et al. 2018). In Europe the number of new species introduced annually is also increasing exponentially (Hulme 2009). Between 2000 and 2008 an average of 19.6 alien species have been established in Europe every year, while 10.9 were introduced between 1950 and 1974 (Roques 2010). In 2009, alien insects registered in Europe were about 1,300 species (Roques et al. 2009), but only 10 years later, there were more than 3,000 non-native species of terrestrial invertebrates in Europe, and about 2,500 of these were insects (European Commission 2019). Biological invasions of arthropods are mainly and positively affected by the increase in speed and volume of international trade (Levine and D'Antonio 2003, Westphal et al. 2008, Hulme 2009) and, on a global scale, the historical accumulation curves of alien species introductions show an increasing trend (Brockerhoff and Liebhold 2017, Seebens et al. 2017). Furthermore, global warming assures insect survival also for tropical species arriving in temperate regions and affects their chances of settling permanently (Walther et al. 2009).

ISO standard shipping containers are largely used in international trade and are now considered one of the main drivers of economic globalization in the 20th century (Bernhofen et al. 2016). Containers on ships carry about 90% of global trade (IMO 2012). In the last forty years, world maritime trade volumes tripled and in 2015 they reached about 10 billion tons per year (UNCTAD 2016). Global containerized trade increased annually by 6.4% in 2015–2017, and future previsions for seaborne trade are still positive (UNCTAD 2018). With such a large volume of commodities transported in containers all around the world, even minimal percentages of container contamination can represent a serious risk of introductions of new alien pests. In this respect, shipping containers are well-known to easily lead to the introduction of alien species in new territories. For instance, in 1,174 containers inspected in Australia in the period between February and August 1996, more than 7,400 insects were found, belonging to 18 orders and at least 114 families, and 19% of them were still alive (Stanaway et al. 2001). In New Zealand, the Ministry of

Agriculture and Forestry conducted a survey of about 11,200 containers arriving at four of their ports in 2001/2002. Live insects, mainly belonged to Coleoptera, Psocoptera, Hymenoptera and Hemiptera orders, were found in 4.1% of loaded containers and in 3.6% of empty ones (MAF 2003). In general, the insect orders most commonly found in containers are Coleoptera, Diptera and Lepidoptera, and they can be found in different life stages, from eggs to adults (Meurisse et al. 2019).

According to the European Council Directive, phytosanitary inspectors of the National Plant Protection Organizations have to check all cargos arriving from non-UE countries or suspected to contain quarantine pests. Nevertheless, no common and optimal survey strategy between all European member states exists yet (Surkov et al. 2008). Moreover, inspectors can sample only a small volume of total consignments of commodities arriving in the international ports (Everett 2000, Surkov et al. 2008). This problem does not just affect Europe; *e.g.*, it is estimated that only 2% of all maritime cargos entering the US is inspected and at most 54% of insect species are detected (Work et al. 2005). Inspectors often use historical records from the interception databases to select shipments to be inspected, but this procedure reduces the number and types of new routes (pathways) checked, increasing the risk of new entries (Bacon et al. 2012). For example, most of the main insect alien species entering Australia in 1986-2005 went unnoticed by phytosanitary controls in the points-of-entry (Caley et al. 2015).

Given the wide variety of alien insects that can easily be introduced in new areas through international trade and the gaps occurring in border phytosanitary controls, new early-detection tools helping inspectors' surveillance are badly needed. The development of nonspecific broad-spectrum traps to be used within shipping containers during the cargo travel could be a simple and effective way for prompt early detection of alien species at the points-of-entry. Species captured during travel, in fact, can help to determine in advance if the load is infested, to direct most efforts only on the lots deemed as riskier. The aim of this study was to test the effectiveness of a sticky light trap to capture different orders of insect pests inside shipping containers. We wanted to verify

a) if light could be an effective broad-spectrum attractant for pests belonging to different insect orders, and b) if container status (empty or loaded with goods) affects the number of captures. We also wanted to investigate if there was an optimal traps density to maximize captures.

Materials and Methods

Tested traps

The experimental trials were conducted using sticky light traps (TransTrap^{TM}, Alpha Scents Inc., West Linn, OR) developed to capture pests potentially occurring inside shipping containers (Mangan and Chapa 2013). This trap model consists of a carton box (15x23x4 cm) made attractive to flying and walking insects by a LED (Light Emitting Diode) light powered by a long-life AA battery. These LEDs emit light that have two peaks, the main at 465 nm (indigo) and a second more broadband included between 525 and 600 nm (between green and yellow) (Alpha Scents Inc., personal communication 2020). The light is positioned in the center of a removable yellow sticky card fixed to the bottom of the box. In our experiment a second yellow sticky card was applied, attached to the inside of the box lid to increase the sticky surface and trap performance (Fig. 1). This trap model is simple to use, easily manageable, potentially attractive to a large number of different insect species, and does not require additional lures.

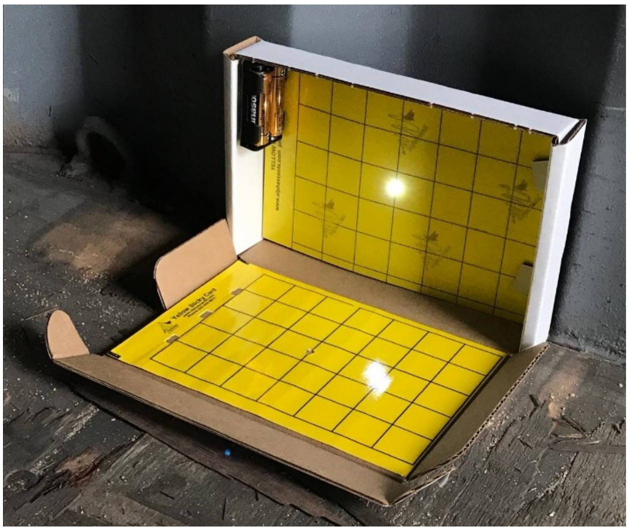

Fig. 1 The trap used for the experiment.

Model species

The tests were conducted on four model species, belonging to 3 different orders of insects. The almond moth, *Cadra cautella* Walker (Lepidoptera: Pyralidae), is a stored food products pest with larvae developing on cereal grains and flour, beans and other dried seeds and fruits (Sedlacek et al. 1995). *Drosophila melanogaster* Meigen (Diptera: Drosophilidae), is a common insect associated with fruits and vegetables (Mallis 1954, Birmingham et al. 2011). The maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), is considered one of the major pests of stored maize (Erenso and Berhe 2016, Nwosu 2018). The European spruce bark beetle, *Ips typographus* L. (Coleoptera: Curculionidae, Scolytinae), is the main European spruce pest developing in stressed or recently dead trees (Wermelinger 2004), and travels in containers used for the international spruce timber trade. These model species were chosen because beetles (Coleoptera), flies (Diptera) and moths (Lepidoptera) are the most common insect orders found inside shipping containers used in international trade (Meurisse et al. 2019).

Ips typographus adults were captured by Theysohn slot-traps (Salzgitter, Germany) set up in clear-cut areas of natural spruce forests of central Alps (Trentino, Italy) infested in 2019. Traps were installed at about 15–20 m from the forest edge, and baited with pheromone dispensers specific to *I. typographus* (Superwood Serbios, Italy). Traps were checked and emptied every second day, and all trapped adults of *I. typographus* were stored in darkness at +4°C in plastic jars containing wet paper and small pieces of spruce bark. The other species (*Cadra cautella*, *Drosophila melanogaster* and *Sitophilus zeamais*) were bought from a company (Entostudio s.r.l., Padua, Italy) specialized in rearing insects of various species and for different uses. Adults of *Cadra cautella* were bred in 5-liter glass jars measuring 16 cm in diameter and 25 cm in height. The jars were positioned upside down with the opening covered of a 2 mm mesh net. The jar was placed above a transparent plastic cup (12 cm in diameter and 6 cm in height) to collect the eggs. These eggs were moved daily into transparent plastic cup (11 cm in diameter and 9 cm in height) that contained a mixture of wheat and corn flour, oat, bran, dry fruit, glycerol, honey and yeast, where larvae can develop. Adults who emerged in these boxes were taken and put inside glass jars. The insects were reared at 25±1°C and 50±5 % R.H. The exposure to light lasted 12 hours during 24 hours and the light intensity was 300 lux at 6,000 K. Adults of *Sitophilus zeamais* were bred in plastic cups measuring 12 cm in diameter and 6 cm in height, closed by a fine net, at 25±1°C and 50±5 % R.H. The photoperiod lasted 12 hours at a solar spectrum artificial light of 6,000 K and 300 lux intensity. Insects were fed with grain. The colony originated in 2014 with insects collected in the field. Adults of *Drosophila melanogaster* were bred in BugDorme cages measuring 32.5x32.5x32.5 cm. The food and oviposition substrate consisted of a mixture of water, pieces of potatoes and fruit, powdered milk and sugar. The insects were reared at 25±1°C and 50±5 % of R.H. The photoperiod lasted 12 hours at a solar spectrum artificial light of 6,000 K and 300 lux intensity.

All insects were tested in the trials only once and within 2 days from their emergence (or trapping) to ensure the highest vitality. We used insects without discriminating between males and females and assuming a sex-ratio 1:1. The reared species *C. cautella*, *D. melanogaster*, and *S. zeamais* reproduce sexually, producing a sex-balanced offspring (Santos et al. 1994, Danho et al. 2002, Soffan et al. 2012). An aggregation pheromone was used to capture *I. typographus*, which attracts both males and females with a sex-ratio slightly unbalanced in favor of females (Faccoli and Buffo 2004).

Trials in container

A blue ISO standard shipping container 1CC (interior size: 5.8 m length, 2.3 m wide, 2.3 m height) with a volume of 32 m^3 (ISO-668 2013) was used for the experiments. The container was placed in a square of the Agripolis Campus, University of Padua (Legnaro, Italy), without any shelter from sun and rain. Specific tests were then conducted between June and August 2019 with the container both empty and loaded.

Empty container. Each model species was tested singly through 7 tests, on 7 consecutive days. In each test 50 individuals per species were released inside the container provided with two sticky traps: one with the light on (activated trap) and the other with the light off (control trap). Insects were put inside a plastic cup with a lid resting on the top and placed at the bottom of the container. With a rope tied to the cup and stretched to the door of the container, it was possible to overturn the cup, releasing the insects and closing the container doors before they escaped. The two traps were placed in corners of the door side of the container, on the floor. Each test, *i.e.* each repetition, lasted about 15 hours (from 6:00 pm to 9:00 am). At the end of each daily trial, before starting a new one, we ventilated the container for many hours and we made sure no survivor was left inside.

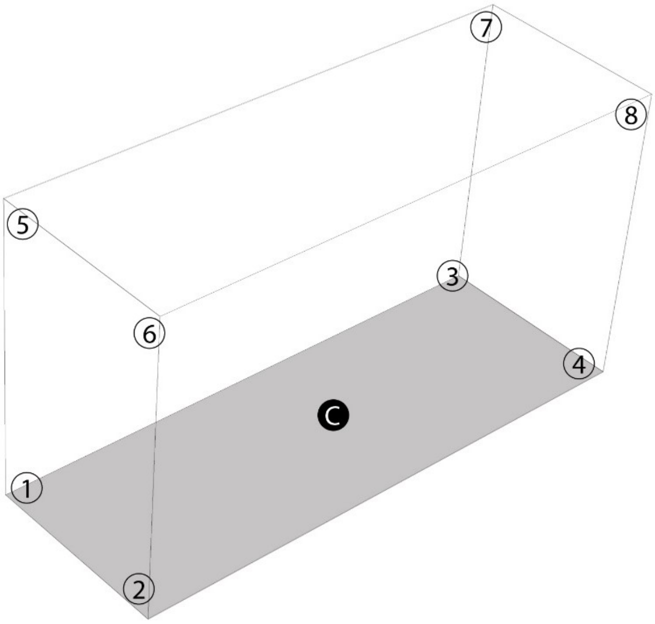

Fig. 2 Position of the traps inside the container (doors were on the left side). One-trap trial: 1. Two-traps trials: 1-2. Four-traps trials: 1-4. Eight-traps trials: 1-8. C is the control trap, always present.

Loaded container. The same tests as those in the empty container were conducted in containers filled with empty cardboard boxes simulating a cargo. In this second group of tests only *C. cautella* and *D. melanogaster* were used (7 tests per species with 50 individuals released per species). We verified that the *S. zeamais* were able to escape the traps and decided not to use them in the following tests, while the *I. typographus* were not used because we did not have enough specimens. Each test lasted about 15 hours (from 6:00 pm to 9:00 am).

Optimal traps density

Optimal trap number maximizing insect catches in the container was also tested in September, on one of the two species that had recorded the best number of catches in previous tests. The captures of *C. cautella* were recorded in loaded containers with 4 different trap densities, using 1, 2, 4, or 8 traps set up in the same container (Fig. 2). For each trap density, 5 tests of 50 insects each were conducted on 5 consecutive days. Each test lasted about 15 hours, with an unlit trap used as a control.

During each test in the container, air temperature was recorded every 15 minutes with 3 data loggers (RC-5 model, Elitech LTD, London, UK) one placed outside and two inside the container, one on the bottom and one at the top.

Escape test

After the first tests on *S. zeamais* in the empty container, given the few specimens captured, the hypothesis was tested that the insects could escape from the trap. Therefore, 10 living *S. zeamais* were placed in each of five traps, marking the insect positions on the sticky card with a circle. Two tests were conducted at 16 °C and at 26 °C constant temperature inside climatic chambers. After 18 hours traps were checked, looking for number and position of the insects placed on the sticky card.

Statistical analysis

Statistical analysis was conducted using R software, version 3.6.1 (R Core Team 2019). Mean catches of *C. cautella* and *D. melanogaster* with activated and control traps were compared using Poisson mixed-effect model, with trap type (activated or control) as fixed variable and tests as random variable. The model was fitted using the "glmer" function in the lme4 package (Bates et al. 2015). For *S. zeamais* and *I. typographus*, for which the use of this model was impossible because catches in control traps were nil, the Wilcoxon test was therefore applied using the "wilcox.test" function in the stat package (R Core Team 2019). Catches made with activated traps in the empty and loaded container were also compared for each single species using Poisson mixed-effect model and, in this case, the container status (empty or loaded) was the fixed variable while the tests were the random variable.

Results

Tests in empty container

Activated traps captured significantly more individuals of *C. cautella* (p-value < 0.001, z-value = 6.68) (Fig. 3), *D. melanogaster* (p-value < 0.001, z-value = 7.27) (Fig. 3), and *I. typographus* (p-value < 0.01) (Fig. 3) than control traps, while for *S. zeamais* activated and control traps showed similar captures (p-value = 0.173), with only a very few specimens in activated traps and nil in control ones (Fig. 3). No abnormal temperature trends were found during the tests, which remained similar during each repetition. The average temperatures recorded during the trials inside the container ranged between 20 and 25 °C, with no significant differences between tests.

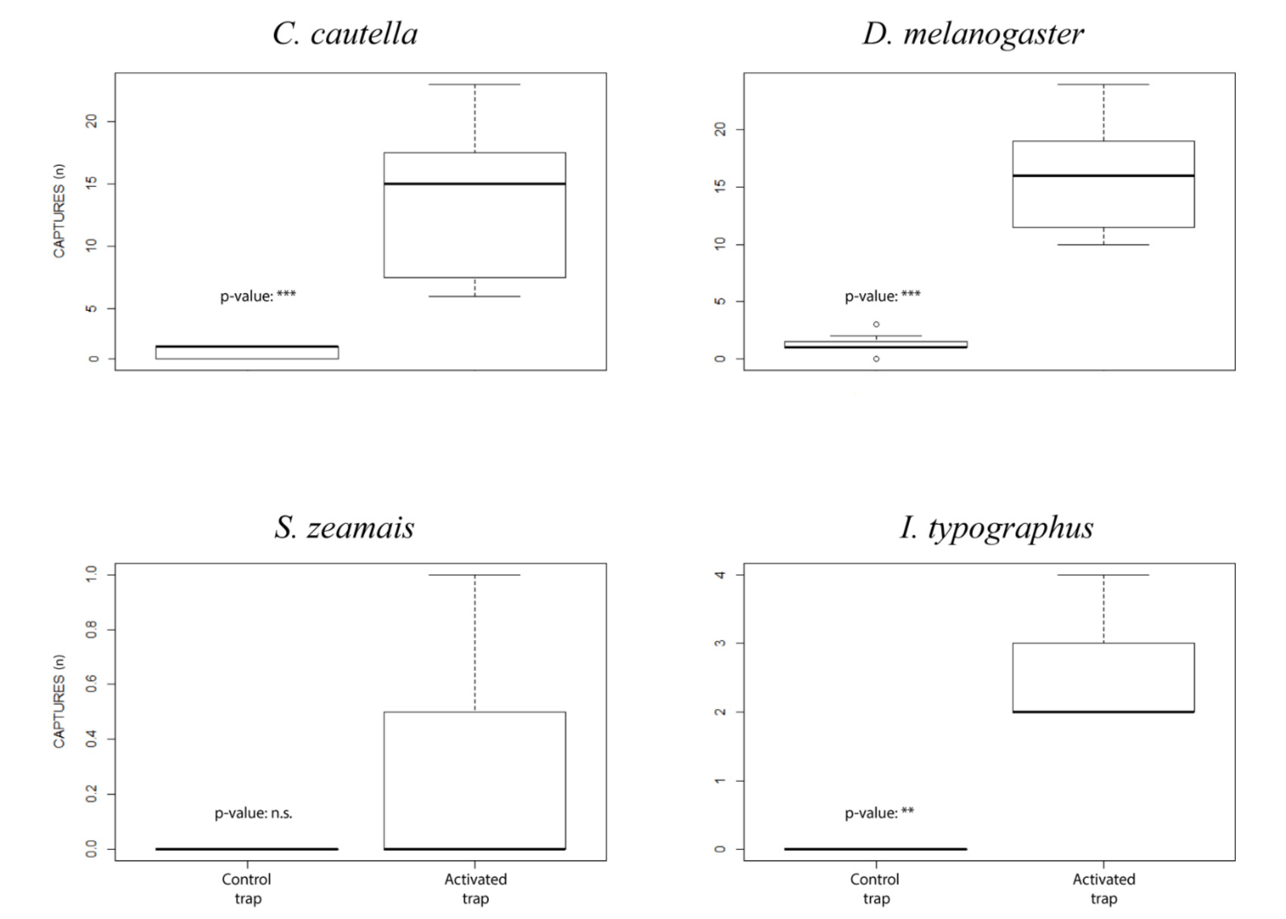

Fig. 3 Catches (± SEM) of activated and control traps for the four model species tested in the empty container. Significant results are displayed within each box (** p-value < 0.01; *** p-value < 0.001).

Tests in loaded container

Activated traps captured significantly more individuals than control traps, for both *C. cautella* (p-value < 0.001, z-value = 5.27) (Fig. 4) and *D. melanogaster* (p-value < 0.001, z-value = 6.81) (Fig. 4). Furthermore, catches of the activated traps were similar in both the empty and loaded container, with no significant differences for either *C. cautella* (p-value = 0.237, z-value = 1.18) (Fig. 5) or *D. melanogaster* (p-value = 0.424, z-value = 0.80) (Fig. 5). Average temperatures recorded during the trials inside the container were about 22 °C, with no significant differences between tests.

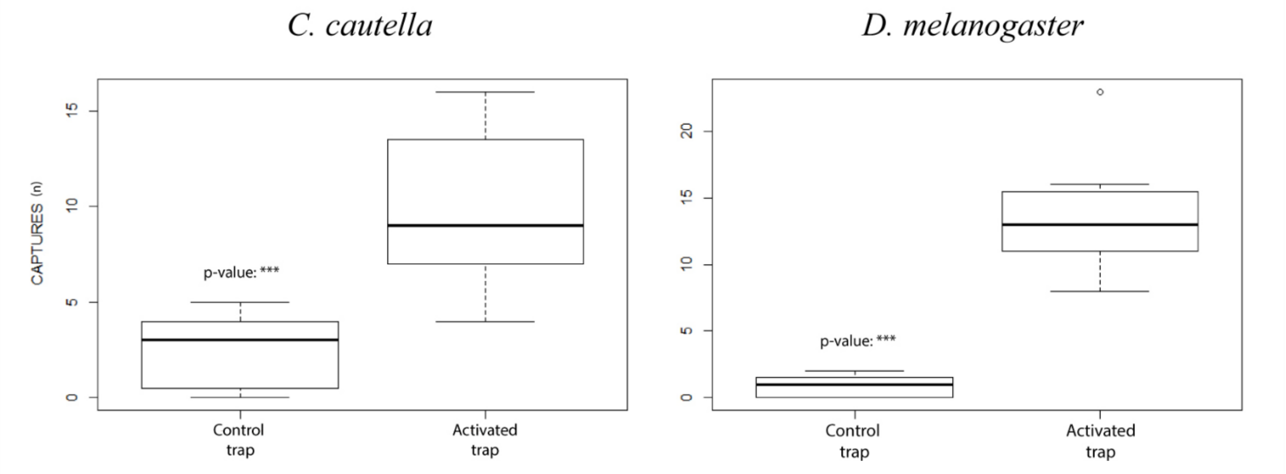

Fig. 1 Catches (± SEM) of activated and control traps for the two model species in the loaded container. Significant results are displayed within each box (*** p-value < 0.001).

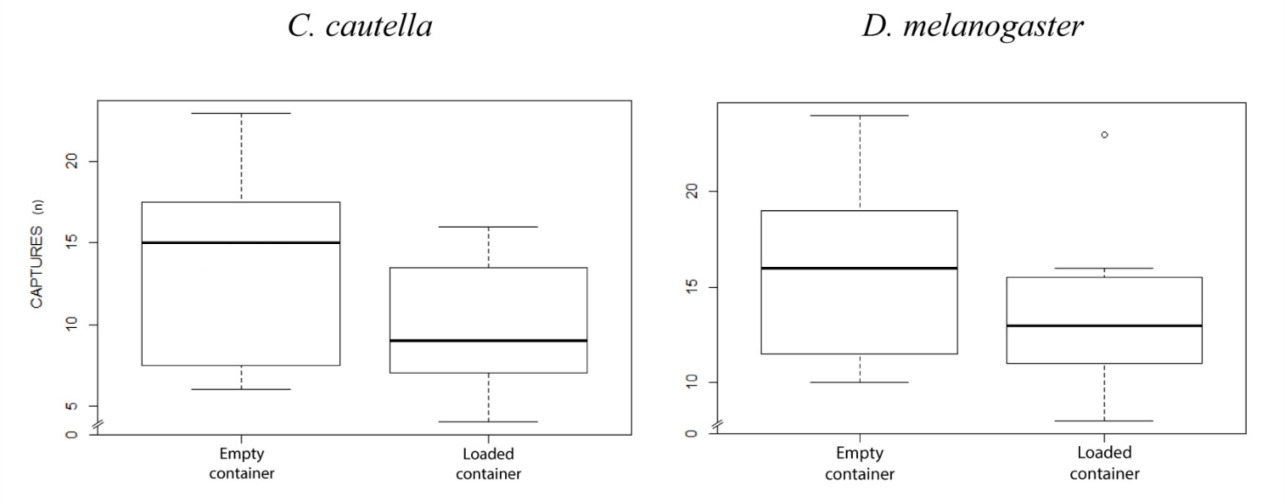

Fig. 2 Catches (± SEM) of activated traps for the two model species in empty and loaded container. There are no significant differences.

Optimal trap density

Densities of 1, 2 and 4 activated traps per container showed mean catches with no significant differences (p-value = 0.556), whereas with 8 traps per container the number of trapped insects more than doubled. Captures of the control traps were not affected by trap density, although they were negatively correlated with captures in the activated traps (p-value < 0.01). The catching trend of the activated traps increases with trap density, but starts to flatten with 8 traps (Fig. 6). The average temperatures recorded during the trials inside the container were around 19-25 °C, with no significant differences between tests.

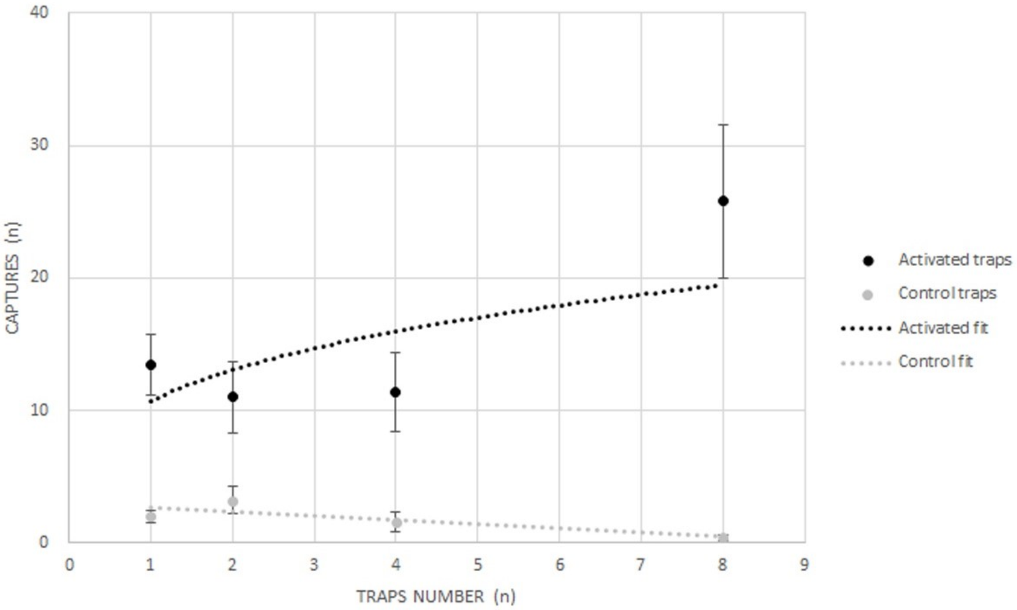

Fig. 3 Captures of *C. cautella* recorded in each test with increasing trap density.

Escape test

Considering the two temperatures separately, the mean proportion of *S. zeamais* escaped from sticky cards were 42% and 62%, for trials at 16 °C and 26 °C respectively.

Discussion

Results show that the tested trap model is effective in catching *C. cautella* and *D. melanogaster*, in both empty and loaded containers. For Coleoptera, instead, and in particular for *S. zeamais*, results are not satisfactory as beetles are able to escape from the sticky card of the trap.

Although results concern only one model species for each tested order, we can assume that similar results would be expected for other species and genera belonging to the same family and having similar size and behavior. In fact, several researches demonstrate the effectiveness of light as an attractant both for Pyralidae (Kanno et al. 1985, Loganathan et al. 2001, Sambaraju and Phillips 2008) and other Lepidoptera families like Crambidae (Keszthelyi and Sáringer 2003, Haihua et al. 2016), and Hyblaeidae (Loganathan et al. 2001). Light traps are already widely used to capture Diptera like Chironomidae (van Grunsven et al. 2014), Culicidae (Burkett et al. 1998, Silva et al. 2019), Psychodidae Phlebotominae (Cohnstaedt et al. 2008, da Silva et al. 2019) and other 14 families (Ndengué et al. 2019). Moreover, this light trap has already been tested on other orders, like *Diaphorina citri* (Hemiptera: Liviidae) (Mangan and Chapa 2013).

Beetles show different results. Although the captures of *I. typographus* in activated traps were very low (only 5% of released insects were captured) they were significantly higher than those recorded in the control traps (no insect). Positive light-responses were also recorded in other scolytines where ethanol baited traps activated with green or UV light are more attractive to *Xylosandrus crassiusculus* than normal traps (Gorzlancyk et al. 2013, 2014). *Sitophilus zeamais*, lastly, shows no significant difference between activated and control traps, with only 2 insects trapped by activated ones and no capture in control traps over a total of 7 replicates (*i.e.*, 350 insects). The low trapping performance of beetles is probably related to the ability of these insects to escape from the traps, verified by the appropriate test showing that 42% and 62% of *S. zeamais* escape from sticky cards at 16 °C and 26 °C respectively. In this context, therefore, it is not clear if the low beetle captures are related to a non-attraction to the light or to their ability to escape. However, light traps are already used for catching beetles, like Tenebrionidae (Duehl et al. 2011), or Curculionidae, Pselaphidae, Silvanidae and other 33 families (Ndengué et al. 2019), and the attractiveness of light − in particular red wave-length (625 nm) − has also been verified for *S. zeamais* in a double-choice test (Park and Lee 2017). The difference between moths/flies and beetles is likely due to their landing strategies. Moths and flies are glued by the wings whereas beetles are somewhat able to avoid wing contact and walk away. To check if the reduce trap performance in catching beetles is related to the ability of these insects to escape from traps, more powerful glues should be tested or the sticky card could be sprayed with contact insecticides to prevent insect's escape after their capture.

It was very difficult to check what happened to insects not captured by traps. We suppose that some of them died during the test, and some others remained alive but undetectable inside the container, which was ventilated and cleaned before running a new test.

In our experiment, the container status (empty or loaded) does not affect the number of captures of the light sticky trap. Trials conducted in the empty container recorded about 27% and 32% of captures versus 21% and 28% in the loaded container for Lepidoptera and Diptera respectively, with no significant differences. This is one of the most interesting results from this study, suggesting the useful application of the light sticky trap also in containers loaded with commodities and, hence, exposed to a major risk of movement and introduction of alien species across countries and continents.

Tests conducted on trap density in the container show that, although using 8 traps (the highest number of traps during this study), the rarefaction curve built on the number of catches per number of traps has not yet reached flattening. So, the more traps that are used the more insects would be expected to be captured. However, the aim of the light sticky trap is not to capture as many insects as possible, but to capture the maximum number of alien species potentially travelling inside the container. In this way, traps could provide information on the status of cargo infestations and allow pre-delivery quarantine measures to prevent the introduction of new alien species in non-native countries. On the other hand, increasing the number of traps also increases the probability of catching species present in low numbers. However, placing a large number of traps inside a container loaded with cargo could be problematic logistically, and considerably increase the survey costs. In this respect, results show that the mean number of captures is similar among 1, 2 and 4 traps per container. For this reason, 1 or 2 traps per container seems to be a sufficiently high number to discover small and flying alien species travelling with the commodities.

This trap technology needs some improvement and more extensive testing, but the preliminary results are very encouraging, especially for small species of Diptera and Lepidoptera infesting seeds, grains, and fruits exported internationally in containers. Although in our tests only a white LED lamp was used, the type of light used to activate the trap could be an important variable to test, as the spectral composition is important to determine the attractiveness of the light to insects (van Grunsven et al. 2014). Insects sensitivity to UV, blue and green light spectrum is well known (Briscoe and Chittka 2001, Cohnstaedt et al. 2008) and, in some cases, also to red light (Peitsch et al. 1992, Park and Lee 2017). In particular, different studies demonstrate the major effectiveness of UV light for catching many different insect species (van Grunsven et al. 2014, Infusino et al. 2017). For example, insects of about 480 species belonging to 10 different orders were captured in a survey conducted in South Korea using UV light (Thein and Choi 2016).

Finally, new tests will be required during real shipments. Tests conducted up to now were in controlled conditions, which simulated reality. However, it is necessary to verify the effective performance of these traps in real situations, where weather, environmental conditions and species involved can be very different from those tested in our trials. The duration of the shipment can also play a key role; we successfully used the light for one month without interruption, so we are pretty sure that this trap is suitable for prolonged use in a container during shipment. In conclusion, light traps set up in containers represent a potentially effective tool for border surveillance and early-detection against biological invasions. This study represents only a first preliminary work dealing with the early-detection of alien species potentially travelling with commodities in containers.

Further and deeper tests about light source and glue type are needed to improve trapping performance and the potential applications of this novel tool of pest interception.

Acknowledgements

The authors thank Giovanni Castellucci for field assistance, Silvia Rossi for checking the pheromone traps of *I. typographus* and providing the insects, Davide Scaccini for comments on the draft of this manuscript, Paolo Paolucci for providing some of the images used in the graphs, Alpha Scents Inc., in particular the President and CEO Dr. Darek Czokajlo, for providing the traps, and Entostudio, in particular Dr. Patrizia Visentin and Dr. Andrea Drago, for providing the insects used in the trials. The study has received funding from the project HOMED within the European Union’s Horizon 2020 Research and Innovation Programme (grant agreement N° 771271).

References

Bacon, S. J., S. Bacher, and A. Aebi. **2012**. Gaps in border controls are related to quarantine alien insect invasions in Europe. PLoS One. 7: 1–9.

Bates, D., M. Mächler, B. Bolker, and S. Walker. **2015**. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67: 1-51.

Bernhofen, D. M., Z. El-Sahli, and R. Kneller. **2016**. Estimating the effects of the container revolution on world trade. J. Int. Econ. 98: 36–50.

Birmingham, A. L., E. Kovacs, J. P. Lafontaine, N. Avelino, J. H. Borden, I. S. Andreller, and G. Gries. **2011**. A new trap and lure for *Drosophila melanogaster* (Diptera: Drosophilidae). J. Econ. Entomol. 104: 1018–1023.

Briscoe, A. D., and L. Chittka. **2001**. The evolution of color vision in insects. Annu. Rev. Entomol. 46: 471–510.

Brockerhoff, E. G., and A. M. Liebhold. **2017**. Ecology of forest insect invasions. Biol. Invasions. 19: 3141–3159.

Burkett, D. A., J. F. Butler, and D. L. Kline. **1998**. Field evaluation of colored light-emitting diodes as attractants for woodland mosquitoes and other diptera in north central Florida. J. Am. Mosq. Control Assoc. 14: 186—195.

Caley, P., R. Ingram, and P. De Barro. **2015**. Entry of exotic insects into Australia: Does border interception count match incursion risk? Biol. Invasions. 17: 1087–1094.

Cohnstaedt, L. W., J. I. Gillen, and L. E. Munstermann. **2008**. Light-emitting diode technology improves insect trapping. J. Am. Mosq. Control Assoc. 24: 331–334.

Danho, M., C. Gaspar, and E. Haubruge. 2002. The impact of grain quantity on the biology of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae): oviposition, distribution of eggs, adult emergence, body weight and sex ratio. J. Stored Prod. Res. 38: 259–266.

Duehl, A. J., L. W. Cohnstaedt, R. T. Arbogast, and P. E. A. Teal. **2011**. Evaluating light attraction to increase trap efficiency for *Tribolium castaneum* (Coleoptera: Tenebrionidae). J. Econ. Entomol. 104: 1430–1435.

Erenso, T. F., and D. H. Berhe. **2016**. Effect of neem leaf and seed powders against adult maize weevil (*Sitophilus zeamais* Motschulsky) Mortality. Int. J. Agric. Res. 11: 90–94.

European Commission. 2019. Joint Research Centre - European Alien Species Information Network (EASIN). https://easin.jrc.ec.europa.eu/.

Everett, R. A. 2000. Patterns and pathways of biological invasions. Trends Ecol. Evol. 15: 177–178.

Faccoli, M., and E. Buffo. 2004. Seasonal variability of sex-ratio in *Ips typographus* (L.) pheromone traps in a multivoltine population in the Southern Alps. J. Pest Sci. **77**: 123-129.

Gorzlancyk, A. M., D. W. Held, D.-J. Kim, and C. M. Ranger. **2013**. Capture of *Xylosandrus crassiusculus* and other Scolytinae (Coleoptera: Curculionidae) in response to visual and volatile cues. Florida Entomol. 96: 1097–1101.

Gorzlancyk, A. M., D. W. Held, C. M. Ranger, Z. Barwary, and D.-J. Kim. **2014**. Capture of *Cnestus mutilatus*, *Xylosandrus crassiusculus*, and other Scolytinae (Coleoptera: Curculionidae) in response to green light emitting diodes, ethanol, and conophthorin. Florida Entomol. 97: 301–303.

van Grunsven, R. H. A., M. Donners, K. Boekee, I. Tichelaar, K. G. van Geffen, D. Groenendijk, F. Berendse, and E. M. Veenendaal. **2014**. Spectral composition of light sources and insect phototaxis, with an evaluation of existing spectral response models. J. Insect Conserv. 18: 225–231.

Haihua, T., T. Naixiong, H. Xiuxia, Y. Zhiwen, W. Guangli, and W. Hongyi. **2016**. LED multispectral circulation solar insecticidal lamp application in rice field. Trans. Chinese Soc. Agric. Eng. 32: 193–197.

Hulme, P. E. 2009. Trade, transport and trouble: managing invasive species pathways in an era of globalization. J. Appl. Ecol. 46: 10–18.

Infusino, M., G. Brehm, C. Di Marco, and S. Scalercio. **2017**. Assessing the efficiency of UV LEDs as light sources for sampling the diversity of macro-moths (Lepidoptera). Eur. J. Entomol. 114: 25–33.

International Maritime Organization. 2012. International shipping facts and figures information resources on trade, safety, security, environment. IMO, London, UK.

International Organization for Standardization. 2013. International Standard ISO/CD 668 Series 1 freight containers - Classification, dimensions and ratings. ISO, Geneva, CH.

Kanno, H., N. Abe, M. Mizusawa, Y. Saeki, K. Koike, S. Kobayashi, S. Tatsuki, and K. Usui. **1985**. Comparison of the trap efficiency and of the fluctuation pattern of moth catches between the synthetic sex pheromone and the light-trap in the rice stem borer moth, *Chilo suppressalis* Walker (Lepidoptera: Pyralidae). Japanese J. Appl. Entomol. Zool. 29: 137–139.

Keszthelyi, S., and G. Sáringer. **2003**. Flight of the European corn borer (*Ostrinia nubilalis* Hübner) as followed by light traps in 2002. Acta Phytopathol. Entomol. Hungarica. 38: 333–340.

Levine, J. M., and C. M. D'Antonio. **2003**. Forecasting biological invasions with increasing international trade. Conserv. Biol. 17: 322–326.

Loganathan, J., P. Soman, and S. Maragatham. **2001**. Monitoring of two major pests of teak in intensively managed plantation through light trap study. Indian For. 127: 1047–1052.

Mallis, A. 1954. Handbook of pest control. The behavior, life history, and control of household pests, 2th ed. MacNair-Dorland Co., New York, N.Y.

Mangan, R. L., and D. Chapa. **2013**. Evaluation of the effects of light source and plant materials on Asian Citrus Psyllid (Hemiptera: Psyllidae) Trapping levels in the transtrap for citrus shipping containers. Florida Entomol. 96: 104–111.

Meurisse, N., D. Rassati, B. P. Hurley, E. G. Brockerhoff, and R. A. Haack. **2019**. Common pathways by which non-native forest insects move internationally and domestically. J. Pest Sci. 92: 13–27.

Ministry of Agriculture and Forestry. 2003. Sea container review New Zealand. MAF Discussion Paper No: 35, Wellington, NZ.

Ndengué, J. D. M., G. Texier, J. Landier, E. De Gavelle, J. Marchi, L. R. Kamgang, M. Kenné, M. Tindo, S. Eyangoh, and P. Le Gall. **2019**. Adapting light trap to catch household insects in central Cameroon: a pilot study. Ann. la Soc. Entomol. Fr. 55: 383–394.

Nwosu, L. C. 2018. Impact of age on the biological activities of *Sitophilus zeamais* (Coleoptera: Curculionidae) adults on stored maize: Implications for food security and pest management. J. Econ. Entomol. 111: 2454–2460.

Park, J. H., and H. S. Lee. 2017. Phototactic behavioral response of agricultural insects and stored-product insects to light-emitting diodes (LEDs). Appl. Biol. Chem. 60: 137–144.

Peitsch, D., A. Fietz, H. Hertel, J. de Souza, D. F. Ventura, and R. Menzel. 1992. The spectral input systems of hymenopteran insects and their receptor-based colour vision. J. Comp. Physiol. A. 170: 23–40.

R Core Team 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, AT. https://www.R-project.org/

Roques, A. 2010. Taxonomy, time and geographic patterns. Chapter 2. BioRisk. 4: 11–26.

Roques, A., W. Rabitsch, J. Rasplus, C. Lopez-vaamonde, W. Nentwig, and M. Kenis. 2009. Alien terrestrial invertebrates of europe, pp. 63–79. *In* DAISIE (ed.), Handb. Alien Species Eur. Springer Netherlands, Dordrecht.

Sambaraju, K. R., and T. W. Phillips. 2008. Responses of adult *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) to light and combinations of attractants and light. J. Insect Behav. 21: 422–439.

Santos, M., K. Fowler, and L. Partridge. 1994. Gene-environment interaction for body size and larvae in *Drosophila melanogaster*: an investigation of effects on development time, thorax length and adult sex ratio. Genet. Soc. Gt. Britain. 72: 515–521.

Sedlacek, J. D., P.A. Weston, and R. J. Barney 1995. Lepidoptera and Psocoptera, pp. 41-70. In Subramanyam B., Hagstrum D.W., Integrated management of insects in stored products. Marcel Dekker, Inc., New York.

Seebens, H., T. M. Blackburn, E. E. Dyer, P. Genovesi, P. E. Hulme, J. M. Jeschke, S. Pagad, P. Pyšek, M. van Kleunen, M. Winter, M. Ansong, M. Arianoutsou, S. Bacher, B. Blasius, E. G. Brockerhoff, G. Brundu, C. Capinha, C. E. Causton, L. Celesti-Grapow, W. Dawson, S. Dullinger, E. P. Economo, N. Fuentes, B. Guénard, H. Jäger, J. Kartesz, M. Kenis, I. Kühn, B. Lenzner, A. M. Liebhold, A. Mosena, D. Moser, W. Nentwig, M. Nishino, D. Pearman, J. Pergl, W. Rabitsch, J. Rojas-Sandoval, A. Roques, S. Rorke, S. Rossinelli, H. E. Roy, R. Scalera, S. Schindler, K. Štajerová, B. Tokarska-Guzik, K. Walker, D. F. Ward, T. Yamanaka, and F. Essl. 2018. Global rise in emerging alien species results from increased accessibility of new source pools. Proc. Natl. Acad. Sci. 115: 2264–2273.

Seebens, H., T. M. Blackburn, E. E. Dyer, P. Genovesi, P. E. Hulme, J. M. Jeschke, S. Pagad, P. Pyšek, M. Winter, M. Arianoutsou, S. Bacher, B. Blasius, G. Brundu, C. Capinha, L. Celesti-Grapow, W. Dawson, S. Dullinger, N. Fuentes, H. Jäger, J. Kartesz, M. Kenis, H. Kreft, I. Kühn, B. Lenzner, A. Liebhold, A. Mosena, D. Moser, M. Nishino, D. Pearman, J. Pergl, W. Rabitsch, J. Rojas-Sandoval, A. Roques, S. Rorke, S. Rossinelli, H. E. Roy, R. Scalera, S. Schindler, K. Štajerová, B. Tokarska-Guzik, M. van Kleunen, K. Walker, P. Weigelt, T. Yamanaka, and F. Essl. **2017**. No saturation in the accumulation of alien species worldwide. Nat. Commun. 8: 14435.

da Silva, A. A., J. M. M. Rebêlo, B. F. Carneiro, M. P. P. Castro, M. de Sousa de Almeida, I. S. Ponte, J. V. C. Aguiar, and F. S. Silva. **2019**. Exploiting the synergistic effect of kairomones and light-emitting diodes on the attraction of Phlebotomine sand flies to light traps in Brazil. J. Med. Entomol. 56: 1441–1445.

Silva, F. S., B. M. Costa-Neta, M. de Sousa de Almeida, E. C. de Araújo, and J. V. C. Aguiar. **2019**. Field performance of a low cost, simple-to-build, non-motorized light-emitting diode (LED) trap for capturing adult Anopheles mosquitoes (Diptera: Culicidae). Acta Trop. 190: 9–12.

Soffan, A., Y. N. Aldryhim, and A. S. Aldawood. 2012. Effects of sex ratio and pairing duration on the biological performance of adult almond moth, *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae). J. Agric. Urban Entomol. 28: 25–33.

Stanaway, M. A., M. P. Zalucki, P. S. Gillespie, C. M. Rodriguez, and G. V Maynard. **2001**. Pest risk assessment of insects in sea cargo containers. Aust. J. Entomol. 40: 180–192.

Surkov, I. V., A. G. J. M. Oude Lansink, O. van Kooten, and W. van der Werf. **2008**. A model of optimal import phytosanitary inspection under capacity constraint. Agric. Econ. 38: 363–373.

Thein, P. P., and S.-W. Choi. **2016**. Forest insect assemblages attracted to light trap on two high mountains (Mt. Jirisan and Mt. Hallasan) in South Korea. J. For. Res. 27: 1203–1210.

United Nations Conference on Trade and Development. 2016. Review of maritime transport 2016. UNCTAD, New York, NYS.

United Nations Conference on Trade and Development. 2018. Review of maritime transport 2018. UNCTAD, New York, NYS.

U.S. Congress, Office of Technology Assessment. 1993. Harmful non-indigenous species in the United States. OTA-F-565, Washington, DC, U.S. Government Printing Office.

Walther, G.-R., A. Roques, P. E. Hulme, M. T. Sykes, P. Pyšek, I. Kühn, M. Zobel, S. Bacher, Z. Botta-Dukát, and H. Bugmann. **2009**. Alien species in a warmer world: risks and opportunities. Trends Ecol. Evol. 24: 686–693.

Wermelinger, B. 2004. Ecology and management of the spruce bark beetle *Ips typographus*—a review of recent research. For. Ecol. Manage. 202: 67–82.

Westphal, M. I., M. Browne, K. MacKinnon, and I. Noble. **2008**. The link between international trade and the global distribution of invasive alien species. Biol. Invasions. 10: 391–398.

Work, T. T., D. G. McCullough, J. F. Cavey, and R. Komsa. **2005**. Arrival rate of nonindigenous insect species into the United States through foreign trade. Biol. Invasions. 7: 323–332.

Chapter 3

Improved light traps for early-detection of insect pests of phytosanitary concern in shipping containers

Paper published as: Marchioro M. & Faccoli M. (2021). Improved light traps for early-detection of insect pests of phytosanitary concern in shipping containers. Journal of Economic Entomology.

JOURNAL OF ECONOMIC ENTOMOLOGY

Improved Light Traps for Early Detection of Insect Pests of Phytosanitary Concern in Shipping Containers

Matteo Marchioro ✉, Massimo Faccoli

Journal of Economic Entomology, toab150, https://doi.org/10.1093/jee/toab150
Published: 29 July 2021

Abstract

The number of introductions of alien insect has been increasing in the last decades, primarily transported in shipping containers. The attraction of light of different wavelengths (white, infrared, ultraviolet, red) applied on sticky traps was tested for the development of new traps for hitchhiker insects. The addition of entomological glue and insecticide on the trap were also tested. Tests were conducted on *Cadra cautella* Walker, *Drosophila melanogaster* Meigen, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), released inside a shipping container. In the first test, one light color at a time was tested setting eight traps in the container, one for each possible combination of the variables: light on or off, glue added or not, insecticide sprayed or not. In the second, five traps were used, all of them coated with the entomological glue: one for each light color and one with light off as control. In all the single color tests (except for infrared) light-on traps captured more, except for *T. castaneum* that was not attracted to white. In the multi-color test, *C. cautella* showed no preference among white, ultraviolet or red; *D. melanogaster* preferred ultraviolet and white over red; beetles had a much greater attraction to red. Lastly, the stronger entomological glue improved catches of beetles whereas insecticide did not. In conclusion, results suggest a possible application of sticky light traps against hitchhiker insects and further studies should verify if the simultaneous use of different light colors can improve the trap performance and does not act as a repellent.

Introduction

Introduction of non-native pests into new territories is a problem that has become of primary importance: driven by trade globalization, the rate of new introductions is increasing year by year (Bertelsmeier et al. 2017, Seebens et al. 2017). In the last centuries, human action has decisively facilitated and increased the processes of settlement of alien species outside their natural range (Hulme et al. 2008, Liebhold and Tobin 2008), with arthropods, and especially insects, considered as the most common and damaging group of invaders (Bradshaw et al. 2016). Invasion science is increasingly recognizing human-mediated dispersal as a pivotal node (Ricciardi et al. 2017, Bullock et al. 2018), demonstrating that the number of new biological invasions is closely related to the increase in international trade (Levine and D'Antonio 2003, Westphal et al. 2008). The most widely used means in international trade are shipping containers, which account for about 90% of global trade (IMO, 2012; Bernhofen et al. 2016).

To try preventing and reducing new introductions, several international agreements have been signed such as the World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures (SPS), the International Plant Protection Convention (IPPC) of the Food and Agricultural Organization of the United Nations and the Convention for Biological Diversity (CBD). All these agreements are based on the assumption that prevention is the most economically sound way to manage biological invasions (Puth and Post 2005, Bogich et al. 2008, Hulme et al. 2009). Nevertheless, there are many major gaps in the regulatory framework for the management of invasive insects, mainly dealing with the difficulty in assessing the effect of potential preventive measures implemented to reduce the risk of new introductions (Hulme et al. 2008, Hulme 2009). In addition, due to the huge volumes of goods passing through points-of-entry every day, phytosanitary inspectors can only check a small part of the commodities, with increasing difficulties in selecting the loads to be sampled (Everett 2000; NRC, 2002; Surkov et al. 2008).

The work of phytosanitary inspectors is a part of the *border surveillance*, applied at the point-of-entry, in order to prevent the settlement of alien species at the initial stage of their possible invasion process (Hulme 2014). In recent years, many tools and techniques have been tested to increase the effectiveness and efficiency of visual inspections (Augustin et al. 2012, Poland and Rassati 2019). Traps activated with pheromones, or volatiles, or other lures (e.g. light, colors) are the most common tools used in bio-surveillance programs, besides sniffer dogs, electronic noses, genetic tools for barcoding, acoustic detection, and laser vibrometry (Augustin et al. 2012, Poland and Rassati 2019). However, baited traps have a limit linked to the specificity of the pheromones used, which are often active only against one or a few species (Augustin et al. 2012, Rassati et al. 2015, 2019). Moreover, pheromone traps are active only during the flight dispersal of the insects in the new area, when adults have already left infested goods and containers. Therefore, traps baited using generic visual (Olenici et al. 2001, Sakalian and Mario 2004) or luminous stimuli (Ndengué et al. 2019, Silva et al. 2019) may have very high potentials in the early detection of unknown alien insect species arriving in international points-of-entry, especially when used inside the containers, *i.e.*, before insect dispersal (Marchioro et al. 2020).

In the field of luminous stimuli, insects can be attracted (positive phototaxis) or repelled (negative phototaxis) to special light sources (Park and Lee 2017). Although the use of light is already widespread in integrated pest management (Garstang 2004), there is still no large scale application of light traps for the interception of alien species. In general, the vision of insect pests ranges from a wavelength of 350 nm (ultraviolet) to 700 nm (red) (Land 1997). In light traps, incandescent or mercury vapor light bulbs are widely used, but LEDs (Light Emitting Diodes) have been used increasingly in recent times (Oh 2011, Mangan and Chapa 2013, Park and Lee 2016). The advantages of LEDs are numerous and include: small size, low weight, low electricity consumption, long lifetime, low temperature, high luminous efficiency, selectivity of specific wavelength and light intensity (Cohnstaedt et al. 2008, Yeh and Chung 2009).

Widely used in agricultural systems (Oh 2011, Park and Lee 2016), light traps were also tested in border surveillance for the interception of pests transported with goods inside containers (Mangan and Chapa 2013, Marchioro et al. 2020). A research conducted by Marchioro et al. (2020)

tested a light trap model inside a container, under different loading conditions, on four model species: *Cadra cautella* Walker (Lepidoptera: Pyralidae), *Drosophila melanogaster* Meigen (Diptera: Drosophilidae), *Sitophilus zeamais* Motschulsky, and *Ips typographus* L. (Coleoptera: Curculionidae). Results showed that trap performance is not affected by the container load and a high number of catches was recorded for Diptera and Lepidoptera. Instead, the trap was scarcely effective against beetles as the glue of the sticky cards of the trap was not strong enough to catch these insects, but a low attractiveness of the light installed in the trap also cannot be excluded. Results of this research have been encouraging and positive, but have also highlighted some gaps to be filled and improvements to be made on traps to improve their performance and effectiveness against more species. In view of these first results, the aim of this study was to investigate 1) how model species belonging to different insect orders respond to different light colors (*i.e.*, wavelength), and 2) whether the synergistic use of more powerful glue and contact insecticides would improve capture performance of traps compared to the use of sticky cards only. This aims to develop a generic light trap efficient in early detection of alien insects belonging to different orders and families.

Materials and Methods

Tested traps

Light-sticky traps (TransTrap^{TM}, Alpha Scents Inc., West Linn, OR) developed for use inside containers during shipment were modified as shown by Marchioro *et al.* (2020). The original device consists of a small carton box (15x23x4 cm) containing a LED (Light Emitting Diode) to attract insects and a yellow sticky card to catch them. The LED is powered by two AA batteries that can keep the light on for at least two consecutive weeks. The sticky card is attached to the bottom of the box and the light is positioned in the center. To increase the sticky surface and, consequently, the catching performance of the trap, we attached a second sticky card inside the box lid (Fig. 1). Sticky cards are produced by Alpha Scents Inc. too, and they are a standard model mainly indicated against flies, aphids, hoppers, psyllids and yellow jackets (Alpha Scents Inc. 2013) and, also considering results obtained by Marchioro et al. (2020), they probably are not stronger enough in order to capture beetles.

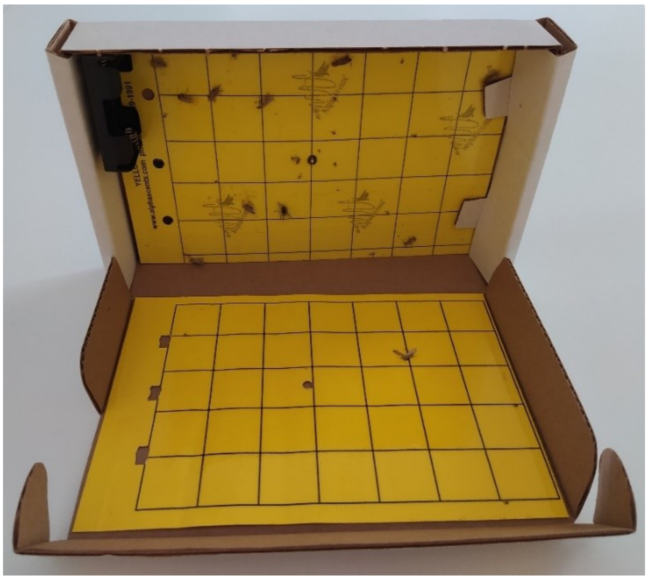

Fig. 1 TransTrap, the trap used for the research.

Standard LED emit light that has two peaks, one at 465 nm (indigo) and the second between 525 and 600 nm (between green and yellow) and the result is white light. Beside the original trap model, in this study we also replaced the manufacturer's LED with LEDs of other three wavelengths: ultraviolet (wavelength 410 nm), red (wavelength 625 nm), and infrared (wavelength 940 nm). In order to prevent beetles from escaping, the inside surfaces of traps were also sprinkled with a strong entomological glue (Temo-O-Cid®, Adama Italia s.r.l., Bergamo, Italy) and a solution composed by 1 ml of deltamethrin-based insecticide (Decis® 15 EW, Bayer AG, Leverkusen, Germany) per 1 liter of water. Temo-O-Cid is a specific glue for the capture of flies and insects that can be spread with a brush. Once applied, the evaporation of the solvent contained makes the product absolutely non-toxic. It does not dry and retains its characteristics even when exposed to atmospheric agents. Temo-O-Cid is used to prepare chromotropic and all kinds of traps, to catch insects in orchards, vineyards, flower crops. The greater strength of this glue, combined with a greater thickness of glue on the sticky card after its addition, should make it easier to catch larger insects.

Model species

The different trap models were tested against four model species belonging to Coleoptera, Lepidoptera, and Diptera orders, the three most common orders found inside shipping containers

(Meurisse et al. 2019). *Sitophilus zeamais*, the maize weevil, is one of the major pests of stored maize in tropical and temperate regions of the world, but it also infests other cereals as alternative hosts (Erenso and Berhe 2016, Nwosu 2018). *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), the red flour beetle, is a stored grain, flour and other cereal product pest (Brown et al. 2009). *Cadra cautella*, the almond moth, is a pest of cereal grains, beans, and other dried seeds (Aldawood et al. 2013, Husain et al. 2017). *Drosophila melanogaster* is a fruit and vegetable pest (Mallis 1954, Birmingham et al. 2011).

All insects were provided by a laboratory (Entostudio s.r.l., Padua, Italy) specialized in the breeding of arthropod species for scientific purposes. The colony of *S. zeamais* was established in 2014 with insects collected in the field. Adults were bred in plastic cups enclosed by a net and fed with grain. The photoperiod lasted 12 hours at a solar spectrum artificial light of 6,000 K and 300 lux intensity and they were bred at 25±1 °C and 50±5% R.H. Similarly, adults of *T. castaneum* were bred in plastic cups enclosed by a fine net, at 25±1 °C and 50±5% R.H. The photoperiod, at a solar spectrum artificial light of 6000 K and 300 lux intensity, lasted 14 hours. Insects were fed with 95% of flour and 5% of beer yeast and a vial filled with water was present in the plastic cup to provide water and humidity to the colony. Adults of *C. cautella* were bred in glass jars positioned upside down with the opening closed by a 2 mm mesh net. The jar was placed above a plastic container to collect the eggs, which were then moved daily into plastic cups containing a mixture of wheat and corn flour, oat, bran, dry fruit, glycerol, honey and yeast. The insects were reared at 25±1 °C and 50±5% R.H. The photoperiod, at a solar spectrum artificial light of 6,000 and 300 lux intensity, lasted 12 hours. Adults of *D. melanogaster* were bred in BugDorme cages. A mixture of water, pieces of potato and fruit, powdered milk, and sugar was used as food and as oviposition substrate. Insects were reared at 25±1 °C and 50±5% of R.H. with a photoperiod of 12 hours at a solar spectrum artificial light of 6,000 K and 300 lux intensity.

All insects were tested only once and within two days after their emergence (for *C. cautella* and *D. melanogaster*) to guarantee highest vitality. We assumed a sex-ratio 1:1 as these four species reproduce sexually and produce a sex-balanced offspring (Englert and Bell 1962, Santos et al. 1994, Danho et al. 2002, Soffan et al. 2012). The insects used in each trial were chosen randomly.

Trials in container

Trials were conducted in an ISO standard shipping container 1CC (interior size: 5.8 m length, 2.3 m width, 2.3 m height) (ISO 2013). The container was placed in the Agripolis Campus, University of Padua (Legnaro, Italy), without any shelter from sun and rain. The container was empty of goods and only the traps and insect releasing device were placed inside. In contrast to the tests conducted the year before (Marchioro et al. 2020), in this case no container load tests were carried out, as the aim of the study was to test the attractiveness of different wavelengths. Traps were positioned inside the container open, with lid and box forming a 90° angle. The lid was resting on the ground, while the box was in a vertical position, as can be seen in Fig. 1. In trials in which some traps had to be placed on the top of the container, the use of metal hooks made it possible to maintain the same conformation as traps placed on the ground. Tests were conducted between May and July 2020.

Single color tests. The first group of tests was conducted using only one light color at a time. We used eight different traps at the same time, one for each of the 8 possible combinations of the three considered variables: light (turned on or off), additional glue (added or not), and insecticide (sprayed or not). A trap with a turned off light and without additional glue or insecticide was used as control (trap “C”). Each different combination of variables corresponds to a different code: "L" if the trap light was on, "G" if glue was added, "I" if insecticide was added. The eight traps were randomly set in the eight corners of the container (changing the traps arrangement at each trial) (Fig. 2): four traps were laid on the ground, while four were hung by hooks from the ceiling. During each trial, we used 50 individuals for each model species released at the same time, for a total of 200 insects. With a device consisting of a cup containing the insects and a rope tied to the lid to free them, it was possible to release the insects just before the doors of the container were closed to prevent their escape. For each LED color (white, infrared, ultraviolet, and red), we conducted seven repetitions, on seven consecutive nights with similar weather conditions; each repetition lasted 18 hours (from 5:00 pm to 11:00 am the following day). At the end of each trial, before starting a new one, we ventilated the container, swept the floor and removed all insects from the walls to make sure there were none left inside.

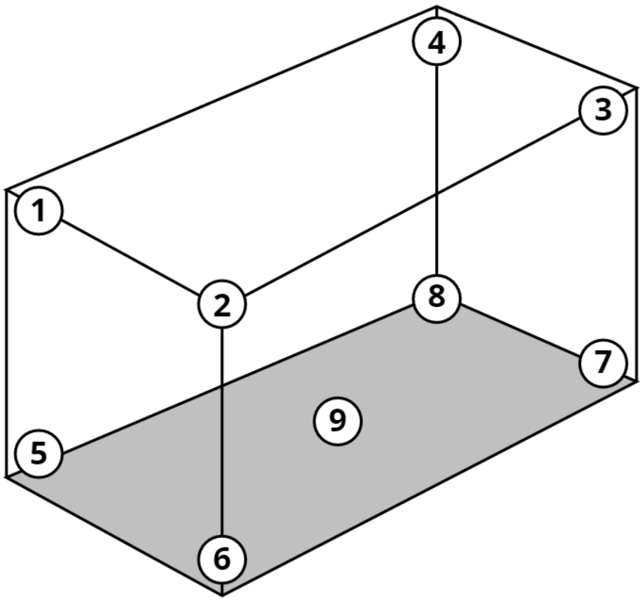

Fig. 2 Disposition of the traps inside the container (doors were on the left side). Single color test: 1-8. Multi-color test: 5–9.

Multi-color tests. Other tests were conducted using, at the same time in the container, all traps with the four different light colors. One trap per light color (white, red, ultraviolet and infrared) and coated with entomological glue was tested, while a trap with a turned off light and without additional glue was used as control (trap “C”). Again, each different light color corresponds to a different code: "W" for white light, "IR" for infrared light, "UV" for ultraviolet light, “R” for red light. The 5 traps were randomly set inside container, on the floor (changing the traps arrangement at each trial) (Fig. 2). Seven repetitions were conducted on seven consecutive nights, with duration of 18 hours (from 5:00 pm to 11:00 am the following day). Fifty individuals per model species were used in each repetition, for a total of 200 insects per day.

Statistical analysis

In the “single color tests”, mean catches per trap of the model species were compared using a mixed-effect model, with trap type (the eight possible combinations of the three tested variables) as fixed variable and repetitions as random variable. The model was fitted using the “lmer” or “glmer” functions in the lme4 package (Bates et al. 2015) and using Poisson distribution or logarithmic transformation as appropriate (Table 1). Multiple comparisons between fixed variables were obtained using Tukey's test ("emmeans" function in the emmeans package) with "Bonferroni correction" (Russell 2019). When the use of this statistical test was inapplicable because of few captures, the Kruskal-Wallis test was applied using the "kruskal.test" function in the stat package (R Core Team 2019).

In the "Multi color tests", mean catches per trap of the model species were compared using a mixed-effect model, with trap type (the five light colors, including control) as fixed variable and repetitions as random variable; Tukey's test with "Bonferroni correction" was used for multiple comparisons between fixed variables. Statistical analysis was performed using R software, version 3.6.1 (R Core Team, 2019).

Results

The main obtained results are presented here briefly according to the tested light color. The number of captures for each model species in each test is reported in Table A1.

Single color test - White light. The four trap combinations with light turned on (trap L, L+G, L+I, and L+G+I) caught similar numbers of *C. cautella* and significantly higher than the light-off traps (C, G, I, and G+I) (Table 1, Table A2, Fig. 3a). The same result was observed for *D. melanogaster*, although in this species trap L captured significantly more individuals than L+G (Table 1, Table A2, Fig. 3b). *S. zeamais* was captured significantly more in traps L+G and L+G+I than all the others. Moreover, in *S. zeamais* the other two light-on traps (L and L+I) caught significantly more than light-off traps (Table A3, Fig. 3c). Only one individual of *T. castaneum* was captured in traps L and L+G+I, numbers too low to allow statistical analysis.

Single color test - Infrared light. The four model species were captured only in very low numbers in traps activated with infrared light. Although for beetles (*S. zeamais* and *T. castaneum*) there were a few captures in traps treated with additional glue or insecticide (G, G+I, L+G, L+I, L+G+I), there were no significant differences between the eight tested trap models (Table A2, Table A3).

Single color test - Ultraviolet light. Similar to the white light test, *C. cautella* and *D. melanogaster* were caught significantly more by light-on traps (L, L+G, L+I, and L+G+I) than light-off traps (Table 1, Table A2, Fig. 3d). In addition, for *D. melanogaster*, L+G+I captured significantly more individuals than L traps (Table 1, Table A2, Fig. 3e). With beetles (*S. zeamais* and *T. castaneum*) L+G and L+G+I captured significantly more insects than the other trap models (Table 1). While for *S. zeamais* the other 6 trap models showed no significant differences (with C and I trapping no individuals (Table A3, Fig. 3f)), for *T. castaneum* L+G+I was the trap type that captured the largest number of insects, while L+G captured significantly more than C, G, I and G+I traps (Table A3, Fig. 3g).

Single color test - Red light. Again, *C. cautella* and *D. melanogaster* were captured significantly more by light-on (L, L+G, L+I, and L+G+I) than light-off traps (Table 1, Table A2, Fig. 3h-i). Similarly, light-on traps coated with additional glue (L+G and L+G+I) caught significantly more individuals of *S. zeamais* ($P < 0.001$, $K = 46.290$) and *T. castaneum* ($P < 0.001$, $K = 45.231$); in both beetle species, C, G, and I traps captured no insects (Table A3, Fig. 3j-k).

Table 1 Results of the statistical models (P-value) used to test the effect of trap type for the four model species in all the tests conducted. L = light on; G = glue added; I = insecticide sprayed.

Model species	Trap type	P-value	t/z-value	Df	Model	Distribution
Single color test - White light						
Cadra cautella	L	<0.001	9.303	48	LMM	Normal
	L+G	<0.001	7.048			
	L+I	<0.001	7.330			
	L+G+I	<0.001	7.893			
Drosophila melanogaster	L	<0.001	4.878	47	GLMM	Poisson
	L+G	<0.001	3.826			
	L+I	<0.001	4.334			
	L+G+I	<0.001	4.366			
Single color test - Infrared light						
Cadra cautella	-	-	-	47	GLMM	Poisson
Drosophila melanogaster	-	-	-	47	GLMM	Poisson
Single color test - Ultraviolet light						
Cadra cautella	L	<0.001	3.660	48	LMM	Normal
	L+G	<0.01	3.253			
	L+I	<0.01	3.186			
	L+G+I	<0.01	3.253			
Drosophila melanogaster	L	<0.001	4.369	47	GLMM	Poisson
	L+G	<0.001	4.872			
	L+I	<0.001	5.546			
	L+G+I	<0.001	6.097			
Single color test - Red light						
Cadra cautella	L	<0.01	3.254	47	GLMM	Poisson
	L+G	<0.01	3.254			
	L+I	<0.001	3.531			
	L+G+I	<0.001	4.542			
Drosophila melanogaster	L	<0.001	4.777	48	LMM	Log-transf.
	L+G	<0.001	4.245			
	L+I	<0.001	5.552			
	L+G+I	<0.001	5.098			
Multi-color test						
Cadra cautella	UV	<0.001	3.791	24	LMM	Normal
Drosophila melanogaster	W	<0.001	6.732	29	GLMM	Poisson
	UV	<0.001	5.988			
	R	<0.001	4.159			
Sitophilus zeamais	W	<0.001	5.727	24	LMM	Log-transf.
	UV	<0.01	3.279			
	R	<0.001	10.370			
Tribolium castaneum	W	<0.05	2.621	24	LMM	Log-transf.
	UV	<0.001	7.474			
	R	<0.001	13.131			

Models = LMM: linear mixed-effects model; GLMM: generalized linear mixed-effects model. Distribution = Normal: normal distribution; Log-transf.: normal on log-transformed data; Poisson: Poisson distribution. t-value is referred to LMM models; z-value is referred to GLMM models.

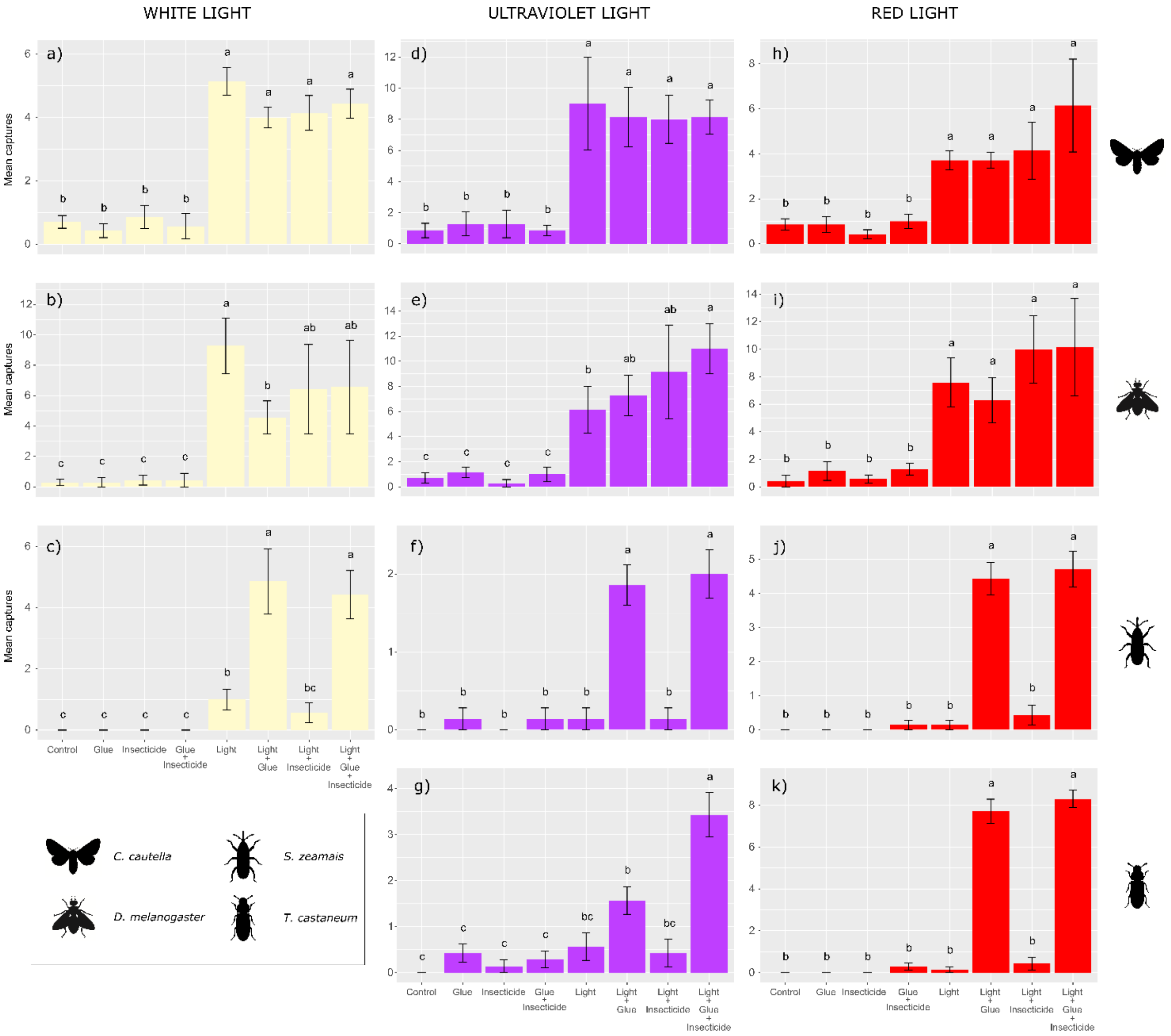

Fig. 3 Mean (± SE) number of insects captured by each trap combination during the "Single color tests" and divided for each model species (rows) and light color (columns). Means with different letters on the same graph were significantly different.

Multi-color tests. Captures of *C. cautella* in ultraviolet light trap were significantly higher than in control (light-off trap) and infrared light traps, but without differences from white and red light traps (Table 1, Table A2, Fig. 4a). For *D. melanogaster* white, ultraviolet and red light traps caught a significantly higher number of individuals than control and infrared light traps. Moreover, white and ultraviolet light traps captured more than the red one (Table 1, Table A2, Fig. 4b). Lastly, for *S. zeamais* and *T. castaneum* red light trap outperformed the others. Ultraviolet and white light traps caught significantly more individuals of *S. zeamais* than control and infrared light traps (Table

1, Table A2, Fig. 4c). Whereas, for *T. castaneum*, ultraviolet light trap outperformed control, infrared and white traps (Table 1, Table A2, Fig. 4d).

Discussion

Results show different phototactic responses for the various tested species. All model species showed a general attraction to light: in fact, in all the single color tests (except for infrared light) light-on traps captured more specimens. Only *T. castaneum* did not present an attraction for white light. In particular, in the multi-color test, we found that *C. cautella* has no preference between white, ultraviolet and red lights; *D. melanogaster* prefers ultraviolet and white over red light; *S. zeamais* and *T. castaneum* have a much greater attraction to red light.

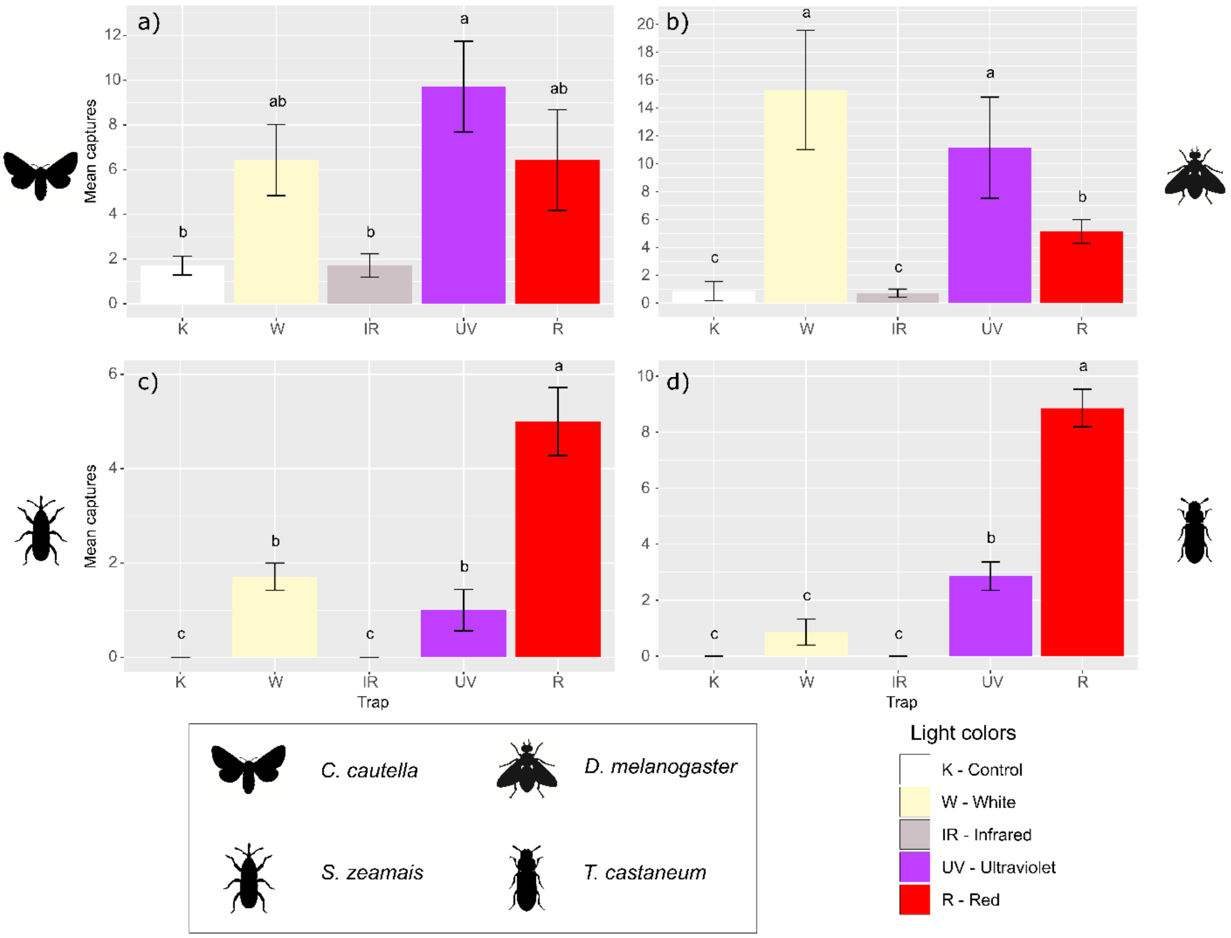

Fig. 4 Mean (± SE) number of insects, divided for each model species, captured by each light color during the “Multi-color test”. Means with different letters on the same graph were significantly different.

Land (1997) observed that, in general, insects can perceive light ranging in wavelength from 350 (ultraviolet) to 700 nm (red) and results of the “single-color tests” agree with him. In fact, for all four model species, we obtained a significant effect of light with ultraviolet, white and red wavelengths, but not with infrared (940 nm). Moreover, light-on traps (with white, ultraviolet and red LED) with the addition of entomological glue captured significantly more beetles (both *S. zeamais* and *T. castaneum*) than normal traps or traps with insecticide only. This result confirms the hypothesis formulated by Marchioro et al. (2020) according to which the standard glue of sticky cards, alone, was unsuitable to retain trapped beetles. Adding insecticide does not improve trap performance, probably because beetles are able to escape before dying. This is also true for Lepidoptera and Diptera: in fact, captures of light-on traps with insecticide are similar to other light-on traps. However, avoiding the use of insecticides may also allow trap use in containers transporting food, without risk of goods contamination.

White light shows among the best results for catching Lepidoptera and Diptera (although with no significant differences from ultraviolet and red light), probably due to its composition of two peaks at indigo and green-yellow wavelength. Measures of spectral efficiency of *C. cautella*, in fact, highlight two regions of high efficiency at 546 nm (yellow-green) and 350 nm (ultraviolet) (Gilburt and Anderson 1996). Moreover, it has been observed by numerous studies that green and blue lights are very effective in catching many Lepidoptera, like for instance *Ephestia kuehniella* (Soderstrom 1970), *Plodia interpunctella* (Soderstrom 1970, Park and Lee 2016), *Sitotroga cerearella* (Soderstrom 1970), *Spodoptera exigua* (Oh 2011), *Spodoptera litura* (Yang et al. 2012) and *Plutella xylostella* (Cho and Lee 2012).Also *D. melanogaster* is most sensitive to short wavelength lights (ultraviolet, blue, and green) with two peaks at 420 nm and 495 nm (de Salomon and Spatz 1983, Kelber and Henze 2013). Light traps with similar wavelengths are largely used for moth monitoring, but they may also intercept Diptera (Kim and Lee 2014a, da Silva et al. 2019, Ndengué et al. 2019, Silva et al. 2019).

Ultraviolet was one of the best wavelengths for Lepidoptera and Diptera (although catches did not differ from those obtained with white and red light), while it provided scarce results for Coleoptera. The general effectiveness of UV as an attraction for several insects is well-known (Hollingsworth et al. 1968, Kirkpatrick et al. 1970, van Grunsven et al. 2014, Thein and Choi 2016), in particular for moths (Cho and Lee 2012, Infusino et al. 2017) and flies (Gaglio et al. 2018, Hogsette 2019). Regarding *S. zeamais* and *T. castaneum*, the literature instead provides conflicting results about the attractiveness of UV. On one hand, Duehl et al. (2011) found that *T. castaneum* was most attracted by UV wavelength and Kirkpatrick *et al.* (1970) found that some species of stored-products beetles preferred UV over green light. On the other, Park et al. (2015) and Song et al. (2016a) found that UV light was the less attractive for *S. zeamais* and *T. castaneum*.

Red wavelength also showed high attractiveness for our model species. For *C. cautella* the number of trapped insects was similar to white and ultraviolet lights, as found for the similar species *P. interpunctella* (Park and Lee 2016). However, for other moth species belonging to different orders, results are different: red light is less attractive than other light colors in *S. litura* (trapped with blue and green (Yang et al. 2012); *S. exigua* (trapped with white light, (Oh 2011), and *S. cerearella* (trapped with ultraviolet light (Kim and Lee 2014b). For *D. melanogaster* captures obtained with red light were lower than white and ultraviolet lights. Also for another Dipteran, *Liriomyza trifolii*, red light was less attractive than green and yellow lights, but more attractive than ultraviolet (Kim and Lee 2014a). Finally, for both beetle species, red light was the most attractive one, with more than twice the catches than those of white and ultraviolet. These results agree with other researches conducted on the phototactic behaviour of *S. zeamais* (Park et al. 2015) and *T. castaneum* (Song et al. 2016a, 2016b), where red light was the best wavelength for both species. However, different results were obtained for other beetles: *S. oryzae*, congeneric of *S. zeamais*, preferred blue and green lights, whereas red and ultraviolet lights showed similarly lower capture performance (Jeon et al. 2012).

Finally, in our trials infrared light was not attractive to any of the tested species. This result is not surprising as insect vision is generally shifted towards ultraviolet and they seem unable to see infrared radiation (Land, 1997). Other studies dealing with the phototactic behaviour of fly and moth species confirm this observation (Cho and Lee 2012, Kim and Lee 2014a, 2014b, Park and Lee 2016). However, certain studies have shown a similar attraction of *S. zeamais* to red, yellow and infrared light (Park et al. 2015), and of *T. castaneum* similar to infrared, white, yellow, green and blue lights, and higher than ultraviolet (Song et al. 2016a).

In conclusion, we found that light is an effective "broad-spectrum" attractant for several insect species belonging to different orders. Moreover, the use of a stronger glue on the sticky cards improves captures of beetles (although it does not improve moth and fly catches), solving the problem highlighted by Marchioro et al. (2020). Instead, the insecticide, in the formulations and doses tested, does not give any improvement in terms of catches. However, we also found that there is a clear response of the different species to the different lights tested: white and ultraviolet lights are the most attractive for *C. cautella* and *D. melanogaster*, while red is the most effective in catching beetles. Moreover, we can hypothesize that using at the same time different traps with different light colors, there must have been some interference in the case of two colors both attractive to one species. Probably, using only one trap, the trap performance will increase. A possible solution could consist in the use of different lights at the same time in the same trap, but further studies should verify that this combination can improve the trap performance and is not a repellent. The aim of this study is to find a trap that can be used in a wide range of shipments, with a wide variety of commodities. The tested glue (Temo-O-Cid®) is non-toxic and this allows the trap to be used in conjunction with any type of food product (grains, flours, fruits and vegetables). However, it can be used with any kind of cargo that can carry hitchhikers' insects. This is only a pilot study that used few model species. In order to obtain more comprehensive and reliable results, other tests must be conducted, possibly during real shipments.

Acknowledgements

The authors thank professor Andrea Battisti for reviewing the draft of this manuscript, Alison Garside for the language review, Paolo Paolucci for providing some of the images used in the graphs, and Entostudio, in particular Dr. Patrizia Visentin and Dr. Andrea Drago, for providing the insects used in the trials. The study has received funding from the project HOMED within the European Union’s Horizon 2020 Research and Innovation Programme (grant agreement N° 771271) and from the DOR projects of the University of Padua.

References

Aldawood, A. S., K. G. Rasool, A. H. Alrukban, A. Soffan, M. Husain, K. D. Sutanto, and M. Tufail. 2013. Effects of temperature on the development of *Ephestia cautella* (Walker) (Pyralidae: Lepidoptera): a case study for its possible control under storage conditions. Pakistan J. Zool. 45: 1573-1578.

Alpha Scents Inc. 2013. Yellow Rectangle Adhesive Traps. https://www.alphascents.com/pub/media/Pdf/Flyer Yellow Cards.pdf

Augustin, S., N. Boonham, W. J. De Kogel, P. Donner, M. Faccoli, D. C. Lees, L. Marini, N. Mori, E. Petrucco Toffolo, S. Quilici, A. Roques, A. Yart, and A. Battisti. 2012. A review of pest surveillance techniques for detecting quarantine pests in Europe. EPPO Bull. 42: 515–551.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. J. Stat. Softw. 67: 1–51.

Bernhofen, D. M., Z. El-Sahli, and R. Kneller. 2016. Estimating the effects of the container revolution on world trade. J. Int. Econ. 98: 36–50.

Bertelsmeier, C., S. Ollier, A. Liebhold, and L. Keller. 2017. Recent human history governs global ant invasion dynamics. Nat. Ecol. Evol. 1: 1-17.

Birmingham, A. L., E. Kovacs, J. P. Lafontaine, N. Avelino, J. H. Borden, I. S. Andreller, and G. Gries. 2011. A new trap and lure for *Drosophila melanogaster* (Diptera: Drosophilidae). J. Econ. Entomol. 104: 1018–1023.

Bogich, T. L., A. M. Liebhold, and K. Shea. 2008. To sample or eradicate? A cost minimization model for monitoring and managing an invasive species. J. Appl. Ecol. 45: 1134–1142.

Bradshaw, C. J. A., B. Leroy, C. Bellard, D. Roiz, C. Albert, A. Fournier, M. Barbet-Massin, J.-M. Salles, F. Simard, and F. Courchamp. 2016. Massive yet grossly underestimated global costs of invasive insects. Nat. Commun. 7: 1-8.

Brown, S. J., T. D. Shippy, S. Miller, R. Bolognesi, R. W. Beeman, M. D. Lorenzen, G. Bucher, E. A. Wimmer, and M. Klingler. 2009. The red flour beetle, *Tribolium castaneum* (Coleoptera): a model for studies of development and pest biology. Cold Spring Harb. Protoc. 4: 1–9.

Bullock, J. M., D. Bonte, G. Pufal, C. da Silva Carvalho, D. S. Chapman, C. García, D. García, E. Matthysen, and M. M. Delgado. 2018. Human-mediated dispersal and the rewiring of spatial networks. Trends Ecol. Evol. 33: 958–970.

Cho, K.-S., and H.-S. Lee. 2012. Visual preference of diamondback moth, *Plutella xylostella*, to light-emitting diodes. J. Korean Soc. Appl. Biol. Chem. 55: 681–684.

Cohnstaedt, L. W., J. I. Gillen, and L. E. Munstermann. 2008. Light-emitting diode technology improves insect trapping. J. Am. Mosq. Control Assoc. 24: 331–334.

da Silva, A. A., J. M. M. Rebêlo, B. F. Carneiro, M. P. P. Castro, M. de Sousa de Almeida, I. S. Ponte, J. V. C. Aguiar, and F. S. Silva. 2019. Exploiting the synergistic effect of kairomones and light-emitting diodes on the attraction of phlebotomine sand flies to light traps in Brazil. J. Med. Entomol. 56: 1441–1445.

Danho, M., C. Gaspar, and E. Haubruge. 2002. The impact of grain quantity on the biology of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae): oviposition, distribution of eggs, adult emergence, body weight and sex ratio. J. Stored Prod. Res. 38: 259–266.

de Salomon, C. H., and H.-C. Spatz. 1983. Colour vision in *Drosophila melanogaster*: wavelength discrimination. J. Comp. Physiol. 150: 31–37.

Duehl, A. J., L. W. Cohnstaedt, R. T. Arbogast, and P. E. A. Teal. 2011. Evaluating light attraction to increase trap efficiency for *Tribolium castaneum* (Coleoptera: Tenebrionidae). J. Econ. Entomol. 104: 1430–1435.

Englert, D. C., and A. E. Bell. 1962. Sex ratio in *Tribolium castaneum* Herbst as influenced by larval starvation. Oikos. 13: 118–124.

Erenso, T. F., and D. H. Berhe. 2016. Effect of neem leaf and seed powders against adult maize weevil (*Sitophilus zeamais* Motschulsky) mortality. Int. J. Agric. Res. 11: 90–94.

Everett, R. A. 2000. Patterns and pathways of biological invasions. Trends Ecol. Evol. 15: 177–178.

Gaglio, G., E. Napoli, F. Arfuso, J. M. Abbate, S. Giannetto, and E. Brianti. 2018. Do different LED colours influence sand fly collection by light trap in the Mediterranean? Biomed Res. Int. 2018: 1–7.

Garstang, R. H. 2004. Mount Wilson observatory: The sad story of light pollution. The Observatory 124, 14-21.

Gilburt, H. L., and M. Anderson. 1996. The spectral efficiency of the eye of *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae). J. Stored Prod. Res. 32: 285–291.

van Grunsven, R. H. A., M. Donners, K. Boekee, I. Tichelaar, K. G. van Geffen, D. Groenendijk, F. Berendse, and E. M. Veenendaal. 2014. Spectral composition of light sources and insect phototaxis, with an evaluation of existing spectral response models. J. Insect Conserv. 18: 225–231.

Hogsette, J. A. 2019. Turning ultraviolet light traps on and off increases their attraction to house flies (Diptera: Muscidae). J. Insect Sci. 19: 22–23.

Hollingsworth, J. P., A. W. Hartstack, and D. A. Lindquist. 1968. Influence of near-ultraviolet output of attractant lamps on catches of insects by light traps. J. Econ. Entomol. 61: 515–521.

Hulme, P. E. 2009. Trade, transport and trouble: managing invasive species pathways in an era of globalization. J. Appl. Ecol. 46: 10–18.

Hulme, P. E. 2014. An Introduction to Plant Biosecurity: Past, Present and Future, pp. 1–25. In G. Gordh and S. McKirdy (eds.), The handbook of plant biosecurity. Springer Netherlands, Dordrecht.

Hulme, P. E., S. Bacher, M. Kenis, S. Klotz, I. Kühn, D. Minchin, W. Nentwig, S. Olenin, V. Panov, J. Pergl, P. Pyšek, A. Roques, D. Sol, W. Solarz, and M. Vilà. 2008. Grasping at the routes of biological invasions: a framework for integrating pathways into policy. J. Appl. Ecol. 45: 403–414.

Hulme, P. E., P. Pyšek, W. Nentwig, and M. Vilà. 2009. Will threat of biological invasions unite the European Union? Science 324: 40–41.

Husain, M., W. S. Alwaneen, K. Mehmood, K. G. Rasool, M. Tufail, and A. S. Aldawood. 2017. Biological traits of *Cadra cautella* (Lepidoptera: Pyralidae) reared on khodari date fruits under different temperature regimes. J. Econ. Entomol. 110: 1923–1928.

Infusino, M., G. Brehm, C. Di Marco, and S. Scalercio. 2017. Assessing the efficiency of UV LEDs as light sources for sampling the diversity of macro-moths (Lepidoptera). Eur. J. Entomol. 114: 25–33.

International Maritime Organization (IMO). 2012. International shipping facts and figures information resources on trade, safety, security, environment. IMO, London, UK.

International Organization for Standardization (ISO). 2013. TC 104. ISO 668: 2013. Series 1 freight containers-classification, dimensions and ratings. Int. Organ. Stand. Geneva, Switz.

Jeon, J.-H., M.-S. Oh, K.-S. Cho, and H.-S. Lee. 2012. Phototactic response of the rice weevil, *Sitophilus oryzae* linnaeus (Coleoptera: Curculionidae), to light-emitting diodes. J. Korean Soc. Appl. Biol. Chem. 55: 35–39.

Kelber, A., and M. J. Henze. 2013. Colour vision: parallel pathways intersect in *Drosophila*. Curr. Biol. 23: R1043–R1045.

Kim, M.-G., and H.-S. Lee. 2014a. Attractive effects of american serpentine leafminer, *Liriomyza trifolii* (Burgess), to light-emitting diodes. J. Insect Behav. 27: 127–132.

Kim, M.-G., and H.-S. Lee. 2014b. Phototactic behavior 5: attractive effects of the angoumois grain moth, *Sitotroga cerealella*, to light-emitting diodes. J. Korean Soc. Appl. Biol. Chem. 57: 259–262.

Kirkpatrick, R. L., D. L. Yancey, and F. O. Marzke. 1970. Effectiveness of green and ultraviolet light in attracting stored-product insects to traps. J. Econ. Entomol. 63: 1853–1855.

Land, M. F. 1997. Visual acuity in insects. Annu. Rev. Entomol. 42: 147–177.

Levine, J. M., and C. M. D'Antonio. 2003. Forecasting biological invasions with increasing international trade. Conserv. Biol. 17: 322–326.

Liebhold, A. M., and P. C. Tobin. 2008. Population ecology of insect invasions and their management. Annu. Rev. Entomol. 53: 387–408.

Mallis, A. 1954. Handbook of Pest Control. The behavior, life history, and control of household pests, 2th ed. MacNair-Dorland Co., New York, N.Y.

Mangan, R. L., and D. Chapa. 2013. Evaluation of the effects of light source and plant materials on asian citrus psyllid (Hemiptera: Psyllidae) trapping levels in the transtrap for citrus shipping containers. Florida Entomol. 96: 104–111.

Marchioro, M., A. Battisti, and M. Faccoli. 2020. Light traps in shipping containers: a new tool for the early detection of insect alien species. J. Econ. Entomol. 113: 1718–1724.

Meurisse, N., D. Rassati, B. P. Hurley, E. G. Brockerhoff, and R. A. Haack. 2019. Common pathways by which non-native forest insects move internationally and domestically. J. Pest Sci. 92: 13–27.

National Research Council (NRC). 2002. Predicting invasions of nonindigenous plants and plant pests. National Academy Press, Washington, DC.

Ndengué, J. D. M., G. Texier, J. Landier, E. De Gavelle, J. Marchi, L. R. Kamgang, M. Kenné, M. Tindo, S. Eyangoh, and P. Le Gall. 2019. Adapting light trap to catch household insects in central Cameroon: a pilot study. Ann. la Société Entomol. Fr. 55: 383–394.

Nwosu, L. C. 2018. Impact of age on the biological activities of *Sitophilus zeamais* (Coleoptera: Curculionidae) adults on stored maize: implications for food security and pest management. J. Econ. Entomol. 111: 2454–2460.

Oh, M. S. 2011. Evaluation of high power light emitting diodes (HPLEDs) as potential attractants for adult *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). J. Korean Soc. Appl. Biol. Chem. 54: 416–422.

Olenici, N., A. Roques, and V. Olenici. 2001. Effectiveness of visual traps for detection and survey of cone flies, *Strobilomya* spp. (Diptera: Anthomyiidae), infesting cones of European larch (Larix decidua Mill.) in Romania. In: Knizek M., Forster B., Grodzki W. (eds.), Methodology of forest insect and disease survey in Central Europe. Proceedings of the IUFRO WP 7.03.10 Workshop, 24–28 September 2000, Busteni, Romania. Forest Research and Management Institute, Bucharest, pp 100–104.

Park, J.-H., and H.-S. Lee. 2016. Phototactic behavior 10: phototactic behavioral effects of *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) adults to different light-emitting diodes of seven wavelengths. J. Appl. Biol. Chem. 59: 95–98.

Park, J.-H., and H.-S. Lee. 2017. Phototactic behavioral response of agricultural insects and stored-product insects to light-emitting diodes (LEDs). Appl. Biol. Chem. 60: 137–144.

Park, J. H., B. K. Sung, and H. S. Lee. 2015. Phototactic behavior 7: phototactic response of the maize weevil, *Sitotroga zeamais* motsch (Coleopter: Curculionidae), to light-emitting diodes. J. Korean Soc. Appl. Biol. Chem. 58: 373–376.

Poland, T. M., and D. Rassati. 2019. Improved biosecurity surveillance of non-native forest insects: a review of current methods. J. Pest Sci. 92: 37–49.

Puth, L. M., and D. M. Post. 2005. Studying invasion: have we missed the boat? Ecol. Lett. 8: 715–721.

R Core Team. 2019. R version 3.6.1: A language and environment for statistical computing. R Found. Stat. Comput. Vienna, Austria. URL https//www. R-project. org.

Rassati, D., M. Faccoli, E. Petrucco Toffolo, A. Battisti, and L. Marini. 2015. Improving the early detection of alien wood-boring beetles in ports and surrounding forests. J. Appl. Ecol. 52: 50–58.

Rassati, D., L. Marini, M. Marchioro, P. Rapuzzi, G. Magnani, R. Poloni, F. Di Giovanni, P. Mayo, and J. Sweeney. 2019. Developing trapping protocols for wood-boring beetles associated with broadleaf trees. J. Pest Sci. 92: 267–279.

Ricciardi, A., T. M. Blackburn, J. T. Carlton, J. T. A. Dick, P. E. Hulme, J. C. Iacarella, J. M. Jeschke, A. M. Liebhold, J. L. Lockwood, H. J. MacIsaac, P. Pyšek, D. M. Richardson, G. M. Ruiz, D. Simberloff, W. J. Sutherland, D. A. Wardle, and D. C. Aldridge. 2017. Invasion science: a horizon scan of emerging challenges and opportunities. Trends Ecol. Evol. 32: 464–474.

Russell, L. 2019. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.5.5-1. https://CRAN.R-project.org/package=emmeans

Sakalian, V., and L. Mario. 2004. Colour traps a method for distributional and ecological investigations of Buprestidae (Coleoptera). Acta Soc. Zool. Bohem. 68: 53–59.

Santos, M., K. Fowler, and L. Partridge. 1994. Gene-environment interaction for body size and larvae in *Drosophila melanogaster*: an investigation of effects on development time, thorax length and adult sex ratio. Genet. Soc. Gt. Britain. 72: 515–521.

Seebens, H., T. M. Blackburn, E. E. Dyer, P. Genovesi, P. E. Hulme, J. M. Jeschke, S. Pagad, P. Pyšek, M. Winter, M. Arianoutsou, S. Bacher, B. Blasius, G. Brundu, C. Capinha, L. Celesti-Grapow, W. Dawson, S. Dullinger, N. Fuentes, H. Jäger, J. Kartesz, M. Kenis, H. Kreft, I. Kühn, B. Lenzner, A. Liebhold, A. Mosena, D. Moser, M. Nishino, D. Pearman, J. Pergl, W. Rabitsch, J. Rojas-Sandoval, A. Roques, S. Rorke, S. Rossinelli, H. E. Roy, R. Scalera, S. Schindler, K. Štajerová, B. Tokarska-Guzik, M. van Kleunen, K. Walker, P. Weigelt, T. Yamanaka, and F. Essl. 2017. No saturation in the accumulation of alien species worldwide. Nat. Commun. 8: 14435.

Silva, F. S., B. M. Costa-Neta, M. de Sousa de Almeida, E. C. de Araújo, and J. V. C. Aguiar. 2019. Field performance of a low cost, simple-to-build, non-motorized light-emitting diode

(LED) trap for capturing adult *Anopheles* mosquitoes (Diptera: Culicidae). Acta Trop. 190: 9–12.

Soderstrom, E. L. 1970. Effectiveness of green electroluminescent lamps for attracting stored-product insects. J. Econ. Entomol. 63: 726–731.

Soffan, A., Y. N. Aldryhim, and A. S. Aldawood. 2012. Effects of sex ratio and pairing duration on the biological performance of adult almond moth, *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae). J. Agric. Urban Entomol. 28: 25–33.

Song, J., S.-G. Lee, and H.-S. Lee. 2016a. Effect of LED trap on controlling *Sitophilus zeamais* and *Tribolium castaneum* in granary. J. Appl. Biol. Chem. 59: 129–132.

Song, J., E.-Y. Jeong, and H.-S. Lee. 2016b. Phototactic behavior 9: phototactic behavioral response of *Tribolium castaneum* (Herbst) to light-emitting diodes of seven different wavelengths. J. Appl. Biol. Chem. 59: 99–102.

Surkov, I. V., A. G. J. M. Oude Lansink, O. van Kooten, and W. van der Werf. 2008. A model of optimal import phytosanitary inspection under capacity constraint. Agric. Econ. 38: 363–373.

Thein, P. P., and S.-W. Choi. 2016. Forest insect assemblages attracted to light trap on two high mountains (Mt. Jirisan and Mt. Hallasan) in South Korea. J. For. Res. 27: 1203–1210.

Westphal, M. I., M. Browne, K. MacKinnon, and I. Noble. 2008. The link between international trade and the global distribution of invasive alien species. Biol. Invasions. 10: 391–398.

Yang, J.-Y., M.-G. Kim, and H.-S. Lee. 2012. Phototactic behavior: attractive effects of *Spodoptera litura* (Lepidoptera: Noctuidae), tobacco cutworm, to high-power light-emitting diodes. J. Korean Soc. Appl. Biol. Chem. 55: 809–811.

Yeh, N., and J.-P. Chung. 2009. High-brightness LEDs - Energy efficient lighting sources and their potential in indoor plant cultivation. Renew. Sustain. Energy Rev. 13: 2175–2180.

SECTION 2

POST-BORDER SURVEILLANCE

Chapter 4

Maximizing bark and ambrosia beetle catches in trapping surveys for longhorn and jewel beetles

Paper published as: Marchioro M., Rassati D., Faccoli M., Van Rooyen K., Kostanowicz C., Webster V., Mayo P. & Sweeney J. (2020). Maximizing bark and ambrosia beetle catches in trapping surveys for longhorn and jewel beetles. Journal of Economic Entomology, 113: 2745-2757.

JOURNAL OF ECONOMIC ENTOMOLOGY

Maximizing Bark and Ambrosia Beetle (Coleoptera: Curculionidae) Catches in Trapping Surveys for Longhorn and Jewel Beetles

Matteo Marchioro ✉, Davide Rassati, Massimo Faccoli, Kate Van Rooyen,
Chantelle Kostanowicz, Vincent Webster, Peter Mayo, Jon Sweeney

Journal of Economic Entomology, toaa181, https://doi.org/10.1093/jee/toaa181
Published: 23 September 2020

Abstract

Bark and ambrosia beetles are commonly moved among continents within timber and fresh wood-packaging materials. Routine visual inspections of imported commodities are often complemented with baited traps set up in natural areas surrounding entry points. Given that these activities can be expensive, trapping protocols that attract multiple species simultaneously are needed. Here we investigated whether trapping protocols commonly used to detect longhorn beetles and jewel beetles can be exploited also for detecting bark and ambrosia beetles. In factorial experiments conducted in 2016 both in Italy (semi-natural and reforested forests) and Canada (mixed forest) we tested the effect of trap color (green vs. purple), trap height (understory vs. canopy), and attractive blend (hardwood-blend developed for broadleaf-associated wood-boring beetles vs ethanol in Italy; hardwood-blend vs. softwood-blend developed for conifer-associated wood-boring beetles, in Canada) separately on bark beetles and ambrosia beetles, as well as on individual bark and ambrosia beetle species. Trap color affected catch of ambrosia beetles more so than bark beetles, with purple traps generally more attractive than green traps. Trap height affected both beetle groups, with understory traps generally performing better than canopy traps. Hardwood-blend and ethanol performed almost equally in attracting ambrosia beetles in Italy, whereas hardwood-blend and softwood-blend were more attractive to broadleaf-associated species and conifer-associated species, respectively, in Canada. In general, we showed that trapping variables suitable for generic surveillance of longhorn and jewel beetles may also be exploited for survey of bark and ambrosia beetles, but trapping protocols must be adjusted depending on the forest type.

Introduction

Ever-increasing national and international trade along with ongoing changes in trade networks is causing an impressive number of forest insect introductions (Brockerhoff and Liebhold 2017; Rassati et al. 2018). This trend is particularly evident for wood-boring Coleoptera, especially bark and ambrosia beetles (Scolytinae), longhorn beetles (Cerambycidae), and jewel beetles (Buprestidae) (Eyre and Haack 2017). These insects may be present in wood-packaging materials, timber and plants for planting (Meurisse et al. 2019), and are difficult to detect by routine inspections as infested materials may show little or no sign of infestation (Humble 2010). Traps baited with attractive lures placed in and around entry points are commonly used to complement visual inspections and improve chances of intercepting incoming species soon after their arrival (Poland and Rassati 2019). Given that surveillance activities can be expensive and budgets are often limited, the main challenge is to develop trapping protocols that can attract multiple species simultaneously (e.g., Wong et al. 2012; Rassati et al. 2014; Chase et al. 2018). This can be achieved by selecting the trap color, trap height and attractive lure blend that maximises detection of target taxa, as recently shown for longhorn and jewel beetles (Rassati et al. 2019). Understanding whether trapping protocols developed for detection of longhorn and jewel beetles can also reliably detect bark and ambrosia beetles requires investigation.

Trap color is a key variable for successfully trapping longhorn beetles and jewel beetles, with many species preferring green traps to purple traps, and some preferring the opposite (Francese et al. 2010; Rhainds et al. 2017; Rassati et al. 2019; Imrei et al. 2020). However, few studies have been published on the effects of trap color on catch of bark and ambrosia beetles. Comparisons of Scolytinae catches among trap colors have produced varied results. Some conifer-infesting bark beetles (e.g., *Dendroctonus* and *Ips* spp.) and ambrosia beetles (e.g., *Trypodendron lineatum* (Olivier)) preferred black-colored traps to white-colored traps (Strom et al. 1999, 2001; Campbell and Borden 2006a,b, 2009), presumably because black traps better resemble the silhouette and color of host tree trunk and bark. Some species preferred dark colors with long wavelengths (e.g., black, brown, red) over light colors such as white and yellow (Dubbel et al. 1985; Strom and Goyer 2001; Chen et al. 2010; Hanula et al. 2011; Werle et al. 2014; Kerr et al. 2017), and other species preferred a specific color, e.g., green by *Hypocryphalus mangiferae* (Stebbing) and *Xylosandrus crassiusculus* (Motschulsky) (Abbasi et al. 2007; Gorzlancyk et al. 2013, 2014), and red by *Hypothenemus hampei* Ferrari (Dufour and Frérot 2008). Thus, further investigation is necessary to understand the effects on Scolytinae detection of trap colors already shown to be suitable for detection of longhorn and jewel beetles, *i.e.*, green, and less so, purple,

Trap height is another key variable affecting trap catches of longhorn (e.g., Graham et al. 2012; Schmeelk et al. 2016; Rassati et al. 2019; Flaherty et al. 2019) and jewel beetles (Imrei et al. 2020). Evidence exists also for Scolytinae, although trends are inconsistent. Several studies reported a higher Scolytinae richness in the understory than in the canopy (Ulyshen and Hanula 2007; Dodds 2014; Hardersen et al. 2014; Flaherty et al. 2019), whereas other studies did not find clear differences among forest strata (Leksono et al. 2005; Wermelinger et al. 2007). In addition, vertical distribution patterns vary between bark beetles and ambrosia beetles: bark beetles are generally more abundant in mid and upper forest strata, whereas ambrosia beetles are generally more abundant in the understory (Sheehan et al. 2019; Ulyshen and Sheehan 2019). However, even the latter patterns are not always consistent, as some ambrosia beetles developing in twigs or branches are more abundant in the canopy and some bark beetles developing in woody debris and stumps are mainly trapped in the understory (Klingeman et al. 2017; Procházka et al. 2018; Miller et al. 2020). Vertical distribution patterns can also change depending on the type, structure and composition of sampled forest. Procházka et al. (2018) found vertical patterns of ambrosia beetles to vary between lowland and montane forests, and Menocal et al. (2018) found absence of vertical stratification for several ambrosia beetles in avocado orchards. Thus, further investigations are needed to better clarify the effect of trap height on bark and ambrosia beetles.

Among factors affecting catch of wood-boring beetles in traps, semiochemicals are the most studied. Significant progress in our knowledge of longhorn beetle chemical ecology (Hanks and

Millar 2016) has led to the development of efficient "*multi-lure*" traps, *i.e.*, single traps baited with multiple longhorn beetle pheromones that attract several longhorn beetle species simultaneously (e.g., Wong et al. 2012; Rassati et al. 2019; Fan et al. 2019). These pheromone blends, which appear to have neutral effect on jewel beetles (Flaherty et al. 2019; Rassati et al. 2019), are often complemented with host volatiles, such as ethanol and α-pinene. The latter volatiles not only synergize longhorn beetle pheromones (Hanks et al. 2012; Miller et al., 2015a; Collignon et al. 2016), but also act as attractants for some jewel beetles (Miller 2006) and bark and ambrosia beetles (Miller and Rabaglia 2009; Ranger et al. 2020). Thus, a blend of pheromones and host volatiles can be potentially used to attract longhorn beetles, jewel beetles, as well as bark and ambrosia beetles simultaneously (Flaherty et al. 2019). The main limitations of this approach are the possible negative interactions that can occur among blend components. For instance, α-pinene can reduce or even interrupt attraction of certain ambrosia beetles to ethanol (Schroeder and Lindelöw 1989; Lindelöw et al. 1993; Miller and Rabaglia 2009; Ranger et al. 2011). Longhorn beetle pheromones can have both positive and negative effects on catch of bark and ambrosia beetles, depending on the species. For example, adding the relatively common cerambycine pheromone, racemic 3-hydroxyoctan-2-one, to ethanol-baited traps significantly reduced catches of *Dryoxylon onoharaensum* (Murayama) (Miller et al. 2015a), *Anisandrus maiche* (Kurenzov), *Xyleborinus attenuatus* (Blandford), and *Trypodendron lineatum* (Sweeney et al. 2016), but significantly increased catches of *Monarthrum scutellare* (LeConte) (Noseworthy et al. 2012) and *Hypothenemus rotundicollis* Wood & Bright (Miller et al. 2015a). Further studies are thus essential to elucidate how different blends can affect overall detection of bark and ambrosia beetles in traps.

Our goal was to investigate whether trapping protocols designed for longhorn beetles and jewel beetles associated either with broadleaf or conifer trees can be exploited also for bark and ambrosia beetles. To this aim, in factorial experiments conducted in Italy and Canada we tested how three key variables commonly exploited for trapping surveys of longhorn and jewel beetles, *i.e.*, trap color, trap height, and attractive blend, can affect species richness and abundance of Scolytinae in traps. These effects were tested separately on bark beetles and ambrosia beetles, as well as on individual species. In addition, in order to test whether the effect of trapping variables change depending on the forest type, these factors were tested both in semi-natural forests and reforested forests in Italy. Based on available literature, we predicted that both bark and ambrosia beetles would be preferentially attracted by purple traps than green traps due to a higher resemblance of the former to host tree bark. In addition, we predicted a mixed response to trap height, with higher richness and abundance in the canopy than in the understory for bark beetles and the opposite trend for ambrosia beetles, although we predict that this pattern may differ between semi-natural and reforested forests. Finally, we predicted that the attractive blend developed for longhorn and jewel beetles associated with broadleaf hosts would reliably attract both bark and ambrosia beetles, whereas the blend developed for conifer-associated species would be more efficient for detecting bark beetles than ambrosia beetles.

Materials and methods

Study sites

In 2016, two trapping studies were conducted, one in Italy and one in Canada. The methods, experimental design, and results regarding effects of trap color, trap height, and lure, on detection of Buprestidae and Cerambycidae have already been described (Rassati et al. 2019) but basic methods are described here for the reader’s convenience. In Italy, the study was carried out at seventeen forest sites (*i.e.*, nine semi-natural forests and eight reforested forests) located in the north-east (Table B1). By “semi-natural forests” we refer to the remains of old oaks (*Quercus* spp.) and hop-hornbeam (*Ostrya carpinifolia* Scopoli) forests that covered the majority of North-Eastern Italian lowlands after the last ice age and that are today present only in small patches embedded in an agriculture-dominated landscape. By “reforested forests” we refer to mixed forests recently established (*i.e.*, last 30 years) to reconvert agricultural fields to woodlands. Both forest types were dominated by oaks, ashes (*Fraxinus* spp.), maples (*Acer* spp.), and hop-hornbeam. Nonetheless, semi-natural forests were characterized by taller and more mature trees than reforested forests, as well as by a higher volume of dead wood as a result of different management strategies. In Canada, the study was carried out at Magazine Hill, Halifax, Nova Scotia (Table B1), in a mixed broadleaf-coniferous forest dominated by *Acer rubrum* L., *Populus tremuloides* Michx., and *Picea rubens* Sarg., with lesser amounts of *Betula papyrifera* Marsh and *Fagus grandifolia* (Ehrh.), located near an industrial park and container facility that receives goods from overseas.

Trap type, trap color, trap height and experimental scheme

In both countries, we used 12-funnel Lindgren traps (Synergy Semiochemical Corp., Burnaby, Canada), which were preferred over panel traps as more resistant, simpler and quicker to set up, and thus more suitable to be used in surveillance programs carried out at entry points (Rassati et al. 2014). All traps were coated by the supplier with a 33% solution of Fluon diluted in water to improve trapping efficacy (Allison et al. 2016). In Italy, trap collecting cups were filled with a 50% solution of ethylene glycol and water, whereas in Canada the cups were filled with a saturated solution of table salt in water, plus a drop of dish detergent to reduce surface tension. We used this technique as traps equipped with wet cups reduce insect escape and help to preserve trapped insects (Allison and Redak 2017). Traps were of two colors: green and purple (known as "EAB green" and "EAB purple," respectively; Synergy Semiochemical Corp., Burnaby, Canada). Half of the traps were set up in the understory and half were set up in the upper one-third of the tree canopy. Understory traps were placed with collecting cups 0.3-1 m above the ground, suspended either from rope tied between two trees or tree branches such as that traps were at least 1 m from the tree bole. Canopy traps were set up following the methodology described by Hughes et al. (2014). Canopy tree stratum ranged from 4–7 m in the youngest reforested forests to 12–15 m in the oldest semi-natural forests in Italy, whereas in Canada, it was around 10–15m.

Traps were deployed in a factorial scheme 2 × 2 × 2 (color × lure × height) both in Italy and Canada. The eight treatments were replicated 17 times in Italy and 6 times in Canada in a randomized complete block design (Fig. B1). In Italy, each block was represented by a different site. In Canada, a distance of at least 30 m was kept among blocks and traps. Traps were active from 9 May to 3 August 2016 in Italy, and from 2 May to 22 August 2016 in Canada. Traps were emptied every three weeks in Italy and every two weeks in Canada. Collected bark and ambrosia beetles were stored in alcohol or at -10°C until processed, identified to species, and classified as either exotic or native to either Italy or Canada according to the available literature (Bright 1976; Balachowsky 1995; Pfeffer 1995; Bousquet et al. 2013). In Italy, only ambrosia beetles were identified, whereas identification was carried out for both bark beetles and ambrosia beetles in Canada. Voucher specimens of Scolytinae species have been deposited in the insect collections at University of Padua, Padua, Italy and the Atlantic Forestry Center, Fredericton, NB, Canada.

Attractive lures

In Italy, traps were baited either with UHR (ultra-high release) ethanol or a blend composed by UHR ethanol and five longhorn beetle pheromones, namely racemic 3-hydroxyhexan-2-one (K6), racemic 3-hydroxyoctan-2-one (K8), *syn*-2,3-hexanediols (D6), (*E/Z*)-fuscumol, and (*E/Z*)-fuscumol acetate (see Table B2 for information on release devices and rates). The latter blend, hereafter referred to as "hardwood-blend", was designed mainly for detecting species infesting hardwoods, and attracts a large number of broadleaf-associated longhorn beetles with apparently neutral effect on jewel beetles (Flaherty et al. 2019; Rassati et al. 2019) and Scolytinae (Flaherty et al. 2019). Ethanol enhances attraction of many longhorn beetle species to the above listed pheromones (Hanks et al. 2012; Miller et al., 2015a; Collignon et al. 2016). Dispenser lures were renewed after 2 months according to their expected field life.

In Canada, traps were baited either with the same blend used in Italy (*i.e.*, "hardwood-blend") or a blend composed of UHR ethanol, UHR α-pinene, ipsenol and 2-undecyloxy-1-ethanol (also known as Monochamol) (see Table B2 for information on release devices and rates). The latter blend, hereafter referred to as "softwood-blend", is designed mainly for species infesting softwoods (Flaherty et al. 2019). α-Pinene is a common host volatile of conifers and attracts several species of wood-boring beetles (Miller and Rabaglia 2009). Ipsenol is a bark beetle pheromone that often increases catches of wood-boring beetles in traps baited with ethanol and α-pinene (Miller et al. 2015b) and *Monochamol* is an aggregation pheromone produced by mature males of *Monochamus* spp. that is attractive to several species in this genus (Boone et al. 2019). The K6, K8, D6, (*E,Z*)-fuscumol, and (*E,Z*)-fuscumol acetate lures were replaced after 2 months; the UHR ethanol, UHR α-pinene, monochamol and ipsenol lures were not replaced as these lures usually last 16 weeks in Eastern Canadian summers.

Statistical analysis

The effects of lure (*i.e.*, hardwood-blend vs ethanol in Italy; hardwood-blend vs. softwood-blend in Canada), trap color (purple vs. green), trap height (canopy vs. understory), and their interactions on ambrosia beetles (both in Canada and Italy) and bark beetles (only in Canada) were tested using either linear mixed-effects models (LMM) with normal distribution or generalized linear-mixed effects models (GLMM) with negative binomial distribution (Table 1 and 2). The total number of species (*i.e.*, species richness) or individuals (*i.e.*, abundance) collected per trap per each lure-height-color combination over the entire trapping period was the response variable. For LMM with normal distribution, abundance was either log- or square root transformed to satisfy the assumption of normality when necessary. Models included site (for Italy) or block (for Canada) as a random factor to account for spatial dependence in the sampling design. In Italy, the effect of the selected variables on ambrosia beetle catches was tested separately for semi-natural forests and reforested forests. Linear mixed-effects models (LMM) were fitted using the “lmer” function in the package “lme4” (Bates et al. 2017) implemented in R (R Core Team 2019), with significance determined by standard F tests. Generalized linear-mixed effects models (GLMM) were fitted using the “glmmTMB” function in the package “glmmTMB” (Magnusson et al. 2017) implemented in R, with significance determined by the Wald χ^2 test. Model overdispersion and residual distribution were checked through the “DHARMa” package (Hartig 2017), which uses a simulation-based approach to create readily interpretable scaled residuals from the fitted LMM and GLMM. iNEXT package (Hsieh et al. 2016) for R was used to generate curves showing the mean number of species detected per number of trap samples for each of the eight different trap color-height-lure combinations in both Canada and Italy.

Table 1 Total number of species and individuals of ambrosia beetles caught in Italy in semi-natural forests and reforested forests, and results of the statistical models (P value) used to test the effect of attractive lure (ethanol vs hardwood-blend), trap color (green vs purple), trap height (canopy vs understory), and their interactions on ambrosia beetle catches.

	Total	Color	Height	Lure	Df	Significant interactions	Model	Distribution
Italy								
Semi-natural forests								
Species richness	8	0.208	<0.001	0.344	1, 60	-	LMM	Normal
Abundance	78,358	0.013	<0.001	0.330	1, 60	-	LMM	Log-transf.
Ambrosiodmus rubricollis (Eichhoff) *	11	-	-	-	-	-	n. t.	-
Ambrosiophilus atratus (Eichhoff) *	10	-	-	-	-	-	n. t.	-
Anisandrus dispar (Fabricius)	1,163	0.517	<0.001	0.473	1, 59	Color x Height	LMM	Log-transf.
Xyleborinus saxesenii (Ratzeburg)	57,311	0.059	<0.001	0.576	1, 60	-	LMM	Log-transf.
Xyleborus dryographus (Ratzeburg)	21	-	-	-	-	-	n. t.	-
Xyleborus monographus (Fabricius)	313	0.833	0.001	0.741	1, 58	-	GLMM	Neg. bin.
Xylosandrus crassiusculus (Motschulsky)*	154	0.376	0.077	0.136	1, 32	-	LMM	Log-transf.
Xylosandrus germanus (Blandford) *	19,375	<0.001	<0.001	0.004	1, 59	-	LMM	Log-transf.
Reforested forests								
Species richness	7	0.158	0.569	0.840	1, 52	Color x Height	LMM	Normal
Abundance	26,586	0.827	0.234	0.018	1, 51	Color x Height, Height x Lure	LMM	Log-transf.
Ambrosiodmus rubricollis (Eichhoff) *	-	-	-	-	-	-	n. t.	-
Ambrosiophilus atratus (Eichhoff) *	12	-	-	-	-	-	n. t.	-
Anisandrus dispar (Fabricius)	1,519	0.218	0.142	0.782	1, 53	-	LMM	Sqrt-transf.
Xyleborinus saxesenii (Ratzeburg)	22,869	0.250	0.112	0.004	1, 51	Color x Height, Height x Lure	LMM	Log-transf.
Xyleborus dryographus (Ratzeburg)	15	-	-	-	-	-	n. t.	-
Xyleborus monographus (Fabricius)	4	-	-	-	-	-	n. t.	-
Xylosandrus crassiusculus (Motschulsky)*	689	0.177	0.097	0.500	1, 25	-	LMM	Log-transf.
Xylosandrus germanus (Blandford) *	1,478	0.870	0.011	0.858	1, 45	Color x Height	LMM	Log-transf.

Models = LMM: linear mixed-effects model; GLMM: generalized linear mixed-effects model. * = exotic species. n.t. = not tested. Distribution = Log-transf: normal on log-transformed data; Neg.bin.: negative binomial; Sqrt-transf.: normal on square root-transformed data. Df= degrees of freedom in form of numerator, denominator. Degrees of freedoms are reported only once for each species as they were the same for all variables in each model

Table 2 Total number of species and individuals of ambrosia beetles and bark beetle caught in Canada, and results of the statistical models (P value) used to test the effect of attractive lure (softwood-blend vs hardwood-blend), trap color (green vs purple), trap height (canopy vs understory), and their interactions on bark and ambrosia beetle catches.

	Total	Color	Height	Lure	Df	Significant interactions	Model	Distribution
Canada								
Ambrosia beetles								
Species richness	10	0.752	<0.001	0.529	1, 44	-	LMM	Normal
Abundance	13,083	0.003	<0.001	<0.001	1, 41	Height x Lure	GLMM	Neg. bin.
Anisandrus dispar (Fabricius) *	18	-	-	-	-	-	-	-
Anisandrus sayi Hopkins	5,240	0.820	0.146	<0.001	1, 38	Color x Lure	LMM	Log-transf.
Gnathotrichus materiarius (Fitch)	10	-	-	-	-	-	n. t.	-
Monarthrum mali (Fitch)	2	-	-	-	-	-	n. t.	-
Trypodendron betulae Swaine	1	-	-	-	-	-	n. t.	-
Trypodendron lineatum (Olivier)	230	0.168	<0.001	<0.001	1, 42	-	GLMM	Neg. bin.
Xyleborinus attenuatus (Blandford) *	1,606	0.096	0.458	0.317	1, 44	-	LMM	Normal
Xyleborinus saxesenii (Ratzeburg) *	11	-	-	-	-	-	n. t.	-
Xylosandrus germanus (Blandford) *	5,833	0.334	<0.001	0.504	1, 38	Color x Height	LMM	Log-transf.
Xyloterinus politus (Say)	132	0.002	0.022	0.030	1, 43	Color x Lure	LMM	Normal
Bark beetles								
Species richness	25	0.185	0.396	0.001	1, 43	Height x Lure	LMM	Normal
Abundance	3,409	0.340	0.015	0.580	1, 38	Height x Lure	LMM	Log-transf.
Conophthorus coniperda (Schwarz)	4	-	-	-	-	-	n. t.	-
Cryphalus ruficollis Hopkins	672	0.167	<0.001	<0.001	1, 43	Color x Lure	LMM	Log-transf.
Crypturgus borealis Swaine	86	0.466	0.164	0.626	1, 42	-	GLMM	Neg. bin.
Crypturgus pusillus (Gyllenhal) *	338	0.241	0.138	<0.001	1, 42	-	GLMM	Neg. bin.
Dendroctonus rufipennis (Kirby)	2	-	-	-	-	-	n. t.	-
Dryocoetes affaber (Mannerheim)	34	-	-	-	-	-	n. t.	-
Dryocoetes autographus (Ratzeburg)	53	0.848	<0.001	0.004	1, 42	-	GLMM	Neg. bin.
Hylastes opacus (Erichson) *	13	-	-	-	-	-	n. t.	-
Hylastinus obscurus (Marsham) *	7	-	-	-	-	-	n. t.	-
Hylesinus aculeatus Say	3	-	-	-	-	-	n. t.	-
Hylurgops pinifex (Fitch)	1	-	-	-	-	-	n. t.	-
Ips grandicollis (Eichhoff)	368	0.982	0.825	<0.001	1, 42	-	GLMM	Neg. bin.
Ips perroti Swaine	1	-	-	-	-	-	n. t.	-
Ips pini (Say)	20	-	-	-	-	-	n. t.	-
Lymantor decipiens (LeConte)	16	-	-	-	-	-	n. t.	-
Orthotomicus caelatus (Eichhoff)	178	0.390	0.001	<0.001	1, 42	-	GLMM	Neg. bin.
Orthotomicus latidens (LeConte)	4	-	-	-	-	-	n. t.	-
Pityogenes hopkinsi Swaine	10	-	-	-	-	-	n. t.	-
Pityokteines sparsus (LeConte)	2	-	-	-	-	-	n. t.	-
Pityophthorus ramiperda Swaine	7	-	-	-	-	-	n. t.	-
Pityophthorus sp.	49	-	-	-	-	-	n. t.	-
Polygraphus rufipennis (Kirby)	121	0.007	0.761	<0.001	1, 42		GLMM	Neg. bin.
Pseudopityophthorus asperulus (LeConte)	626	0.048	<0.001	0.029	1, 44	-	LMM	Log-transf.
Pseudopityophthorus minutissimus (Zimmermann)	791	0.972	0.006	<0.001	1, 42	-	GLMM	Neg. bin.
Scolytus piceae (Swaine)	3	-	-	-	-	-	n. t.	-

Model= LMM: linear mixed-effects model; GLMM: generalized linear mixed-effects model. * = exotic species. n.t. = not tested. Distribution = Log-transf: normal on log-transformed data; Neg.bin.: negative binomial. Df= degrees of freedom in form of numerator, denominator. Degrees of freedoms are reported only once for each species as they were the same for all variables in each model.

Results

General results

In Italy, a total of 104,944 individuals from 8 ambrosia beetle species were caught. Among them, four were exotic species known to be established in Italy (Table 1). Both ambrosia beetle species richness and abundance were higher in semi-natural forests than in reforested forests (8 vs. 7 species and 78,358 vs. 26,586 individuals, respectively) (Table 1). The native *Xyleborinus saxesenii* (Ratzeburg) was the most abundant species in both semi-natural and reforested forests (57,311 and 22,869 individuals, respectively), whereas differences among forest types emerged for abundance of the other species collected. In semi-natural forests, the exotic *Xylosandrus germanus* (Blandford) was the second most abundant species (19,375 individuals), whereas in reforested forests, its abundance was a tenth of that, and was lower than that of the native *Anisandrus dispar* (Fabricius) (Table 1). In addition, the native *Xyleborus monographus* (Fabricius) was considerably more abundant in semi-natural forests than in reforested forests (313 vs 4 individuals), whereas the opposite trend was found for the exotic *Xylosandrus crassiusculus* (154 vs. 689 individuals).

In Canada, a total of 16,492 individuals from 35 scolytine species were caught (Table 2). Among them, seven were exotic species (Table 2). Bark beetles were more species-rich than ambrosia beetles (25 vs 10), whereas the trend was opposite for abundance (13,083 for ambrosia beetles and 3,409 for bark beetles) (Table 2). Among ambrosia beetles, the exotic *X. germanus* was the most abundant species (5,833 individuals), followed the native *Anisandrus sayi* Hopkins (5,240 individuals) and exotic *Xyleborinus attenuatus* (Blandford) (1,606 individuals). Among bark beetles, *Pseudopityophthorus minutissimus* (Zimmermann) was the most abundant species (791 individuals), followed by *Cryphalus ruficollis* Hopkins (672 individuals) and *Pseudopityophthorus asperulus* (LeConte) (626 individuals) (Table 2). These were the first Canadian records for *P. asperulus* and the first Nova Scotia records for the exotic bark beetle, *Hylastes opacus* Erichson (Webster et al. 2020). Two ambrosia beetle species and 10 bark beetle species were represented by less than 10 individuals

Effect of trap color, trap height and attractive lure on bark and ambrosia beetles

In Italy, the effects of trap color, height and lure on ambrosia beetle species richness and abundance differed in semi-natural vs. reforested forests. In semi-natural forests, species richness was significantly affected by trap height (F=36.40) (Table 1), with more species detected in understory traps than canopy traps (Fig. 1A and Table B3). In reforested forests, species richness was significantly affected by the interaction between trap color and trap height (F=4.96; $P<0.030$): purple traps outperformed green traps in the understory but not in the canopy (Fig. 1C and Table B3). The species accumulation curves show that in both forest types, purple understory traps baited with ethanol detected the most ambrosia beetle species per trap, whereas green canopy traps baited with the hardwood-blend detected the fewest (Fig. 2A and 2B).

In semi-natural forests, ambrosia beetle abundance was significantly affected by trap color (F=6.43) and trap height (F=85.05) (Table 1); understory traps outperformed canopy traps, and purple traps outperformed green traps (Fig. 1B and Table B3). In reforested forests, ambrosia beetle abundance was significantly affected by attractive blend (F=5.91), and the interactions between trap color and trap height (F=8.08; $P=0.006$) and trap height and attractive blend (F=5.77; $P=0.019$) (Table 1). Ethanol-baited traps captured more ambrosia beetles than traps baited with the hardwood-blend (Fig. 1D and Table B3) and this was more evident in the canopy of reforested forests than in the understory. Purple traps performed better than green traps in the understory of reforested forests but not in the canopy (Fig. 1D).

In Canada, ambrosia beetle species richness was significantly affected only by trap height (F=16.97) (Table 2), with understory traps outperforming canopy traps (Fig. 1E and Table B4). Species accumulation curves showed that green understory traps baited with the softwood-blend detected the most species of ambrosia beetles per trap, whereas the same traps placed in the canopy detected the fewest (Fig. 2C). Ambrosia beetle abundance, instead, was significantly affected by all tested variables (Table 2), plus the interaction between trap height and attractive lure ($\chi^2= 28.09$; $P<0.001$). In general, purple traps, understory traps, and hardwood-baited traps performed better than green traps, canopy traps, and softwood-baited traps, respectively (Fig. 1F and Table B4). In addition, the higher attractiveness of hardwood-baited traps than softwood-baited traps was more evident in the canopy than in the understory (Fig. 1F).

Bark beetle species richness was affected by attractive blend (F=11.71), and marginally by the interaction between trap height and attractive blend (F=3.54; *P*=0.066) (Table 2). Softwood-baited traps outperformed hardwood-baited traps and this trend was more evident in the understory than in the canopy (Fig. 1G and Table B4). The species accumulation curves showed that understory traps baited with the softwood-blend detected the most species per trap, with purple and green traps performing almost equally (Fig. 2D). Bark beetle abundance was significantly affected by trap height (F=6.36) and the interaction between trap height and attractive blend (F=12.63; *P*=0.001) (Table 2). Canopy traps caught more individuals than understory traps (Fig. 1H) and softwood-baited traps performed better than hardwood-baited traps in the understory but not in the canopy (Fig. 1H).

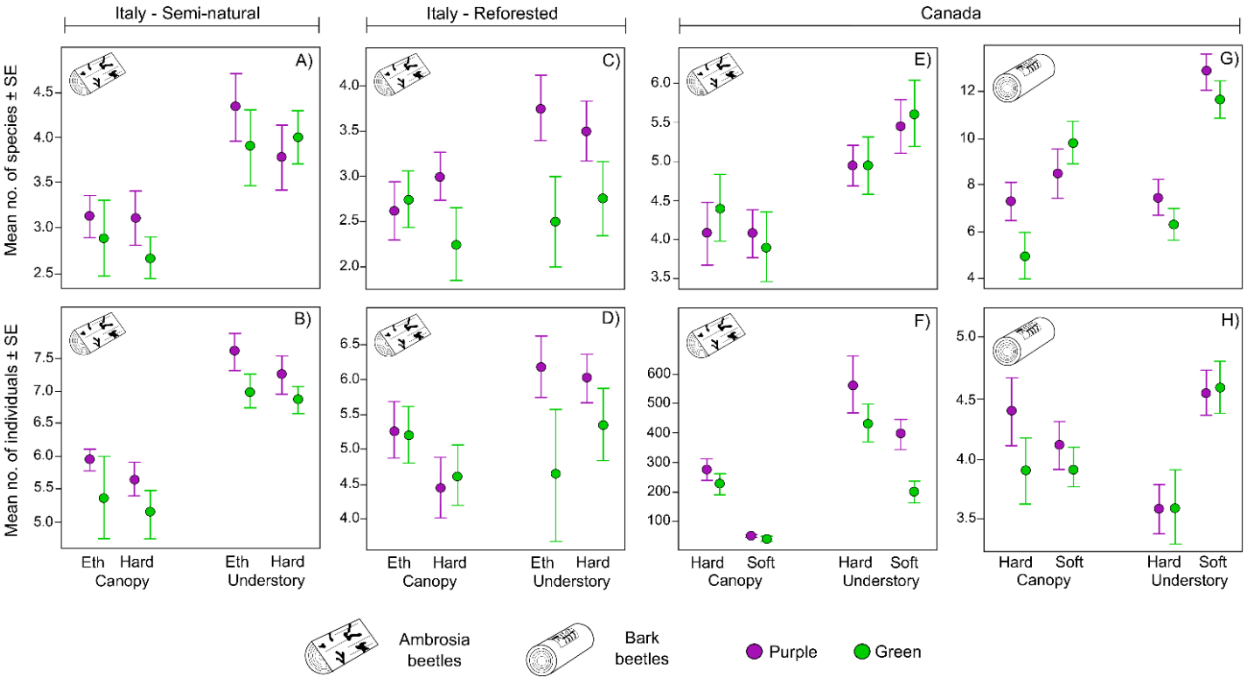

Fig. 1 Effect of trap color, trap height, attractive lures, and their interactions on species richness and abundance of bark and ambrosia beetles caught in Italy (semi-natural forests vs reforested forests) and Canada. Abundance of both bark beetles and ambrosia beetles (except for Canada) is log-transformed according to data transformation used in statistical analysis. Eth = ethanol; Hard = hardwood-blend; Soft = softwood-blend.

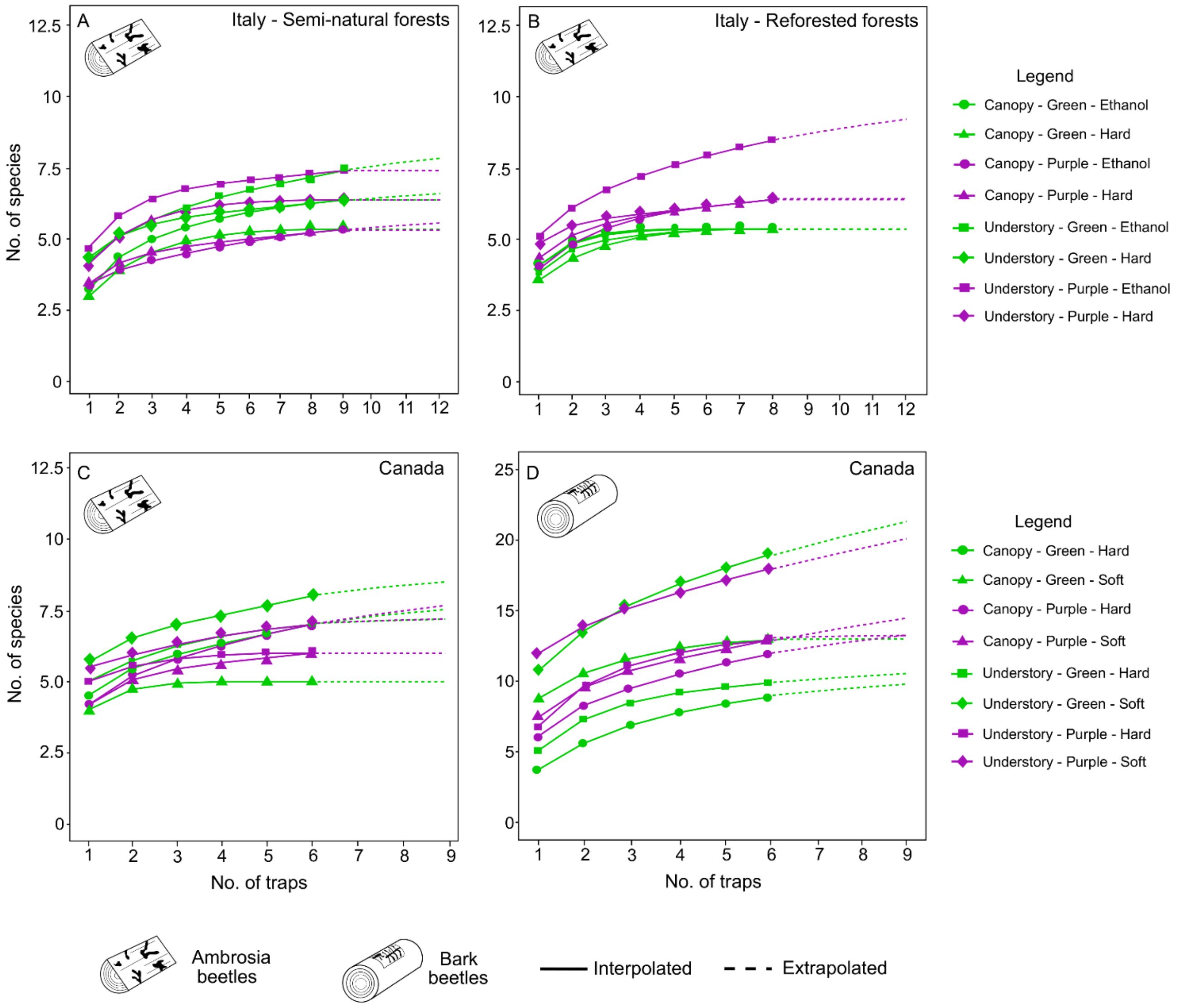

Fig. 2 Rarefaction curves showing mean number of bark and ambrosia beetle species detected per number of trap samples in green vs purple funnel traps placed in the upper canopy vs understory and baited with two different lures. In Italy, the lures were ethanol vs hardwood-blend; in Canada, hardwood-blend vs. softwood-blend. See text for details on multi-lure components.

Effect of trap color, trap height and attractive lure on individual species

In Italy, tested variables significantly affected abundance of five ambrosia beetle species in semi-natural forests and three ambrosia beetle species in reforested forests (Table 1), but with mixed responses. In semi-natural forests, understory traps performed significantly better than canopy traps in detecting *A. dispar* (F=19.11) (Fig. 3A), *X. saxesenii* (F=43.56) (Fig. 3B), *X. germanus* (F=131.52) (Fig. 3D), *X. monographus* (χ^2= 10.43) (Fig. 3E), and marginally *X. crassiusculus* (F=3.31) (Fig. 3C). In reforested forests, the same pattern was found only for *X. germanus* (F=6.95) (Fig. 3I) and marginally *X. crassiusculus* (F=2.97) (Fig. 3H). Trap color significantly affected mean catch of *X. germanus* (*F*=20.58) (Fig. 3D) and marginally *X. saxesenii* (F=3.68) (Fig. 3B) in semi-natural forests, with purple traps outperforming green traps, but had no effects on catch of any species in reforested forests (Table 1 and Table B3). Three species were significantly affected by the interaction between trap color and trap height. In reforested forests, purple traps caught significantly more *X. saxesenii* and *X. germanus* individuals than green traps in the understory but not in the canopy (F=12.27; $P<0.001$ and F=3.92; $P=0.053$, respectively) (Fig. 3G and 3I and Table B3). In semi-natural forests, however, green traps caught more *A. dispar* individuals than purple traps in the understory but not in the canopy (F=2.99; $P=0.088$) (Fig. 3A). Finally, ethanol-baited traps performed better than hardwood-baited traps at detecting *X. germanus* in semi-natural forests (*F*=8.83) (Fig. 3D) and *X. saxesenii* in reforested forests (F=8.92) (Fig. 3G and Table B3).

In Canada, tested variables significantly affected five ambrosia beetle species and seven bark beetle species (Table 2). Among ambrosia beetles, *A. sayi* (F=252.26), *T. lineatum* (χ^2=56.07), and *Xyloterinus politus* (Say) (F=5.02) were significantly affected by the attractive blend (Table 2); hardwood-baited traps caught more *A. sayi* (Fig. 4A) and *X. politus* (Fig. 4E) individuals than did softwood-baited traps (Table B4), whereas the opposite trend was found for *T. lineatum* (Fig. 4B, Table B4). Trap height affected abundance of *T. lineatum* (χ^2=41.16), *X. germanus* (F=132.50) and *X. politus* (F=5.57): in all cases, understory traps outperformed canopy traps (Fig. 4B, 4D and 4E, Table B4). Purple traps caught more individuals than green traps only for *X. politus* (*F*=10.55) (Fig. 4E) and marginally *X. attenuatus* (F=2.87) (Fig. 4C). The latter pattern was found in the understory but not in the canopy for *X. germanus* (F=5.32; $P=0.026$) (Fig. 4D), and in softwood-baited traps but not in hardwood-baited traps for *A. sayi* (F=4.83; $P=0.034$) (Fig. 4A).

Among bark beetles, seven species were significantly affected by attractive blend, although with mixed patterns (Table 2). Softwood-baited traps performed better than hardwood-baited traps for *C. ruficollis* (*F*=20.05) (Fig. 4F and Table B4), *Crypturgus pusillus* (Gyllenhal) (χ^2=48.70) (Fig. 4G), *Dryocoetes autographus* (Ratzeburg) (χ^2=8.13) (Fig. 4H), *Ips grandicollis* (Eichhoff) (χ^2=87.74) (Fig. 4I), *Orthotomicus caelatus* (Eichhoff) (χ^2=24.03) (Fig. 4J) and *Polygraphus* *rufipennis* (Kirby) (χ^2=12.75) (Fig. 4K), whereas hardwood-baited traps performed better than softwood-baited traps for *P. asperulus* (F=5.03) (Fig. 4L) and *P. minutissimus* (χ^2=38.32) (Fig. 4M). Trap height affected abundance of five bark beetle species (Table 2), but again with mixed responses. Understory traps performed better than canopy traps for *C. ruficollis* (F=27.49) (Fig. 4F), *D. autographus* (χ^2=20.67) (Fig. 4H), and *O. caelatus* (χ^2=10.50) (Fig. 4J), whereas the opposite trend was found for *P. asperulus* (F=14.64) (Fig. 4L) and *P. minutissimus* (χ^2=7.29) (Fig. 4M). Lastly, purple traps performed better than green traps in detecting *P. rufipennis* (χ^2=7.04) (Fig. 4K) and *P. asperulus* (F=4.13) (Fig. 4L).

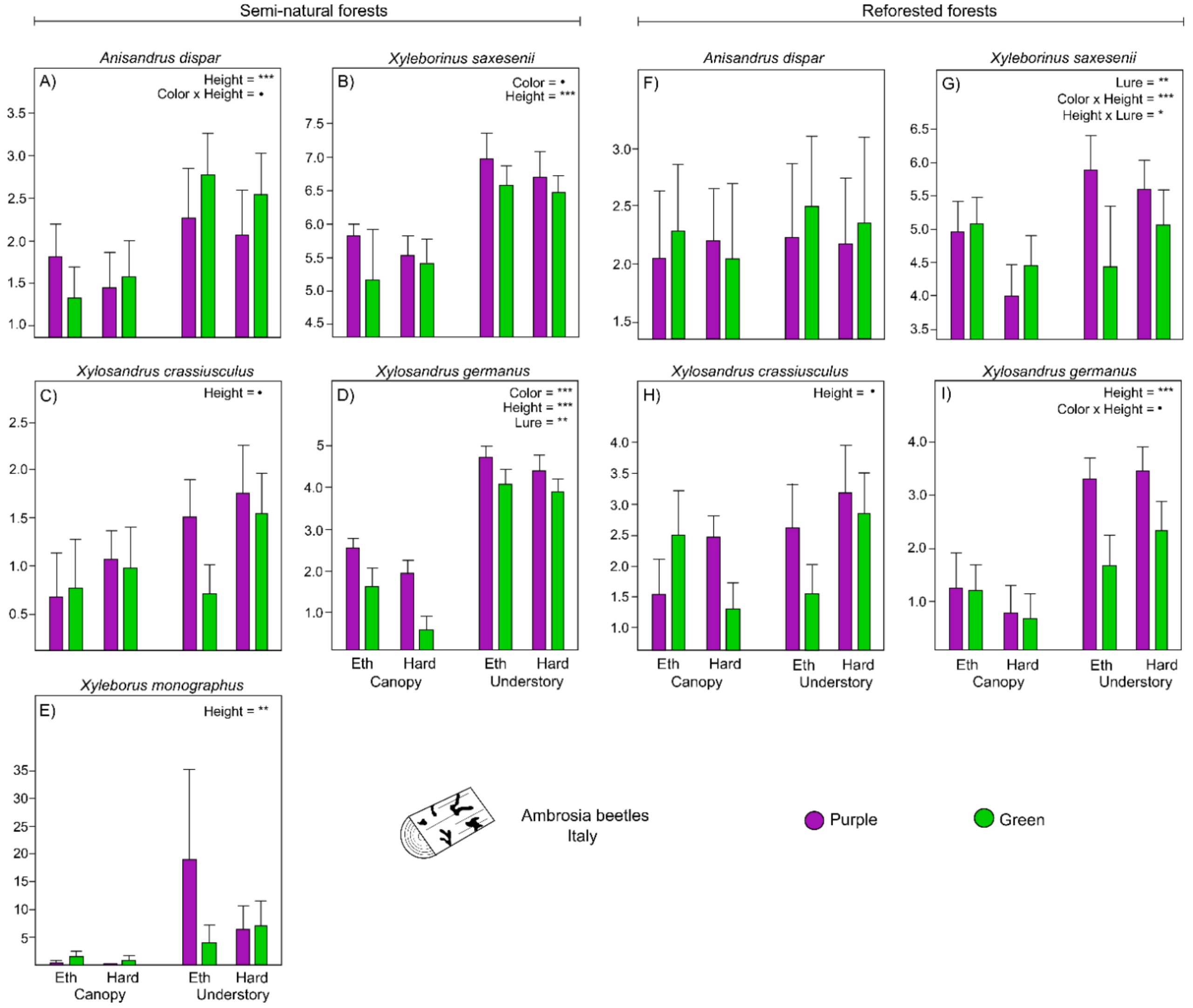

Fig. 3 Effect of trap color, trap height, attractive lures, and their interactions on abundance of ambrosia beetle species caught in Italy in semi-natural forests and reforested forests. Significant explanatory variables and/or interactions are displayed within each box with black asterisk/s or black circle depending on the p-value: *** = P<0.001; ** = P<0.01; * P<0.05; ● = P<0.1. Except for *X. monographus*, abundance is transformed according to data transformation used in statistical analysis (A, B, C, D, G, H, I = log-transformed; F = square root transformed). Eth = ethanol; Hard = hardwood-blend.

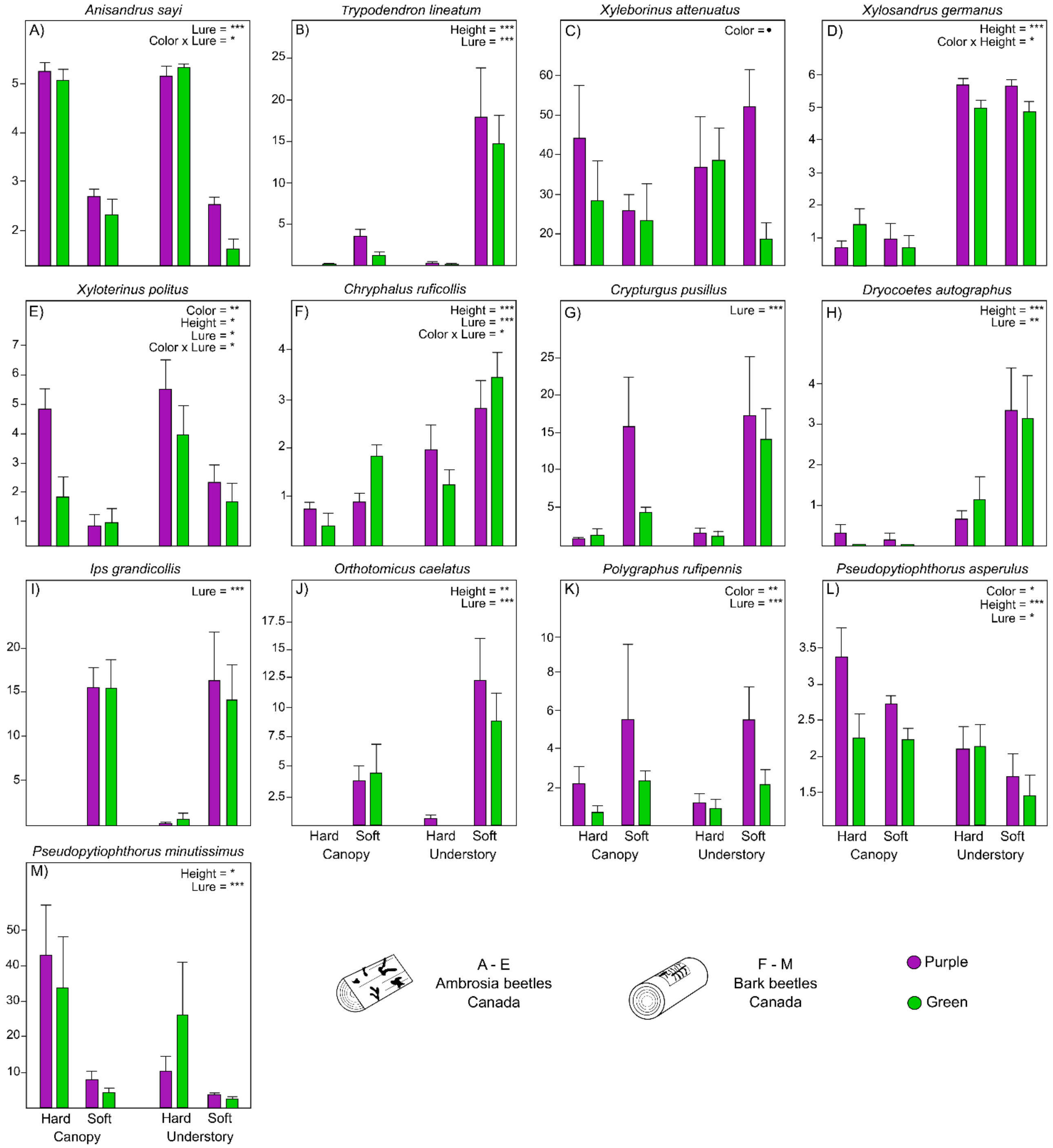

Fig. 4 Effect of trap color, trap height, attractive lures, and their interactions on abundance of ambrosia beetles and bark beetles caught in Canada. Significant explanatory variables and/or interactions are displayed within each box with black asterisk/s or black circle depending on the p-value: *** = P<0.001; ** = P<0.01; * P<0.05; ● = P<0.1. Abundance of *A. sayi*, *X. germanus*, *C. ruficollis*, and *P. asperulus* is log-transformed according to data transformation used in statistical analysis. Hard = hardwood-blend; Soft = softwood-blend.

Discussion

Traps baited with attractive lures are set up in or around international points of entry to improve the chance of intercepting incoming wood-boring beetles and complement visual inspections of imported commodities (Poland and Rassati 2019). Trapping protocols that simultaneously detect longhorn beetles and jewel beetles have been recently developed for both broadleaf- and conifer-associated species (Flaherty et al. 2019; Rassati et al. 2019), but their efficacy of detecting bark and ambrosia beetles is less known, particularly the effects of trap color and longhorn beetle pheromones. Here we show the effects of trap color, trap height, and attractive lure blend on catches of bark and ambrosia beetles, and propose two trapping protocols for generic surveillance of longhorn beetles, jewel beetles and bark and ambrosia beetles at risk of arriving and establishing in broadleaf or mixed-forests close to entry points.

Trap color generally affected abundance of ambrosia beetles in traps but not species richness, and had almost no effect on catch of bark beetles. Previous studies reported that bark and ambrosia beetle abundance was greater in dark-colored traps (resembling bark of host trees) than in light colored traps (Dubbel et al. 1985; Chen et al. 2010; Hanula et al. 2011; Allison and Redak 2017) and that yellow and white traps were avoided (Strom and Goyer 2001; Werle et al. 2014; Kerr et al. 2017). Here we confirmed the lack of response to color by most bark beetles, and showed that ambrosia beetles were generally detected more often in purple traps than in green traps. The latter result was largely due to an apparent preference for purple traps by the dominant ambrosia beetle species, *i.e.*, *X. saxesenii* and *X. germanus* in Italy, and *X. germanus, A. sayi,* and *X. attenuatus* in Canada. However, two species (the ambrosia beetle *A. dispar* and the bark beetle *C. ruficollis*) preferred green traps to purple traps, similar to trends reported for some other bark and ambrosia beetles (Abbasi et al. 2007; Gorzlancyk et al. 2013, 2014). Why certain bark and ambrosia beetle species displayed an apparent color preference while other species did not might be explained by differences in their daily flight activity pattern. For instance, diurnal species of hawk moths are known to perceive colors differently than crepuscular or nocturnal species (Balkenius et al. 2006).

Nonetheless, understanding whether this is valid also for bark and ambrosia beetles require further investigation, as daily flight activity pattern is known for only a limited number of species (Mendel 1991; Seo et al. 2017; Menocal et al. 2018). Our results also showed that the effect of trap color differed depending on the forest type. This was evident in Italy for *X. germanus* and *X. saxesenii*: these two species preferred purple traps to green traps both in the understory and the canopy of semi-natural forests, whereas this preference was evident only in the understory of reforested forests. Our results support those of Niemeyer (1985) who found that beetle response to trap color was affected by the environment in which the traps were located. Semi-natural forests had taller and more mature trees than reforested forests, and this may have affected light levels and relative reflectance of traps in the understory and canopy, which may have, in turn, induced a different response of beetles to trap colors. Unfortunately, we did not measure light levels or reflectance from traps in our plots so this is purely speculative.

Trap height significantly affected species richness and abundance of ambrosia beetles, but only abundance of bark beetles. For ambrosia beetles, understory traps outperformed canopy traps in most cases, whereas for bark beetles we observed a less clear pattern. A higher species richness and abundance in understory traps was expected for ambrosia beetles, which are known to be more active in the lower forest strata (Flaherty et al. 2019; Sheehan et al. 2019; Ulyshen and Sheehan 2019), whereas the lack of a clear response was less expected for bark beetles, which have been reported to be more active in the upper forest strata (Sheehan et al. 2019; Ulyshen and Sheehan 2019). Nonetheless, there is increasing evidence that vertical stratification is mostly species-specific (Procházka et al. 2018; Miller et al. 2020). Preference for a given forest stratum is mainly linked to the main ecological habit of a species, and this should be taken into account when planning a species-specific surveillance program. The bark beetles *P. asperulus* and *P. minutissimus*, for example, typically colonize small branches and twigs (Ambourn et al. 2006), and this may explain why we caught more in canopy traps than in understory traps. The ambrosia beetles, *X. germanus* and *T. lineatum,* usually attack the lower part of stressed or dying trees (Foit et al. 2010; Ranger et al. 2020), and this may explain why they were caught more in understory traps than in canopy traps. Trap catch of other ambrosia beetles, such as *A. sayi* and *X. attenuatus*, and bark beetles, such as *I. grandicollis*, did not differ between canopy and understory traps, confirming that for some species trap height is not an important variable (Klingeman et al. 2017; Miller et al. 2020). Our results also confirmed that vertical distribution patterns can differ depending on forest type and structure (Ulyshen 2011; Procházka et al. 2018). This was particularly evident for *A. dispar*, for which trap catches were affected by trap height in semi-natural forests but not in reforested forests. This pattern might be explained by the greater height of both trees and canopy traps in semi-natural forests than in reforested forests; tree height is in fact considered one of the key variables affecting arthropod vertical stratification in deciduous temperate forests (Ulyshen 2011).

In Italy, ambrosia beetle catches were similar in traps baited with either ethanol or the hardwood-blend, suggesting the longhorn beetle pheromones in our hardwood-blend had little effect on species of ambrosia beetles present in our sites. Nonetheless, total ambrosia beetle abundance and abundance of *X. saxesenii* in reforested forests, as well as abundance of *X. germanus* in semi-natural forests, were higher in ethanol-baited traps than hardwood-blend baited traps. Negative effects of racemic 3-hydroxyoctan-2-one (K8) on certain ambrosia beetle species, including *X. attenuatus*, congeneric of *X. saxesenii*, have been observed in previous studies (Noseworthy et al. 2012; Miller et al. 2015a; Sweeney et al. 2016), and its presence in the hardwood-blend may account for our results. Why these effects were evident in one forest type and not the other, depending on the species, is unknown. However, differences among sites in the effects of lure on trap catches have been previously observed for longhorn beetles (Rassati et al. 2020) and other saproxylic beetles (Bouget et al. 2009). We speculate that two mechanisms may be involved. First, forest structure may affect the microclimate near traps, in particular temperature, which, in turn, affects release rates of lures and, potentially, the attraction of certain ambrosia beetle species. Release rates of ethanol and α-pinene have had both positive (Ranger et al. 2011) and negative effects (Bakke 1983, Montgomery and Wardo 1983; Salom and McLean 1990) on trap catches of ambrosia beetles, depending on the species. Second, the amount of dead or dying trees was higher in semi-natural forests than in reforested forests, and dead or dying trees can act as source of ethanol and host volatiles that could potentially compete with baited traps.

In Canada, traps baited with the hardwood-blend generally detected more ambrosia beetle individuals than traps baited with the softwood-blend, whereas the softwood-blend was generally more attractive to bark beetles than the hardwood-blend. This was not surprising and was explained mainly by host associations. Most of the ambrosia beetles we captured are polyphagous on broadleaf-trees, whereas 18 of 25 bark beetles we captured are associated with conifers. Differences from this pattern were exhibited by some species and were also explained by host association. For example, catches of the ambrosia beetles, *A. sayi* and *X. politus,* associated mainly with broadleaf trees (https://www.barkbeetles.info/index.php), were significantly lower in traps baited with the softwood-blend than the hardwood-blend. This was likely due to the high release rate lure of α-pinene in the softwood-blend that is presumably perceived as a non-host signal by these species (Miller and Rabaglia 2009; Ranger et al. 2011; Flaherty et al. 2019). By the same token, catch of the conifer-associated ambrosia beetle, *T. lineatum,* was greater in traps baited with the softwood-blend than the hardwood-blend. Similarly, conifer-associated bark beetle species, such as *C. pusillus*, *C. ruficollis*, *I. grandicollis*, *O. caelatus*, and *P. rufipennis* preferred the more conifer-like softwood-blend, whereas broadleaf-associated species (e.g., *P. asperulus* and *P. minutissimus*) preferred the hardwood-blend. However, catches of some species known to be associated with broadleaf trees (e.g., *X. attenuatus* and *X. germanus*), were not affected by lure blend, as previously observed by Flaherty et al. (2019).

In conclusion, we showed that the same trapping variables exploited for detection of longhorn beetles and jewel beetles (Flaherty et al. 2019; Rassati et al. 2019) can also be used to detect bark and ambrosia beetles, but the trapping protocol should be adjusted depending on the forest targeted nearby entry points. In a broadleaf forest, managers should consider baiting multi-funnel traps with the hardwood-blend lures with green traps in the canopy and purple traps in the understory. The green canopy traps can reliably detect longhorn beetles and jewel beetles (Rassati et al. 2019), as well as bark beetles living in the upper forest strata that are not affected by trap color. The purple understory traps can reliably attract longhorn beetles living in the lowest forest strata (Flaherty et al. 2019; Rassati et al. 2019) as well as ambrosia beetles, which mostly forage near the ground, tend to prefer dark colors, and are not affected by longhorn-beetle pheromones present in the hardwood-blend. In a mixed-forest, managers should instead complement the above mentioned protocol with understory and canopy purple traps baited with the softwood-blend, which can increase chances of detecting wood-boring beetles associated with conifer trees (Flaherty et al. 2019), including bark- and ambrosia beetles. It is important to point out that both in Canada and Italy ambrosia beetle communities were largely dominated by a few species, and it is thus necessary to understand if the proposed trapping protocols can be considered efficient also for ambrosia beetle species having different ecological habits. Furthermore, it is important to underline that these trapping protocols are valid for generic surveillance programs aimed at trapping as many species as possible (Poland and Rassati 2019), whereas species-specific surveillance programs have to be adjusted depending on the biological and ecological habits of the target species. Further studies should also investigate whether multi-lure blends developed to simultaneously attract both conifer- and broadleaf-associated longhorn beetles (Fan et al. 2019) are also suitable for detecting jewel beetles and bark and ambrosia beetles. Similarly, future studies should investigate the differential efficacy of purple and green traps vs. the commonly used black traps (Brockerhoff et al. 2006; Rassati et al. 2015a,b; Rabaglia et al. 2019). This is an important aspect as the adoption of colored traps for surveillance of wood-boring beetles requires specific data to justify the additional costs. In addition, the potential efficacy of multi-colored traps (e.g., half-green/half-purple multi-funnel traps) is worth investigating, as this could decrease the number of traps needed and the overall costs of the surveillance program. Lastly, given that this study mainly targeted natural areas embedded in a urban-dominated landscape, it would be interesting to understand performance of the proposed protocols in forest-dominated areas.

Acknowledgments

The authors thank Cory Hughes, Natasha Higgins, Deepa Abeysekara, Alessandro Ferrari, Filippo Rigodanza for technical assistance in the laboratory, Peter Silk, Gaetan Leclair, Troy Kimoto and Joe Francese for logistical support, the Canadian Department of National Defense for granting us permission to work at the Magazine Hill site, and two anonymous reviewers for their helpful suggestions. This study was supported by funding from Natural Resources Canada, US Department of Agriculture—Animal and Plant Health Inspection Service—Plant Protection Quarantine (15-8130-0395-CA), the Canadian Food Inspection Agency, the Ontario Ministry of Natural Resources and Nova Scotia Department of Natural Resources through SERG International, and by "Dotazione Ordinaria Ricerca (DOR)", University of Padova.

References

Abbasi, Q. D., N. D. Jan, A. N. Maher, R. D. Khuhro, S. M. Nizamani, and A. Panhwar. 2007. Monitoring of ambrosia bark beetle through installation of sticky color traps at different heights in mango trees. Int. J. Fruit Sci. 7: 65–79

Allison, J. D., E. E. Graham, T. M. Poland, and B. L. Strom. **2016**. Dilution of fluon before trap surface treatment has no effect on longhorned beetle (Coleoptera: Cerambycidae) captures. J. Econ. Entomol. 109: 1215–1219.

Allison, J. D., and R. A. Redak. **2017**. The impact of trap type and design features on survey and detection of bark and woodboring beetles and their associates: a review and meta-analysis. Annu. Rev. Entomol. 62: 127–146.

Ambourn, A. K., J. Juzwik, and J. E. Eggers. 2006. Flight periodicities, phoresy rates, and levels of *Pseudopityophthorus minutissimus* branch colonization in oak wilt centers. For. Sci. 52: 243–250.

Balachowsky, A. 1949. Faune de France: Coléoptéres Scolytides. Librairie de la Faculté des Sciences, France.

Bakke, A. 1983. Dosage response of the ambrosia beetle, *Trypodendron lineatum* Olivier (Coleoptera: Scolytidae) to semiochemicals. J. Appl. Entomol. 95: 158–161.

Balkenius, A., W. Rosén, and A. Kelber A. 2006. The relative importance of olfaction and vision in a diurnal and a nocturnal hawkmoth. J. Comp. Physiol. A. 192: 431–437.

Bates, D., M. Maechler, B. Bolker et al. 2017. Linear mixed-effects models using ‘Eigen’ and S4. R package, version 1.1–15.1–117. URL https://cran.r-project.org/web/packages/lme4/index.html

Boone, C. K., J. Sweeney, P. Silk, C. Hughes, R. P. Webster, F. Stephen, L. Maclauchlan, B. Bentz, A. Drumont, B. Zhao, N. Berkvens, H. Casteels, and J. C. Grégoire. 2019. *Monochamus* species from different continents can be effectively detected with the same trapping protocol. J. Pest Sci. 92: 3–11.

Bouget, C., H, Brustel, A. Brin, and L. Valladares. 2009. Evaluation of window flight traps for effectiveness at monitoring dead wood-associated beetles: the effect of ethanol lure under contrasting environmental conditions. Agric. For. Entomol. 11: 143–152.

Bousquet, Y., P. Bouchard, A. E. Davies, and D. Sikes. 2013. Checklist of beetles (Coleoptera) of Canada and Alaska. ZooKeys. 360: 1–44

Brockerhoff, E. G. and A. M. Liebhold. 2017. Ecology of forest insect invasions. Biol. Invasions. 19: 3141–3159.

Brockerhoff, E. G., D. C. Jones, M. O. Kimberley, D. M. Suckling, T. Donaldson. 2006. Nationwide survey for invasive wood-boring and bark beetles (Coleoptera) using traps with pheromones and kairomones. For. Ecol. Manag. 228: 234–240.

Bright, D. E., Jr. 1976. The insects and Arachnids of Canada, Part 2: The bark beetles of Canada and Alaska (Coleoptera: Scolytidae). Biosystematics Research Institute, Research Branch, Canada Department of Agrilculture, Ottawa ON, Publication No. 1576, 241 pp.

Campbell, S. A., and J. H. Borden. **2006a**. Integration of visual and olfactory cues of hosts and non-hosts by three bark beetles (Coleoptera: Scolytidae). Ecol. Entomol. 31: 437–449.

Campbell, S. A., and J. H. Borden. **2006b**. Close-range, in-flight integration of olfactory and visual information by a host-seeking bark beetle. Entomol. Exp. Appl. 120: 91–98.

Campbell, S. A. and J. H. Borden. 2009. Additive and synergistic integration of multimodal cues of both hosts and non-hosts during host selection by woodboring insects. Oikos. 118: 553–563.

Chase, K. D., L. D. Stringer, R. C. Butler, A. M. Liebhold, D. R. Miller, P. W. Shearer, and E. G. Brockerhoff. 2018. Multiple-lure surveillance trapping for *Ips* bark beetles, *Monochamus* longhorn beetles, and *Halyomorpha halys* (Hemiptera: Pentatomidae). J. Econ. Entomol. 111: 2255–2263.

Chen, G., Q. H. Zhang, Y. Wang, G. T. Liu, X. Zhou, J. Niu, and F. Schlyter. **2010**. Catching *Ips duplicatus* (Sahlberg) (Coleoptera: Scolytidae) with pheromone-baited traps: optimal trap type, colour, height and distance to infestation. Pest Manag. Sci. 66: 213–219.

Collignon, R. M., I. P. Swift, Y. Zou, J. S. McElfresh, L. M. Hanks, and J. G. Millar. 2016. The influence of host plant volatiles on the attraction of longhorn beetles to pheromones. J. Chem. Ecol. 42: 215–229.

Dodds, K. J. 2014 Effects of trap height on captures of arboreal insects in pine stands of northeastern United States of America. Can. Entomol. 146: 80–89.

Dubbel, V., K. Kerck, M. Sohrt, and S. Mangold. **1985**. Influence of trap color on the efficiency of bark beetle pheromone traps. J. Appl. Entomol. 99: 59–64.

Dufour, B. P., and B. Frérot. 2008. Optimization of coffee berry borer, *Hypothenemus hampei* Ferrari (Col., Scolytidae), mass trapping with an attractant mixture. J. Appl. Entomol. 132: 591–600.

Eyre, D. and R. A. Haack. 2017. Invasive cerambycid pests and biosecurity measures, pp. 563–618. *In* Q. Wang (ed.), Cerambycidae of the world: biology and pest management. CRC Press, Boca Raton.

Fan, J. T., O. Denux, C. Courtin, A. Bernard, M. Javal, J. G. Millar, L. M. Hanks, and A. Roques. **2019**. Multi-component blends for trapping native and exotic longhorn beetles at potential points-of-entry and in forests. J. Pest Sci. 92: 281–297.

Flaherty, L., J. M. G. Gutowski, C. Hughes, P. Mayo, T. Mokrzycki, G. Pohl, P. Silk, K. Van Rooyen, and J. Sweeney. **2019**. Pheromone-enhanced lure blends and multiple trap heights improve detection of bark and wood-boring beetles potentially moved in solid wood packaging. J. Pest Sci. 92: 309–325.

Foit, J. 2010. Distribution of early-arriving saproxylic beetles on standing dead Scots pine trees. Agric. For. Entomol. 12: 133–141.

Francese, J. A, D. J. Crook, I. Fraser, D. R. Lance, A. J. Sawyer, and V. C. Mastro. 2010. Optimization of trap color for emerald ash borer (Coleoptera: Buprestidae). J. Econ. Entomol. 103:1235–1241

Gorzlancyk, A. M., D. W. Held, D. J. Kim, and C. M. Ranger. 2013. Capture of *Xylosandrus crassiusculus* and other scolytinae (Coleoptera: Curculionidae) in response to visual and volatile cues. Fla. Entomol. 96: 1097–1101.

Gorzlancyk, A. M., D. W. Held, C. M. Ranger, Z. Barwary, and D. J. Kim. 2014. Capture of *Cnestus mutilatus*, *Xylosandrus crassiusculus*, and other Scolytinae (Coleoptera: Curculionidae) in response to green light emitting diodes, ethanol, and conophthorin. Fla. Entomol. 97: 301–303.

Graham, E. E., T. M. Poland, D. G. McCullough, and J. G. Millar. 2012. A comparison of trap type and height for capturing cerambycid beetles (Coleoptera). J. Econ. Entomol. 105: 837–846.

Hanks, L.M., and J. G. Millar. 2016. Sex and aggregation-sex pheromones of cerambycid beetles: basic science and practical applications. J. Chem. Ecol. 42: 631–654.

Hanks, L. M., J. G. Millar, J. A. Mongold-Diers, J. C. Wong, L. R. Meier, P. F. Reagel, and R. F. Mitchell. 2012. Using blends of cerambycid beetle pheromones and host plant volatiles to simultaneously attract a diversity of cerambycid species. Can. J. For. Res. 42: 1050–1059.

Hanula, J. L., M. D. Ulyshen, and S. Horn. 2011. Effect of trap type, trap position, time of year, and beetle density on captures of the redbay ambrosia beetle (Coleoptera: Curculionidae: Scolytinae). J. Econ. Entomol. 104: 501–508.

Hardersen, S., G. Curletti G, L. Leseigneur, G. Platia, G. Liberti, P. Leo, P. Cornacchia, and E. Gatti. 2014. Spatio-temporal analysis of beetles from the canopy and ground layer in an Italian lowland forest. Bull. Insectology 67: 87–97.

Hartig, F. 2017. Package'DHARMa' residual diagnostics for hierarchical (multi-level/mixed) regression models. Version 0.1.5. URL: https://cran.r-project.org/web/packages/DHARMa/DHARMa.pdf.

Hsieh, T. C., K. H. Ma, and A. Chao. 2016. iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). Meth. Ecol. Evol. 7: 1451–1456.

Humble, L. 2010. Pest risk analysis and invasion pathways – insects and wood packing revisited: what have we learned? N Z J. For. Sci. 40(Suppl.): S57–S72.

Hughes, C. C., R. C. Johns, and J. D. Sweeney. **2014**. A technical guide to installing beetle traps in the upper crown of trees stand and tree selection considerations. J. Acad. Entomol. Soc. 10: 12–18.

Kerr, J. L., D. Kelly, M. K. F. Bader, and E. G. Brockerhoff. 2017. Olfactory cues, visual cues, and semiochemical diversity interact during host location by invasive forest beetles. J. Chem. Ecol. 43: 17–25.

Klingeman, W. E., A. M. Bray, J. B. Oliver, C. M. Ranger, and D. E. Palmquist. 2017. Trap style, bait, and height deployments in black walnut tree canopies help inform monitoring strategies for bark and ambrosia beetles (Coleoptera: Curculionidae: Scolytinae). Environ. Entomol. 46: 1120–1129.

Imrei, Z., Z. Lohonyai, G. Csóka, J. Muskovits, S. Szanyi, G. Vétek, J. Fail, M. Toth, and M. J. Domingue. 2020. Improving trapping methods for buprestid beetles to enhance monitoring of native and invasive species. Forestry: Int. J. For. Res. 93: 254–264.

Leksono, A. S., K. Takada, S. Koji, N. Nakagoshi, T. Anggraeni, and K. Nakamura. **2005**. Vertical and seasonal distribution of flying beetles in a suburban temperate deciduous forest collected by water pan trap. Insect Sci. 12: 199–206.

Lindelöw, Å., H. H. Eidmann, and H. Nordenhem. **1993**. Response on the ground of bark beetle and weevil species colonizing conifer stumps and roots to terpenes and ethanol. J. Chem. Ecol. 19: 1393–1403.

Magnusson, A., H. Skaug, A. Nielsen, C. Berg, K. Kristensen, M. Maechler, K. Van Bentham, B. Bolker, and M. Brooks. 2017. glmmTMB: Generalized linear mixed models using a template model builder. R package version 0.1.3.

Mendel, Z., O. Boneh, Y. Shenhar, and J. Riov. 1991. Diurnal flight patterns of *Orthotomicus erosus* and *Pityogenes calcaratus* in Israel. Phytoparasitica. 19: 23–31.

Menocal, O., P. E. Kendra, W. S. Montgomery, J. H. Crane, and D. Carrillo. **2018**. Vertical distribution and daily flight periodicity of ambrosia beetles (Coleoptera: Curculionidae) in Florida avocado orchards affected by laurel wilt. J. Econ. Entomol. 111: 1190–1196.

Meurisse, N., D. Rassati, B. P. Hurley, E. G. Brockerhoff, and R. A. Haack. **2019**. Common pathways by which non-native forest insects move internationally and domestically. J. Pest Sci. 92: 13–27.

Miller, D. R. 2006. Ethanol and (−)-α-pinene: attractant kairomones for some large wood-boring beetles in southeastern USA. J. Chem. Ecol. 32: 779–794.

Miller, D. R., and R. J. Rabaglia. 2009. Ethanol and (−)-α-pinene: attractant kairomones for bark and ambrosia beetles in the southeastern US. J. Chem. Ecol. 35: 435–448.

Miller, D. R., C. M. Crow, P. D. Mayo, P. J. Silk, and J. D. Sweeney. 2015a. Responses of Cerambycidae and other insects to traps baited with ethanol, 2,3-hexanediol, and 3,2-hydroxyketone lures in northcentral Georgia. J. Econ. Entomol. 108: 2354–2365.

Miller, D. R., C. M. Crowe, K. J. Dodds, L. D. Galligan, P. De Groot, E. R. Hoebeke, A. E. Mayfield III, T. M. Poland, K. F. Raffa, and J. D. Sweeney. 2015b. Ipsenol, ipsdienol, ethanol, and α-pinene: trap lure blend for Cerambycidae and Buprestidae (Coleoptera) in pine forests of eastern North America. J. Econ. Entomol. 108: 1837–1851.

Miller, D. R., C. M. Crowe, and J. D. Sweeney. 2020. Trap height affects catches of bark and woodboring beetles (Coleoptera: Curculionidae, Cerambycidae) in baited multiple-funnel traps in southeastern United States. J. Econ. Entomol. 113: 273–280.

Montgomery, M. E., and P. M. Wargo. 1983. Ethanol and other host-derived volatiles as attractants to beetles that bore in hardwoods. J. Chem. Ecol. 9: 181–190.

Niemeyer, H.V. 1985. Field response of *Ips typographus* L. (Col., Scolytidae) to different trap structures and white versus black flight barriers. Z. Angew. Entomol. 99: 44–51.

Noseworthy, M. K., L. M. Humble, J. Sweeney, P. J. Silk, and P. Mayo. 2012. Attraction of *Monarthrum scutellare* (Coleoptera: Curculionidae: Scolytinae) to hydroxy ketones and host volatiles. Can. J. For. Res. 42: 1851–1857.

Pfeffer, A. 1995. Zentral- und Westpalärktische Borken- und Kernkäfer (Coleoptera, Scolytidae, Platypodidae). Entomol. Basil. 17:5–310.

Poland, T. M., and D. Rassati. 2019. Improved biosecurity surveillance of non-native forest insects: a review of current methods. J. Pest Sci. 92: 37–49.

Procházka, J., L. Cizek, and J. Schlaghamerský. 2018. Vertical stratification of scolytine beetles in temperate forests. Insect Conserv. Divers. 11: 534–544.

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Austria.

Rabaglia, R. J., A. I. Cognato, E. R. Hoebeke, C. W. Johnson, J. R. LaBonte, M. E. Carter, and J. J. Vlach. 2019. Early detection and rapid response: a 10-Year summary of the USDA forest service program of surveillance for non-native bark and ambrosia beetles. Am. Entomol. 65: 29–42.

Ranger, C. M., M. E. Reding, K. J. Gandhi, J. B. Oliver, P. B. Schultz, L. Cañas, and D. A. Herms. 2011. Species dependent influence of (−)-α-pinene on attraction of ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) to ethanol-baited traps in nursery agroecosystems. J. Econ. Entomol. 104: 574–579.

Ranger C. M., M. E. Reding, K. Addesso, M. Ginzel, and D. Rassati. 2020. Semiochemical-mediated host selection by *Xylosandrus* spp. ambrosia beetles (Coleoptera: Curculionidae) attacking horticultural tree crops: a review of basic and applied science. Can. Entomol. (in press).

Rassati, D., E. Petrucco Toffolo, A. Roques, A. Battisti, and M. Faccoli. **2014**. Trapping wood boring beetles in Italian ports: a pilot study. J. Pest Sci. 87: 61–69.

Rassati, D., M. Faccoli, E. Petrucco Toffolo, A. Battisti, and L. Marini. 2015a. Improving the early detection of alien wood-boring beetles in ports and surrounding forests. J. Appl. Ecol. 52: 50–58.

Rassati, D., M. Faccoli, L. Marini, R. A. Haack, A. Battisti, E. Petrucco Toffolo. 2015b. Exploring the role of wood waste landfills in early detection of non-native wood-boring beetles. J. Pest Sci. 88: 563–572.

Rassati, D., R. A. Haack, M. Knížek, and M. Faccoli. 2018. National trade can drive range expansion of bark-and wood-boring beetles. J. Econ. Entomol. 111: 260–268.

Rassati, D., L. Marini, M. Marchioro, P. Rapuzzi, G. Magnani, R. Poloni, F. Di Giovanni, P. Mayo, and J. Sweeney. **2019**. Developing trapping protocols for wood-boring beetles associated with broadleaf trees. J. Pest Sci. 92: 267–279.

Rassati, D., M. Marchioro, L. Flaherty, R. Poloni, S. Edwards, M. Faccoli, J. Sweeney. 2020 Response of native and exotic longhorn beetles to common pheromone components provides partial support for the pheromone-free space hypothesis. Insect Sci. doi: 10.1111/1744-7917.12790

Rhainds, M., T. Kimoto, J. Galko, C. Nikolov, K. Ryall, G. Brodersen, and V. Webster. 2017. Survey tools and demographic parameters of Slovakian *Agrilus* associated with beech and poplar. Entomol. Exp. Appl. 162: 328–335.

Salom, S. M., and J. A. McLean. 1990. Flight and landing behavior of *Trypodendron lineatum* (Coleoptera: Scolytidae) in response to different semiochemicals. J. Chem. Ecol. 16: 2589–2604.

Schmeelk, T. C., J. G. Millar, and L. M. Hanks. 2016. Influence of trap height and bait type on abundance and species diversity of cerambycid beetles captured in forests of east-central Illinois. J. Econ. Entomol. 109: 1750–1757.

Schroeder, L. M., and Å. Lindelöw. **1989**. Attraction of scolytids and associated beetles by different absolute amounts and proportions of α-pinene and ethanol. J. Chem. Ecol. 15: 807–

817.

Seo, M., X. Martini, M. J. Rivera, and L. L. Stelinski. 2017. Flight capacities and diurnal flight patterns of the ambrosia beetles, *Xyleborus glabratus* and *Monarthrum mali* (Coleoptera: Curculionidae). Environ. Entomol. 46: 729–734.

Sheehan, T. N., M. D. Ulyshen, S. Horn, and E. R. Hoebeke. 2019. Vertical and horizontal distribution of bark and woodboring beetles by feeding guild: is there an optimal trap location for detection? J. Pest Sci. 92: 327–341.

Strom, B. L., L. M. Roton, R. A. Goyer, and J. R. Meeker. **1999**. Visual and semiochemical disruption of host finding in the southern pine beetle. Ecol. Appl. 9: 1028–1038.

Strom, B. L., and R. A. Goyer. **2001**. Effect of silhouette color on trap catches of *Dendroctonus frontalis* (Coleoptera: Scolytidae). Ann. Entomol. Soc. Am. 94: 948–953.

Strom, B. L., R. A. Goyer, and P. J. Shea. **2001**. Visual and olfactory disruption of orientation by the western pine beetle to attractant-baited traps. Entomol. Exp. Appl. 100: 63–67.

Sweeney, J. D., P. J. Silk, V. Grebennikov, and M. Mandelshtam 2016. Efficacy of semiochemicals-baited traps for detection of Scolytinae species (Coleoptera: Curculionidae) in the Russian Far East. Eur. J. Entomol. 113: 84–97.

Ulyshen, M. D. 2011. Arthropod vertical stratification in temperate deciduous forests: implications for conservation-oriented management. For. Ecol. Manag. 261: 1479–1489.

Ulyshen, M. D., and J. L. Hanula. 2007. A comparison of the beetle (Coleoptera) fauna captured at two heights above the ground in a North American temperate deciduous forest. Am. Mid. Nat. 158: 260–278.

Ulyshen, M. D., and T. N. Sheehan. 2019. Trap height considerations for detecting two economically important forest beetle guilds in southeastern US forests. J. Pest Sci. 92: 253–265.

Webster, R. P., P. de Tonnancour, J. D. Sweeney, V. L. Webster, C. A. Kostanowicz, C. Hughes, R. S. Anderson, J. Klymko, C. Chantal, and R. Vigneault. 2020. New Coleoptera records from eastern Canada, with additions to the fauna of Manitoba, British Columbia, and Yukon Territory. ZooKeys 946: 56–112.

Werle, C. T., A. M. Bray, J. B. Oliver, E. K. Blythe, and B. J. Sampson. 2014. Ambrosia beetle (Coleoptera: Curculionidae: Scolytinae) captures using colored traps in southeast Tennessee and south Mississippi. J. Entomol. Sci. 49: 373–382.

Wermelinger, B., P. F. Flückiger, M. K. Obrist, and P. Duelli. **2007**. Horizontal and vertical distribution of saproxylic beetles (Col., Buprestidae, Cerambycidae, Scolytinae) across sections of forest edges. J. Appl. Entomol. 131: 104–114.

Wong, J. C., R. F. Mitchell, B. Striman, J. G. Millar, and L. M. Hanks. 2012. Blending synthetic pheromones of cerambycid beetles to develop trap lures that simultaneously attract multiple species. J. Econ. Entomol. 105: 906–915.

Chapter 5

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

Paper submitted to the *Journal of Applied Entomology* as: Marchioro M., Bianchi A., Ciampitti M. & Faccoli M. Testing trapping protocols for detecting the Citrus Longhorn Beetle, *Anoplophora chinensis* (Coleoptera: Cerambycidae).

Abstract

The Citrus Longhorn Beetle (CLB), *Anoplophora chinensis* (Coleoptera: Cerambycidae) is a highly polyphagous species native to eastern and south-eastern Asia. Since 2000, *A. chinensis* was accidentally introduced in Lombardy Region (Italy). In 2017 an extensive trapping experiment was conducted in 3 infested sites in order to evaluate the effectiveness of three variables: trap model (Econex soft cross-vane trap, Witasek cross-vane trap and Witasek multi-funnel trap), trap position (on a wooden pole in open space or in the canopy of a host tree) and type of lure (ChemTica, Synergy and Glabriwit pheromones). Five traps for each variables combination were deployed in each site, used as replicates, for a total amount of 270 traps. At the end of the study, 162 adults were caught, with catches gradually increasing during the month of June to peak in early July. The two cross-vane traps outperformed the multi-funnel one and Econex trap capture more females than Witasek trap, probably due to the structure of the collecting funnel. The three lures had similar catch performance, although the best combination was the Econex trap with Synergy blend, due to the remarkable variations in catches observed with Glabriwit blend. Lastly, traps set in the trees canopy outperformed traps set on wooden poles. In conclusion, the best protocol was the use of Econex cross-vane traps baited with Sinergy blend and deployed on host trees.

Introduction

The Citrus Longhorn Beetle (CLB), *Anoplophora chinensis* (Coleoptera: Cerambycidae), is a pest native to eastern and south-eastern Asia, widely distributed in China, Korea, Japan, and locally present in Indonesia, Malaysia, Myanmar, Taiwan, Vietnam, and the Philippines (Lingafelter and Hoebeke 2002; Haack et al. 2010; Hoppe et al. 2019). The large CLB adults (≤ 4 cm long) lay eggs under the bark of apparently healthy trees making characteristic T-shaped oviposition wounds in the bark near the ground level or even in the more superficial roots (Van Der Gaag et al. 2010). Larvae develop at ground level or lower taking about 1 or 2 years, or even more, according to the latitudes (Haack et al. 2010; Van Der Gaag et al. 2010). Larvae bore long galleries initially in the phloem and then deeply in the wood. Insect oviposition may affect the same plant also for several years finally killing the tree.

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

Due to the market globalization and the quick international trade, in the last 40 years CLB has been intercepted several times in USA (EPPO 1999; Haack et al. 2010), where four outbreaks were successfully eradicated; to date, the pest is absent from Americas (Hoppe et al. 2019; EPPO 2020a). In EPPO region CLB was first reported in 1980 in the Netherlands (Haack et al. 2010). Since 2000, several outbreaks have been reported and successfully eradicated in Netherlands (2003, eradicated in 2010), Germany (2008, eradicated in 2017), Denmark (2011, eradicated in 2015) and Switzerland (2014, eradicated in 2019) (EPPO 2010, 2015, 2020b; Hoppe et al. 2019); whereas the pest is considered transient under eradication in France (2003 and 2008), Croatia (2007) and Turkey

(2015) (EPPO 2020a). Finally, in Italy CLB is present with restricted distributions in Lombardy Region (2000) and in Tuscany Region (2014, 2017 and 2019), whereas in Lazio Region (2008) the pest was successfully eradicated in 2019 (EPPO 2019, 2020c). CLB is considered one of the major pests of the urban forests, gardens and parks infesting mainly *Acer*, *Platanus*, *Betula*, *Carpinus*, *Fagus*, *Corylus*, *Lagerstroemia*, *Malus* and *Pyrus* (Hoppe et al. 2019; EPPO 2020a).

In addition to control measures carried out according to EU legislation, Lombardy Region implemented a specific survey of the regional territory based on the use of traps baited with lures attractive to CLB. Nevertheless, very few information is available about the best trap model and lure effective for CLB detection. Similarly, no data is known concerning the best trap position – i.e., height from ground, type of trap support, and distance from possible host trees – for CLB interception. Moreover, there is any pheromone blend specific for CLB except those applied against the Asian Longhorn Beetle (ALB), *Anoplophora glabripennis*, which, apparently, is sharing the same male-produced volatile pheromones 4-(n-heptyloxy)butan-1-ol and 4-(n-heptyloxy) butanal (Hansen et al. 2015).

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

Materials and methods

Experimental sites and periods

The study was conducted in 3 CLB populations occurring in northern Italy (Fig. 1), used as replicates:

a) the infestation of Milan city, including the South Milan Agricultural Park;

b) the infestation of Nerviano (Milan province), including the neighboring municipalities;

c) the infestation occurring in the municipality of Gussago (Brescia province).

All the experimental sites have been chosen within CLB infested areas ensuring the presence of the species and, therefore, to be suitable to evaluate the effectiveness of the tested protocols.

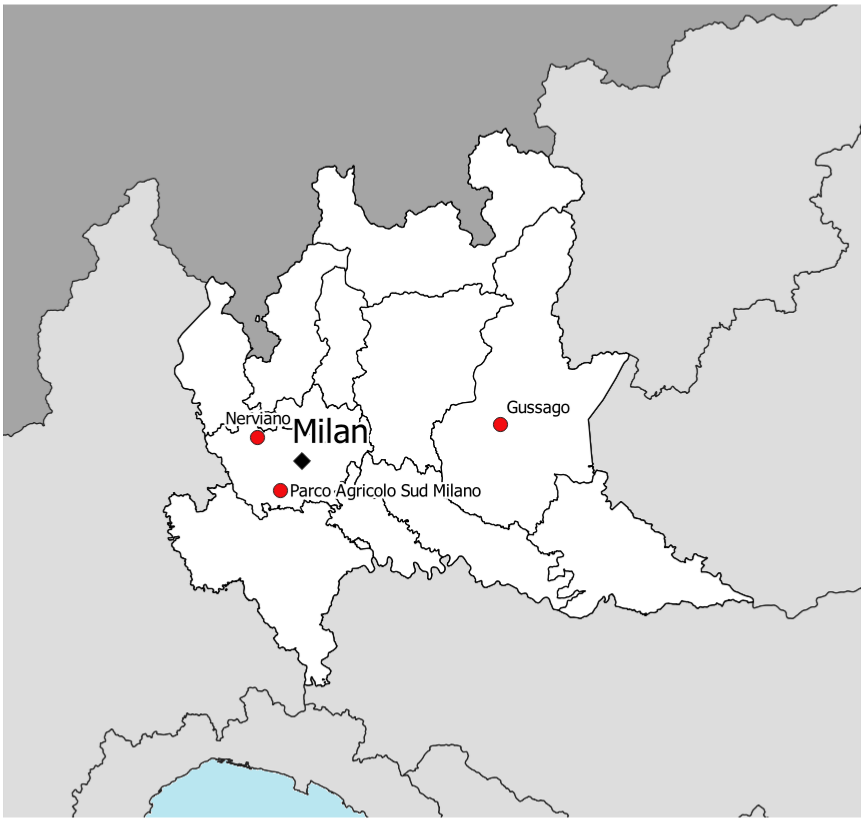

Fig. 1 Map of the 3 CLB infestations where the study was conducted.

For the same reasons, the study was conducted during the period of maximum CLB flight activity of the emerging beetles which in northern Italy is generally concentrated in June and July. Traps were therefore set up and baited with lures between 31 May and 7 June 2017, and withdrawn between 31 July and 3 August 2017, covering 2 months. All the traps were checked and emptied every second week, i.e. four times at mid and end of June and July, recording the number of CLB males and females collected in each date and trap.

Trap setting

In each of the three experimental sites, traps were set up in 5 different locations each containing 18 traps covering all combinations of trap model (3), lure composition (3), and trap position (2) (see experimental design below). The locations were chosen as far as possible from each other to avoid overlapping effects, and never closer than 500-600 meters.

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

Experimental design

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

In this regard, 3 different models of traps were tested (Fig. 2):

- Black cross-vane traps produced by the Austrian company Witasek (cross-vane Witasek): panel-traps with rigid crossed panels mounted on a collector funnel communicating with a collector cup;
- Black soft cross-vane traps produced by the Spanish company Econex (cross-vane Econex): new model of panel-traps consisting of two soft crossed panels, longer than the classic cross-vane traps (approximately 120 cm in height compared to 80 cm of the Witasek model), mounted on a collector funnel communicating with a collector glass;
- Black multi-funnel traps produced by the Austrian company Witasek (multi-funnel): traps made up of 12 overlapping funnels communicating with a collector cup.

Each trap model was individually activated with 3 different blends of aggregation pheromones produced and sold by the companies Synergy Semiochemicals (Canada), ChemTica (Costa Rica), and Witasek (Glabriwit, Austria) for trapping the Asian Longhorn Beetle, *Anoplophora glabripennis*, as no specific pheromone is commercially available for CLB.

Finally, each trap and pheromone combination was tested either on wooden poles placed in open areas or directly on the canopy of suitable host plants, for a total of 18 different combinations tested in each location (Table 1). Based on this experimental design, 90 traps for each trap model and lure were set up in each of the 3 monitored infested sites for a total of 270 traps.

Fig. 2 The traps used in this study. From left to right: soft cross-vane (Econex), standard cross-vane (Witasek), multi-funnels (Witasek) on the support wooden poles.

Statistical analysis

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

Table 1 Experimental design adopted in each of the 3 monitored sites, testing the 18 possible combinations of trap model (3), lure composition (3), and trap position (2). In each site, all combinations were replicated 5 times in 5 different locations for a total of 90 traps.

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

Results

Total captures and flight period

A total of 162 CLB adults were captured in June-July 2017, divided into 84 males and 78 females, without significant differences between sexes (Anova, df = 1; 268, F = 2.13, P> 0.05). Catches gradually increased during the month of June to peak in early July and then gradually dropping in the following month (Fig. 3).

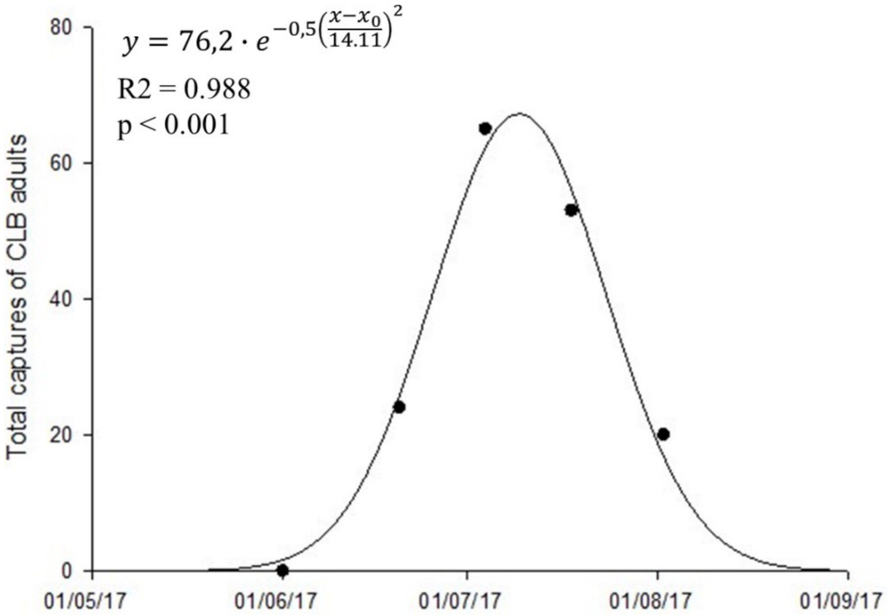

Fig. 3 Temporal trend of the CLB adults trapped in Lombardy (N Italy) in June-July 2017, and descriptive model of the flight curve of the species. Traps were checked and emptied every second week.

Captures of different trap models

CLB catches show significant differences between the 3 trap models tested in the present study (Anova, df = 2; 267, F = 1.39, P <0.05). In particular, Econex cross-vane traps showed the best performance with average catches of 0.86 adults per trap. A 22% lower value, although not statistically different, was provided by Witasek cross-vane traps, with average catches of 0.67 adult per trap. Multi-funnel traps showed the worst catching performance with only 0.48 insects per trap, i.e. about half (-44%) of the captures had with the Econex cross-vane traps from which they differ significantly.

Males and females of CLB respond differently to the various traps (Anova, df = 2; 267, F = 1.43, P<0.05). While Econex cross-vane and multi-funnel traps do not show significant differences in capture's sex-ratio, Witasek cross-vane traps catch significantly fewer females than males (Fig. 4). The latter, in fact, had capture levels high and similar to those observed in the Econex cross-vane traps, whereas the female catches drop to low values similar to those found in the multi-funnel traps.

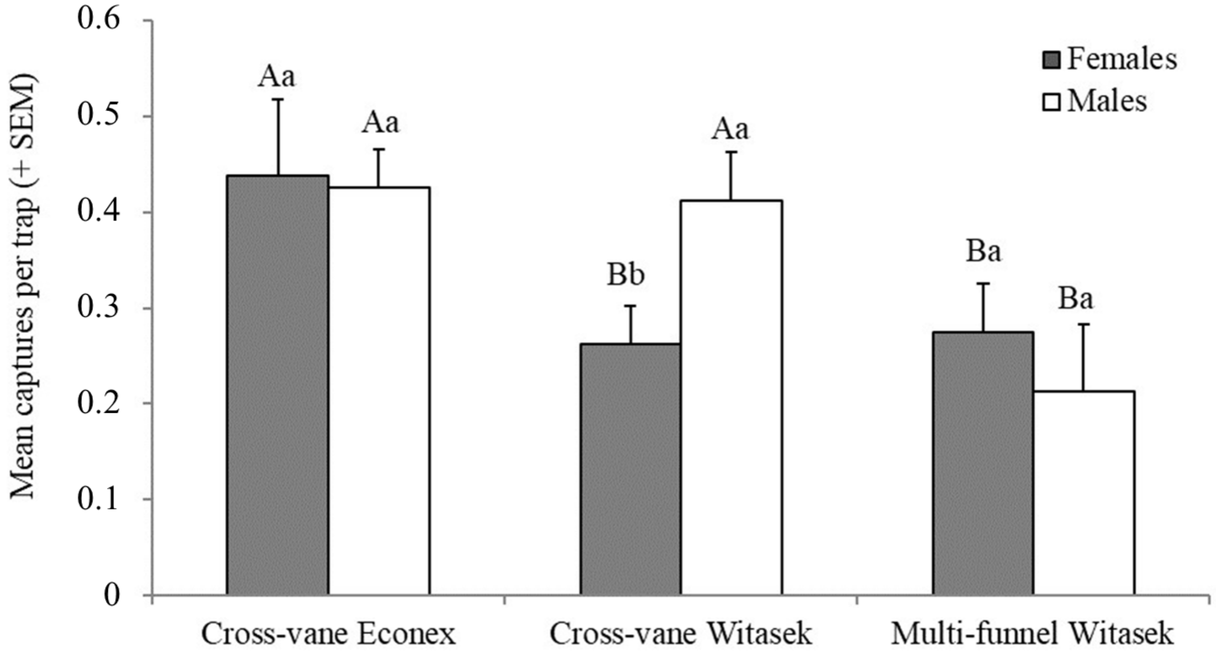

Fig. 4 Mean CLB captures per trap (+ SEM) in relation to trap model and insect sex. Different letters correspond to significant differences at the analysis of variance (Tukey test, P <0.05). Capital letters indicate differences among trap models, lower case letters indicate differences between genders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

Attractiveness of different lures

The 3 different tested lures do not show significant differences in the mean number of trapped CLB (Anova, df = 2; 267, F = 0.49, P = 0.61), although the Canadian Synergy pheromone – with 0.83 adults per trap – catches approximately 25% more insects than Chemtica and Witasek lures (both with average catches of 0.62 insects per trap) (Fig. 5).

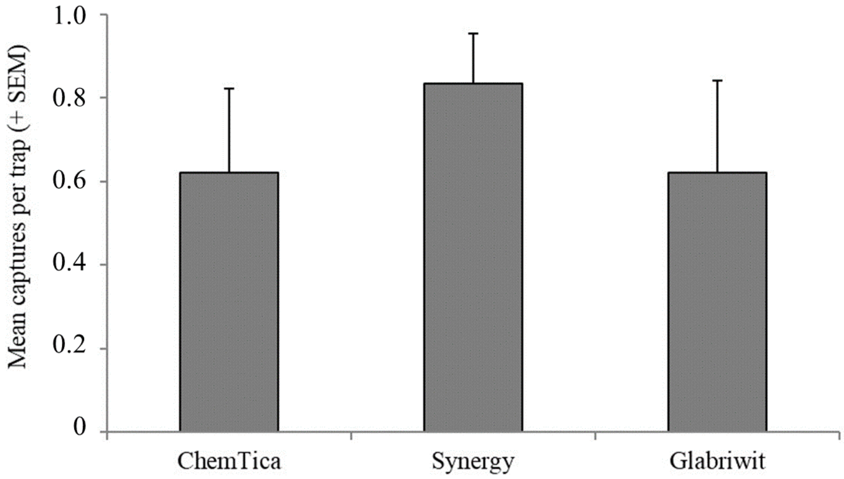

Fig. 5 Mean captures of CLB per trap (+ SEM) in relation to pheromone blend.

Although there are no significant differences in the total number of males (Anova, df = 2; 267, F = 1.14, P = 0.32) or females (Anova, df = 2; 267, F = 0.02, P = 0.97) captured with the various lures, Synergy blend captures a number of males (0.5 per trap) 50% higher than females (0.33 per trap). On the other hand, catches of males and females had with the ChemTica and Witasek blends are lower and almost identical to each other (about 0.3 per trap).

Trap-lure interactions

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

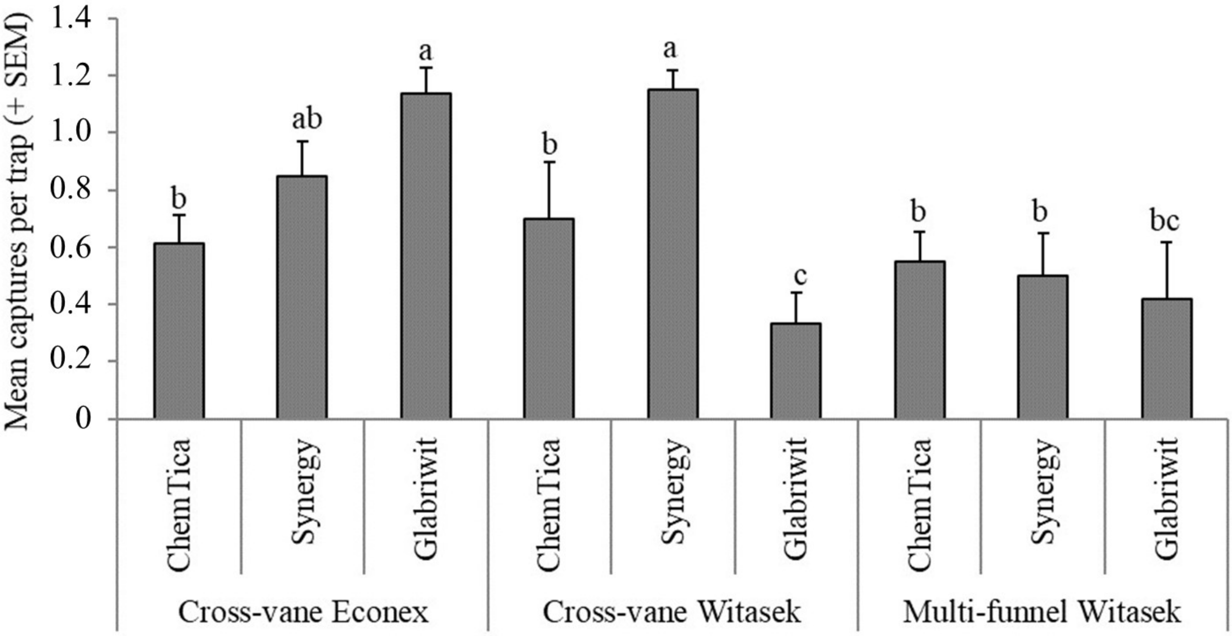

Fig. 6 Mean CLB captures (+ SEM) according to trap model and pheromone blend. Different letters correspond to significant differences to the analysis of variance (Tukey test, P <0.05).

Effect of the trap position

Cross-vane traps installed on tree crowns of the host plants showed average catch levels significantly higher – and almost double – than traps installed on wooden poles placed far from the trees (Anova, df = 1; 268, F = 5, 68, P <0.01). Canopy effect was however non-significant in multi-funnel traps, which have mean catches similar between canopies and poles (Anova, df = 2; 267, F = 2.59, P <0.05) (Fig. 7).

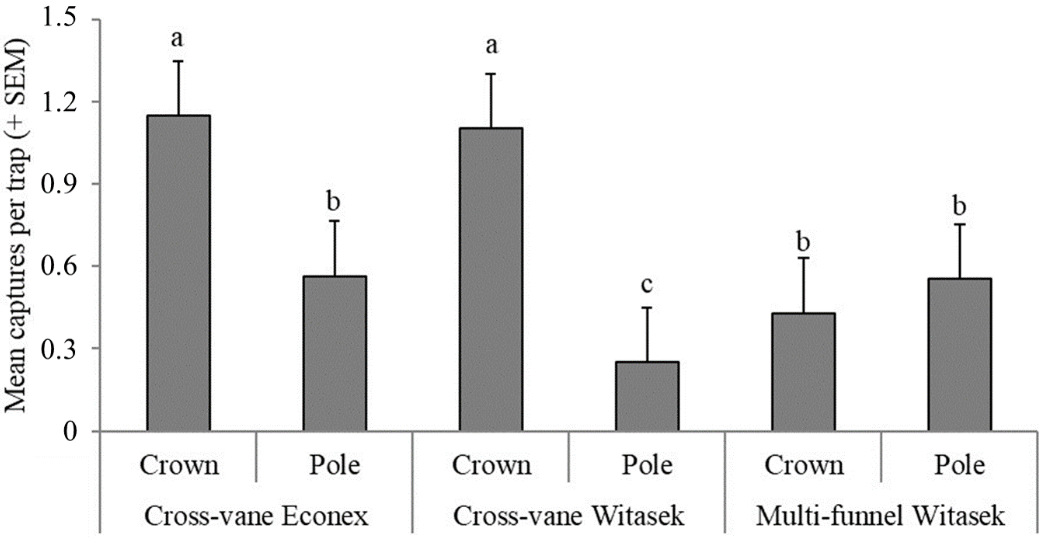

Fig. 7 Mean CLB captures per trap (+ SEM) according to trap model and trap position. Different letters correspond to significant differences at the analysis of variance (Tukey test, P <0.05).

Discussion

The results concerning the trapping protocols of CLB populations tested in the present study indicate the cross-vane traps as the best trap model to be used to increase the probability of CLB catching. This result is in agreement with previous reports for similar species: cross-vane traps are more effective than multi-funnel traps in catching many families of bark and wood-boring beetles, including long-horn beetles (Allison and Redak 2017). Moreover, even in ALB monitoring and eradication protocols, mainly cross-vane traps were used (Nehme et al. 2014; Eyre and Barbrook 2021). Although non-significant differences occur between the catches of Econex and Witasek cross-vane traps, the former showed higher mean level of captures. Actually, Econex cross-vane traps are structurally different from standard cross-vane traps as they consist of 2 longer panels made by soft and very slippery rubber, with a greater interception surface (approximately 4270 cm^2 compared to 3960 cm^2 of the Witasek model). The differences in the mean adult catches observed between Econex and Witasek cross-vane traps are essentially based on the deeply reduced number of CLB females captured by the Witasek model, or to a higher ability of females to escape from traps. This phenomenon led to an overall reduction of captures recorded in the Witasek cross-vane traps compared to Econex ones. In average, CLB females are sensibly larger than males (37 mm Vs 21 mm) (Hoppe et al. 2019), increasing the possibility for females to escape from catching when the funnel posed at the base of the cross panels shows a connection hole towards the collector jar too small and easily clogged up with leaves and debris. In this respect, the diameter of the funnel hole of the Witasek cross-vane trap is a bit larger (4 - 7 cm compared to the 2.5 - 4.5 cm of those of the Econex), but the structure of the Witasek trap is different. In fact, the two panels penetrate the funnel at the base causing a narrowing, and could facilitate the escape of the insects, especially for large females. Besides having a greater insect interception surface, Econex cross-vane traps allow also a considerable space-saving for winter storage as the panels fold over themselves. Overall, the Econex soft cross-vane trap appears to be a more efficient model for CLB trapping.

Among lures tested in the present study, the Synergy ones provided the best results with high mean capture levels, although it does not differ statistically from the other two blends. In this respect, the best trap-lure combination for catching CLB adults are the Econex cross-vane trap baited with Synergy pheromones. It is interesting to underline the remarkable variation in adult catching showed by the Glabriwit pheromone when tested in different trap models, even when used in the same trap type (cross-vane), suggesting its variable and unreliable performance.

On average, traps hooked on tree crowns had greater catches than traps hung on the wooden poles. This result can be explained by the visual and chemical attractiveness induced on CLB adults by the canopy silhouette and the host-tree volatiles, which integrate and enhance the action of the aggregation pheromones used to bait the traps. A similar result was observed also for ALB, which was caught in higher number by traps hanging from trees than from bamboo poles (Nehme et al. 2010). Other researchers as well found traps placed under the canopy or in the forest edges to be more effective in catching long-horned beetles than traps set-up in clearing and open fields (Dodds 2011; Sweeney et al. 2020). According to the results of our study, the protocol that provides the highest levels of CLB captures is therefore the installation of cross-vane traps in the crowns of CLB host trees. In this context, however, there is a risk of an over-spilling effect, *i.e.* insects attracted by the pheromone to a host plant and then not caught by the trap but directly infesting the tree. In this respect, healthy plants used to install traps must be carefully surveyed to avoid the beginning of new local infestations.

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

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

Finally, although the mean catches per trap recorded in the present study are in general particularly low, they are in line with ALB catches reported in other works (Nehme et al. 2014; Eyre and Barbrook 2021). Moreover, the use of pheromone traps against CLB allows the species detection and the spatial and temporal survey of its populations, giving crucial information concerning the presence of the species and the success of the applied eradication protocols.

Acknowledgements

We thank Lombardy Region for partially funding the research, data providing and their help in field-works. A special thanks to University of Padua for partially funding the research (DOR projects).

References

Adachi, I. 1988. Reproductive biology of the White-Spotted Longicorn Beetle, *Anoplophora malasiaca* THOMSON (Coleoptera : Cerambycidae), in citrus trees. Appl. Entomol. Zool. 23: 256–264.

Adachi, I. 1989. Spatial distribution and mortality process of *Anoplophora malasiaca* (Coleoptera: Cerambycidae) eggs in citrus groves. Res. Popul. Ecol. (Kyoto). 31: 343–352.

Adachi, I. 1990. Population studies of *Anoplophora malasiaca* adults (Coleoptera: Cerambycidae) in a citrus grove. Res. Popul. Ecol. (Kyoto). 32: 15–32.

Adachi, I. 1994. Development and life cycle of *Anoplophora malasiaca* (THOMSON) (Coleoptera: Cerambycidae) on citrus trees under fluctuating and constant temperature regimes. Appl. Entomol. Zool. 29: 485–497.

Adachi, I., and R. Korenaga. **1989**. Control methods for *Anoplophora malasiaca* THOMSON (Coleoptera : Cerambycidae) in citrus groves. : comparison of effects in several methods for preventing oviposition. Appl. Entomol. Zool. 24: 315–318.

Allison, J. D., and R. A. Redak. **2017**. The impact of trap type and design features on survey and detection of bark and woodboring beetles and their associates: a review and meta-analysis. Annu. Rev. Entomol. 62: 127–146.

Dodds, K. J. 2011. Effects of habitat type and trap placement on captures of bark (Coleoptera: Scolytidae) and longhorned (Coleoptera: Cerambycidae) beetles in semiochemical-baited traps. J. Econ. Entomol. 104: 879–888.

EPPO. **1999**. *Anoplophora chinensis* found under glasshouse in Georgia (US). EPPO Report. Serv. 6.

EPPO. **2010**. *Anoplophora chinensis* eradicated from the Netherlands. EPPO Report. Serv. 7.

EPPO. **2015**. *Anoplophora chinensis* eradicated from Denmark. EPPO Report. Serv. 10.

EPPO. **2019**. Update on the situation of *Anoplophora chinensis* in Italy. EPPO Report. Serv. 11.

EPPO. **2020a**. *Anoplophora chinensis* (ANOLCN)[Datasheet]| EPPO Global Database. (https://gd.eppo.int/taxon/ANOLCN/datasheet).

EPPO. **2020b**. Eradication of *Anoplophora chinensis* from Switzerland. EPPO Report. Serv. 1.

EPPO. **2020c**. Update on the situation of *Anoplophora chinensis* in Italy. EPPO Report. Serv. 10.

Eyre, D., and J. Barbrook. **2021**. The eradication of Asian Longhorned Beetle at Paddock Wood, UK. CABI Agric. Biosci. 2: 12.

Haack, R. A., F. Hérard, J. Sun, and J. J. Turgeon. **2010**. Managing invasive populations of Asian Longhorned Beetle and Citrus Longhorned Beetle: a worldwide perspective. Annu. Rev. Entomol. 55: 521–546.

Hansen, L., T. Xu, J. Wickham, Y. Chen, D. Hao, L. M. Hanks, J. G. Millar, and S. A. Teale. **2015**. Identification of a male-produced pheromone component of the Citrus Longhorned Beetle, *Anoplophora chinensis*. PLoS One. 10: e0134358.

Hoppe, B., G. Schrader, M. Kinkar, and S. Vos. **2019**. Pest survey card on *Anoplophora chinensis*. EFSA Support. Publ. 16.

Lingafelter, S. W., and E. R. Hoebeke. **2002**. Revision of the genus *Anoplophora* (Coleoptera: Cerambycidae). Entomological Society of Washington, Washington DC.

Mitomi, M., E. Kuroda, and H. Okamoto. **1990**. Ecological study of the white-spotted longicorn beetle, *Anoplophora malasiaca* Thomson (Coleoptera: Cerambycidae). Investigation of adult emergence holes in citrus orchards in Kagawa Prefecture. Japanese J. Appl. Entomol. Zool. 34: 7–13.

Nehme, M. E., M. A. Keena, A. Zhang, T. C. Baker, Z. Xu, and K. Hoover. **2010**. Evaluating the use of male-produced pheromone components and plant volatiles in two trap designs to monitor *Anoplophora glabripennis*. Environ. Entomol. 39: 169–176.

Nehme, M. E., R. T. Trotter, M. A. Keena, C. McFarland, J. Coop, H. M. Hull-Sanders, P. Meng, C. M. De Moraes, M. C. Mescher, and K. Hoover. **2014**. Development and evaluation of a trapping system for *Anoplophora glabripennis* (Coleoptera: Cerambycidae) in the United States. Environ. Entomol. 43: 1034–1044.

Sjöman, H., J. Östberg, and J. Nilsson. **2014**. Review of host trees for the wood-boring pests *Anoplophora glabripennis* and *Anoplophora chinensis*: an urban forest perspective. Arboric. Urban For. 40: 143–164.

Sweeney, J., C. Hughes, V. Webster, C. Kostanowicz, R. Webster, P. Mayo, and J. D. Allison. **2020**. Impact of horizontal edge–interior and vertical canopy–understory gradients on the abundance and diversity of bark and woodboring beetles in survey traps. Insects. 11: 573.

Van Der Gaag, D. J., G. Sinatra, P. F. Roversi, A. Loomans, F. Hérard, and A. Vukadin. **2010**.

Evaluation of eradication measures against *Anoplophora chinensis* in early stage infestations in Europe. EPPO Bull. 40: 176–187.

Zar, J. H. 1999. Biostatistical Analysis., 4th edn.(Prentice-Hall: Englewood Cliffs, NJ.).

SECTION 3

CONTAINMENT AND ERADICATION PROGRAMS

Chapter 6

Dispersal and colonization-risk of the Walnut Twig Beetle, *Pityophthorus juglandis*, in southern Europe

Paper published as: Marchioro M. & Faccoli M. (2021). Dispersal and colonization-risk of the Walnut Twig Beetle, *Pityophthorus juglandis*, in southern Europe. Journal of Pest Science.

Journal of Pest Science

Original Paper | Open Access | Published: 11 April 2021

Dispersal and colonization risk of the Walnut Twig Beetle, *Pityophthorus juglandis*, in southern Europe

Matteo Marchioro & Massimo Faccoli

Journal of Pest Science (2021)

Abstract

The Walnut Twig Beetle (WTB), *Pityophthorus juglandis* Blackman, is a small bark beetle native to Mexico and South-Western USA recorded for the first time in Europe (NE Italy) in 2013. WTB attacks walnut (*Juglans* spp.) and wingnut trees (*Pterocarya* spp.) and is the vector of *Geosmithia morbida* Kolarík et al., a pathogen causing the Thousand Cankers Disease (TCD). WTB and TCD represent a serious threat for walnut orchards in Europe. Spatio-temporal data of the WTB-TCD infestations recorded from an 8-year long (2013-2020) monitoring conducted in 106 walnut orchards of NE Italy, were used to develop a model in order to analyse: (i) the effective dispersal capacity of WTB, (ii) the factors affecting dispersal, and (iii) the colonization-risk of healthy walnut orchards. We registered a mean annual dispersal of 9.4 km, with peaks of about 40 km. Pest dispersal is affected by distance of suitable hosts from the nearest infested site, number of walnut orchards in the surroundings (both infested and healthy), orchard size, and walnut species in the orchard. Using the model, it was also possible to calculate the colonization-risk of a specific walnut orchard according to its characteristics showing, for instance, that a medium size (5,000 trees) black walnut orchard located at 25 km from the nearest infested orchard has an infestation risk of about 50% of probability.

Introduction

International trade, moving large amounts of goods globally, leads to inadvertent global translocation of invasive insects (Seebens et al. 2018). Wood-boring beetles, like bark and ambrosia beetles (Scolytinae), longhorn beetles (Cerambycidae) and jewel beetles (Buprestidae), are one of the most successful guilds of alien species invasive of forest habitats (Eyre and Haack 2017). Bark and ambrosia beetles, especially, represent a serious threat to European forests and wood orchards, due both to the ease with which they are transported all over the world inside wood-packaging materials, timber, and woody plants (Meurisse et al. 2019), and the plant pathogens they may carry (Kirisits 2007, Six 2012, Ploetz et al. 2013, Carrillo et al. 2014, Malacrinò et al. 2017). Every year new alien species of bark and ambrosia beetles are recorded in Europe (Kirkendall and Faccoli 2010, Barnouin et al. 2020). An example is the Walnut Twig Beetle (WTB), *Pityophthorus juglandis* Blackman (Coleoptera: Curculionidae, Scolytinae), found for the first time in Europe in 2013 in North-Eastern Italy (Montecchio and Faccoli 2014).

Pityophthorus juglandis is a small (1.5-1.9 mm long) bark beetle native to Mexico and southwest USA (Wood and Bright 1992). Adults colonize and reproduce in the phloem of walnut (*Juglans* spp.) and wingnut trees (*Pterocarya* spp.). In spring, with a mean air temperature of about 18 °C, overwintering adults disperse and infest new hosts mainly at the base of twigs, although large branches and the warmer side of the trunk can also be attacked (Newton and Fowler 2009). In southern Europe, the first-generation development takes about 7-9 weeks with new adult emergence occurring at mid-end July, whereas the second generation is complete at the end of September (Faccoli et al. 2016). In both North America and Europe, *P. juglandis* is the vector of *Geosmithia morbida* Kolarík, an aggressive pathogen causing the Thousand Cankers Disease (TCD) (Kolařík et al. 2011). The conidia of the pathogen are carried on the elytra of WTB adults (Newton and Fowler 2009) and introduced into the host plant during beetle bark colonization (Tisserat et al. 2009).

P. juglandis is native to Mexico, New Mexico, Arizona and California (Bright and Stark 1973, Wood and Bright 1992). Nevertheless, since 2009 WTB and the associated pathogen *G.*

morbida have spread to north-western US states (Colorado, Idaho, Oregon, Utah and Washington) and the east coast, reaching Tennessee (2010), Nevada (2011), Virginia (2011), Pennsylvania (2011), Ohio (2012), North Carolina (2012), Indiana (2013) and Maryland (2013) (Cranshaw 2011, Seybold et al. 2012, 2013, Wiggins et al. 2014, Rugman-Jones et al. 2015).

In 2013 the species crossed the Atlantic Ocean reaching Europe, where both *P. juglandis* and *G. morbida* were found in a black walnut orchard in Vicenza province (Veneto region, north-eastern Italy) (Montecchio and Faccoli 2014, Montecchio et al. 2014). During a preliminary visual survey conducted immediately in the surrounding walnut orchards, a further four infested sites (*i.e.*, orchards) were found in the same province (Faccoli et al. 2016). The following year (2014), a second more extensive survey was done in the whole Veneto region (where the first finding was recorded) and in the two west and east bordering regions (Lombardy and Friuli-Venezia Giulia), to check the real distribution of WTB in NE Italy. During the second survey, the pest was found in other nine orchards, including one located in Lombardy (Faccoli et al. 2016). Since 2013, WTB has spread in many other regions of central-northern Italy, and to date the species has been recorded in Friuli-Venezia Giulia (2015) (Montecchio et al. 2016), Piedmont (2015) (Bosio and Cooke-McEwen 2018), Tuscany (2018) (Moricca et al. 2019), and Emilia Romagna (2019) (EPPO 2019). The dispersal of *P. juglandis* and its associated pathogen *G. morbida* in Italy and Europe is considered a serious threat for walnut orchards (mainly *J. nigra*) largely used in the last decades for wood production (Eichhorn et al. 2006). Massive attacks of this pest may have great impacts on the economy and landscape of many areas of southern Europe, as occurred in the USA (Leslie et al. 2009, Seybold et al. 2019).

Since its discovery, and in less than 6 years, WTB has colonized almost 13 American States and reached another continent, representing one of the fastest and most successful invaders among forest insect species. Nevertheless, little is known about the active and passive dispersal capacity of *P. juglandis*. In a recent laboratory experiment, Kees et al. (2017) found that the maximum total active flight distance covered by WTB adults was about 3.6 km in 24 hours, but mean and median distances flown by beetles were much lower (about 372 m and 158 m respectively). However, the contribution of natural dispersal (for instance by wind) to the insect's spread across the western United States remains unknown (Cranshaw 2011). A recent study carried out on more than 60 American populations of WTB demonstrated that the expansion of the insect is, in part, facilitated by anthropogenic movement of infested wood (Rugman-Jones et al. 2015), which probably also allowed the pest to reach Europe.

In this study, we present the results of the WTB survey conducted for eight consecutive years in NE Italy (2013-2020) since the first discovery of the pest in Europe. The aim of the study is to investigate the annual increase in invaded range of *P. juglandis* based on data from the yearly survey, and to present a descriptive model assessing the spreading capacity of WTB populations according to specific environmental parameters. In particular, we hypothesize that the dispersal of WTB is affected by the presence and distribution of walnut orchards, by the walnut species, and by the orchard size, as bigger suitable sites are easier to find and may promote greater proliferation of the pest. Based on the historical epidemiological data collected in NE Italy during the last 8-year survey, this model is intended to quantify a) the real dispersal capacity of the insect, b) the factors affecting dispersal, and c) the risk of infestation (probability) of healthy walnut orchards in a given area.

Materials and Methods

Monitored areas and survey protocol

The survey of WTB occurrence was conducted in the Veneto region (NE Italy) since 2013 when the pest and associated pathogen were found for the first time at Bressanvido (province of Vicenza). In the year of discovery, a first inspection was made on 19 walnut orchards within about 10 km of the infested area. Since 2014, a more extensive survey has been conducted in all the provinces of the Veneto region where walnut orchards occur. Initially, the customer lists of the major forest tree nurseries of the region were used to identify all the main walnut orchards created in Veneto in the previous 15 years. Then, more than 100 walnut orchards identified from the lists and other public parks and private gardens with walnut trees were monitored annually by visual inspections looking for symptoms of insect colonization or disease infection according to the regional protocol for detection of alien species (Montecchio et al. 2016). In addition, 40-50 orchards (depending on the year) homogeneously distributed in the region were monitored annually also with a 12-black-multifunnel trap (Econex, Murcia, Spain) baited with a 400 mg dispenser of a pheromone specific for WTB (3-methyl-2-buten-1-ol; Contech Enterprises, Delta, BC, Canada; Seybold et al. 2013) (Table 1). The release rate was about 1 mg/day with a temperature of 20 °C and generally doubled with every 5 °C rise in temperature. Average daily temperature in the study area in spring-summer is about 25 °C, so the estimated duration of the blend was about 200 days. One trap per site was set up in the last weeks of July, in the middle of the walnut orchard at about 2 m above the ground, fixed to a tree branch and emptied every second week. Traps were removed in the last week of October, 80-90 days later, assuring that they were active during the main emergence period and dispersal of adults of the second generation (Faccoli et al. 2016). Orchards where the presence of WTB was confirmed by captures with the traps or visual inspection were then excluded from the survey in the following years and replaced with new uninfected ones.

Table 1 Sites monitored with traps: total number of sites monitored in each year and number of sites for each province monitored.

Year	Monitored sites	Sites per province					
		Vicenza	Padova	Rovigo	Venezia	Treviso	Verona
2013	19	13	6	-	-	-	-
2014	17	3	4	1	3	4	2
2015	50	4	12	8	11	7	8
2016	51	9	12	8	11	7	4
2017	50	4	12	9	11	7	7
2018	50	4	13	9	11	7	6
2019	50	4	11	9	10	13	3
2020	40	2	18	4	10	4	2

Dataset

The dataset used for the analyses was obtained from 106 sites (walnut orchards, public parks and private gardens) visually inspected or monitored with traps in Veneto since 2013 (Table C1). Data collected through different detection methods were handled in the same way, because they only served to ascertain the presence of WTB in the site. Sites from other Italian regions where the WTB presence was recorded in the following years were not included in the dataset because they were isolated points not belonging to a widespread and intensive monitoring network, and were recorded only following an accidental finding. For each monitored site, identified by a unique code, the beginning of monitoring, spatial coordinates (UTM zone 32N), number of trees, species composition (*J. nigra*, *J. regia*, or "mixed"), and year of first attack (from that year onwards the site was considered infected) were reported (Table C1). The dataset was then updated year-by-year with information on new sites considered infested by WTB according to trap captures and visually detected symptoms. WTB has been added in the EPPO A2 Quarantine Pest list just in 2019 (EPPO 2021) and, to date, no clear-cut or pesticide treatments were performed in infested sites. For this reason, an "infected site" of our database was considered infected for all subsequent years.

Statistical analysis

A probabilistic model was constructed through the analysis of the binomial (infested or not infested) individual site's data using a generalized linear model (GLM) with a probit link function (McCullagh and Nelder 1989). The independent variables included in the model tested were: attack index (*ati*), available-host index (*ahi*), distance to nearest attacked site (*dna*), orchard size (number of trees in the site), and walnut species (*J. nigra*, *J. regia*, or mixed orchards). The dependent variable was the probability (P) that an orchard or group of trees can be attacked by WTB as a function of its characteristics. The dependent variable and the first four independent variables (*ati*, *ahi*, *dna*, orchard size) were considered continuous, whereas walnut species was discrete variable.

The attack index (*ati*) and the available-host index (*ahi*) were introduced by Favaro et al. (2015) in a similar study on the dispersal of invasive populations of the Asian longhorn beetle, *Anoplophora glabripennis*. The attack index for site i considers how many sites attacked in the previous years by WTB occur nearby, and is calculated as:

$$ati_i = \sum\left[\exp\left(-\, d_{ij}/c_a\right)\right] \qquad (1)$$

where d_{ij} is the distance between site *i* and each site (*j*) attacked in the previous years, whereas c_a is a constant (see below).

The available-host index for site i, instead, considers how many available sites (site with available uninfested hosts) occur nearby, and is calculated as:

$$ahi_i = \sum\left[\exp\left(-\, d_{ij}/c_h\right)\right] \qquad (2)$$

where d_{ij} is the distance between site *i* and each monitored non-attacked site (*j*) in the current year, and c_h is a constant. The two indices ati and ahi are calculated for each site and year, for a range of values of c. The constants c_a and c_h are instead estimated using an iterative approach as they represent a threshold distance: all distances d_{ij} greater than c have little influence on the final value of the index; the terms related to shorter distances will strongly affect the final value. In order to obtain a biologically plausible model, the constants c_a and c_h were set on the basis of the displacement capacity of the insect. Because there is currently no precise information about *P. juglandis* dispersal, we set a surrogate limit value of 80 km, corresponding to the maximum distance covered actively by the six-toothed bark beetle *Pityogenes chalcographus*, a similar size bark beetle (Nilssen 1984). This distance was considered a feasible estimate also for the WTB by the expert working group who drew up the EPPO report for *P. juglandis* (EPPO 2015). The best values of c_a and c_h were chosen using Akaike's information criterion (AIC) (Akaike 1974), choosing the couple that minimizes the AIC index. Initially, all possible pairs of c_a and c_h were tested in the model in a range from 1 to 80,000 meters (the threshold-value presented), with intervals of 2,000 meters. Subsequently, the model was gradually refined by narrowing the range of the two constants around the best value, and reducing the dispersal interval.

All the independent variables and their interactions were considered in the preliminary model. During each iteration the least significant variable or interaction, evaluated on the basis of the significance level, was removed from the model. The process continued until all non-significant variables were eliminated and all variables left were significant at 1% (P<0.01). Data were analyzed using the GLM routine in R software (R Core Team 2019).

Results

Of the 106 sites monitored over the 8-year survey (2013-2020), 44 walnut orchards (41.5%) were found infested by WTB (Fig. 1). Among the attacked sites, 34 (77%) were black walnut orchards (corresponding to 45% of all black walnut monitored orchards), 6 (14%) were English walnut orchards (corresponding to 29% of all English walnut monitored orchards), and 4 (9%) had a mixed composition. The median size of infested orchards was 92 trees and 25% of attacked sites had at least 160 trees. The year-by-year evolution of the survey, with sites monitored and sites found infected, is shown in Figure 2.

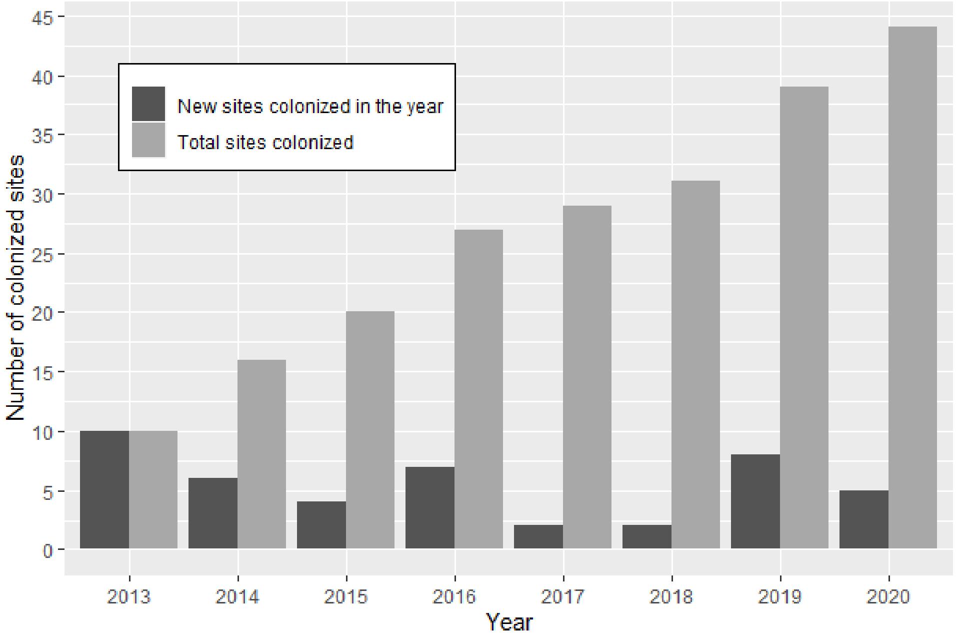

Fig. 1 Year-by-year number of new colonized sites and total amount of colonized sites.

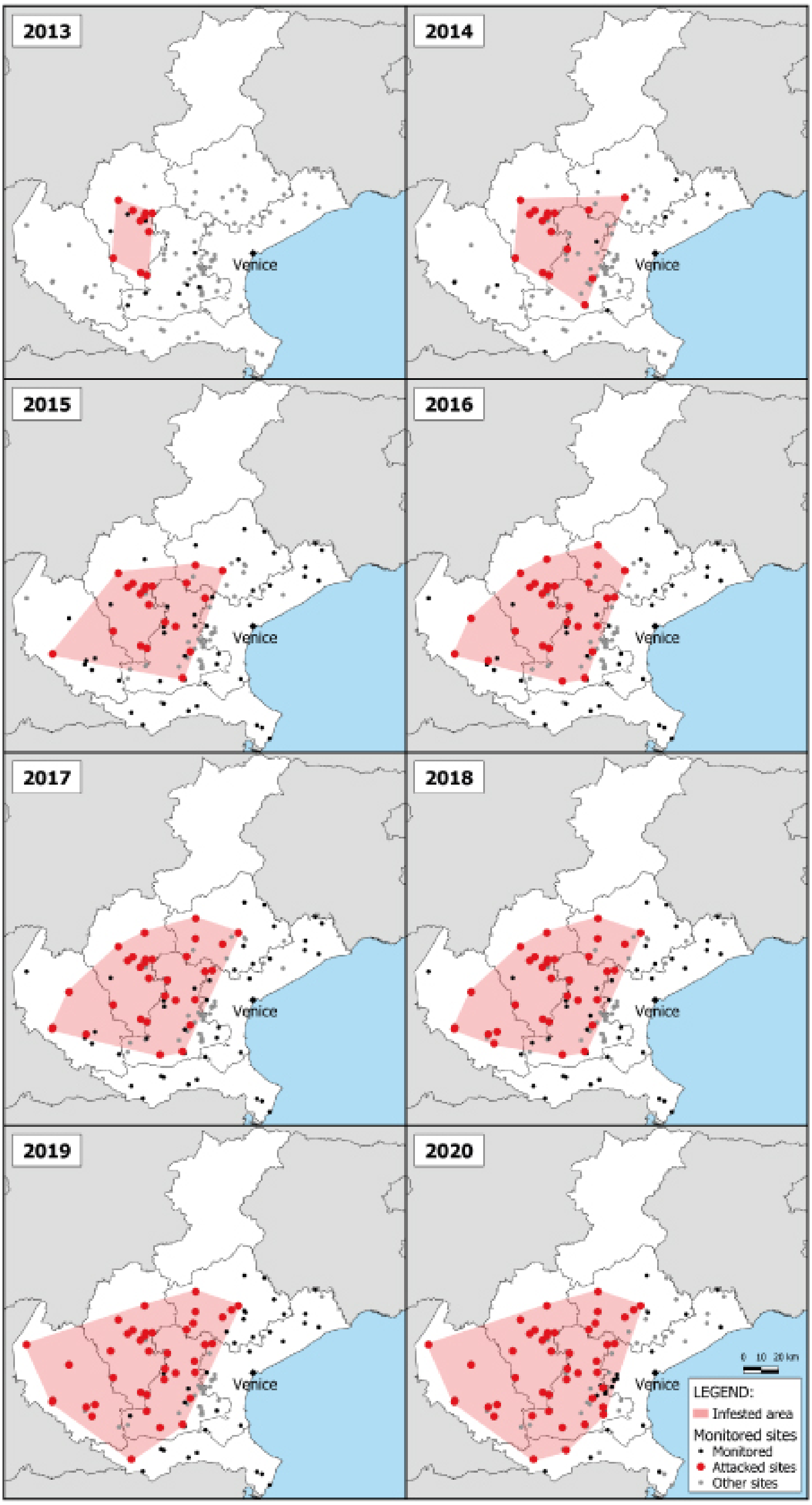

Fig. 2 Year-by-year evolution of the WTB infestation in the Veneto region. Maps show the sites attacked (bigger red dots), monitored with traps (smaller black dots), monitored visually (smaller grey dots), and the approximate spread area of the pest (red shape).

The cumulative distribution of *dna* covered annually by WTB indicates a non-linear trend: the minimum *dna* registered is about 830 m, whereas the maximum is 40.91 km, with a mean distance of 9.43±0.49 km. However, 25% of newly infested sites were located at least 12.89 km from the closest infested ones (Fig. 3).

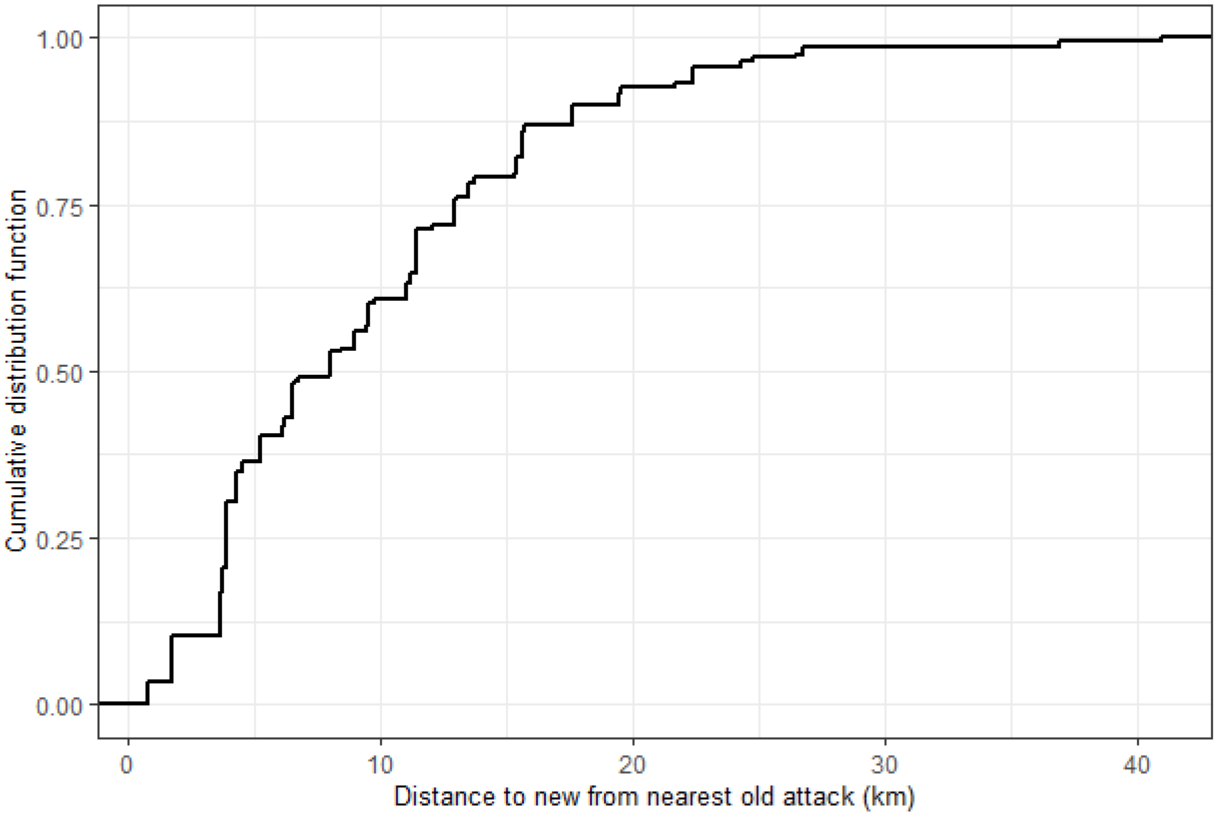

Fig. 3 Cumulative distribution function of minimum distance to each new infested orchard from the nearest infested orchard recorded in the previous year.

All variables considered were significant in the model, even if all the interactions considered were rejected because they did not produce significant effects. The final GLM model, therefore, is the following:

$$P \sim ati + ahi + dna + host\ species + n\_trees \qquad (3)$$

The estimated constants c_a and c_h for the attack (*ati*) and available-host indexes (*ahi*) are 21 km and 14 km, respectively. The final GLM fit summary statistic for the model (equation 3) is reported in Table 2.

Table 2 Summary of GLM fit for the model (3).

Variable	Estimate	SE	z-value	P
Intercept	1.955E-01	3.017E-01	0.648	0.52
Ati	1.957E-01	3.971E-02	4.927	8.33E-07
Ahi	-1.086E-01	1.348E-02	-8.053	8.07E-16
Dna	-3.949E-02	8.679E-03	-4.550	5.36E-06
species *J. regia*	-1.281	2.631E-01	-4.868	1.13E-06
species mixed	-6.088E-01	2.016E-01	-3.020	2.53E-03
n_trees	1.549E-04	5.527E-05	2.803	5.06E-03

Using the model of equation (3) it is possible to predict the attack risk of a healthy walnut orchard as a function of its characteristics and distance from the closest attacked site (Fig. 4). According to the model, the probability of attack of a walnut orchard increases with its size (increasing number of trees) (z-value=2.803; P<0.001), but decreases inversely to the distance from the closest infested site (source orchard) (z-value=-4.550; P<0.001). Moreover, the risk of being infested is higher in orchards composed of *J. nigra* (Fig. 4a) rather than *J. regia* (z-value=-4.868; P<0.001; Fig. 4b), whereas it has an intermediate value for mixed orchards, but still significantly lower than black walnut (z-value=-3.020; P<0.001).

Discussion

The study analyzes spatial and temporal data related to the dispersal of WTB in NE Italy since the species discovery in 2013. Our analysis shows that dispersal of WTB in this region reached up to 41 km in one year, although the probability of new infestations at a given distance depends on the number of walnut orchards occurring around the healthy ones (*i.e.*, attack index and available-host index, respectively), its size (*i.e.*, the number of trees), its dominant walnut species (*J. nigra* or *J. regia*), and its distance from the site attacked during the previous year.

The model shows WTB preference for black walnut (*J. nigra*) over English walnut (*J. regia*), consistent with the literature (Newton and Fowler 2009, Wilstermann et al. 2020). In the sites monitored during this study, more than 45% of black walnut orchards were attacked by WTB, compared to 29% of English walnut orchards. Even in mixed composition sites, the risk of attack was significantly lower than in pure black walnut orchards, indicating a greater preference of the pest for this species. Black and English walnuts are largely cultivated in Europe both for nuts and noble hardwood timber production (EPPO 2015), and they can be found in most of Europe, apart from northern regions (de Rigo et al. 2016). Moreover, walnut trees are also widely spread as ornamental plants in parks, gardens and street tree lines (Eichhorn et al. 2006). This capillary presence of host plants could therefore facilitate the spread and stabilization of WTB over much of the continent.

Our study clearly shows that the risk of WTB attack increases with orchard size. An orchard of 10,000 black walnuts should be easy to find by dispersing beetles, showing a risk of attack reaching almost 100% when infested sites occur in the immediate proximity, and remaining high (about 50%) even at distances greater than 40 km; instead for a small group of trees the risk of attack is just over 50% even for very short distances (Fig. 4a). For English walnut, the risk of attack is lower but not negligible (Fig. 4b). The size of the source orchard may also have a crucial role moderating insect dispersal. Faccoli et al. (2016) report that an infestation in a walnut orchard can persist for many years, with a progressive increase of the population density of *P. juglandis* that will then disperse to other nearby walnut orchards looking for new host-trees. Thus, a large walnut orchard can sustain a longer infestation with a large WTB population and a higher dispersal probability.

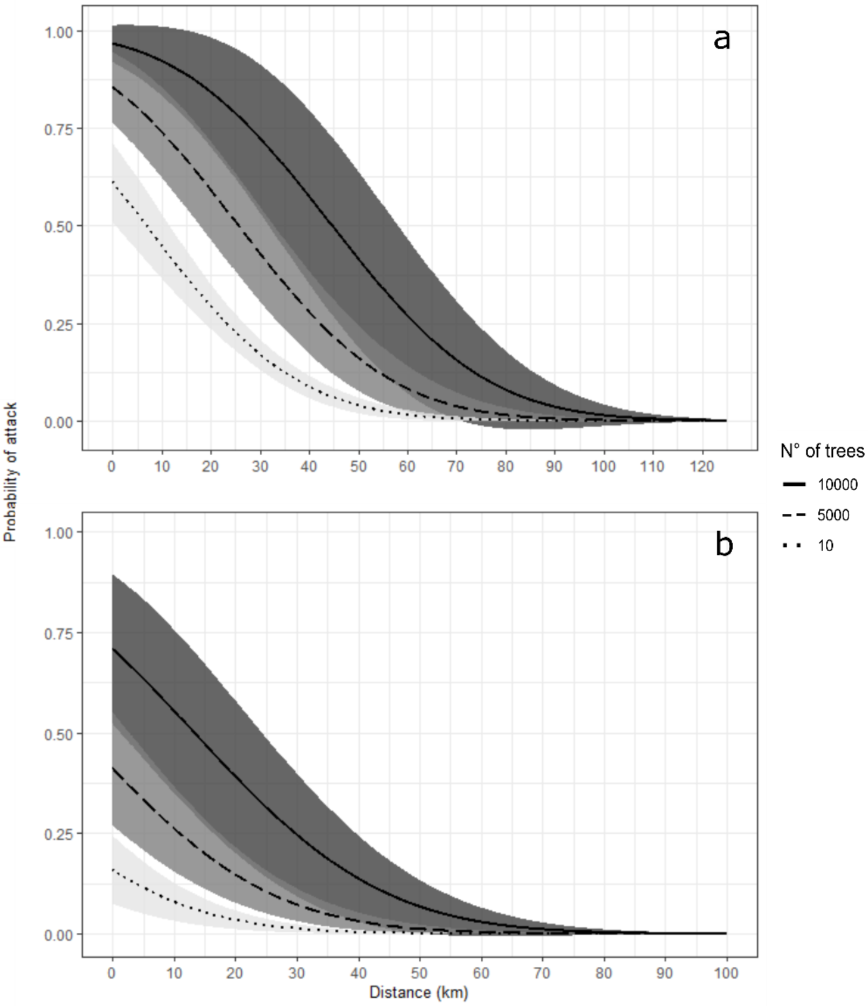

Fig. 4: Probability of attack for a black walnut (*J. nigra*) (a) and English walnut (*J. regia*) (b) plantation as a function of its size (increasing number of trees) and distance from the closest infested site (dna), predicted by the model in equation (3) with the parameter estimates in Table 2.

Another interesting result regards the decrease of infestation risk with increasing availability of potential host trees, *i.e.* high available-host index (ahi). This means that a site in an area with a large number of other host orchards or groups of trees has a lower probability of being infested than an isolated one. This result can be explained by the colonization strategies of bark beetles. Typically, bark beetles feed and breed in recently dead or severely weakened host trees which are located through specific plant volatiles released by dying trees; nevertheless, when no suitable hosts are available, the insects may also attack healthy hosts massively (using aggregation pheromones), to overcome the plant's defenses (Wood 1982, Kausrud et al. 2011). Walnut orchards are usually maintained in healthy and vigorous conditions to maximize wood and nut production. In this context, a pest large dispersal would have little chance of colonization (with a few individuals attacking vigorous hosts); for this reason, even with many sites available in the area, the insect colonization remains concentrated only in one or a few orchards, in order to maximize the attack effectiveness. Moreover, with a small WTB population density, a greater number of available host sites reduces the risk of each one being colonized.

Finally, data collected during the survey highlight a high spread of WTB, with dispersal capacity of over 40 km per year (the longest distance between an attacked site and the closest infested one in the previous years) and with 75% of newly infested sites located at least 4 km away from the nearest source sites. Considering the results of Kees et al. (2017), which presented an active flight distance covered by WTB estimated at less than 2 km, additional factors other than active dispersal must be considered to explain such a spread of the pest. As previously observed by Seybold et al. (2012), a fundamental contribution to spread of beetle population is made by the human-mediated movement of walnut logs or wood products. Cases have been observed where even the wind has favored the dispersal of WTB (Cranshaw and Tisserat 2012), although this is not considered as the main dispersal mechanism of diffusion of this pest (EPPO 2015). Such a large active and passive dispersal capacity causes many problems for pest management. The containment measures currently applied in the Veneto region were based on the creation of a buffer zone with 2 km range beyond the infested area (Regione del Veneto 2014a, 2014b, 2015). According to our model, a 2 km buffer around the infested site corresponds to an infestation risk higher than 80% and 40% for medium size orchards (5,000 trees) of black and English walnut, respectively (Fig. 4). This remains, hence, a very high risk of colonization for walnuts growing also outside the buffer area, despite the considerable size of the buffer proposed. Adjustment of buffer zones, with an increase in radius, can be used in synergy with other techniques to achieve greater protection of still healthy orchards. An example are the recent works by Audley et al. (2020a,b) on the repellent effect of some semiochemicals against WTB: volatiles such as (R)-(+)-limonene, trans-conophthorin, (R)-(+)-verbenone and α-pinene can be used to repel the pest from the sites to be protected.

In conclusion, this model has been built using the data obtained from an eight-year long surveillance program of the main walnut orchards of Veneto region to explain the natural dispersal of WTB in NE Italy. Although some of the most important factors affecting WTB presence and its dispersal probability have been clarified (distance from nearest attacked site, attack index, available-host index, orchard size and tree species), the role of other variables - such as environmental conditions (e.g., dominant winds) and human-related activities (e.g., main trade routes, volume and type of potentially infected goods) - should be carefully considered to better understand the potential spread of this relatively new invasive pest across Europe and to establish effective containment and local eradication measures. Help in this regard may come from the recent work of Chen et al. (2020) presenting the effects of climatic variables (*i.e.*, precipitation, solar radiation, vapor pressure, air temperature, relative humidity, and wind speed) on the flight activity of WTB. Considering also the other Italian regions where WTB was found, since after its first record in 2013 the insect traveled over 320 km westwards reaching the Piedmont region in 2014 (Bosio and Cooke-McEwen 2018), and about 200 km southwards reaching the Tuscany region in 2018 (Moricca et al. 2019). These distances are incompatible with our results (320 km in one year to reach Piedmont) or extremely unlikely (a mean of 40 km per year to reach Tuscany), but several hypotheses can be explored. First, the human-mediated movement of infested materials can play a key role in the dispersal of WTB, allowing the insect to cover hundreds or thousands of kilometers in a few days. This dispersal pathway was well documented in the USA (Newton and Fowler 2009, Jacobi et al. 2012). Another possibility is that *P. juglandis* was already present in Italy long before the year of discovery (2013) and it was found only when an active and specific survey had been implemented. This would have given the insect more time to disperse slowly into new areas and regions. Nevertheless, although certainly possible, this hypothesis does not explain how the presence of such aggressive species as WTB and TCD was not detected earlier. Lastly, reports of the presence of *P. juglandis* in various regions of Italy, even far from the first record, may be due to several independent introductions. This latter hypothesis seems extremely probable and could be tested by performing specific genetic analyses to compare specimens collected from populations sampled in the Italian regions where the pest has been recorded in the last years.

Pityophthorus juglandis represents only one of the insect alien species recently introduced in Europe that attack Juglans species. The walnut husk fly, *Rhagoletis completa* Cresson (Diptera: Tephritidae), is a pest originating from North-America and introduced in Europe in the early 1990s (Duso and Dal Lago 2006, Verheggen et al. 2017). *Megaplatypus mutatus* (Chapuis) (Coleoptera: Curculionidae) is an ambrosia beetle native to South America (Wood 1993), accidentally introduced to Italy in 1998 (EPPO 2004), polyphagous on a wide range of forest and fruit tree species, including walnuts (Gonzalez-Audino et al. 2013). The black timber bark beetle, *Xylosandrus germanus* (Blandford) (Coleoptera: Curculionidae), is an ambrosia beetle native to Eastern Asia, Russian Far East and China introduced to both Europe (Groschke 1953) and USA, where is causing large damage to American walnut orchards (Weber and McPherson 1984). For the future protection of walnut orchards and to prevent further spreading of quarantine pests, phytosanitary measures for Juglans plants and their products are required (EPPO 2020) and specific and accurate monitoring of these environments have to be implemented.

Acknowledgements

We thank Iris Bernardinelli, Giovanni Zanini, Marco Vettorazzo, Tiziano Visigalli, Giovanni Bosio, Dino Calzavara, Giovanni Raffaelli, Riccardo Favaro, and Lars Wichmann for providing information and for their help during the field and laboratory work. We especially thank Alison Garside for proofreading the manuscript, the editor and four anonymous reviewers for their useful comments.

References

Akaike, H. 1974. A new look at the statistical model identification. IEEE Trans. Automat. Contr. 19: 716–723.

Audley, J. P., R. M. Bostock, and S. J. Seybold. 2020a. Trap assays of the Walnut Twig Beetle, *Pityophthorus juglandis* Blackman (Coleoptera: Curculionidae: Scolytinae), reveal an effective semiochemical repellent combination. Journal of Chemical Ecology. 46: 1047-1058.

Audley, J. P., P. Dallara, L. J. Nelson, S. M. Hamud, R. M. Bostock, and S. J. Seybold. 2020b. Trapping failure leads to discovery of potent semiochemical repellent for the Walnut Twig Beetle. Journal of Economic Entomology. 113: 2772-2784.

Barnouin, T., F. Soldati, A. Roques, M. Faccoli, L. R. Kirkendall, R. Mouttet, J.-B. Daubree, and T. Noblecourt. **2020**. Bark beetles and pinhole borers recently or newly introduced to France (Coleoptera: Curculionidae, Scolytinae and Platypodinae). Zootaxa. 4877: 51–74.

Bosio, G., and C. Cooke-McEwen. **2018**. Insects collected from wood infested with *Pityophthorus juglandis* Blackman (Coleoptera Curculionidae Scolytinae) in the Piemonte region, Northwestern Italy. Boll. della Soc. Entomol. Ital. 150: 21–30.

Bright, D. E., J., and R. W. Stark. **1973**. The bark and ambrosia beetles of California (Coleoptera: Scolytidae and Platypodidae). Bull. Calif. Insect Surv. 16.

Carrillo, D., R. E. Duncan, J. N. Ploetz, A. F. Campbell, R. C. Ploetz, and J. E. Peña. **2014**. Lateral transfer of a phytopathogenic symbiont among native and exotic ambrosia beetles. Plant Pathol. 63: 54–62.

Chen, Y., B. H. Aukema, and S. J. Seybold. 2020. The effects of weather on the flight of an invasive bark beetle, *Pityophthorus juglandis*. Insects. 11: 156.

Cranshaw, W. 2011. Recently recognized range extensions of the Walnut Twig Beetle, *Pityophthorus juglandis* Blackman (Coleoptera: Curculionidae: Scolytinae), in the Western United States. Coleopt. Bull. 65: 48–49.

Cranshaw, W., and N. Tisserat. **2012**. Questions and answers about thousand cankers disease of walnut. Color. State Univ. Fort Collins, CO. Available online http//goquestions_answers. pdf.

de Rigo, D., C. M. Enescu, T. Houston Durrant, W. Tinner, and G. Caudullo. **2016**. Juglans regia in Europe: distribution, habitat, usage and threats. *In* San-Miguel-Ayanz, J., de Rigo, D., Caudullo, G., Houston Durrant, T., Mauri, A. (eds.), Eur. Atlas For. Tree Species. Publ. Off.

EU, Luxembourg.

Duso, C., and G. Dal Lago. 2006. Life cycle, phenology and economic importance of the walnut husk fly *Rhagoletis completa* Cresson (Diptera: Tephritidae) in northern Italy. Ann. la Société Entomol. Fr. 42: 245-254.

Eichhorn, M. P., P. Paris, F. Herzog, L. D. Incoll, F. Liagre, K. Mantzanas, M. Mayus, G. Moreno, V. P. Papanastasis, D. J. Pilbeam, A. Pisanelli, and C. Dupraz. **2006**. Silvoarable systems in Europe – Past, present and future prospects. Agrofor. Syst. 67: 29–50.

European and Mediterranean Plant Protection Organization (EPPO). **2004**. EPPO Reporting Services no.4.

European and Mediterranean Plant Protection Organization (EPPO). **2015**. Pest Risk Analysis for Thousand cankers disease (*Geosmithia morbida* and *Pityophthorus juglandis*). Paris.

European and Mediterranean Plant Protection Organization (EPPO). **2019**. EPPO Reporting Services no.5 - Disease.

European and Mediterranean Plant Protection Organization (EPPO). **2020**. PM 8/12 (1) *Juglans*. EPPO Bulletin 50: 107-119.

European and Mediterranean Plant Protection Organization (EPPO). **2021**. *Pityophthorus juglandis*. EPPO datasheets on pests recommended for regulation. Available online. https://gd.eppo.int

Eyre, D., and R. A. Haack. **2017**. Invasive cerambycid pests and biosecurity measures, pp. 563–618. *In* Wang, Q. (ed.), Cerambycidae World Biol. Pest Manag. CRC Press, Boca Raton, FL.

Faccoli, M., M. Simonato, and D. Rassati. **2016**. Life history and geographical distribution of the walnut twig beetle, Pityophthorus juglandis (Coleoptera: Scolytinae), in southern Europe. J. Appl. Entomol. 140: 697–705.

Favaro, R., L. Wichmann, H. P. Ravn, and M. Faccoli. **2015**. Spatial spread and infestation risk assessment in the Asian longhorned beetle, Anoplophora glabripennis. Entomol. Exp. Appl. 155: 95–101.

Jacobi, W. R., J. G. Hardin, B. A. Goodrich, and C. M. Cleaver. **2012**. Retail firewood can transport live tree pests. J. Econ. Entomol. 105: 1645–1658.

Gonzalez-Audino, P., R. Griffo, P. Gatti, G. Allegro, and E. Zerba. 2013. Pheromone detection of the introduced forest pest *Megaplatypus mutatus* (*=Platypus mutatus*) (Chapuis)

(Platypodinae, Curculionidae) in Italy. Agrofor. Syst. 87: 109–115.

Groschke, F. 1953. Der "schwarze nutzholzborkenkäfer", *Xylosandrus germanus* Blandf., ein neuer schädling in Deutschland. Z. Angew. Entomol. 34: 297–302.

Kausrud, K. L., J.-C. Grégoire, O. Skarpaas, N. Erbilgin, M. Gilbert, B. Økland, and N. C. Stenseth. **2011**. Trees wanted—dead or alive! Host selection and population dynamics in tree-killing bark beetles. PLoS One. 6: e18274.

Kees, A. M., A. R. Hefty, R. C. Venette, S. J. Seybold, and B. H. Aukema. **2017**. Flight capacity of the Walnut Twig Beetle (Coleoptera: Scolytidae) on a laboratory flight mill. Environ. Entomol. 46: 633–641.

Kirisits, T. 2007. Fungal associates of european bark beetles with special emphasis on the ophiostomatoid fungi, pp. 181–236. *In* Bark wood boring insects living trees Eur. a Synth. Springer Netherlands.

Kirkendall, L., and M. Faccoli. **2010**. Bark beetles and pinhole borers (Curculionidae, Scolytinae, Platypodinae) alien to Europe. Zookeys. 56: 227–251.

Kolařík, M., E. Freeland, C. Utley, and N. Tisserat. **2011**. *Geosmithia morbida* sp. nov., a new phytopathogenic species living in symbiosis with the walnut twig beetle (*Pityophthorus juglandis*) on Juglans in USA. Mycologia. 103: 325–332.

Leslie, C. A., S. J. Seybold, A. D. Graves, W. Cranshaw, and N. Tisserat. **2009**. Potential impacts of thousand cankers disease on commercial walnut production and walnut germplasm conservation, pp. 431–434. *In* VI Int. Walnut Symp. 861.

Malacrinò, A., D. Rassati, L. Schena, R. Mehzabin, A. Battisti, and V. Palmeri. **2017**. Fungal communities associated with bark and ambrosia beetles trapped at international harbours. Fungal Ecol. 28: 44–52.

McCullagh, P., and J. Nelder. **1989**. Generalized Linear Models, 2nd editio. ed. Chapman and Hall, London, UK.

Meurisse, N., D. Rassati, B. P. Hurley, E. G. Brockerhoff, and R. A. Haack. **2019**. Common pathways by which non-native forest insects move internationally and domestically. J. Pest Sci. (2004). 92: 13–27.

Montecchio, L., and M. Faccoli. **2014**. First record of Thousand Cankers Disease *Geosmithia morbida* and Walnut Twig Beetle *Pityophthorus juglandis* on *Juglans nigra* in Europe. Plant

Dis. 98: 696–696.

Montecchio, L., G. Fanchin, M. Simonato, and M. Faccoli. **2014**. First record of thousand cankers disease fungal pathogen *Geosmithia morbida* and walnut twig beetle *Pityophthorus juglandis* on *Juglans regia* in Europe. Plant Dis. 98: 1445.

Montecchio, L., M. Vettorazzo, and M. Faccoli. **2016**. Thousand cankers disease in Europe: an overview. EPPO Bull. 46: 335–340.

Moricca, S., M. Bracalini, A. Benigno, B. Ginetti, F. Pelleri, and T. Panzavolta. **2019**. Thousand Cankers Disease caused by *Geosmithia morbida* and its insect vector *Pityophthorus juglandis* first reported on *Juglans nigra* in Tuscany, Central Italy. Plant Dis. 103: 369–369.

Newton, L., and G. Fowler. **2009**. Pathway assessment: *Geosmithia* sp. and *Pityophthorus juglandis* Blackman movement from the western into the eastern United States. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Washington, D.C.

Nilssen, A. C. 1984. Long-range aerial dispersal of bark beetles and bark weevils (Coleoptera, Scolytidae and Curculionidae) in northern Finland. Ann. Entomol. Fenn. 50: 37–42.

Ploetz, R. C., J. Hulcr, M. J. Wingfield, and Z. W. de Beer. **2013**. Destructive tree diseases associated with ambrosia and bark beetles: black swan events in tree pathology? Plant Dis. 97: 856–872.

R Core Team. **2019**. R version 3.6.1: A language and environment for statistical computing. R Found. Stat. Comput. Vienna, Austria. URL https//www. R-project. org.

Regione del Veneto. 2014a. Decreto del dirigente del settore servizi fitosanitari n. 30 del 14 agosto 2014. Misure fitosanitarie di controllo ed eradicazione di Geosmithia morbida in Regione Veneto. Bur n. 83 del 26/08/2014.

Regione del Veneto. 2014b. Decreto n. 43 del 6 novembre 2014. Misure fitosanitarie di controllo di Geosmithia morbida in Regione Veneto. Aggiornamento della zona delimitata

Regione del Veneto. 2015. Decreto n. 08 del 6 Febbraio 2015. Misure fitosanitarie di controllo di Geosmithia morbida in Regione Veneto. Aggiornamento della zona delimitata.

Rugman-Jones, P. F., S. J. Seybold, A. D. Graves, and R. Stouthamer. **2015**. Phylogeography of the Walnut Twig Beetle, *Pityophthorus juglandis*, the vector of Thousand Cankers Disease in North American walnut trees. PLoS One. 10: e0118264.

Seebens, H., T. M. Blackburn, E. E. Dyer, P. Genovesi, P. E. Hulme, J. M. Jeschke, S. Pagad,

P. Pyšek, M. Winter, M. Arianoutsou, S. Bacher, B. Blasius, G. Brundu, C. Capinha, L. Celesti-Grapow, W. Dawson, S. Dullinger, N. Fuentes, H. Jäger, J. Kartesz, M. Kenis, H. Kreft, I. Kühn, B. Lenzner, A. Liebhold, A. Mosena, D. Moser, M. Nishino, D. Pearman, J. Pergl, W. Rabitsch, J. Rojas-Sandoval, A. Roques, S. Rorke, S. Rossinelli, H. E. Roy, R. Scalera, S. Schindler, K. Štajerová, B. Tokarska-Guzik, M. van Kleunen, K. Walker, P. Weigelt, T. Yamanaka, and F. Essl. **2017**. No saturation in the accumulation of alien species worldwide. Nat. Commun. 8: 14435.

Seybold, S. J., T. W. Coleman, P. L. Dallara, N. L. Dart, A. D. Graves, L. A. Pederson, and S.-E. Spichiger. **2012**. Recent collecting reveals new state records and geographic extremes in the distribution of the walnut twig beetle, *Pityophthorus juglandis* Blackman (Coleoptera: Scolytidae), in the United States. Pan-Pac. Entomol. 88: 277–280.

Seybold, S. J., D. Haugen, J. O'Brien, and A. D. Graves. **2013**. Thousand cankers disease. USDA Forest Service, Northeastern Area State and Private Forestry Pest Alert. NA-PR-02-10, originally published May 2010, reprinted Aug. 2010, Oct. 2011 ….

Seybold, S. J., W. E. Klingeman, S. M. Hishinuma, T. W. Coleman, and A. D. Graves. **2019**. Status and impact of Walnut Twig Beetle in urban forest, orchard, and native forest ecosystems. J. For. 117: 152–163.

Six, D. L. 2012. Ecological and evolutionary determinants of bark beetle - fungus symbioses. Insects. 3: 339–366.

Tisserat, N., W. Cranshaw, D. Leatherman, C. Utley, and K. Alexander. **2009**. Black Walnut mortality in Colorado caused by the Walnut Twig Beetle and Thousand Cankers Disease. Plant Heal. Prog. 10: 10.

Verheggen, F., A. Verhaeghe, P. Giordanengo, X. Tassus, and A. Escobar-Gutiérrez. 2017. Walnut husk fly, *Rhagoletis completa* (Diptera: Tephritidae), invades Europe: invasion potential and control strategies. Appl. Entomol. Zool. 52: 1-7.

Weber, B. C., and J. E. McPherson. 1984. Attack on Black Walnut trees by the ambrosia beetle *Xylosandrus germanus* (Coleoptera: Scolytidae). Forest Science 30: 864–870.

Wiggins, G., J. Grant, P. Lambdin, P. Merten, K. Nix, D. Hadziabdic, and M. Windham. **2014**. Discovery of Walnut Twig Beetle, *Pityophthorus juglandis*, associated with forested Black Walnut, *Juglans nigra*, in the Eastern U.S. Forests. 5: 1185–1193.

Wilstermann, A., B. Hoppe, G. Schrader, A. Delbianco, and S. Vos. **2020**. Pest survey card on

Geosmithia morbida and its vector *Pityophthorus juglandis*. EFSA Support. Publ. 17.

Wood, D. L. 1982. The role of pheromones, kairomones, and allomones in the host selection and colonization behavior of bark beetles. Annu. Rev. Entomol. 27: 411–446.

Wood, S. L., and D. E. Bright. **1992**. A catalog of Scolytidae and Platypodidae (Coleoptera), Part 2: taxonomic index. Gt. Basin Natur. Mem. 13: 1–1553.

Wood. S. L. 1993. Revision of the genera of Platypodidae (Coleoptera). Great Basin Nat 53:259–281.

Chapter 7

Successful eradication of the Asian Longhorn Beetle, *Anoplophora glabripennis*, from North-eastern Italy: protocol, techniques and results

Paper published as: Marchioro M. & Faccoli M. (2021). Successful eradication of the Asian Longhorn Beetle, *Anoplophora glabripennis*, from North-eastern Italy: protocol, techniques and results. Insects.

insects MDPI

Article

Successful eradication of the Asian Longhorn Beetle, *Anoplophora glabripennis*, from North-eastern Italy: protocol, techniques and results

Matteo Marchioro* and Massimo Faccoli

Abstract

The Asian Longhorn Beetle (ALB), *Anoplophora glabripennis* (Coleoptera: Cerambycidae), is an important and extremely polyphagous wood-boring beetle native to Asia. Since the 1990s, ALB was accidentally introduced in North America and Europe. In 2009 a large ALB infestation was found in Veneto Region (North-eastern Italy), in the municipality of Cornuda (Treviso province). Eradication actions were immediately undertaken, based on delimitation of infested and buffer zones, tree visual inspections, felling and chipping of infested trees, trapping protocols and citizen alert. 36,361 trees, belonging to 16 genera, were surveyed twice a year over an area of 7,594 hectares. In 2020, after 11 years of eradication measures, the ALB population of Cornuda has been declared eradicated. Overall, 2,361 trees belonging to 8 genera were felled and destroyed, of which 1,157 were found to be infested by ALB. This paper describes all the actions carried out and the procedures applied in order to eradicate ALB from North-eastern Italy, providing a useful example for current and future ALB eradication programs.

Introduction

The Asian Longhorn Beetle (ALB), *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae), is a wood-boring beetle native to China and the Korean Penin-sula (Lingafelter and Hoebeke 2002). Although in its native range *A. glabripennis* mainly infests *Populus* spp., *Salix* spp., *Ulmus* spp. and *Acer* spp. (Wang 2004), *A. glabripennis* is an extremely polyphagous pest able to develop on woody broadleaves of 34 tree species belonging to 14 genera in 10 families (Lingafelter and Hoebeke 2002, Haack et al. 2010). *A. glabripennis* attacks both young and mature trees growing in urban and peri-urban areas (Hu et al. 2009, Haack et al. 2010, Dodds and Orwig 2011). Unlike most longhorn beetles, *A. glabripennis* develops in apparently healthy plants, although it can infest also stressed trees and fresh logs (He and Huang 1993, Ruitong et al. 1993). For these reasons, the introduction of *A. glabripennis* in new areas represents an enor-mous threat to urban parks and rural forests (Haugen 2000, Nowak et al. 2001).

Accidentally introduced with infested wood packaging material associated with international trade (Haack et al. 2010), *A. glabripennis* was first found outside its native range in New York City (NY, USA) in 1996 (Haack et al. 1996, Cavey et al. 1998). Then, the pest was found also in other states of the USA (Illinois, New Jersey, Massachusetts, Ohio, and South Carolina) (Poland 1998, Haack 2003, Hu et al. 2009, Shatz et al. 2013, Coyle et al. 2021), in Canada (Ontario) (Pedlar et al. 2020) and in Europe, where the first presence of *A. glabripennis* was recorded in Austria (2001) (Tomiczek et al. 2002), followed by France (continental) (2003) (Hérard et al. 2006), Germany (2004) (Benker et al. 2004), Italy (2007) (EPPO 2007), Netherlands (2010) (EPPO 2010), Switzerland (2011) (EPPO 2011), UK (2012) (EPPO 2012), Corse (2013) (EPPO 2013a), Finland (2015) (EPPO 2015), and Montenegro (2015) (EPPO 2017). After eradication measures undertaken by different states, to date, infestations of the pest still occur in France (both continental and Corse), Germany, Italy and USA (EPPO 2021).

In Italy *A. glabripennis* was found in the northern regions of Lombardy (2007) (EPPO 2007), Veneto (2009) (EPPO 2009), Piedmont (2018) (EPPO 2018) and in the central region of Marche (2013) (EPPO 2013b). At these latitudes the whole *A. glabripennis* life cycle lasts 12-18 months (Faccoli, pers. observ.). Adults emerge in summer, mainly in late June - early July (Faccoli et al. 2015). After the maturation feedings and mating, the female lays eggs in oviposition pits chewed out under the bark of the host tree (Faccoli et al. 2015). Young larvae initially feed on phloem and, starting from the 3rd instar, they complete the development into the wood (Faccoli and Favaro 2016). Pupation lasts a couple of weeks, in a pupal chamber created by mature larvae in the sapwood and the new adult emerges through a circular hole (about 10 mm diameter, smaller in males) (Faccoli and Favaro 2016). In Europe, complete development of *A. glabripennis* has been recorded on many genera of woody broadleaves.

The *A. glabripennis* infestation occurring in North-eastern Italy (Veneto Region) was discovered in June 2009 in the municipality of Cornuda (EPPO 2009, Faccoli et al. 2011). A maple was found to be infested in a private garden of the city center following a report of the garden owner to the local phytosanitary office of the Regional Plant Protection Organ-ization (RPPO). The infested tree showed all the typical symptoms of the *A. glabripennis* infestation, with large and circular emergence holes, sectorial dieback of the canopy with dead branches, and oviposition pits along the stem. Immediately after the discovery of the pest, a large-scale intensive monitoring and eradication program started. Eradication program was based on the establishment of buffer zones, visual inspections, felling and destruction of infested trees, trapping protocol and citizen alert, following the official guidelines issued by EPPO (EPPO 2013c). The *A. glabripennis* population of Cornuda has been declared eradicated in 2020 (EPPO 2020), after 11 years of application of specific control measures. The aim of this paper is to present and describe all the actions and procedures successfully carried out in order to eradicate *A. glabripennis* from north-eastern Italy.

Materials and Methods

Site description

The municipality of Cornuda (45°830N, 12°010E; Treviso province, NE Italy), is located along the southern border of the Italian Alps, approximately 160 m a.s.l., in a transition from continental to Mediterranean climatic conditions, with temperate summer and winter. Annual precipitation ranges from 1100 to 1200 mm, concentrated in the spring and autumn. Cornuda is a small village of about 6200 inhabitants located in a suburban area fallowing within a hilly landscape with patches of agricultural land. The village structure consists mainly of small isolated houses surrounded by small private gardens often containing ornamental trees represented by woody native or exotic broadleaves. Public tree-lined parks and rows of trees along the streets are also common.

The village is closely surrounded by mixed deciduous forests and riparian habitats which develop along the Piave river. Natural forests are primarily composed of *Acer pseudoplatanus* L., *Carpinus betulus* L., *Fagus sylvatica* L., *Fraxinus excelsior* L. and *Quercus robur* L. on shady damp slopes, as well as *Betula pendula* Roth., *Fraxinus ornus* L., *Ostrya carpinifolia* Scopoli and *Quercus pubescens* Willd. on sunny dry slopes.

Tree database

Since the first record of the *A. glabripennis* in June 2009, an extensive monitoring program started, visually checking one-by-one of all the possible host trees occurring in Cornuda and in the surrounding municipalities (see next paragraphs), with search directions radially oriented from the point of first discovery of the pest. All host trees were also geo-referenced creating a specific database reporting information about the plant (genera, age, general physiological conditions), geographic position (coordinates), and ownership data (owner name, address and phone number). Some import-export companies occur in the Cornuda territory, with frequent commercial trade with China. As the infestation may have originated from these companies, particular attention was paid to survey their surrounding areas.

Visual tree survey

After the first survey carried out in summer 2009, with the creation of the tree database, a systemic and continuative survey of the host-plants started, choosing the most susceptible tree genera according to literature: *Acer*, *Aesculus*, *Alnus*, *Betula*, *Carpinus*, *Cercidiphyllum*, *Corylus*, *Fagus*, *Fraxinus*, *Ostrya*, *Platanus*, *Populus*, *Prunus*, *Salix*, *Tilia* and *Ulmus* (Hu et al. 2009, Haack et al. 2010). The aim of the survey was to discover the presence of *A. glabripennis* infested plants, and update the infested and buffer areas accordingly. The survey was conducted by 3 teams of operators each composed by 2 trained technicians of the Regional Plant Protection Organization that checked one-by-one all the host-trees from the ground, with the use of binoculars looking for *A. glabripennis* infestation symptoms. All host-trees were geo-referenced and checked twice a year, in summer and late-autumn, looking for adult exit holes, larval frass emission, oviposition scars, maturation feedings on twigs, tree decline with branch dieback and presence of adults on branches and canopies (Figure 1). On one hand, some symptoms are recognizable more easily in summer, such as the presence of recently emerged adults, fresh oviposition pits on the bark, maturation feedings performed by immature adults on twigs, and occurrence of dying branches. On the other hand, the presence of emerging holes is more visible in late-autumn after the plants lost leaves and the upper branches and canopy are checkable from the ground easily. In case of large trees or plants having trunk and branches covered with ivy, difficult to check from ground with accuracy, the inspectors of the Phytosanitary Service were supported both in summer and winter by a team of 4 tree-climbers of the Treviso Forest Service, who checked the potential presence of symptoms also in the upper part of the canopy.

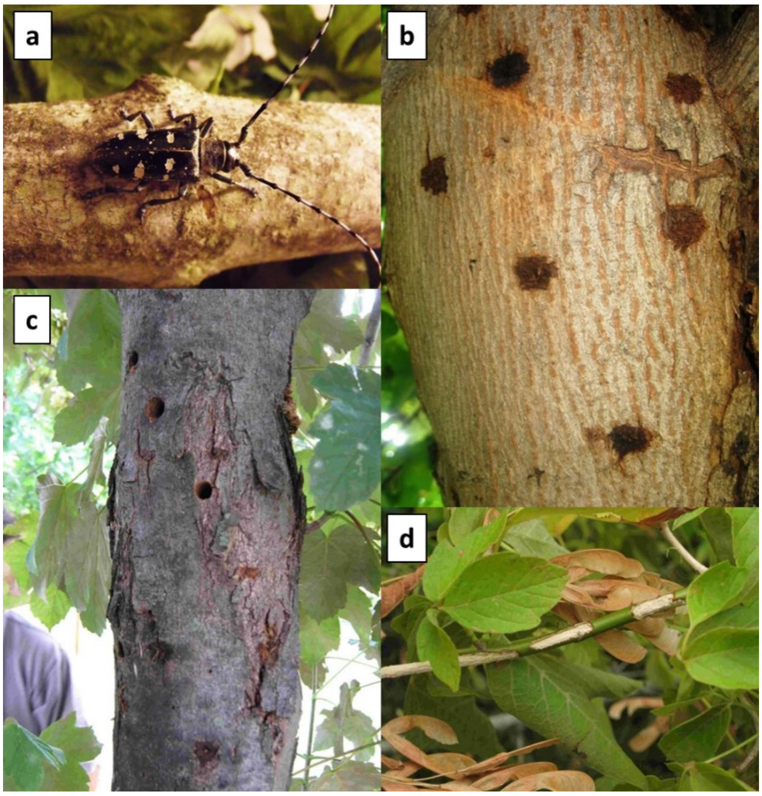

Fig. 1 Main symptoms of *A. glabripennis* infestation: presence of adults (a), adult exit holes (b), oviposition scars (c), maturation feedings on twigs (d).

Forested areas neighboring the village of Cornuda and falling within the infested or the buffer areas were surveyed once per year, along the main forest edges (i.e., external edges, forest tracks, and clear-cut edges). A strip at least 30 m deep inside the forest was monitored. Studies conducted in native regions of South Korea suggest that *A. glabripennis* is not a true forest species but is adapted to riparian habitats characterized by long edges (Williams et al. 2004). Similarly, in the countries of introduction, *A. glabripennis* infestations are usually limited to urban trees that are isolated, growing in small groups or rows, in small rural stands or along forest edges (Hu et al. 2009, Haack et al. 2010).

Zone delimitation

After the checking of all host plants growing in the area, the infested and the buffer zones were established (Figure 2). The "infested zone" consists in a polygon including all the infested plants where the vertices of the polygon were the more external infected plants. The infested zone was included in the territory of six municipalities (Cornuda, Pederobba, Crocetta del Montello, Maser, Montebelluna and Caerano di San Marco). Then, a "buffer zone" was established with a radius of 2 km around the infested zone, *i.e.* around the most external infested trees, according to European regulations (EPPO 2013c). Year-by-year, when new infested trees were found, the delimited zones (infested and buffer zones) have been officially extended (or restricted) several times, arriving to include also the bordering municipalities where satellite infestations were discovered since 2010.

Pheromone traps

During the eradication program, trapping protocols were carried out in the buffer zone in order to verify the presence of *A. glabripennis* in the territory and to test the effectiveness of different trapping tools (i.e. trap models and lures). The sites for trap-setting were chosen in relation to the concentration of susceptible hosts and in suitable areas near the edge of the delimited zone.

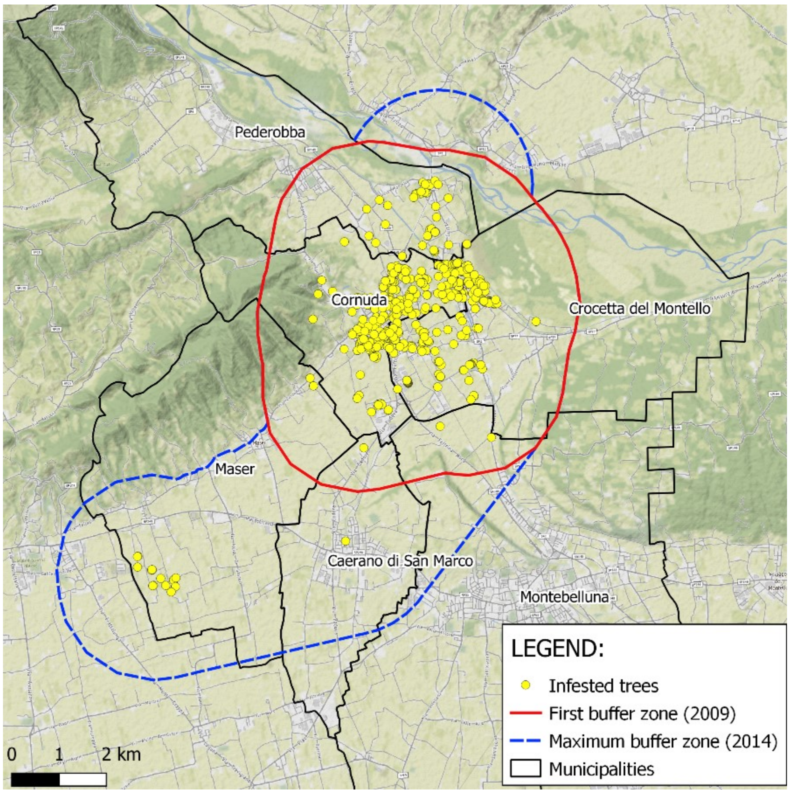

Fig. 2 Map of the *A. glabripennis* infestation, with position of infested trees, involved municipalities border and first and maximum buffer zones extension.

In 2011, 14 traps of different type, size and color were placed for one month (August) and checked weekly (Table 1). Traps were baited with a blend produced by ChemTica International

(Heredia, Costa Rica). In 2012, 27 black cross-vane traps were set up and baited with either the ChemTica blend used in 2011, or a Russian experi-mental blend provided in six different formulations by Dr. Oleg Kulinich of the De-partment of Forest Quarantine, of the All-Russian Center of Plant Quarantine of Mos-cow. Traps were checked three times during July, the month with the highest emer-gence of *A. glabripennis* adults (Faccoli et al. 2015). In 2013, 24 traps were placed: 6 control unbaited traps, and others 6 for each of three different blends equally divided (ChemTica blend and two new formulations of the Russian blend). Lastly, in 2019, ten black cross-vane traps were set in the delimited zone and checked every 2 weeks from middle June to middle September, in order to support the action of visual inspections and verify if *A. glabripennis* was successfully eradicated. Traps were baited with the ChemTica blend tested in the previous year. The used traps types and lure formulations are summarized in Table 1.

Table 1 Information about monitoring protocols conducted during the eradication program. For each year were reported information about the trap model (type and color), the trap number, and the type of lure.

Year	Trap type	Trap color	Trap number	Lure
2011	Cross-vane, long	Black	2	CHEMTICA
	Cross-vane, long	Transparent	1	
	Cross-vane, short	Black	1	
	Cross-vane, short	Transparent	3	
	Multi-funnel, long	Black	3	
	Multi-funnel, short	White	4	
2012	Cross-vane, long	Black	3	CHEMTICA
			4	RUSSIAN (I)
			4	RUSSIAN (II)
			4	RUSSIAN (III)
			4	RUSSIAN (IV)
			4	RUSSIAN (V)
			4	RUSSIAN (VI)
2013	Cross-vane, long	Black	3	CONTROL
			3	CHEMTICA
			3	RUSSIAN (VII)
			3	RUSSIAN (VIII)
	Multi-funnel, long	Black	3	CONTROL
			3	CHEMTICA
			3	RUSSIAN (VII)
			3	RUSSIAN (VIII)
2019	Cross-vane, long	Black	10	CHEMTICA

Blends legend:
CHEMTICA = 1:1 ratio of 4-(n-heptyloxy)butanal (0.3 mg) and 4-(n-heptyloxy)butan-1-ol (0.3 mg) + (−)-linalool (3 mg) + trans-caryophyllene (3 mg) + (Z)-3-hexen-1-ol (3 mg)
RUSSIAN (I) = 1:1 ratio of 4-(n-heptyloxy)butanal (5 µl) and 4-(n-heptyloxy)butan-1-ol (5 µl)
RUSSIAN (II) = 1:1 ratio of 4-(n-heptyloxy)butanal (50 µl) and 4-(n-heptyloxy)butan-1-ol (50 µl)
RUSSIAN (III) = 1:1 ratio of 4-(n-heptyloxy)butanal (500 µl) and 4-(n-heptyloxy)butan-1-ol (500 µl)
RUSSIAN (IV) = 1:1 ratio of 4-(n-heptyloxy)butanal (5 µl) and 4-(n-heptyloxy)butan-1-ol (5 µl) + (Z)-3-hexen-1-ol (100 µl)
RUSSIAN (V) = 1:1 ratio of 4-(n-heptyloxy)butanal (50 µl) and 4-(n-heptyloxy)butan-1-ol (50 µl) + (Z)-3-hexen-1-ol (1000 µl)
RUSSIAN (VI) = 1:1 ratio of 4-(n-heptyloxy)butanal (500 µl) and 4-(n-heptyloxy)butan-1-ol (500 µl) + (Z)-3-hexen-1-ol (3000 µl)
RUSSIAN (VII) = 1:1 ratio of 4-(n-heptyloxy)butanal (0.3 mg) and 4-(n-heptyloxy)butan-1-ol (0.3 mg) + (−)-linalool (3 mg)
RUSSIAN (VIII) = 1:1 ratio of 4-(n-heptyloxy)butanal (0.6 mg) and 4-(n-heptyloxy)butan-1-ol (0.6 mg) + (−)-linalool (6 mg)

Sanitation felling and tree destruction

All trees detected during the visual survey as showing *A. glabripennis* infestation symptoms were cut and destroyed (details below). Trees reporting unclear symptoms possibly confused with those potentially caused by others urban pests infesting the same host-trees (e.g., *Cossus cossus* (Lepidoptera: Cossidae), *Zeuzera pyrina* (Lepidop-tera: Cossidae), *Saperda carcharias* (Coleoptera: Cerambycidae)) were cut and destroyed as well.

A. glabripennis adults leave the tree where they emerged rarely, when they are strongly disturbed for example by tree felling. Moreover, adults were found to be active from end of May to November. Therefore, infested trees found during spring-summer survey were marked but not cut immediately, to avoid adult dispersal during tree felling and movement of infested wood through the village. Tree felling, carried out by workers of the Regional Forest Service, was hence postponed every year to winter, between December and April, during insect hibernation as mature larvae (Faccoli et al. 2015). Infested trees, from both public and private properties, were felled at ground level, leaving only stumps, and moved to a fenced and paved storage area falling within the infestation zone, but away from host plants. In winter, cut trees were then chipped in 2-cm long chips, and chips sold to a biomass power station falling outside the infes-tation area. Wood chips have been submitted to an entomological analysis by the University of Padua in order to exclude the presence of live larvae. However, chips were moved to the biomass power station and burned by the end of winter to reduce the risk of dispersal outside the infestation area of woody material potentially infested with active *A. glabripennis* stages.

The first 4 years of eradication (2009-2012), tree cutting and chipping concerned only infested plants and few infested trees growing close to the attacked ones. Then, in order to make the eradication protocol more effective, the Regional Decree no. 33 of September 10, 2012 of the Veneto Region introduced the “Clear-cut” measure, which involves identification and cutting of all susceptible plants, even if apparently unin-fested, occurring in the area within a 50 m radius from each infested plant. Since 2015, considering the imminent approval of the Decision UE/2015/893 of the European Commission, the clear-cut radius has been increased to 100 m.

Mitigation plan

In accordance with the owner's will, plants felled in private gardens were re-placed for free with new trees belonging to non-host species. Young trees (3-4 years old) were chosen by the citizens among the species available for reforestation and urban design programs at the forest nursery of the Regional Forest Service, including *Cercis siliquastrum*, *Clerodendrom trychotomum*, *Ginko biloba*, *Liquidambar styraciflua*, *Quercus robur*, and *Quercus pubescens*.

Communication

Municipalities and other territorial authorities, such as schools, park administrators and citizens' associations, were immediately involved. Public meetings were organized to inform citizens, with the distribution of informative brochures and poster hanging, and releases were sent to local newspapers. Moreover, specific technical meetings were organized to inform and train local nurserymen, pruners, gardeners and other professional stakeholders. Lastly, brief lessons were organized for students of primary and secondary schools of the territory, with projection of slides and photos concerning pest biology and symptoms. In this way, citizens were informed about the threat and how to recognize and report signs of the pest presence. A toll-free number has been also activated to allow citizens to report suspect symptoms or *A. glabripennis* sightings. Finally, an internet site was activated providing information for citizens and a platform to upload reports.

Results

The eradication plan started in summer of 2009 and ended in 2020 when, according to Commission regulation EU/2015/893, the species has been declared eradicated from Cornuda and surrounding municipalities (EPPO 2020), following 4-years of no new record of infested plants. Indeed, neither insects nor plants showing infestation symptoms (oviposition pits, emerging holes or maturation feedings) have been found since 2016.

Visual tree survey

Since the beginning of monitoring in 2009, more than 36,000 trees were surveyed singly in twelve years (Table 2). Among the 16 surveyed genera, the most abundant were *Acer* (10,277 trees, 28%), *Ulmus* (6,227 trees, 17%), *Salix* (5,271 trees, 15%), *Carpinus* (4,837 trees, 13%), and *Betula* (2,067 trees, 6%); other genera occurred in per-centages lower than 5% (Table 2).

During the survey carried out from 2009 to 2020, 1,157 trees belonging to 8 genera were found to be infested (3% of the total amount of surveyed trees). The most attacked genera were *Acer* (431 trees infested), *Ulmus* (337 trees infested), *Betula* (210 trees infested), and *Salix* (149 trees infested), even if, looking at the ratio between infested and monitored trees, the most susceptible genera were *Cercidiphyllum* (18.2%), *Aesculus* (11.6%) and *Betula* (10.2%), followed by *Ulmus* (5.4%), *Acer* (4.2%), and *Salix* (2.8%) (Table 2). Monitored but never found infested genera were *Alnus*, *Carpinus*, *Corylus*, *Fagus*, *Fraxinus*, *Ostrya*, *Platanus* and *Tilia* (Table 2).

Table 2 Number of monitored and infested trees, with relative percentage of infestation, divided for genera.

Tree genus	Monitored plants (n)	Infested plants (n)	Ratio (%)
Cercidiphyllum	11	2	18.18
Aesculus	147	17	11.56
Betula	2,067	210	10.16
Ulmus	6,227	337	5.41
Acer	10,277	431	4.19
Salix	5,271	149	2.83
Prunus	1,361	9	0.66
Populus	1,709	2	0.12
Alnus	59	0	-
Carpinus	4,837	0	-
Corylus	1,238	0	-
Fagus	486	0	-
Fraxinus	680	0	-
Ostrya	43	0	-
Platanus	908	0	-
Tilia	1,040	0	-
Total	**36,361**	**1,157**	**3.18**

Zone delimitation

In 2009, the year of the infestation discovery, the delimited zone had an area of 4,105 ha which grew in the subsequent years, reaching the maximum size of 7,594 ha in 2013 (Figure 2). In 2016, as a result of the application of the eradication protocol, both infestation and buffer zone began to decline reaching the minimum value of 1,843 ha in 2018 (Table 3).

Table 3 Extension of buffer zones, number of monitored and felled trees (divided in "infested" and susceptible trees), and clear-cut radius adopted for each year.

Year	Delimited zone (ha)	Monitored trees (n)	Infested trees (n)	Susceptible trees (Clear-cut) (n)	Total felled trees (n)	Clear-cut radius (m)
2009/10	4105	12816	576	54	630	-
2010/11	5625	20366	327	198	525	-
2011/12	7214	24292	163	82	245	-
2012/13	7214	24292	67	679	746	50
2013/14	7594	25223	15	83	98	50
2014/15	7594	30990	5	52	57	100
2015/16	7594	36361	4	56	60	100
2016/17	4555	24511	0	0	0	100
2017/18	4555	24511	0	0	0	100
2018/19	1843	13041	0	0	0	100
2019/20	1843	13041	0	0	0	100
Total (n)		**36361***	**1157**	**1204**	**2361**	

*Healthy trees were monitored every year, so the total is not the sum of monitored trees for each year, but the total amount of monitored trees during the eradication period.

Susceptible trees: healthy host-trees growing close to the infested one. During the first three years no clear-cut were applied systematically; however, in case of polychromic trees, or trees very close to infested ones, or trees damaged by felling infested trees, felling was also carried out on plants that were not directly infested.

Pheromone traps

During the 3-years monitoring with traps (2011- 2013) only two *A. glabripennis* females were caught in 2013. The two individuals were caught by one multi-funnel and one cross-vane trap both baited with the ChemTica blend. In 2019, after three years without finding any attacked plants, traps were placed to provide further confirmation that eradication had taken place and no individuals were caught.

Sanitation felling, tree destruction and mitigation plan

Beside the 1,157 infested trees, other 1,204 trees were felled because falling inside the "clear-cut area" (Table 3). Overall, a total of 2,361 trees were cut in 12 years during the eradication plan applied in Cornuda, of which only 220 plants from private gardens. The highest peak of felling occurred during the first year (2009), with 630 cut trees. Subsequently, the number was steadily decreasing in time, until no new plants were felled since 2016.

Two samples of wood chips, taken in 2010 and in 2011, have been submitted to an entomological analysis by the University of Padua. The analysis showed that the size of the wood pieces from the chipping operations is incompatible with the development and survival of *A. glabripennis* larvae in the wood: almost all of the material analysed was less than 2 cm in length, compared to 4-5 cm in length for mature larvae. In fact, several remains of crushed larvae were found during the analysis. Moreover, the few larger elements are subject to rapid deterioration due to tissue dehydration or fermentation depending on the humidity conditions. In conclusion, the product tested was found to be biologically safe and free from risk of spreading *A. glabripennis* infestation.

Main costs incurred in carrying out eradication program are:

- € 380,000 used by Regional Plant Protection Organization for the annual trees survey (twice per year)
- € 520,000 used by Regional Forest Services for felling and chipping trees
- € 20,000 used by University of Padua for scientific support and research activities.

The program was initially financed by funds from the Veneto Region, which then accessed European funds for the management and eradication of invasive species.

As compensation for the felling of infested trees occurring in private properties, owners could choose a new tree to plant as a replacement. A total of 217 new trees (over 220 cut) were planted, including *Cercis siliquastrum* (65), *Liquidambar styraciflua* (41), *Ginko biloba* (35), *Clerodendrom trychotomum* (29), *Quercus robur* (27), and *Quercus pubescens* (20). There was no financial compensation.

Discussion

Eradication is the numerical reduction of a population in a specific geographic area to prevent its reproduction and, therefore, bring it to the local extinction (Myers et al. 1998, Liebhold and Tobin 2008). Conditions that support a higher probability of successful eradication include early detection of the pest (*i.e.*, limited spatial distribution), ability to detect and identify the invader or the infested plants, availability of effective tools of pest monitor and con-trol, and public support (Brockerhoff et al. 2010, Tobin et al. 2014). Moreover, the target species should have all or most of the following characteristics: low rate of reproduction and dispersal, ease of detection at low population density, and limited host range (Brockerhoff et al. 2010).

Early detection of the pest plays a key role on a successful eradication program. The earlier the parasite is discovered from the time of actual arrival, the higher the chances of success, as the parasite will have less time to reproduce and spread. In fact, the probability of successful eradication declines with the increase of the infested area (Rejmánek and Pitcairn 2002, Pluess et al. 2012). In particular, Rejmánek and Pitcairn (Rejmánek and Pitcairn 2002), analyzing data from 53 infestations of 18 pest species, showed that eradication success probability is about 50% between 0.1 and 1.0 hectares and about 25% between 100 and 1000 hectares of infested area. Moreover, as the area of eradication increases, the required efforts (*i.e.*, costs) also increase and the operation may no longer be economically viable (Tobin et al. 2014). The Cornuda infestation initially measured about 4,000 ha (infested and buffer zones) and expanded to a maximum extension of about 7,600 ha. Although the infestation was discovered in 2009, it was verified, by dating the exit holes from the host trees, that the infestation started at least five years previously, in 2005 (Faccoli et al. 2015). This delay in starting eradication program caused an effort of 8 years of active eradication (2009-2016) and other 4 years (2017-2020) of survey in order to eradicate *A. glabripennis* from the territory. The monitoring protocol involved more than 36,000 trees checked one-by-one twice a year for 11 years; 2,361 of these trees were felled because found to be infested or simply because falling within the clear-cut radius. Another example of successful *A. glabripennis* erad-ication is at Paddock Wood (Kent, UK), although in this case the infestation was much smaller (with an infested zone of only 11.4 ha). After just one year (and other seven years of survey) the pest was eradicated and about 2,200 trees were felled, of which 66 infested (Straw et al. 2015, Eyre and Barbrook 2021). In contrast, a large infestation was detected in Worcester (Massachusetts, USA) in 2008 (Dodds and Orwig 2011), and still active (Santos and Bond 2021). Until 2015, the extension of infestation is larger than 20,000 ha with more than 5 million monitored trees, of which approximately 34,000 removed (both infested, and those deemed to be high-risk) (Trotter and Hull-Sanders 2015). Such a wide spread makes an eradication success challenging (Dodds and Orwig 2011).

Beside early detection, a successful eradication is based on the possibility to easily identify the pest or its infestation symptoms and to dispose of effective tools for its de-tection. Visual inspections have proved to be effective against *A. glabripennis*, but they lose effectiveness for recently infested trees (Eyre and Barbrook 2021), or in case of large trees or trunks covered by ivy (Faccoli, pers. observ.). Pheromone traps are often used alongside the work of phytosanitary inspectors, both to find pests and for their eradication by mass-trapping and lure-and-kill techniques (El-Sayed et al. 2006, El-Sayed et al. 2009, Suckling et al. 2014, Sanchez-Husillos et al. 2015). Unfortunately, no long-range pheromone has been reported for *A. glabripennis*, although both male-produced short-range and female-produced contact recognition pheromones were identified (Zhang et al. 2002, Zhang et al. 2003, Hu et al. 2009, Haack et al. 2010). Several studies have tested the effectiveness of these pheromones combined with some host-volatiles (*e.g.*, Z-3-hexen-1-ol and Linalool) in trapping protocols, showing some positive outcomes, but with few catches despite the dozens of traps used (Nehme et al. 2009, Nehme et al. 2010, Nehme et al. 2014). During the eradication program carried out in Cornuda, only 2 *A. glabripennis* females were caught by traps in 2013 and no *A. glabripennis* individuals were caught by traps used at Paddock Wood (Eyre and Barbrook 2021). Despite the use of *A. glabripennis* pheromones remains indicated for pest interception in areas where it is not yet been detected (Hoppe et al. 2019), our results corroborate the hypothesis that the attraction of pheromones is not strong enough to be used for active eradication actions by mass-trapping, and probably not even for a reliable monitoring. The low effectiveness of pheromone-based trapping techniques is probably due to the fact that they mainly attract virgin females and, at close range, females used also other visual and chemicals stimuli which require further studies (Branco et al. 2021).

Despite *A. glabripennis* has many of the ecological and biological characteristics indicated by Brockerhoff et al. (Brockerhoff et al. 2010) as facilitating the eradication, the effective *A. glabripennis* eradication is never easy because of its extreme polyphagy and the generic symptoms caused to host plants. First of all, *A. glabripennis* has a low fecundity rate (Haack et al. 2010). In China, under natural conditions, were estimated 25-40 viable eggs per female (Hu et al. 2009), whereas in USA that fecundity was estimated varying between 30-178 eggs per female (Keena 2002, Keena 2006). Also the limited active dispersal capacity is an important factor. The potential dispersal of *A. glabripennis* adults was estimated in about 2,000 m, with a realistic annual spread of about 300 m from the closest infested tree (Smith et al. 2004, Favaro et al. 2015, Hoppe 2019). Moreover, the tendency to reinfest the same tree for several years was usually observed (Hu et al. 2009, Haack et al. 2010). Lastly, according to climatic conditions *A. glabripennis* takes 1-3 years or even more to fully complete its life cycle (Hu et al. 2009, Haack et al. 2010). All these characteristics (*i.e.*, fecundity, active fly and life cycle duration) strictly depend on temperatures (Kappel et al. 2017). In Cornuda, annual temperatures ranges between -2 and 29 °C, with a mean temperature of 23 °C during warmer months (ARPAV 2021). In Paddock Wood annual temperatures vary between 2 and 23 °C, with a mean temperature of 17 °C during warmer months (Straw et al. 2015). The first effects of the different climatic conditions at the two sites can be observed on the life cycle. In northern Italy *A. glabripennis* was considered univoltine (Favaro et al. 2015), whereas for UK a 2-3 years life cycle was estimated (Straw et al. 2015). Moreover, a research carried out on the effects of temperature on *A. glabripennis* fecundity estimated that the optimum temperature for maximum fecundity is about 25 °C (Keena 2006). Another study showed that the adult's flight capacity increases with temperature from 15 to 30 °C, and that no flight occurs under 15 °C (Keena 2018). In conclusion, lower temperatures of Paddock Wood caused a lower *A. glabripennis* adults fecundity, a lower flight propensity (*i.e.*, lower dispersion of the infestation) and lengthen the development time, doubling or even tripling it, compared to Cornuda infestation. All these factors probably contributed to keeping the pest infestation low in UK, despite the eradication began about ten years after the estimated arrival of the pest (Straw et al. 2016), whereas in Italy only five years had elapsed.

One of the major problems dealing with *A. glabripennis* eradication concerns the extreme polyphagy on woody broadleaves. An extensive investigation conducted in Yinchuan region (China) found damage on trees belonging to 14 genera of broadleaves, although complete development has not been confirmed on all species listed as hosts (Hu et al. 2009). However, hosts suitability differs in different continents: *Populus* and *Salix* are more suitable than *Ulmus* in China (Hu et al. 2009); *Acer* and *Ulmus* are generally more suitable than *Fraxinus* in USA (Haack et al. 2006, Koiyle et al. 2021); in Europe the most suitable genera are, in decreasing order, *Acer*, *Betula*, *Salix*, *Aesculus* and *Populus* (Hérard et al. 2006). In Cornuda infestation the most infested genera (in percentage) were *Betula*, *Ulmus*, *Acer*, and *Salix* (excluding *Cercidiphyllum* and *Aesculus* because of the small number of present trees), similarly to in-festations in other Italian regions (Lombardy, Marche and Piedmont) (EPPO 2020) and to infestation at Paddock Wood, where the most attacked genera were *Acer*, *Salix*, and *Betula* (Straw et al. 2015, Eyre and Barbrook 2021). Interesting, in both Cornuda and Paddock Wood infestations the number of infested poplars is very low; this is particularly evident in Italy, were only 2 out 1,709 poplars (0.1%) were found infested. Differently, poplars are among the most suitable hosts for *A. glabripennis* in China, even if not all *Populus* species are equally susceptible to *A. glabripennis* (Yin and Lu 2005). *Acer*, instead, is confirmed as one of the main hosts for *A. glabripennis*. Such a large polyphagy has important consequences in the management of *A. glabripennis* infestations. A higher number of potential hosts means, on one hand, higher chances for pest to reproduce and proliferate and, on the other hand, a higher number of trees to be surveyed and, if infested, to be felled and replaced. Moreover, *A. glabripennis* infests healthy and vigorous trees (Hu et al. 2009, Haack et al. 2010), which makes prevention more difficult, as keeping plants healthy and in good physiological condition does not prevent infestations.

The eradication of *A. glabripennis* from the municipality of Cornuda shows the importance of taking prompt, coordinate and effective actions to contain the spread of the pest and to proceed with its systematic elimination from the infested area. Despite the considerable large size of the Italian infestation, the benefits obtained from the eradication of the pest have far exceeded the costs incurred for its implementation (Faccoli and Gatto 2016). Of course, different scenarios may occur. For example, the *A. glabripennis* infestation occurring in Worcester (Massachusetts) seems to be too widespread now and the eradication, although it remains the goal, is of uncertain outcome and is taking enormous effort (Trotter and Hull-Sanders 2015).

Of all the actions undertaken, those that proved most effective were visual survey of susceptible trees (in order to find signs of infestation) and the felling and destruction of all the infested trees and nearby ones (in order to prevent the diffusion of the pest). Also the destruction of felled trees by chipping is a very useful practice in order to kill all the larvae present inside the wood, as demonstrated by the wood chips analyses conducted by University of Padua and already reported in other works (Wang et al. 2000, Branco et al. 2021). On the other hand, the use of pheromone traps proved useless, as was also the case during eradication in Paddock Wood (Eyre and Barbrook 2021). Finally, the involvement and active participation of citizens and stakeholders is also of paramount importance (Brockerhoff et al. 2010, Porth et al. 2015). If not properly motivated, 'unpopular' actions such as felling private trees and killing insects may lead to protests and non-cooperation by the population, jeopardizing the outcome of the whole operation. In this respect, the first report of the presence of *A. glabripennis* in Cornuda was made by a private citizen, demonstrating the importance of citizens' cooperation in the interception of alien species.

Acknowledgements

We thank phytosanitary office of the Regional Plant Protection Organization (RPPO) for partially funding the research, data provided and their help in field-works; the staff of U.O. Forestale of Treviso for data provided and their help in field-works; Dr. Oleg Kulinich (Department of Forest Quarantine, of the All-Russian Center of Plant Quarantine of Moscow) for providing Russian lures; University of Padua for partially funding the research (DOR projects).

References

ARPAV 2021. Dati meteorologici ultimi anni. (https://www.arpa.veneto.it/bollettini/storico/).

Benker, U., C. Bogel, and M. Blaschke. **2004**. Tree fellings due to black longicorn beetle: the asiatic longicorn beetle has been found in the district of Passau [Bavaria, Germany]. AFZ/Der Wald, Allg. Forst Zeitschrift für Waldwirtschaft und Umweltvorsorge. 59: 1112.

Branco, S., M. Faccoli, E.G. Brockerhoff, G. Roux, H. Jactel, N. Desneux, E. Gachet, R. Mouttet, J.C. Streito and M. Branco. 2021. Preventing invasions of Asian longhorn beetle and citrus longhorn beetle: are we on the right track? J. Pest Sci. 2021, doi:10.1007/s10340-021-01431-x.

Brockerhoff, E. G., A. M. Liebhold, B. Richardson, and D. M. Suckling. **2010**. Eradication of invasive forest insects: Concepts, methods, costs and benefits. New Zeal. J. For. Sci. 40: 117–135.

Cavey, J. F., E. R. Hoebeke, S. Passoa, and S. W. Lingafelter. **1998**. A new exotic threat to North American hardwood forests: an Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae). I. Larval description and diagnosis. Proc. Entomol. Soc. Washingt. 100: 373–381.

Coyle, D. R., R. T. Trotter, M. S. Bean, and S. E. Pfister. **2021**. First recorded Asian Longhorned Beetle (Coleoptera: Cerambycidae) infestation in the Southern United States. J. Integr. Pest Manag. 12: 10–11.

Dodds, K. J., and D. A. Orwig. **2011**. An invasive urban forest pest invades natural environments - Asian longhorned beetle in northeastern US hardwood forests. Can. J. For. Res. 41: 1729–1742.

El-Sayed, A. M., D. M. Suckling, J. A. Byers, E. B. Jang, and C. H. Wearing. **2009**. Potential of “Lure and Kill” in long-term pest management and eradication of invasive species. J. Econ. Entomol. 102: 815–835.

El-Sayed, A. M., D. M. Suckling, C. H. Wearing, and J. A. Byers. **2006**. Potential of mass trapping for long-term pest management and eradication of invasive species. J. Econ. Entomol. 99: 1550–1564.

EPPO. **2007**. First record of *Anoplophora glabripennis* in Italy. EPPO Report. Serv. 9.

EPPO. **2009**. *Anoplophora glabripennis* detected in the Veneto region, Italy. EPPO Report. Serv. 8.

EPPO. **2010**. First record of *Anoplophora glabripennis* in the Netherlands. EPPO Report. Serv. 11.

EPPO. **2011**. First report of *Anoplophora glabripennis* in Switzerland. EPPO Report. Serv. 9.

EPPO. **2012**. First outbreak of *Anoplophora glabripennis* in the United Kingdom. EPPO Report. Serv. 4.

EPPO. **2013a**. *Anoplophora glabripennis* found in Corse (FR). EPPO Report. Serv. 7.

EPPO. **2013b**. *Anoplophora glabripennis* found for the first time in Marche region, Italy. EPPO Report. Serv. 9.

EPPO. **2013c**. PM 9/15 (1) *Anoplophora glabripennis*: procedures for official control. EPPO Bull. 43: 510–517.

EPPO. **2015**. First report of *Anoplophora glabripennis* in Finland. EPPO Report. Serv. 10.

EPPO. **2017**. First report of *Anoplophora glabripennis* in Montenegro. EPPO Report. Serv. 1.

EPPO. **2018**. *Anoplophora glabripennis* found in Piemonte, Italy. EPPO Report. Serv. 10.

EPPO. **2020**. Update on the situation of *Anoplophora glabripennis* in Italy. EPPO Report. Serv. 10.

EPPO. **2021**. EPPO Global Database. (https://gd.eppo.int/taxon/ANOLGL/distribution).

Eyre, D., and J. Barbrook. **2021**. The eradication of Asian longhorned beetle at Paddock Wood, UK. CABI Agric. Biosci. 2: 12.

Faccoli, M., and R. Favaro. **2016**. Host preference and host colonization of the Asian long-horned beetle, *Anoplophora glabripennis* (Coleoptera: Cerambycidae), in Southern Europe. Bull. Entomol. Res. 106: 359–367.

Faccoli, M., R. Favaro, M. T. Smith, and J. Wu. **2015**. Life history of the Asian longhorn beetle *Anoplophora glabripennis* (Coleoptera Cerambycidae) in southern Europe. Agric. For. Entomol. 17: 188–196.

Faccoli, M., and P. Gatto. **2016**. Analysis of costs and benefits of Asian longhorned beetle eradication in Italy. Forestry. 89: 301–309.

Faccoli, M., M. Vettorazzo, M. Zampini, G. Zanini, M. Coppe, and A. Battisti. **2011**. An outbreak of *Anoplophora glabripennis* (Coleoptera: Cerambycidae) in NE Italy: first results of pest management and eradication attempt. Proc. IUFRO Work. Party. 7: 3–5.

Favaro, R., L. Wichmann, H. P. Ravn, and M. Faccoli. **2015**. Spatial spread and infestation risk assessment in the Asian longhorned beetle, *Anoplophora glabripennis*. Entomol. Exp. Appl. 155: 95–101.

Haack, R. A. 2003. Exotics, exotics, exotics: recently detected bark-and wood-boring insects in the US. Newsl Mich Entomol Soc. 48: 16–17.

Haack, R. A., J. F. Cavey, E. R. Hoebeke, and K. R. Law. **1996**. *Anoplophora glabripennis*: a new tree-infesting exotic cerambycid invades New York. Newsl. Mich. Entomol. Soc. 41: 1–3.

Haack, R. A., F. Hérard, J. Sun, and J. J. Turgeon. **2010**. Managing invasive populations of Asian Longhorned Beetle and Citrus Longhorned Beetle: A worldwide perspective. Annu. Rev. Entomol. 55: 521–546.

Haack, R., L. Bauer, R.-T. Gao, J. McCarthy, D. Miller, T. Petrice, and T. Poland. **2006**. *Anoplophora glabripennis* within-tree distribution, seasonal development, and host suitability in China and Chicago. Gt. Lakes Entomol. 39: 169–183.

Haugen, D. A. 2000. Update on Asian longhorned beetle infestations in the US. Newsl. Mich. Entomol. Soc. 45: 2–3.

He, P., and J. Huang. **1993**. Adult behavior of *Anoplophora glabripennis*. Acta Entomol. Sin. 36: 51–55.

Hérard, F., M. Ciampitti, M. Maspero, H. Krehan, U. Benker, C. Boegel, R. Schrage, L. Bouhot-Delduc, and P. Bialooki. **2006**. *Anoplophora* species in Europe: infestations and management processes. EPPO Bull. 36: 470–474.

Hoppe, B., G. Schrader, M. Kinkar, and S. Vos. **2019**. Pest survey card on *Anoplophora glabripennis*. EFSA Support. Publ. 16: 1750E.

Hu, J., S. Angeli, S. Schuetz, Y. Luo, and A. E. Hajek. **2009**. Ecology and management of exotic and endemic Asian longhorned beetle *Anoplophora glabripennis*. Agric. For. Entomol. 11: 359–375.

Kappel, A. P., R. T. Trotter, M. A. Keena, J. Rogan, and C. A. Williams. **2017**. Mapping of the Asian longhorned beetle's time to maturity and risk to invasion at contiguous United States extent. Biol. Invasions. 19: 1999–2013.

Keena, M. A. 2002. *Anoplophora glabripennis* (Coleoptera: Cerambycidae) fecundity and longevity under laboratory conditions: Comparison of populations from New York and Illinois on *Acer saccharum*. Environ. Entomol. 31: 490–498.

Keena, M. A. 2006. Effects of temperature on *Anoplophora glabripennis* (Coleoptera: Cerambycidae) adult survival, reproduction, and egg hatch. Environ. Entomol. 35: 912–921.

Keena, M. A. 2018. Factors that influence flight propensity in *Anoplophora glabripennis* (Coleoptera: Cerambycidae). Environ. Entomol. 47: 1233–1241.

Liebhold, A. M., and P. C. Tobin. **2008**. Population ecology of insect invasions and their management. Annu. Rev. Entomol. 53: 387–408.

Lingafelter, S. W., and E. R. Hoebeke. **2002**. Revision of the genus *Anoplophora* (Coleoptera: Cerambycidae). Entomological Society of Washington, Washington DC.

Myers, J. H., A. Savoie, and E. van Randen. **1998**. Eradication and pest management. Annu. Rev. Entomol. 43: 471–491.

Nehme, M. E., M. A. Keena, A. Zhang, T. C. Baker, and K. Hoover. **2009**. Attraction of *Anoplophora glabripennis* to male-produced pheromone and plant volatiles. Environ. Entomol. 38: 1745–1755.

Nehme, M. E., M. A. Keena, A. Zhang, T. C. Baker, Z. Xu, and K. Hoover. **2010**. Evaluating the use of male-produced pheromone components and plant volatiles in two trap designs to monitor *Anoplophora glabripennis*. Environ. Entomol. 39: 169–176.

Nehme, M. E., R. T. Trotter, M. A. Keena, C. McFarland, J. Coop, H. M. Hull-Sanders, P. Meng, C. M. De Moraes, M. C. Mescher, and K. Hoover. **2014**. Development and evaluation of a trapping system for *Anoplophora glabripennis* (Coleoptera: Cerambycidae) in the United States. Environ. Entomol. 43: 1034–1044.

Nowak, D. J., J. E. Pasek, R. A. Sequeira, D. E. Crane, and V. C. Mastro. **2001**. Potential effect of *Anoplophora glabripennis* (Coleoptera: Cerambycidae) on urban trees in the United States. J. Econ. Entomol. 94: 116–122.

Pedlar, J. H., D. W. McKenney, D. Yemshanov, and E. S. Hope. **2020**. Potential economic impacts of the Asian Longhorned Beetle (Coleoptera: Cerambycidae) in Eastern Canada. J. Econ. Entomol. 113: 839–850.

Pluess, T., V. Jarošík, P. Pyšek, R. Cannon, J. Pergl, A. Breukers, and S. Bacher. **2012**. Which factors affect the success or failure of eradication campaigns against alien species? PLoS One. 7: e48157.

Poland, T. 1998. Chicago joins New York in battle with the Asian longhorned beetle. Newsl. Mich.

Entomol. …. 43: 15–17.

Porth, E. F., N. Dandy, and M. Marzano. **2015**. "My garden is the one with no trees:" residential lived experiences of the 2012 Asian Longhorn Beetle eradication programme in Kent, England. Hum. Ecol. 43: 669–679.

Rejmánek, M., and M. Pitcairn. **2002**. When is eradication of exotic pest plants a realistic goal?, pp. 249–253. *In* Veitch, C., Clout, M. (eds.), Turn. Tide Erad. Invasive Species. IUCN.

Ruitong, G., Q. Xixiang, C. Dejun, and C. Weipei. **1993**. A Study on the damage of poplar caused by *Anoplophora glabripennis*. For. Res. 2.

Sanchez-Husillos, E., I. Etxebeste, and J. Pajares. **2015**. Effectiveness of mass trapping in the reduction of *Monochamus galloprovincialis* Olivier (Col.: Cerambycidae) populations. J. Appl. Entomol. 139: 747–758.

Santos, R., and S. Bond. **2021**. USDA Announces 2021 plans for Asian Longhorned Beetle eradication efforts in Massachusetts, New York, Ohio and South Carolina. Anim. Plant Heal. Insp. Serv. (https://www.aphis.usda.gov/aphis/newsroom/news/sa_by_date/sa-2021/alb-eradication-efforts).

Shatz, A. J., J. Rogan, F. Sangermano, Y. Ogneva-Himmelberger, and H. Chen. **2013**. Characterizing the potential distribution of the invasive Asian longhorned beetle (*Anoplophora glabripennis*) in Worcester County, Massachusetts. Appl. Geogr. 45: 259–268.

Smith, M. T., P. C. Tobin, J. Bancroft, G. Li, and R. Gao. **2004**. Dispersal and spatiotemporal dynamics of Asian Longhorned Beetle (Coleoptera: Cerambycidae) in China. Environ. Entomol. 33: 435–442.

Straw, N. A., N. J. Fielding, C. Tilbury, D. T. Williams, and T. Cull. **2016**. History and development of an isolated outbreak of Asian longhorn beetle *Anoplophora glabripennis* (Coleoptera: Cerambycidae) in southern England. Agric. For. Entomol. 18: 280–293.

Straw, N. A., N. J. Fielding, C. Tilbury, D. T. Williams, and D. Inward. **2015**. Host plant selection and resource utilisation by Asian longhorn beetle *Anoplophora glabripennis* (Coleoptera: Cerambycidae) in southern England. Forestry. 88: 84–95.

Straw, N. A., C. Tilbury, N. J. Fielding, D. T. Williams, and T. Cull. **2015**. Timing and duration of the life cycle of Asian longhorn beetle *Anoplophora glabripennis* (Coleoptera: Cerambycidae) in southern England. Agric. For. Entomol. 17: 400–411.

Suckling, D. M., L. D. Stringer, A. E. Stephens, B. Woods, D. G. Williams, G. Baker, and A. M. El-Sayed. **2014**. From integrated pest management to integrated pest eradication: technologies and future needs. Pest Manag. Sci. 70: 179–189.

Tobin, P. C., J. M. Kean, D. M. Suckling, D. G. McCullough, D. A. Herms, and L. D. Stringer. **2014**. Determinants of successful arthropod eradication programs. Biol. Invasions. 16: 401–414.

Tomiczek, C., H. Krehan, and P. Menschhorn. **2002**. Dangerous Asiatic longicorn beetle found in Austria: new danger for our trees. Allg. Forst Zeitschrift fur Waldwirtschaft und Umweltvorsorge. 57: 52–54.

Trotter, R. T., and H. M. Hull-Sanders. **2015**. Quantifying dispersal of the Asian longhorned beetle (*Anoplophora glabripennis*, Coleoptera) with incomplete data and behavioral knowledge. Biol. Invasions. 17: 3359–3369.

Wang, B., V.C. Mastro and W.H. McLane. 2000. Impacts of cchipping on surrogates for the longhorned beetle *Anoplophora glabripennis* (Coleoptera: Cerambycidae) in logs. J. Econ. Entomol. 93: 1832–1836.

Wang, Z. G. 2004. Study on the occurrence dynamics of *Anoplophora glabripennis* (Coleoptera: Cerambycidae) and its control measures.

Williams, D. W., H-P. Lee, and I-K. Kim. **2004**. Distribution and abundance of *Anoplophora glabripennis* (Coleoptera: Cerambycidae) in natural acer stands in South Korea. Environ. Entomol. 33: 540–545.

Yin, W. L., and W. Lu. **2005**. Review of tree selection and afforestation for control of Asian longhorned beetle in North China. Forest Health & Biosecurity Working Paper FBS/7E. Food and Agriculture Organization of the United Nations. For. Dep. Rome, Italy.

Zhang, A., J. E. Oliver, J. R. Aldrich, B. Wang, and V. C. Mastro. **2002**. Stimulatory beetle volatiles for the Asian Longhorned Beetle, *Anoplophora glabripennis* (Motschulsky). Zeitschrift für Naturforsch. C. 57: 553–558.

Zhang, A., J. E. Oliver, K. Chauhan, B. Zhao, L. Xia, and Z. Xu. **2003**. Evidence for contact sex recognition pheromone of the Asian longhorned beetle, *Anoplophora glabripennis* (Coleoptera: Cerambycidae). Naturwissenschaften. 90: 410–413.

Chapter 8

General conclusions

Insect invasive species cause economic and ecologic impacts in the invaded areas (Kenis and Branco 2010, Vaes-Petignat and Nentwig 2014) and the number of new introductions, which has risen steadily in recent decades, is set to increase further in the next years (Seebens et al. 2017, 2021). When an alien species establishs and expands in a new area, its management becomes more difficult and more expensive (Liebhold and Tobin 2008). The early-detection of an invasive species at the initial stage of the invasion process increases the chances of its prompt eradication, reducing costs and damages (Epanchin-Niell and Liebhold 2015). For this reason, the biosecurity surveillance is an evolving field, both at *border* and *post-border* levels, constantly searching for ever more effective means of detection and interception of alien species.

At *border* level several tools are used for the interceptions of alien insects: baited traps, sniffer dogs and E-nooses, acoustic detection and laser vibrometry (Poland and Rassati 2019). Traps can be used at points-of-entry against insects arriving in imported commodities for their interception before establishment (Rassati et al. 2015, Fan et al. 2019). A recent innovation in the biosecurity surveillance consists in the use of sniffer dogs, in order to detect specific odours produced by insects (Suma et al. 2014, Hoyer-Tomiczek et al. 2016). A similar approach consists in the use of electronic tools, such as the E-noses, to find insects through the perception of odours (Lampson et al. 2014, Nouri et al. 2019). Others electronic tools were used for the detection of alien insects by picking up their acoustic signals (Mankin et al. 2011, Ekramirad et al. 2021). A particular evolution of these tools is represented by laser Doppler vibrometer (LDV), that are able to perceive and measure vibrations without any contact with tested surface (Zorović and Čokl 2015). In this context, in Chapter 2 and Chapter 3 new trap, designed to be used within containers during shipment, was tested. This trap, that use a LED light as attractor and a sticky card to capture insects, is an aspecific and broadspectrum tool for the border surveillance. The first research showed that the trapping performance is not affected by the container status (empty or loaded). Furthermore, in relation to the trap density, it verified that the use of one or two traps is the best compromise to maximise catching while minimising costs. Lastly, during the study it was found that the glue on the sticky cards was not strong enough to retain beetles. In fact, in the next research, the use of a stronger entomological glue made it possible to increase the number of catches for this group of insects as well. Moreover, tests conducted with different light colors, found that red, white and ultraviolet lights are the most attractive for the tested species.

At *post-border* level the most common applied strategies is based on the use of baited traps in points-of-entry surrounding areas, in order to detect the possible presence of alien species that eluded border controls (Kean and Stringer 2019, Rassati et al. 2019). Another strategy is to use remote sensing and hyperspectral imagery to detect changes in structural and temporal characteristics of vegetation, which may indicate plant damage induced by the presence of alien species (Corcoran and Hamilton 2021, Kaivosoja et al. 2021). Lastly, the involvment of non-scientists in territory survey and sample collection (Citizen Science) can be particularly usefull: is cheap, has wide application and can be conducted in a capillary manner over large areas (Poland and Rassati 2019). In Chapter 4 the effectes of variables tested in the detection protocols applied against longhorn and jewel beetles were investigate also for bark and ambrosia beetles. Variables such as trap colour, trap position and the type of bait are of crucial importance for setting effective monitoring protocols and, if not carefully controlled, could also be repulsive (*e.g.*, use of the wrong attractant). As the species that may arrive are unknown, it is essential to have monitoring protocols effective for a broad range of species, even belonging to different families. Moreover, finding the right protocol also allows reducing costs without compromising the effectiveness of the survey. In this respect, we found that the best general trapping protocol that maximize catches of all the wood-boring beetles (*i.e.*, Cerambycidae, Buprestidae and Scolytinae) must use black or purple multi-funnel traps set in the understory (especially for cerambicids and scolitids) and green multi-funnel traps set in the canopy (for buprestids and some cerambicidis and scolitids species). Furthermore, the use of a multi-lure blend composed by different cerambicid pheromones and general host volatiles (ethanol and alfa-pinene) guarantees a high number of catches both in terms of species richness and abundance. If the detection is developed for a specific species, instead, knowledge of the exact responses of the target species is equally important. In this respect, in Chapter 5 the carching performance of different trap models, trap positions and type of lures were tested in order to find the best combination maximizing caught of *Anoplophora chinensis*. This invasive longhorn beetle was intercepted several times both in North America and Europe (Haack et al. 2010, EPPO 2021) and specific knowledge about effective trapping protocols can help in future discovery of new infestations. The research showed that the Econex soft cross-vane trap is the best model, thanks to its higher capture performance and the possibility of saving space during winter storage. Furthermore, traps set on tree crowns had greater catches than traps hung on the wooden poles and Synergy blend provided the best results with high mean capture levels, although it does not differ statistically from the other two blends. In conclusion, the best trapping protocol is the use of Econex cross-vane traps baited with Synergy blend and set on tree crowns, planning periodic checks in order to avoid the over-spilling effect.

Lastly, when biosecurity surveillance fails and a new pest species establishes in a new ecosistem, eradication or, as a second choice, containment are necessary for all the harmful species. In this context, in-depth knowledge of the target species is required in order to adopt effective actions for its eradication and to prevent further spread. In Chapter 6 the dispersal capacity of *Pityophthorus juglandis* was studied using a spatio-temporal model built on infestation data recorded from an 8-year long monitoring carried out in the Veneto region. Factors affecting the WTB dispersal were analysed (*i.e.*, orchards distribution, orchards size and walnut species) providing usefull information for future eradication and containment programs. The mean annual dispersal distance registered for the pest is 9.4 km, with peaks over 40 km. Pest dispersal is affected by distance from the nearest infested site, by the number of walnut orchards in the surroundings (both infested and healthy) and by the orchard size (*i.e.*, the number of trees). Moreover, the black walnut (*Juglans nigra*) is more susceptible than the English walnut (*J. regia*). Lastly, the infestation risk estimated with the model shows the inadequacy of current containment measures based on 2 km buffer zones around infested orchards. Lastly, Chapter 7 presents the eradication program conducted in Cornuda and neighbouring municipalities against *Anoplophora glabripennis*. In this chapter, all the actions undertaken are described in order to provide a useful practical guide for the management of other ALB infestations. During the eradication program, more than 36,000 trees were surveyed, 1,157 trees were found infested, 2,361 trees were felled and the more susceptible genera were *Cercidiphyllum*, *Aesculus*, *Betula*, *Ulmus* and *Acer*. Lastly, 217 new trees from species non-susceptible to the pest were planted in private garden in order to mitigate plants felling actions.

In conclusion, this thesis addresses the different stages of biosecurity surveilance, bringing innovations and expanding current knowledge on the subject. This work presents both general researches aimed at studying wide-range tools and protocols for general surveillance, and specific studies on the containment of certain species of particular interest (*i.e.*, *A. chinensis*, *P. juglandis* and *A. glabripennis*), to improve current management and eradication protocols. However, new developments and research can be carried out on the basis of the results obtained. For example, performance of the container traps can be further improved, and the same approach used in the study on dispersal capacity of *P. juglandis* could be applied to other species of similar biology.

References

Corcoran, E., and G. Hamilton. **2021**. The future of biosecurity surveillance, pp. 261–275. *In* Barker, K., Francis, R.A. (eds.), Routledge Handb. Biosecurity Invasive Species. Routledge, London (UK).

Ekramirad, N., A. Y. Khaled, C. A. Parrish, K. D. Donohue, R. T. Villanueva, and A. A. Adedeji. **2021**. Development of pattern recognition and classification models for the detection of vibro-acoustic emissions from codling moth infested apples. Postharvest Biol. Technol. 181: 111633.

Epanchin-Niell, R. S., and A. M. Liebhold. **2015**. Benefits of invasion prevention: Effect of time lags, spread rates, and damage persistence. Ecol. Econ. 116: 146–153.

EPPO. **2021**. EPPO Global Database (available online). (https://gd.eppo.int).

Fan, J. ting, O. Denux, C. Courtin, A. Bernard, M. Javal, J. G. Millar, L. M. Hanks, and A. Roques. **2019**. Multi-component blends for trapping native and exotic longhorn beetles at potential points-of-entry and in forests. J. Pest Sci. (2004). 92: 281–297.

Haack, R. A., F. Hérard, J. Sun, and J. J. Turgeon. **2010**. Managing invasive populations of Asian Longhorned Beetle and Citrus Longhorned Beetle: a worldwide perspective. Annu. Rev. Entomol. 55: 521–546.

Hoyer-Tomiczek, U., G. Sauseng, and G. Hoch. **2016**. Scent detection dogs for the Asian longhorn beetle, *Anoplophora glabripennis*. EPPO Bull. 46: 148–155.

Kaivosoja, J., J. Hautsalo, J. Heikkinen, L. Hiltunen, P. Ruuttunen, R. Näsi, O. Niemeläinen, M. Lemsalu, E. Honkavaara, and J. Salonen. **2021**. Reference measurements in developing UAV systems for detecting pests, weeds, and diseases. Remote Sens. 13: 1238.

Kean, J. M., and L. D. Stringer. **2019**. Optimising the seasonal deployment of surveillance traps for detection of incipient pest invasions. Crop Prot. 123: 36–44.

Kenis, M., and M. Branco. **2010**. Impact of alien terrestrial arthropods in Europe. Chapter 5. BioRisk. 4: 51–71.

Lampson, B. D., Y. J. Han, A. Khalilian, J. K. Greene, D. C. Degenhardt, and J. O. Hallstrom. **2014**. Development of a portable electronic nose for detection of pests and plant damage. Comput. Electron. Agric. 108: 87–94.

Liebhold, A. M., and P. C. Tobin. **2008**. Population ecology of insect invasions and their management. Annu. Rev. Entomol. 53: 387–408.

Mankin, R. W., D. W. Hagstrum, M. T. Smith, A. L. Roda, and M. T. K. K. Kairo. **2011**. Perspective and promise: a century of insect acoustic detection and monitoring. Am. Entomol. 57: 30–44.

Nouri, B., K. Fotouhi, S. S. Mohtasebi, A. Nasiri, and S. H. Goldansaaz. **2019**. Detection of different densities of *Ephestia kuehniella* pest on white flour at different larvae instar by an electronic nose system. J. Stored Prod. Res. 84: 101522.

Poland, T. M., and D. Rassati. **2019**. Improved biosecurity surveillance of non-native forest insects: a review of current methods. J. Pest Sci. (2004). 92: 37–49.

Rassati, D., M. Faccoli, E. Petrucco Toffolo, A. Battisti, and L. Marini. **2015**. Improving the early detection of alien wood-boring beetles in ports and surrounding forests. J. Appl. Ecol. 52: 50–58.

Rassati, D., L. Marini, M. Marchioro, P. Rapuzzi, G. Magnani, R. Poloni, F. Di Giovanni, P. Mayo, and J. Sweeney. **2019**. Developing trapping protocols for wood-boring beetles associated with broadleaf trees. J. Pest Sci. (2004). 92: 267–279.

Seebens, H., S. Bacher, T. M. Blackburn, C. Capinha, W. Dawson, S. Dullinger, P. Genovesi, P. E. Hulme, M. Kleunen, I. Kühn, J. M. Jeschke, B. Lenzner, A. M. Liebhold, Z. Pattison, J. Pergl, P. Pyšek, M. Winter, and F. Essl. **2021**. Projecting the continental accumulation of alien species through to 2050. Glob. Chang. Biol. 27: 970–982.

Seebens, H., T. M. Blackburn, E. E. Dyer, P. Genovesi, P. E. Hulme, J. M. Jeschke, S. Pagad, P. Pyšek, M. Winter, M. Arianoutsou, S. Bacher, B. Blasius, G. Brundu, C. Capinha, L. Celesti-Grapow, W. Dawson, S. Dullinger, N. Fuentes, H. Jäger, J. Kartesz, M. Kenis, H. Kreft, I. Kühn, B. Lenzner, A. Liebhold, A. Mosena, D. Moser, M. Nishino, D. Pearman, J. Pergl, W. Rabitsch, J. Rojas-Sandoval, A. Roques, S. Rorke, S. Rossinelli, H. E. Roy, R. Scalera, S. Schindler, K. Štajerová, B. Tokarska-Guzik, M. van Kleunen, K. Walker, P. Weigelt, T. Yamanaka, and F. Essl. **2017**. No saturation in the accumulation of alien species worldwide. Nat. Commun. 8: 14435.

Suma, P., A. La Pergola, S. Longo, and V. Soroker. **2014**. The use of sniffing dogs for the detection of *Rhynchophorus ferrugineus*. Phytoparasitica. 42: 269–274.

Vaes-Petignat, S., and W. Nentwig. **2014**. Environmental and economic impact of alien terrestrial arthropods in Europe. NeoBiota. 22: 23–42.

Zorović, M., and A. Čokl. **2015**. Laser vibrometry as a diagnostic tool for detecting wood-boring beetle larvae. J. Pest Sci. (2004). 88: 107–112.

Appendix

Appendix A (Chapter 3)

Table A1 Mean (± SE) number of insects captured by each trap combination during the “Single color tests”, divided for each light color. C = control; L = light on; G = glue added; I = insecticide sprayed.

		K	G	I	L	L + G	L + I	G + I	L + G + I
WHITE	*Cadra cautella* Walker	0.71 ± 0.18	0.42 ± 0.20	0.85 ± 0.34	5.14 ± 0.40	4.00 ± 0.30	4.14 ± 0.50	0.57 ± 0.36	4.42 ± 0.42
	Drosophila melanogaster Meigen	0.28 ± 0.18	0.28 ± 0.28	0.42 ± 0.29	9.28 ± 1.68	4.57 ± 0.99	6.42 ± 2.71	0.42 ± 0.42	6.57 ± 2.84
	Sitophilus zeamais Motschulsky	-	-	-	1.00 ± .030	4.85 ± 0.98	0.57 ± 0.29	-	4.42 ± 0.71
	Triboliu castaneum (Herbst)	-	-	-	0.14 ± 0.14	-	-	-	0.14 ± 0.14
INFRARED	*Cadra cautella* Walker	1.42 ± 0.52	1.14 ± 0.55	1.85 ± 0.76	1.28 ± 0.60	1.14 ± 0.50	1.71 ± 0.42	1.71 ± 0.35	2.28 ± 0.60
	Drosophila melanogaster Meigen	1.57 ± 0.71	2.42 ± 0.57	2.00 ± 0.48	1.42 ± 0.71	2.14 ± 0.55	1.42 ± 0.64	1.85 ± 0.70	2.28 ± 0.56
	Sitophilus zeamais Motschulsky	-	0.28 ± 0.18	-	-	-	-	0.28 ± 0.28	0.28 ± 0.28
	Triboliu castaneum (Herbst)	-	0.57 ± 0.28	-	-	0.14 ± 0.14	0.28 ± 0.28	-	1.00 ± 0.48
ULTRAVIOLET	*Cadra cautella* Walker	2.00 ± 1.52	1.28 ± 0.74	1.28 ± 0.89	9.00 ± 2.98	7.00 ± 2.26	6.71 ± 2.02	0.85 ± 0.34	6.00 ± 1.77
	Drosophila melanogaster Meigen	0.71 ± 0.42	1.14 ± 0.40	0.28 ± 0.28	6.14 ± 1.84	7.28 ± 1.61	9.14 ± 0.14	1.00 ± 0.57	11.00 ± 2.00
	Sitophilus zeamais Motschulsky	-	0.14 ± 0.14	-	0.14 ± 0.14	1.85 ± 0.26	0.14 ± 0.14	0.14 ± 0.14	2.00 ± 0.30
	Triboliu castaneum (Herbst)	-	0.42 ± 0.20	0.14 ± 0.14	0.57 ± 0.29	1.57 ± 0.29	0.42 ± 0.29	0.28 ± 0.18	2.42 ± 0.71
RED	*Cadra cautella* Walker	0.85 ± 0.26	0.85 ± 0.34	0.42 ± 0.20	3.71 ± 0.42	3.28 ± 0.42	4.14 ± 1.26	1.00 ± 0.30	6.14 ± 2.06
	Drosophila melanogaster Meigen	0.42 ± 0.42	1.14 ± 0.67	0.57 ± 0.29	5.57 ± 2.22	6.28 ± 1.65	8.14 ± 3.00	1.28 ± 0.42	10.10 ± 3.52
	Sitophilus zeamais Motschulsky	-	-	-	0.14 ± 0.14	4.42 ± 0.48	0.42 ± 0.29	0.14 ± 0.14	4.71 ± 0.52
	Triboliu castaneum (Herbst)	-	-	-	0.14 ± 0.14	7.71 ± 0.56	0.42 ± 0.29	0.28 ± 0.18	8.28 ± 0.42

Table A2 Output (P-value) of the multiple comparisons between different trap combinations conducted with Tukey's test in the "Single color tests" and "Multi-color test". * P < 0.05; ** P < 0.01; *** P < 0.001. Single color tests: C = control; L = light on; G = glue added; I = insecticide sprayed. Multi-color test: C = control; W = white light; IR = infrared light; UV = ultraviolet light; R = red light.

Model species		C	G	I	L	L+G	L+I	G+I
Single color test - White light								
Cadra cautella	G	-						
	I	-	-					
	L	***	***	***				
	L+G	***	***	***	0.823			
	L+I	***	***	***	-	-		
	G+I	-	-	-	***	***	***	
	L+G+I	***	***	***	-	-	-	***
Drosophila melanogaster	G	-						
	I	-	-					
	L	***	***	***				
	L+G	**	**	**	*			
	L+I	***	***	***	-	-		
	G+I	-	-	-	***	**	***	
	L+G+I	***	***	***	-	-	-	***
Single color test - Infrared light								
Cadra cautella	G	-						
	I	-	-					
	L	-	-	-				
	L+G	-	-	-	-			
	L+I	-	-	-	-	-		
	G+I	-	-	-	-	-	-	
	L+G+I	-	-	-	-	-	-	-
Drosophila melanogaster	G	-						
	I	-	-					
	L	-	-	-				
	L+G	-	-	-	-			
	L+I	-	-	-	-	-		
	G+I	-	-	-	-	-	-	
	L+G+I	-	-	-	-	-	-	-
Single color test - Ultraviolet light								
Cadra cautella	G	-						
	I	-	-					
	L	*	*	*				
	L+G	*	*	*	-			
	L+I	*	*	*	-	-		
	G+I	-	-	-	*	*	*	
	L+G+I	*	*	*	-	-	-	*
Drosophila melanogaster	G	-						
	I	-	-					
	L	***	***	***				
	L+G	***	***	***	-			
	L+I	***	***	***	-	-		
	G+I	-	-	-	***	***	***	
	L+G+I	***	***	***	0.062	0.629	-	***
Single color test - Red light								
Cadra cautella	G	-						
	I	-	-					
	L	*	*	*				
	L+G	*	*	*	-			
	L+I	*	*	**	-	-		
	G+I	-	-	-	*	*	*	
	L+G+I	***	***	***	-	-	-	***

Drosophila melanogaster	G	-						
	I	-	-					
	L	***	***	***				
	L+G	***	**	***	-			
	L+I	***	***	***	-	-		
	G+I	-	-	-	**	*	***	
	L+G+I	***	***	***	-	-	-	**

Multi-color test		C	W	IR	UV
Cadra cautella	W	0.351			
	IR	-	0.351		
	UV	**	-	**	
	R	0.351	-	0.351	-
Drosophila melanogaster	W	***			
	IR	-	***		
	UV	***	0.328	***	
	R	***	***	***	**
Sitophilus zeamais	W	***			
	IR	-	***		
	UV	*	0.220	*	
	R	***	**	***	***
Tribolium castaneum	W	0.150			
	IR	-	0.150		
	UV	***	***	***	
	R	***	***	***	***

Table A3 Output (P-value) of the multiple comparisons between different trap combinations conducted with Kruskal-Wallis test in the "Single color tests". * P < 0.05; ** P < 0.01. C = control; L = light on; G = glue added; I = insecticide sprayed.

Model species		C	G	I	L	L+G	L+I	G+I
Single color test - White light								
Sitophilus zeamais	G	-						
	I	-	-					
	L	*	*	*				
	L+G	**	**	**	*			
	L+I	0.08	0.08	0.82	0.35	*		
	G+I	-	-	-	*	**	0.08	
	L+G+I	**	**	**	**	0.56	**	**
Single color test - Infrared light								
Sitophilus zeamais	G	0.47						
	I	-	0.47					
	L	-	0.47	-				
	L+G	-	0.47	-	-			
	L+I	-	0.47	-	-	-		
	G+I	0.47	0.76	0.47	0.47	0.47	0.47	
	L+G+I	0.47	0.76	0.47	0.47	0.47	0.47	-
Tribolium castaneum	G	0.21						
	I	-	0.21					
	L	-	0.21	-				
	L+G	0.43	0.43	0.43	0.43			
	L+I	0.43	0.43	0.43	0.43	-		
	G+I	-	0.21	-	-	0.43	0.43	
	L+G+I	0.21	0.70	0.21	0.21	0.43	0.43	0.21
Single color test - Ultraviolet light								
Sitophilus zeamais	G	0.53						
	I	-	0.53					
	L	0.53	-	0.53				
	L+G	**	**	**	**			
	L+I	0.53	-	0.53	-	**		
	G+I	0.53	-	0.53	-	**	-	
	L+G+I	**	**	**	**	-	**	**
Tribolium castaneum	G	0.14						
	I	0.55	0.43					
	L	0.14	0.92	0.40				
	L+G	**	*	**	0.09			
	L+I	0.29	0.88	0.69	0.79	0.05		
	G+I	0.29	0.75	0.72	0.69	*	0.94	
	L+G+I	**	**	**	**	*	**	**
Single color test - Red light								
Sitophilus zeamais	G	-						
	I	-	-					
	L	0.47	0.47	0.47				
	L+G	**	**	**	**			
	L+I	0.29	0.29	0.29	0.58	**		
	G+I	0.47	0.47	0.47	-	**	0.58	
	L+G+I	**	**	**	**	0.77	**	**
Tribolium castaneum	G	-						
	I	-	-					
	L	0.47	0.47	0.47				
	L+G	**	**	**	**			
	L+I	0.24	0.24	0.24	0.58	**		
	G+I	0.24	0.24	0.24	0.62	**	0.94	
	L+G+I	**	**	**	**	0.54	**	**

Appendix B (Chapter 4)

Table B1 Locality, country, geographic coordinates, and forest type for each of the 18 sites (17 in Italy and 1 in Canada) where sampling occurred in 2016.

Locality	Country	Lat.	Long.	Forest type
Carlino	Italy	45.792788°	13.206675°	Reforested forest
Carlino	Italy	45.785140°	13.214767°	Semi-natural forest
Cavalier	Italy	45.763795°	12.552077°	Semi-natural forest
Cessalto	Italy	45.700941°	12.617732°	Semi-natural forest
Gaiarine	Italy	45.860650°	12.492083°	Semi-natural forest
Gaiarine	Italy	45.861352°	12.493672°	Reforested forest
Malisana	Italy	45.806015°	13.240315°	Semi-natural forest
Meolo	Italy	45.635649°	12.437976°	Reforested forest
Merlo	Italy	45.746736°	12.742403°	Semi-natural forest
Muzzana del Turgnano	Italy	45.798868°	13.109849°	Reforested forest
Muzzana del Turgnano	Italy	45.791784°	13.120926°	Semi-natural forest
Palazzolo dello Stella	Italy	45.767133°	13.079461°	Reforested forest
Pampaluna	Italy	45.853809°	13.195429°	Semi-natural forest
Precenicco	Italy	45.776864°	13.063894°	Semi-natural forest
Sacile	Italy	45.781893°	13.201817°	Reforested forest
San Stino di Livenza	Italy	45.750036°	12.704410°	Reforested forest
Santa Maria di Campagna	Italy	45.705331°	12.579475°	Reforested forest
Halifax	Canada	44.42847°	63.37975°	Mixed forest

Fig. B1 Experimental scheme used in Italy and Canada in 2016 to test for the effect of trap color, trap height and attractive blend on bark and ambrosia beetle catches in traps. In Italy, red dots indicate semi-natural forests, yellow dots indicate reforested forests.

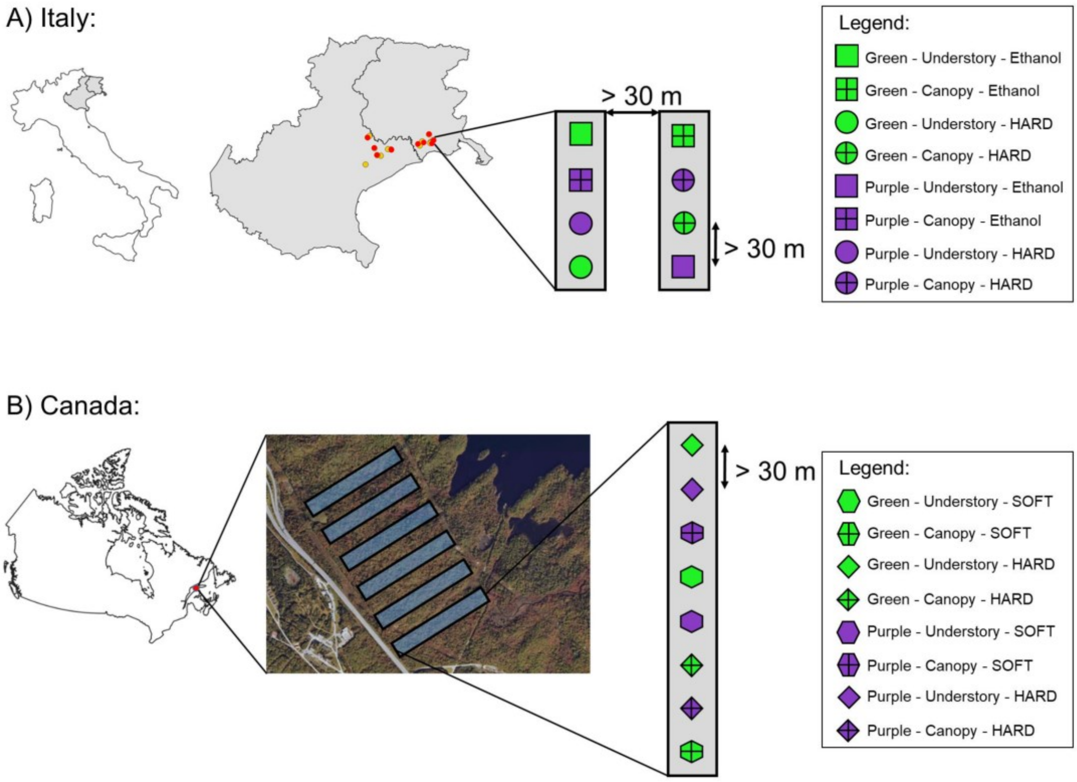

Table B2 List of pheromones and host volatile lures used in Italy (A. hard-blend) and Canada (A. hard-blend and B. soft-blend) with percentage purity, release rates, and sources.

Attractants	Release device	Purity (%)	Release rate (mg/day) at 20°C	Source
A. Hard-blend				
Racemic 3-hydroxyhexan-2-one	Pouch	99%^{1}	20-25	Bedoukian Research Danbury, CT/Contech Enterprises, Delta, BC
Racemic 3-hydroxyoctan-2-one	Pouch	99%^{1}	20-25	Bedoukian Research/Contech Enterprises
Racemic *syn*-2,3-hexanediols	Pouch	99%^{1}	1-2	Atlantic Forestry Centre, Fredericton, NB/Contech Enterprises
(*E/Z*)-fuscumol	Rubber septa	99%^{2}	0.5-2	Sylvar Technologies, Fredericton, NB
(*E/Z*)-fuscumol acetate	Rubber septa	99%^{2}	0.5-2	Sylvar Technologies
Ethanol UHR lure	Pouch	99%^{2}	300-400	Contech Enterprises
B. Soft-blend				
Alpha-pinene (+25/-75) UHR lure	Pouch	98%^{2}	2000	Contech Enterprises
Ethanol UHR lure	Pouch	98%^{2}	300-400	Contech Enterprises
Ipsenol (racemic)	Bubble cap	92%^{2}	0.300	Synergy Semiochemicals, Delta, BC
Monochamol	Bubble cap	97%^{2}	0.750	Synergy Semiochemicals, Delta, BC

1 Determined at Canadian Forest Service, Atlantic Forest Centre, Fredericton, NB
2 Percent purity and release rate provided by the supplier

Table B3 Mean (± standard error) number of species and individuals trapped by each lure (ethanol vs hard-blend), height (canopy vs understory), color (green vs purple) combination in the two different forest types (semi-natural forests vs reforested forests) in Italy.

	Italy							
	Ethanol				Hard-blend			
	Canopy		Understory		Canopy		Understory	
	Green	Purple	Green	Purple	Green	Purple	Green	Purple
Semi-natural forests								
Ambrosia richness	2.88 ± 0.42	3.11 ± 0.26	3.88 ± 0.42	4.33 ± 0.37	2.66 ± 0.23	3.11 ± 0.30	4.00 ± 0.28	3.77 ± 0.36
Ambrosia abundance	512.00 ± 171.74	427.77 ± 72.26	1359.77 ± 282.41	2639.22 ± 669.10	361.44 ± 107.17	351.77 ± 72.55	1080.00 ± 163.18	1974.44 ± 625.76
Ambrosiodmus rubricollis	-	-	0.22 ± 0.22	1.00 ± 1.00	-	-	-	-
Ambrosiophilus atratus	0.33 ± 0.33	-	-	-	-	-	0.77 ± 0.77	-
Anisandrus dispar	4.88 ± 1.60	8.55 ± 2.79	32.00 ± 12.03	27.66 ± 13.67	7.22 ± 2.36	7.33 ± 3.85	25.11 ± 9.93	16.44 ± 5.89
Xyleborinus saxesenii	485.55 ± 168.18	386.22 ± 74.78	889.88 ± 140.14	1791.88 ± 615.62	347.11 ± 107.91	327.22 ± 72.97	794.00 ± 159.29	1346.00 ± 522.31
Xyleborus dryographus	-	-	0.22 ± 0.22	1.22 ± 0.64	-	-	-	0.88 ± 0.58
Xyleborus monographus	1.44 ± 0.80	0.33 ± 0.33	3.44 ± 2.96	16.88 ± 14.55	0.88 ± 0.56	0.11 ± 0.11	6.11 ± 4.18	5.55 ± 3.88
Xylosandrus crassiusculus	1.55 ± 1.22	1.22 ± 0.99	0.88 ± 0.38	3.22 ± 1.07	1.88 ± 0.78	1.33 ± 0.60	2.77 ± 1.19	4.22 ± 2.30
Xylosandrus germanus	18.22 ± 6.66	31.44 ± 9.39	433.11 ± 191.52	797.33 ± 373.45	4.33 ± 3.97	15.77 ± 4.82	251.22 ± 88.92	601.33 ± 202.41
Reforested forests								
Ambrosia richness	2.75 ± 0.31	2.62 ± 0.32	2.75 ± 0.36	3.75 ± 0.36	2.25 ± 0.41	3.00 ± 0.26	2.50 ± 0.53	3.50 ± 0.32
Ambrosia abundance	301.50 ± 98.22	321.87 ± 121.20	518.50 ± 187.06	874.00 ± 332.39	178.75 ± 60.71	149.12 ± 53.12	390.87 ± 145.45	588.62 ± 172.20
Ambrosiodmus rubricollis	-	-	-	-	-	-	-	-
Ambrosiophilus atratus	-	0.37 ± 0.37	-	0.62 ± 0.62	-	-	-	0.50 ± 0.50
Anisandrus dispar	22.00 ± 9.81	17.87 ± 7.86	31.50 ± 16.86	24.50 ± 10.64	20.00 ± 8.54	16.00 ± 7.35	38.25 ± 17.27	19.75 ± 9.55
Xyleborinus saxesenii	263.12 ± 85.80	283.37 ± 122.08	443.12 ± 184.25	801.37 ± 327.52	153.00 ± 55.21	115.00 ± 47.57	328.25 ± 123.87	471.37 ± 152.37
Xyleborus dryographus	-	-	-	0.37 ± 0.37	-	1.50 ± 1.50	-	-
Xyleborus monographus	-	-	-	0.50 ± 0.37	-	-	-	-
Xylosandrus crassiusculus	11.00 ± 6.66	2.87 ± 1.60	9.25 ± 6.28	12.25 ± 7.44	1.75 ± 0.94	6.50 ± 3.03	12.87 ± 8.91	29.62 ± 24.31
Xylosandrus germanus	5.37 ± 2.14	17.37 ± 13.56	34.62 ± 20.43	34.37 ± 10.03	4.00 ± 2.93	10.12 ± 9.69	11.50 ± 4.09	67.37 ± 35.16

Table B4 Mean (± standard error) number of ambrosia beetle and bark beetle species and individuals trapped by each lure (hard-blend vs soft-blend), height (canopy vs understory), and color (green vs purple) combination in Canada.

	Canada							
	Hard-blend				Soft-blend			
	Canopy		Understory		Canopy		Understory	
	Green	Purple	Green	Purple	Green	Purple	Green	Purple
Ambrosia beetles								
Species richness	4.50 ± 0.42	4.16 ± 0.40	5.00 ± 0.36	5.00 ± 0.25	4.00 ± 0.44	4.16 ± 0.30	5.66 ± 0.42	5.50 ± 0.34
Abundance	225.66 ± 31.40	268.50 ± 41.35	435.50 ± 64.33	565.66 ± 98.09	40.16 ± 10.49	50.00 ± 4.48	199.16 ± 35.84	395.83 ± 51.18
Anisandrus dispar	0.33 ± 0.33	-	1.33 ± 0.80	1.16 ± 0.40	-	-	0.16 ± 0.16	-
Anisandrus sayi	188.00 ± 26.92	217.66 ± 37.49	222.83 ± 16.15	200.16 ± 35.50	12.33 ± 4.02	15.16 ± 2.88	4.66 ± 0.88	12.50 ± 1.60
Gnathotrichus materiarius	-	0.16 ± 0.16	-	-	-	0.16 ± 0.16	1.00 ± 0.44	0.33 ± 0.21
Monarthrum mali	-	0.33 ± 0.21	-	-	-	-	-	-
Trypodendron betulae	-	-	-	-	-	-	0.16 ± 0.16	-
Trypodendron lineatum	0.16 ± 0.16	-	0.16 ± 0.16	0.33 ± 0.21	1.33 ± 0.33	3.50 ± 0.88	14.83 ± 3.28	18.00 ± 5.84
Xyleborinus attenuatus	28.33 ± 10.02	44.00 ± 13.39	38.50 ± 8.26	36.66 ± 12.81	23.50 ± 9.15	25.83 ± 4.15	18.66 ± 3.96	52.16 ± 9.53
Xyleborinus saxesenii	1.00 ± 0.44	0.33 ± 0.33	0.33 ± 0.21	-	-	-	-	0.16 ± 0.16
Xylosandrus germanus	6.00 ± 3.28	1.16 ± 0.40	168.33 ± 46.03	321.83 ± 63.79	2.00 ± 1.41	4.50 ± 3.71	158.00 ± 36.18	310.33 ± 57.02
Xyloterinus politus	1.83 ± 0.70	4.83 ± 0.70	4.00 ± 0.96	5.50 ± 1.02	1.00 ± 0.44	0.83 ± 0.40	1.66 ± 0.66	2.33 ± 0.61
Bark beetles								
Species richness	5.00 ± 1.00	7.33 ± 0.80	6.33 ± 0.66	7.50 ± 0.76	9.83 ± 0.90	8.50 ± 1.05	11.66 ± 0.80	12.83 ± 0.79
Abundance	60.12 ± 17.40	98.49 ± 27.01	46.90 ± 15.14	39.56 ± 7.66	54.52 ± 8.14	67.83 ± 12.62	113.50 ± 28.46	105.00 ± 22.26
Conophthorus coniperda	-	0.16 ± 0.16	-	0.16 ± 0.16	-	-	0.16 ± 0.16	0.16 ± 0.16
Cryphalus ruficollis	0.83 ± 0.65	1.16 ± 0.30	3.33 ± 1.30	10.83 ± 4.62	6.33 ± 2.02	1.66 ± 0.55	56.00 ± 26.31	31.83 ± 15.97
Crypturgus borealis	4.00 ± 3.02	2.00 ± 0.85	1.16 ± 0.65	1.16 ± 0.47	1.83 ± 0.54	1.33 ± 0.71	1.33 ± 0.80	1.50 ± 0.34
Crypturgus pusillus	1.33 ± 0.88	0.66 ± 0.21	1.33 ± 0.49	1.50 ± 0.76	4.16 ± 0.94	15.83 ± 6.53	14.16 ± 3.99	17.33 ± 7.76
Dendroctonus rufipennis	-	0.16 ± 0.16	-	-	-	-	0.16 ± 0.16	-
Dryocoetes affaber	-	-	0.33 ± 0.21	0.33 ± 0.21	-	-	1.66 ± 0.66	3.33 ± 0.71
Dryocoetes autographus	-	0.33 ± 0.21	1.16 ± 0.54	0.66 ± 0.21	-	0.16 ± 0.16	3.16 ± 1.04	3.33 ± 1.05
Hylastes opacus	-	-	-	-	-	-	1.33 ± 0.88	0.83 ± 0.30
Hylastinus obscurus	-	-	-	-	-	-	0.50 ± 0.50	0.66 ± 0.42
Hylesinus aculeatus	0.16 ± 0.16	-	-	-	0.33 ± 0.21	-	-	-
Hylurgops rugipennis	-	-	-	-	-	-	-	0.16 ± 0.16
Ips grandicollis	-	-	0.66 ± 0.66	0.16 ± 0.16	15.16 ± 3.32	15.33 ± 2.21	14.00 ± 3.85	16.00 ± 5.60
Ips perroti	-	-	-	-	-	-	0.16 ± 0.16	-
Ips pini	-	0.16 ± 0.16	-	-	0.83 ± 0.30	0.66 ± 0.33	1.16 ± 0.47	0.50 ± 0.22
Lymantor decipiens	0.16 ± 0.16	-	-	-	0.50 ± 0.34	0.66 ± 0.33	0.33 ± 0.21	1.00 ± 0.36
Orthotomicus caelatus	-	-	-	0.50 ± 0.34	4.33 ± 2.59	3.66 ± 1.40	8.83 ± 2.41	12.33 ± 3.62
Orthotomicus latidens	-	-	-	-	-	0.16 ± 0.16	-	-
Pityogenes hopkinsi	-	0.50 ± 0.50	-	0.33 ± 0.21	0.33 ± 0.21	0.16 ± 0.16	0.16 ± 0.16	0.16 ± 0.16
Pityokteines sparsus	-	-	-	-	0.16 ± 0.16	-	0.16 ± 0.16	-
Pityophthorus ramiperda	0.16 ± 0.16	0.50 ± 0.34	0.16 ± 0.16	0.33 ± 0.21	-	-	-	-
Pityophthorus sp.	0.83 ± 0.47	3.66 ± 1.28	0.50 ± 0.22	1.16 ± 0.40	0.33 ± 0.21	0.16 ± 0.16	1.16 ± 0.79	0.33 ± 0.33
Polygraphus rufipennis	0.70 ± 0.34	2.17 ± 0.86	0.86 ± 0.50	1.17 ± 0.47	2.34 ± 0.51	5.50 ± 3.93	2.16 ± 0.74	5.50 ± 1.72
Pseudopityophthorus asperulus	11.66 ± 4.63	39.66 ± 13.50	10.16 ± 4.31	9.16 ± 2.68	8.83 ± 1.55	14.66 ± 1.96	4.16 ± 1.35	6.00 ± 1.96
Pseudopityophthorus minutissimus	34.16 ± 13.83	42.83 ± 14.24	26.50 ± 14.69	10.16 ± 4.60	4.16 ± 1.47	7.83 ± 2.77	2.66 ± 0.61	3.50 ± 0.95
Scolytus piceae	0.16 ± 0.16	0.16 ± 0.16	0.16 ± 0.16	-	-	-	-	-

Appendix C (Chapter 6)

Table C1 List of the sites monitored from 2013 to 2020, reporting year of survey beginning, ID code, province and municipality, coordinates (UTM 32N), tree species ("Mixed" refers to a mixed *J. nigra* and *J. regia* orchard), number of trees (orchard size), year of infestation, and number of WTB caught (v.i. = visual inspection, for the sites where the pest presence was verified without the use of traps).

Surveyed from	Site ID	Province	Municipality	Latitude	Longitude	Tree species	N° of trees	Attack year	N° of catches
2013	PR82	PD	Bovolenta	5016344.84	733083.72	Mixed	500		-
2013	PR75	PD	Cartura	5017809.45	726463.12	Mixed	30		-
2013	PR70	PD	Ospedaletto Euganeo	5013073.67	702558.99	Mixed	420		-
2013	PR69	PD	San Pietro Viminario	5012691.72	721563.76	Mixed	200		-
2013	PR30	PD	Urbana	5005996.54	692377.60	Mixed	500		-
2013	PR32	PD	Vigonza	5035891.33	730755.76	*J. nigra*	110		-
2013	PR08	VI	Agugliaro	5023192.77	703333.75	*J. nigra*	92	2013	v.i.
2013	S1	VI	Bressanvido	5058515.27	706454.91	*J. nigra*	35	2013	276
2013	PR21	VI	Campiglia dei Berici	5024652.19	699761.81	*J. nigra*	140	2013	164
2013	PR45	VI	Dueville	5054136.44	699477.28	Mixed	160	2013	v.i.
2013	PR63	VI	Lonigo	5032877.69	684157.98	Mixed	27	2013	13
2013	PR27	VI	Monticello Conte Otto	5054307.38	702680.43	Mixed	15		-
2013	PR99	VI	Quinto Vicentino	5047951.82	704535.11	Mixed	15	2013	v.i.
2013	S2	VI	Sandrigo	5056794.12	702124.52	*J. nigra*	15	2013	504
2013	S3	VI	Sandrigo	5058511.19	702567.79	*J. nigra*	60	2013	2311
2013	S5	VI	Schio	5065905.83	687052.82	*J. nigra*	20	2013	1054
2013	S4	VI	Thiene	5060075.11	695208.03	*J. nigra*	30	2013	52
2013	PR40	VI	Trissino	5048108.72	682642.16	*J. nigra*	60	2019	v.i.
2013	PR79	VI	Villaverla	5057916.78	692295.37	Mixed	30	2013	v.i.
2014	ID017	PD	Campodarsego	5042245.45	730361.81	*J. nigra*	275	2019	14
2014	ID043	PD	Casalserugo	5021259.08	727993.87	*J. nigra*	108	2014	v.i.
2014	ID001	PD	Mestrino	5038032.55	713688.23	*J. nigra*	790	2014	199
2014	ID013	PD	Tribano	5006285.82	723619.13	*J. nigra*	382	2014	v.i.
2014	ID073	RO	Stienta	4979554.9	701046.23	*J. nigra*	75		-
2014	ID098	TV	Gorgo al Monticano	5077480.78	775627.72	*J. nigra*	80		-
2014	ID110	TV	Pederobba	5081925.98	731134.73	*J. nigra*	30	2016	v.i.
2014	ID077	TV	Postioma di Paese	5067357.03	746397.23	*J. nigra*	1550	2014	36
2014	ID102	TV	Treville	5060271.71	725900.91	*J. nigra*	70	2014	87
2014	ID129	VE	Cavarzere	5003169.26	736619.95	*J. nigra*	120		-
2014	ID128	VE	Meolo	5056181.14	769539.22	*J. nigra*	154		-
2014	ID124	VE	S Stino di Livenza	5069297.02	793512.47	*J. nigra*	200		-
2014	ID143	VR	Isola Rizza	5017529.71	673782.31	*J. nigra*	75	2018	31
2014	ID140	VR	Nogarole Rocca	5019116.24	649437.17	*J. nigra*	180	2017	27
2015	ID019	PD	Bagnoli di Sopra	5005308.9	724935.69	*J. nigra*	262		-
2015	ID040	PD	Cartura	5018808.78	725016.77	*J. nigra*	114		-
2015	ID030	PD	Codevigo	5014118.58	743046.86	*J. nigra*	170		-
2015	ID044	PD	Codevigo	5020053.46	744168.26	*J. nigra*	105		-
2015	JR08	PD	Piazzola sul Brenta	5046872.42	714964.47	*J. regia*	11000	2016	395
2015	ID048	PD	Rubano	5035538.81	719743.14	*J. nigra*	103	2015	1715
2015	ID015	PD	Saccolongo e Veggiano	5031902.53	713239.94	*J. nigra*	334	2019	v.i.

2015	ID049	PD	Trebaseleghe	5051727.57	736364.76	*J. nigra*	100	2015	v.i.
2015	ID039	PD	Veggiano	5035273.57	712991.82	*J. nigra*	118		-
2015	ID058	PD	Villa Estense	5004465.53	710856.23	*J. nigra*	79	2016	215
2015	ID075	RO	Ceregnano	4990246.1	731892.84	*J. nigra*	32		-
2015	JR04	RO	Costa di Rovigo	4991649.21	712989.34	*J. regia*	2800	2020	v.i.
2015	ID065	RO	Crespino	4987485.59	726966.04	*J. nigra*	400		-
2015	ID064	RO	Porto Tolle	4971823.19	773177.32	*J. nigra*	430		-
2015	ID074	RO	Porto Tolle	4979339.85	765962.91	*J. nigra*	34		-
2015	ID076	RO	Porto Tolle	4978030.24	768802.24	*J. nigra*	17		-
2015	ID068	RO	Trecenta e Salara	4986515.83	694484.28	*J. nigra*	225	2019	15
2015	ID081	TV	Altivole	5070491.2	730978.78	*J. nigra*	300	2015	19
2015	ID109	TV	Gaiarine	5089706.03	770119.01	*J. nigra*	38		-
2015	ID085	TV	Roncade	5056010.98	763616.73	*J. nigra*	230		-
2015	ID091	TV	San Fior	5091054.12	758835.15	*J. nigra*	150		-
2015	ID093	TV	Spresiano	5073815.66	755371.77	*J. nigra*	130	2017	v.i.
2015	ID114	VE	Caorle	5061653.82	800991.03	*J. nigra*	510		-
2015	ID122	VE	Chioggia	5005712.39	756746.00	*J. nigra*	200		-
2015	ID131	VE	Dolo	5035743.35	737520.70	*J. nigra*	102		-
2015	ID123	VE	Fossalta di Piave	5079118.33	802843.39	*J. nigra*	200		-
2015	ID120	VE	Fossalta di Portogruaro	5059003.01	773279.47	*J. nigra*	240		-
2015	ID133	VE	Gruaro	5082864.6	799178.47	*J. nigra*	55		-
2015	ID127	VE	Marcon	5047751.91	761798.65	*J. nigra*	179		-
2015	ID112	VE	San Stino di Livenza	5063902.69	793174.24	*J. nigra*	620		-
2015	ID136	VE	Scorzè	5052311.58	740582.67	*J. nigra*	20	2016	138
2015	TM03	VI	Conco	5073854.74	702082.27	*J. nigra*	40	2016	v.i.
2015	ID137	VR	Bovolone	5013929.01	667991.20	*J. nigra*	350		-
2015	ID145	VR	Oppeano	5016126.97	668771.18	*J. nigra*	35	2016	371
2015	ID141	VR	San Pietro di Morubio	5010861.16	672088.61	*J. nigra*	100	2018	293
2015	ID139	VR	Terrazzo	5004748.03	687565.87	*J. nigra*	210		-
2015	ID144	VR	Verona	5040229.32	658997.30	*J. nigra*	50	2016	32
2015	ID142	VR	Vigasio	5019906.82	649680.52	*J. nigra*	100	2015	21
2016	ID302	VR	Castion	5051678.59	634807.48	*J. nigra*	9	2019	v.i.
2017	ID055	PD	Ospedaletto Euganeo	5013729.9	703160.85	*J. nigra*	91	2019	v.i.
2017	ID072	RO	Costa di Rovigo	4992227.37	711423.54	*J. nigra*	80		-
2017	ID088	TV	Breda di Piave	5066264.49	758349.33	*J. nigra*	185		-
2017	ID089	TV	Preganziol	5052615.32	753160.49	*J. nigra*	170		-
2017	ID113	VE	Noale	5046714.4	739023.78	*J. nigra*	550		-
2018	ID061	PD	Montagnana	5011239.07	693755.89	*J. nigra*	70	2020	33
2018	ID028	PD	Padova	5026717.51	726859.37	*J. nigra*	180		-
2018	ID121	VE	San Donà di Piave	5053548.59	780806.93	*J. nigra*	216		-
2019	JR11	TV	Castelfranco	5063898.71	729681.77	*J. regia*	119	2019	25
2019	JR12	TV	Cessalto	5069064.51	781335.65	*J. regia*	5791		-
2019	JR13	TV	Maserada sul Piave	5070020.53	759490.72	*J. regia*	832		-
2019	JR14	TV	Maserada sul Piave	5070949.87	758800.66	*J. regia*	109		-
2019	JR15	TV	Treviso	5059135.18	748605.04	*J. regia*	271		-
2019	JR16	TV	Villorba	5071552.48	751456.86	*J. regia*	543	2019	72
2020	ID150	PD	Bovolenta	5016309.93	733159.72	*J. regia*	9		-

2020	V17	PD	Bovolenta	5016099.18	734040.51	*J. regia*	6000	2020	v.i.
2020	ID018	PD	Candiana	5013791.15	734687.48	*J. nigra*	266		-
2020	ID032	PD	Candiana	5011996.09	734271.97	*J. nigra*	160	2020	v.i.
2020	ID147	PD	Candiana	5012066.58	735057.25	*J. nigra*	322		-
2020	ID400	PD	Polverara	5022787.31	730547.99	*J. regia*	10		-
2020	ID026	PD	S. Angelo di Piove di Sacco	5023884.95	735213.18	*J. nigra*	204		-
2020	V01	PD	Saonara	5027396.15	735073.43	*J. regia*	615		-
2020	V02	PD	Saonara	5028456.69	732592.88	*J. regia*	1750		-
2020	V03	PD	Saonara	5027234.10	733971.67	*J. regia*	800	2020	v.i.
2020	V06	PD	Saonara	5027313.82	734269.95	*J. regia*	1800		-
2020	V08	PD	Saonara	5026837.09	733873.39	*J. regia*	600		-
2020	V09	PD	Saonara	5027195.74	735149.53	*J. regia*	600		-
2020	V14	PD	Saonara	5026517.69	734633.38	*J. regia*	150		-
2020	V19	PD	Saonara	5024916.11	735538.38	*J. regia*	3000		-
2020	ID135	VE	Camponogara	5031897.85	740145.08	*J. nigra*	52		-
2020	V11	VE	Camponogara	5031168.02	740168.46	*J. regia*	900		-
2020	ID117	VE	Dolo	5034780.70	741970.12	*J. nigra*	289		-
2020	ID119	VE	Dolo	5032332.90	742070.82	*J. nigra*	275		-
2020	ID130	VE	Fossò	5027802.25	739145.07	*J. regia*	6		-