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Invasive wood borers: Detection, Establishment and Spread

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Padova, 18 January 2015

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Riassunto

L'incremento di scambi commerciali internazionali avvenuto ultimi decenni ha portato ad un aumento del rischio di introduzione di specie invasive, organismi che vengono introdotti in nuovi ambienti al di fuori del loro areale d'origine. Questi sono soprattutto piante ed insetti. Tra gli insetti, di particolare interesse forestale risultano essere le specie xilofaghe. Queste introduzioni hanno gravi effetti economici ed ecologici, ed è un obiettivo prioritario l'intercettazione e l'eradicazione di queste specie nei siti di introduzione.

Nel presente lavoro viene presa in considerazione il coleottero cerambicide di origine asiatica *Anoplophora glabripennis* (Motschulsky). Questo cerambicide rappresenta una grande minaccia per le foreste temperate dell'emisfero boreale, e ricorre in molte aree di infestazione in Europa e Nord America. La consistente bibliografia esistente, la sua biologia e la sua accezione cosmopolita ne fanno una specie modello estremamente interessante. Un'area di infestazione presente in Nord Italia (Regione Veneto) è stato il sito di studio della specie.

La tesi si struttura in quattro parti. La prima (capitolo 1) è un'introduzione sulle specie invasive e su *A. glabripennis* in particolare, fornendo le conoscenze necessarie alla comprensione dei capitoli successivi.

La seconda parte (capitolo 2) presenta un'analisi delle dinamiche di introduzione di *A. glabripennis* tramite uno studio dendrocronologico delle piante colpite, che identifica i punti di più antica datazione e ne spiega la causa dell'introduzione.

La terza parte è costituita da tre capitoli (3-4-5) che considerano l'insediamento di *A. glabripennis* in un nuovo ambiente e l'adattamento a nuove piante ospiti, investigando inoltre quali sono gli effetti di diversi ambienti e ospiti. Il capitolo 3 indaga la fenologia e biologia di *A. glabripennis* in nord Italia; il capitolo 4 tratta delle preferenze di *A. glabripennis* a le diverse specie ospiti; il capitolo 5 indaga infine l'interazione tra ambiente e pianta ospite, ricercando le cause della mancata colonizzazione delle aree forestali confinanti l'infestazione considerata nello studio.

La quarta e ultima parte presenta un'analisi delle dinamiche di dispersione di *A. glabripennis* negli ambienti di neocolonizzazione, sviluppando un approccio basato sulla probabilità di attacco di un nuovo ospite in relazione alla distanza da una pianta colpita.

Nel complesso, lo studio contribuisce alle conoscenze sulla specie, fornendo dati provenienti da una nuova area di infestazione. Non solo, ricerca anche spiegazioni ad alcune caratteristiche di *A. glabripennis*, quali la scarsa attitudine alla colonizzazione di aree boschive e la variabilità intrinseca

nella scelta delle specie ospiti. Viene introdotto, infine, un nuovo approccio alla stima di dispersione della specie che potrà essere applicato anche ad altri organismi invasivi.

Abstract

The increasing international trade occurred in the last decades has raised the risk of introduction of invasive species. These are organisms introduced in new environments located out of their native area. Plants and insects represent the most common invasive species. Concerning insects, wood borers are the most important for forest ecosystems. Introductions of invasive alien species have strong ecological and economic effects on native fauna and their interception and eradication are prior goals.

In the present work, the Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky), recently discovered in north Italy has been considered. This longhorned beetle is a threat for temperate forests of the northern hemisphere, accounting many infestation sites in Europe and North America. The rich literature, its biological characteristics and the wide distribution makes of *A. glabripennis* an appropriate model species. The thesis is divided into four main parts: the first (chapter 1) is an introduction about invasive species with special attention on *A. glabripennis*, providing the knowledge for understanding the following chapters.

The second part (chapter 2) investigated the beetle introduction and spreading pathways in the infestation site through a dendrochronological study of the infested plants, identifying the oldest spot and the possible causes of introduction.

The third part is composed of three chapters (3-4-5) focused on establishment into a new environment and adaptation to novel hosts, analysing the interactions between different environments and host preference. Chapter 3 studies insect phenology and life history in N Italy. Chapter 4 investigates the host-plant preferences. Effect of habitats and host plants is addressed in chapter 5, where the colonization failure of forest areas nearby the infestation is investigated.

A last part (chapter 6) presents an analyses of the adult dispersal, developing an approach based on the probability of attack from an attacked host to an healthy one.

The study contributes on the global knowledge of *Anoplophora glabripennis*, providing data from a new infestation area. Moreover, it looks for plausible explanation of species traits, such as the variation on host preference between populations. Finally, it is proposed a new approach to dispersal evaluation, which can be developed also on other invasive organisms.

Chapter 1

Introduction

INVASIVE ALIEN SPECIES

World biota has been depicted by evolution of species over millions of years and insects themselves evolved in the last 400 millions of years. Population isolation is the main cause involved in speciation and geographical barriers maintain species isolated one to other. Sometimes changes occur determining a species to be introduced in a new community where it was not present before. This has normally happened in the past, but the increasing human activity led to an abrupt increase of the frequency of new introductions (Roques et al., 2009), never seen before (Hulme, 2009). These introductions are either intentional, such as new crop plants, or incidental, as their parasites. In particular, the international trade characterizing the last 200 years of human history led to the incidental introduction of many species (di Castri, 1989). While most of the species introduced fail to survive in the new environment, sometimes establishment can take place (Mack et al., 2000).

Once an organism is introduced into new environments located out of its natural range of distribution either intentionally or unintentionally is defined as invasive alien species (IUCN 2000). Alien invasive insects affect the hoisting environment through direct interactions, for instance feeding on native plants, or indirect interactions, such as competition. The ecological effects vary at different levels of the system and at different spatial scales (Kenis et al., 2009). Enormous damages may be caused both to ecosystems and economies and invasive alien species are well recognized as one of the biggest threat for biodiversity (Kenis et al., 2009).

A biological invasion can be divided into three main phases: arrival, establishment and spread (Liebhold and Tobin 2008). Whereas the arrival is primary associated with the management activities of quarantines and inspections, the other two phases concern adaptation to the new environment and interactions with the native hosts. In this study, attention has been posed mainly on the establishment and the spread, which represent key periods in which an invading population adapts, grows up and expand its distributional ranges.

This work aims on the study of alien wood borers introduced in new areas as a consequence of the increasing goods trade. Establishment into a new environment, adaptation to new host plants and spreading dynamics have been the main aspects considered. Because of the large number of invasive species reported in the last decades (Hulme, 2009), the attention was focused on a study species, which represents, in terms of hazards and eradication efforts, an excellent example of invasive species.

The “Asian Longhorn Beetle” (ALB), *Anoplophora glabripennis* (Motschulsky) (Coleoptera Cerambycidae), is a wood borer belonging to the subfamily of Lamiinae, polyphagous on broad-leaf species. This species, native from China, displays a broad polyphagia on alive, healthy trees and it is involved in many invasions over the temperate areas of Asia, Europe and North America, representing a great threat for their broadleaf forests. Because of it, this pest is attracting a significantly increasing attention from the scientific world (Hu et al. 2009, Haack et al. 2010).

***Anoplophora glabripennis* (The Asian Longhorned Beetle)**

Description

ALB has glossy black adults with 10-20 distinct irregular-shaped patches on the elytra (Fig. 1), although in rare instances the number of patches ranges from 0 to 60. Patch colour is usually white, but can be shades of yellow to orange in the *nobilis* form. Body length usually ranges between 17 and 40 millimetres. Antennae are composed of 11 segments, with a banding pattern in which the basal portion of each antennomer is pale blue or white and the distal portion is black (Fig. 2). The ratio of antennal length to body length is about 2 for males and 1.5 for females (Hajek et al., 2004). Eggs are oblong, white, and 5-7 mm long (Fig. 3). Larvae are legless, cream-colored and 30-50 mm long when mature (Fig. 3). Larvae have a pigmented pronotal shield characteristic of the specie Pupae are whitish and 27-38 mm long.



Fig. 1: Adult of *Anoplophora glabripennis* (photo by author).



Fig. 2: Detail of elytra and antennae of ALB (Photo by pest and disease image library)

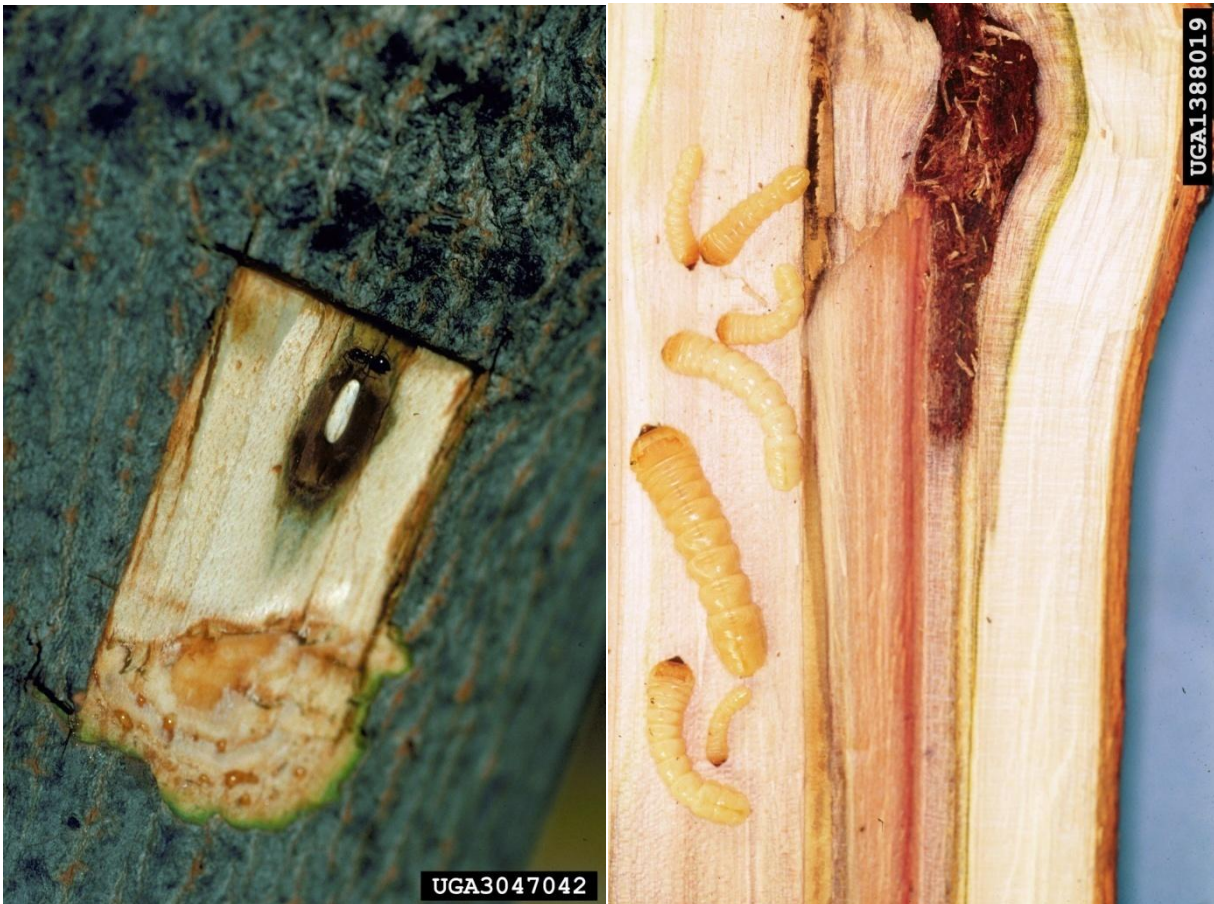


Fig. 3: Egg and larvae at different instars of *Anoplophora glabripennis* (Photos by L.R. Barber)

Taxonomy

The genus *Anoplophora* is composed of 36 species (Lingafelter & Hoebeke, 2002) that occur throughout Asia, with the highest diversity in tropical and subtropical regions. Elytra characters are important morphological characters used for distinguishing *A. glabripennis* from other similar species. The similarities among all the *Anoplophora* species have often resulted in taxonomic confusion (Fukaya, 2003). It has been suggested that *A. glabripennis* from Northern China and from Southern China are actually two separate species (Chen, 1989), although this is not generally accepted. In recent years, the relationship between the two most closely-related species within *Anoplophora*, *A. glabripennis* and *A. nobilis* Ganglbauer has been investigated (Gao et al., 2000; Tang et al., 2004). A study of external morphology shows that they have the same microreticulations at the elytral base, although there is a variation in colour of the elytral spots (white in *A. glabripennis*, yellow in *A. nobilis*). These species are usually found in the same areas and they utilize similar host species (An et al., 2004). Analyses of peroxidases and esterase enzymes were compared and no significant differences were found (Zhou et al., 1995). Results obtained from studies using RAPD also suggest they belong to the same specie (An et al., 2004). To solve the uncertainty, cross-mating experiments were conducted between *A. glabripennis* and *A. nobilis*. The results obtained showed that the F1 generation could produce a viable F2 generation (Gao et al., 2000). Therefore, *A. glabripennis* and *A. nobilis* are now regarded as two forms of *A. glabripennis*, in agreement with Lingafelter & Hoebeke (2002).

Geographic distribution

A. glabripennis is native from China and South Korea (Cavey et al. 1998; Lingafelter & Hoebeke, 2002; Williams et al. 2004a). In China *A. glabripennis* occurs in the regions of Liaoning, Jiangsu Shanxi, Henan and Hubei (Yan, 1985) where it is known since the Qing dynasty (1644 - 1912).

Anoplophora glabripennis was originally restricted to Asia, being present in four climatic zones of China and Korea (Cavey *et al.*, 1998; Lingafelter & Hoebeke, 2002; Williams *et al.*, 2004a). In China, this beetle was first detected in the eastern regions, extending from Liaoning to Jiangsu and to Shanxi, Henan, and Hubei (Yan, 1985). After exotic tree species (e.g. *Populus*) that were suitable breeding sites for *A. glabripennis* had been planted over large areas, especially in the north, and had matured (Zhao *et al.*., 2007), this beetle was recorded throughout most of the whole country, with the exception of the central Asian provinces of Qinghai, Xinjiang and Tibet (Li & Wu, 1993). However, in recent years, *A. glabripennis* was also found in Tibet (Wang *et al.*, 2003) and Xinjiang Province (Y.Q. Luo, unpublished data).

Studies in natural forests in South Korea, where *A. glabripennis* is native but not common, suggest that these beetles are naturally adapted to riparian habitats and, thus, edges of habitats; this hypothesized specialization for edges of habitats helps to account for the ready adaptability of *A. glabripennis* to fragmented and disturbed habitats (Williams *et al.*, 2004a).

An unresolved question is whether *A. glabripennis* is also indigenous to Japan. On the basis of a collection record from 100 years ago (Cavey *et al.*, 1998), Japan has been included in the indigenous area of *A. glabripennis* (Yan & Qin, 1992; Zhang *et al.*, 2002). However, according to Makihara (2002), *A. glabripennis* is not present in Japan at the present time, lending support to suggestions that it is not native to Japan.

Life history

Anoplophora glabripennis usually takes one year to complete its life cycle, although two years is common (Keena MA, 2005). In China, ALB requires 1-2 years to develop from egg to adult and generally overwinters as a larva, although it has been found on rare occasions to overwinter as an egg or pupa (Li & Wu, 1993). Voltinism may vary as a function of local climatic conditions, and a significant correlation has been established with altitude. For example, in inner Mongolia (northern China), a single generation takes 2 years to develop, whereas, in Taiwan, one generation per year has been documented. In Shandong Province (central-eastern China), approximately 90 % of individuals complete one generation in one year. It has been estimated that overall in China about 80% of individuals can complete their development within one year and less than 20% require 2 years. However, the time to complete one generation may vary among populations in a single area, depending on the type of host in which the larvae develop.

Under field conditions, it has been calculated that, to complete metamorphosis, *A. glabripennis* needs 1264.2 accumulated degree-days (DD) at a developmental threshold of 13.4°C (Yang *et al.*, 2000).

Depending on local temperatures, adults have been observed over all their areas from April to December, with peak activity usually during May to July.

The initiation of adult emergence is influenced by accumulated annual temperature (Zhao & Yoshida, 1999). Studies in China showed that male adults live for 3 - 50 days, and females live for 14 - 66 days (Li & Wu, 1993), whereas laboratory studies in the U.S.A. reported a longevity of approximately 80 and 100 days at 25°C in males and females, respectively (Keena, 2006).

Adults conduct maturation feeding for 10-15 days before initiating oviposition, usually feeding on twigs, petioles, and veins of leaves (Fig. 4).



Fig. 4: Adult maturation feeding signs (Photo by D. Morewood).

Mate-finding is mediated by contact and short range pheromones (He & Huang , 1993). ALB typically initiates oviposition along the upper trunk and main branches. The females usually chew a distinct funnel-shaped oviposition pit through the bark and inject a single egg beneath the bark (Fig 5). Chinese studies have reported that eggs need 8-12 days to mature (Li & Wu, 1993).



Fig. 5: *Anoplophora glabripennis* oviposition pit on *Salix* sp. Tree (photo by author).

After eggs are laid and before they hatch, the inner part of the bark surrounding the oviposition site becomes degraded. After eggs hatch, the larvae begin to feed on the decayed phloem around the oviposition site. As they progressively move under the bark, away from the egg

niche, the second instars larvae feed primarily on the healthy phloem and feed little on the nearby xylem.

They start tunneling into the xylem in the late third or early fourth instars. The larval galleries are at first horizontal and slightly curved but galleries later turn upward, usually away from the roots. As the larvae tunnel, they expel frass from the initial oviposition site. At pupation, each larva creates a chamber near the outer bark. The prepupal stage is followed by a pupal stage (average 19.6 days). After melanization, adults spend several days resting before chewing the exit hole. It typically measures 10-15 mm in diameter, but can range from 6 to 20 mm (Lingafelter & Hoebeke, 2002)

The larger larvae are always well protected inside the heartwood and are scarcely affected by the outside environment, which includes being protected from chemical pesticides to tree surfaces.

Host trees

Anoplophora glabripennis is a highly polyphagous species. Many tree species from several family (at least 15) have been reported as hosts in Asia, Europe and North America (Gaag & Loomans, 2014). However, complete development has not been confirmed on all tree species listed as possible hosts (Smith et al., 2009). In its native range, ALB infests trees primarily in the genera *Acer* (Sapindaceae), *Populus* (Salicaceae), *Salix* (Salicaceae), and *Ulmus* (Ulmaceae). Several other genera have been reported as occasional hosts in Asia (Lingafelter & Hoebeke, 2002).

In the United States, ALB has completed development on species of *Acer* (Aceraceae), *Aesculus* (Sapindaceae), *Albizia* (Fabaceae), *Betula* (Betulaceae), *Cercidiphyllum* (Cercidiphyllaceae), *Fraxinus* (Oleaceae), *Platanus* (Platanaceae), *Populus*, *Salix* (Salicaceae), *Sorbus* (Rosaceae), and *Ulmus* (Ulmaceae) (Haack et al., 2006). *Acer* was the most commonly infested tree genus in the United States, followed by *Ulmus* and *Salix*. In Canada, complete development has been confirmed only on *Acer*, *Betula*, *Populus*, and *Salix*, although oviposition has occurred on other tree genera. *Acer* was the most commonly infested tree genus in Canada (Turgeon et al., 2007). In Europe, complete development has been recorded on *Acer*, *Aesculus*, *Alnus*, *Betula*, *Carpinus*, *Fagus*, *Fraxinus*, *Platanus*, *Populus*, *Prunus*, *Salix*, and *Sorbus*. The top five host genera infested in Europe, in decreasing order, are *Acer*, *Betula*, *Salix*, *Aesculus*, and *Populus* (Herard et al., 2006).

Host - plant finding and selection

The ability of perceive and respond to volatile organic compounds (VOCs) released by host trees plays an important role in the host selection and reproduction of cerambycid species (Allison et al., 2004). Several research projects have focused on the effects of VOCs released from the trunks or leaves of host species on adults. For example, an attraction test was performed using the high susceptible host *A. negundo* and adults of *A. glabripennis* (Wen et al., 1999). More than 66% of the beetles within a radius of 100 meters were attracted to feed on *A. negundo*. In the same study, the greatest distance from which *A. glabripennis* could be attracted was found to be 240 meters. To determine when the higher quantity of VOCs is released by *A. negundo*, a study on the VOCs releasing was performed at different times in summer (Li et al., 2003). The quantities of VOCs released during the day varied throughout the summer: in July, the highest peak was found at 14.00 h, whereas in August, the peak was at 10.00 h (Li et al., 2002).

Because elevated damage by ALB has been reported to occur on water stressed *A. negundo* trees, the VOCs released from water stressed plants were investigated (Jin et al., 2004). The results obtained showed that, three compounds (butyl-alcohol, pentyl-alcohol, cis-3-hexenol) play an important role in signaling to *A. glabripennis* the presence of water stressed trees susceptible to the insect attack. Moreover, these results are in agreement with the general observations from Chinese poplar forests indicating that *A. glabripennis* populations increase the outbreak levels after trees have undergone several years of drought stress (Gao et al., 1997).

Host selection by *A. glabripennis* may also be driven by some repellent volatiles that signal the presence of non-host trees. One compelling example is given by the high resistance of callery pear (*Pirus calleryana*), a native of China that is resistant to both larvae and adults of ALB (Morewood et al., 2004). Compared with other tree species, the insect laid few eggs on this plant, and the few larvae that hatched on callery pear fail to survive. Adults beetles feeding on callery pear had reduced longevity and females feeding only on callery pear failed to develop any eggs.

These negative effects may be caused by the chemical composition of the tree, including toxic compounds and/or secondary metabolites that interfere with normal beetle development (Morewood et al., 2004). In fact, two other plants, *Ailanthus altissima* and *Melia azedarach* have strong negative effects on the larval growth of *A. glabripennis* (Zhao et al., 1994). The monoterpenes β -pinene and β -caryophyllene have been identified from these trees and effectively repel ALB adults (Tang et al., 1999).

Pheromones

Studies have been conducted to characterize the pheromones of *A. glabripennis*. Preliminary experiments have shown that male orientation is influenced by volatiles released by females (Li et al., 1999). Further investigations revealed that two dialkyl ether volatiles are potential male produced pheromones in this specie (Zhang et al., 2002). They are secreted by males in a ratio of 1:1 and they elicit strong responses in both males and females. Olfactometer experiments showed that they were significantly attractive to adults, although they do not seem to be involved in sex recognition (Zhang et al., 2002).

GC-MS (Gas Chromatography – Mass Spectroscopy) analysis of female cuticular extracts, showed that five monounsaturated compounds were constantly more abundant in females than in males (Zhang et al., 2003). Males attempted to mate when contacting a surface coated with a synthetic mixture of these compounds, indicating that the blend effectively elicits copulatory behavior in males (Zhang et al., 2003).

It is often very difficult to distinguish between sex, aggregation and defense pheromones in coleopteran species, and individual compounds can function in several ways (e.g., see Suzuki et al., 1988). Thus, it is possible that, for *A. glabripennis*, the female-produced contact sex pheromone or the male-produced pheromone resulting in attraction may also have additional functions.

Symbiotic relationships

A. glabripennis is a woodborer that feeds for the most part of its life on durable plant materials, mainly lignified and nutritionally poor. The mouth system and the digestion apparatus are the morphologic elements that allow the insects to exploit these substances.

The mouth is characterized by a pair of strong and well developed mandibles, both in the larval and adult stages, with the function of grind and chew the hard tissues of the trees. Although the important role of this system, the main function in the assumption of nutrients is performed by the gut. In fact, the broad variety of organic substances that the insects feed requires many enzymes to make all the nutrients available.

An additional resource on the food exploitation is the mutualistic symbiosis. Insects often exploit beneficial symbiotic relationships to augment their physiological capabilities and facilitate their expansion into challenging niches. Many insects harbor specific bacteria in their digestive tract, and these gut microbiota often play important roles in digestion and nutrient provisioning.

Obligate mutualists are usually intracellular, transmitted vertically, and enable survival of many insects on nutritionally deficient diets such as blood, plant sap, or wood (Aksoy S, 2000; Buchner P, 1965; Moran et al., 2003). In addition to intracellular obligates, many insects harbor secondary facultative symbionts that display a wider range of tissue tropism and can be intracellular (Oliver et al., 2006), located in the gut of the insect (Bauer et al., 2000; Broderick et al., 2004; Cazemier et al., 1997; Tholen, 1997), or associated externally (Abril & Bucher, 2002; Cardoza et al., 2006; D’Ettore et al., 2002). Secondary symbionts primarily serve a nutrient provisioning role in their hosts, which may include cellulose digestion, nitrogen fixation, and synthesis of vitamins, amino acids, lipids, and sterols (Breznak, 2000; Brune, 2003).

The bacterial communities and the cellulose digestion have been analyzed on ALB larvae reared on different host plants: *Acer saccharinum*, *Quercus palustris* (Morewood et al., 2005) and a high resistant species, *Pyrus calleryana* (Morewood et al., 2004b). The obtained results have been compared against the bacterial communities of insects collected from a population of *Anoplophora glabripennis* in New York. The larvae reared on the resistant specie, callery pear (*Pyrus calleryana*), have shown a totally reduced cellulose digestion. The results prove the difference amongst host specie, on their influence on the bacterial community complexity and cellulose digestion activity.

Risk of introduction and outbreaks in non-native regions

The increasing in the international trade, as a part of the globalization mechanism, has brought to a high risk of pests spreading, treating both cultivated and wild plants.

Especially the recent exchanges among China and western countries led to the introduction of *Anoplophora glabripennis* in many areas over the world. The main ways of incoming are related to the products that require wood packages, probably containing young larvae. For this reason, more controls have been applied on the traded goods.

Anoplophora glabripennis was detected outside Asia for the first time in 1996 in New York City (Haack et al., 1996), although it had probably already arrived in that area at least by 1990. It is likely that it was transported from China to New York City within SWPM. After 1996, this species was found in an increasing number of places in North America (Chicago, 1998; New Jersey, 2002; Toronto, Ontario, Canada, 2003; Massachusetts, 2008). In August 2008, a new infestation site was discovered in Worcester, Massachusetts, as the first occurrence in New England (EPPO, 2008b). Outside North America, the first discovery was made in Braunau

am Inn, Austria (Tomiczek *et al.*, 2002) followed by a discovery in 2002 at Yokohama in Japan (where it was considered as an invasive species) (Takahashi & Ito, 2005). In France, the first infestation was discovered in 2003, at Gien, and a second infestation in 2004, at Sainte-Anne-sur-Brivet (Hérard *et al.*, 2006). In Germany, the first infestation was discovered in 2004; at Neukirchen am Inn, and a second infestation in 2005, at Bornheim (EPPO, 2008a). In Italy, a small infestation was discovered in 2007, at Corbetta (Maspero *et al.*, 2007). In May 2008, *A. glabripennis* was officially declared as present and under eradication in France and in Germany (EPPO, 2008a). The same year, two beetles were found in Belgium but, since that discovery, no other ALB has been found in the surrounding (IPPC, 2009). Since 2011, repeated ALB discoveries have been reported in different sites of Switzerland (EPPO 2014c). A first infestation in England occurred in 2012 (EPPO 2012), whereas in Corse (France), ALB has been found in 2013 and is still under eradication (EPPO 2013). Another infestation in Italy has been reported in Marche Region in 2013 (EPPO 2013). Indeed, *A. glabripennis* has been intercepted in many more locations but, in these cases, beetles were detected before they dispersed into the landscape; such locations include detections inside warehouses after emerging from SWPM (USDA-APHIS, 2008b) and after emergence from imported bonsai (Poland; Białooki, 2003).

Several models have been developed to predict the potential survival of *A. glabripennis* worldwide. The climate-matching model CLIMEX has been employed to match the climate where the beetle is native with potential introduction areas (MacLeod *et al.*, 2002).

The Italian populations

In Italy, three cases have been reported in 2007, 2009 and 2013 respectively. In June 2007, the Asian longhorned beetle *Anoplophora glabripennis* (Motschulsky) was found at Corbetta, in Lombardy, 23 km west of Milan. The specie was observed in four host trees, one sycamore maple *Acer pseudoplatanus* L. and three silver birches *Betula pendula* Rothmahler. During summer 2007, 20 living and 107 dead *A. glabripennis* beetles were collected on or around the infested trees. The dissection of the infested material showed that 287 beetles emerged from the trees during previous years and 158 living larvae of varied ages were still developing in April, 2008. After the destruction of the plants, no more ALB or symptomatic tree has been reported.

In August 2013, an infestation has been detected in Grottazzollina municipality, Marche region (EPPO 2013). After one year, ALB has been detected in more than 80 sites over the area. Attacked plants belonged to the genera *Ulmus*, *Acer*, *Aesculus*, *Salix*, *Populus* and *Betula* (EPPO 2014b).

In summer 2009, many insects and infested trees have been found in Treviso (NE Italy), where an eradication plan is still in act. In June 2009, in a garden placed in the municipality of Cornuda (TV), an *Acer ginnala* tree has been found infested of many insect of the family Cerambycidae. These were recognized from the University of Padua, Department of Agronomy, Food, Natural Resources, Animals and Environment, as *Anoplophora glabripennis*. The tree was destroyed immediately from the agencies of Servizio Forestale di Treviso and Servizio Fitosanitario Regionale.

Since the detection of the pest in the area, a first action was taken thanks to the collaboration between the regional Forest Service, Phytosanitary Service, University of Padova and Municipality of Cornuda. First, a monitoring plan was set to outline the infested area detecting the presence of the insect on all the plants around the first recognition. During this controls, the attack to many trees on a wide scale has been confirmed and was assessed the outbreak of ALB. The infested area encompasses three municipalities: Cornuda , Crocetta del Montello and Pederobba. Because of its extension it seems to be the greatest in Europe.

Many tree genera were infested from the beetle (*Acer*, *Aesculus*, *Prunus*, *Salix*, *Betula*, *Populus*, *Ulmus*, *Cercidiphyllum*) but only four of them are the main involved in the attack (*Acer*, *Ulmus*, *Betula*, *Salix*). According to the other infestations, *Anoplophora glabripennis* infests primarily the genus *Acer* (Lingafelter & Hoebeke, 2002) and several other genera are reported as possible hosts, but the host preference shows to be variable (Turgeon et al., 2007; Herard et al., 2006; Lingafelter & Hoebeke, 2002; Haack et al., 2006).

The eradication plan arranged from the authorities aims to recognize the presence of *Anoplophora glabripennis* distinguish tree by tree. Moreover, the plan considers each plant that reveals the presence of the insect as infested and then it must be destroyed. During winter the selected trees are cut, harvested in a collection point and minced in chips for burning.

The infestation has been initially detected in a urban area, characterized by patches of industrial, housing and agricultural zones. Despite the presence of two big forest areas aside the outbreak, beetle presence has been only recorded in the urban area. By now, only one isolated tree (birch) has been found attacked at forest edge, and no other insect signs has been detected nearby, along forest borders or inside it.

After five years since the first record and the creation of the eradication plan, the number of infested trees has strongly reduced and seems to be decreasing again (EPPO Reporting Service, 2014a). In summer 2010, a new infested area has been found in Maser, five kilometres far from the edge of Cornuda infestation. Genetic analysis have shown that there is no significant difference between the population and the new one can be considered derived from the first.

Objectives and content of the thesis

This thesis focuses on the study of invasive wood borers, considering different aspects of the model species *Anoplophora glabripennis*. Three main aspects are here investigated: detection, establishment and spread.

Detection, as first, plays a crucial role in the understanding of arrival and spreading pathways of an alien species. In this thesis, a study (Chapter II) based on dendrochronological analyses is proposed as a reliable method for dating wood borers infestations and assess the introduction pathways in the area.

After arrival, establishment involves alien species adaptation to new environment (Chapter III) and interactions with native host plants (Chapter IV and V). In Chapter III, biology, phenology and survival of ALB in the new introduction area are investigated, while host plant preferences and reproductive performance are explored in Chapter IV, with a focus on the effects of different environments in Chapter V.

Once the invasive species population begins to grow, spreading toward new hosts takes place. In the last part (Chapter VI), I analysed the spreading of ALB through a probability-based approach.

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Chapter 2

Dating *Anoplophora glabripennis* introduction in NE Italy by growth-ring analysis

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I collected the data, drafted the paper and contributed to data analysis.



Dating *Anoplophora glabripennis* introduction in North-East Italy by growth-ring analysis

R. Favaro, A. Battisti, M. Faccoli

Introduction

The Asian Longhorn Beetle (ALB), *Anoplophora glabripennis* (Coleoptera: Cerambycidae), is a quarantine species in the whole EU (EPPO, 2004; 2010). In June 2009, an outbreak of ALB was detected in the province of Treviso (municipality of Cornuda, NE Italy). In less than one year (June 2009 - May 2010), 576 trees were found to be infested by ALB, over a total of about 10,000 inspected trees. As ALB usually needs several years to kill healthy trees, the exit holes bored by the beetles are often sealed by the reaction tissues produced by the cambium in the years following the emergence. In this respect, the reaction tissues produced by the tree can be dated by growth-ring analyses (Sawyer, 2007). In addition, in some tree species the transition from early- to latewood is readily recognizable as a change from lighter to darker wood. In order to identify time and location of ALB arrival in NE Italy, we carried out a retrospective analysis based on the dating of exit holes bored by the emerging ALB beetles.

Materials and methods

In summer 2009, 46 trees belonging to 4 genera (*Acer*, *Ulmus*, *Betula* and *Aesculus*) infested by ALB were randomly sampled from the whole of the infested area, which extended over an urban area of about 2 x 2 km in the village of Cornuda (Treviso) (coordinates 45° 49' 56" N 12° 0' 19" E). The coordinates of each sampled tree were recorded by GPS and their position mapped. Infested trees were cut and a number of branch sections bearing exit holes variable according to the infestation density was cut from each host tree and taken to the laboratory. Each section was then cut orthogonally to the branch axis in the centre of the exit hole. The resulting surfaces were smoothed by sandpaper and the tissue layers around the hole were dated by analysis of the growth

rings. It was also noted whether the first tissue layer around the edge of the exit hole was of early- or latewood type, to indicate the timing of the ALB adult emergence.

Results and discussion

More than 91% (310) of the exit holes (339) dated 2009, whereas about 7% (24) were from 2008. Two holes (0.5%) were from 2007 and 2006, while the oldest exit hole dated 2005 (Fig. 1). Most of the exit holes (78.9%) were surrounded by latewood. The oldest emergence hole (2005) suggests that ALB was occurring in the sampling site at least from 2004, and that the ALB infestation was discovered at least 5 years after the insect introduction. Similar results were found by Sabbatini *et al.* (2012) on *A. chinensis* in Rome. The main occurrence of the exit holes in latewood suggests that the largest part of the beetles emerged from the host trees during the second part of the growing season (summer), in accordance with literature (Hu et al., 2009). Lastly, all the oldest emerging holes were located near to companies involved in international trade, confirming that wood packaging materials is probably the way by which ALB was introduced in the area (Hu et al., 2009).

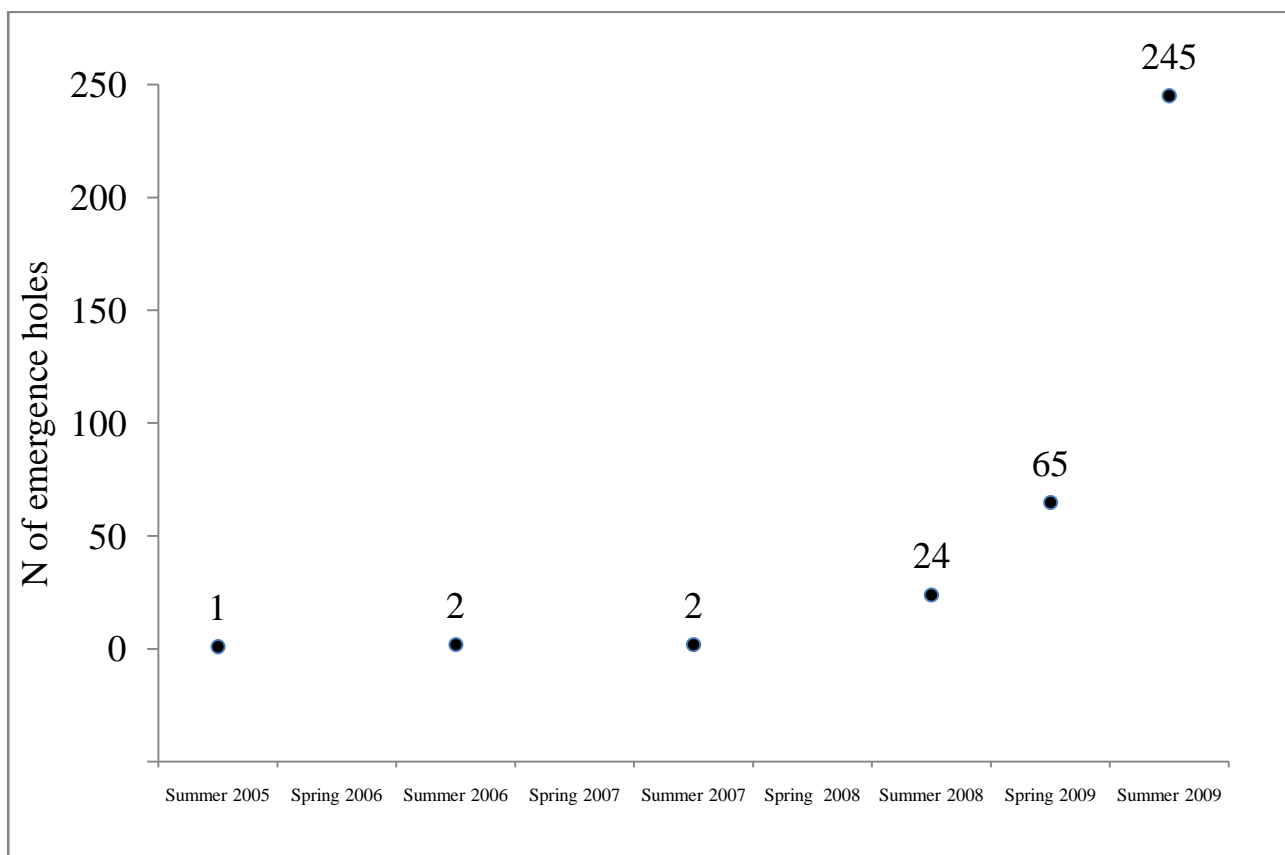


Fig. 1: Estimated age of the analyzed exit holes.

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Chapter 3

Life history of the Asian longhorn beetle

Anoplophora glabripennis (Coleoptera

Cerambycidae) in southern Europe

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I collected the data, contributed to data analysis and drafted the paper.



Abstract

- 1 The Asian longhorn beetle *Anoplophora glabripennis* is highly polyphagous and widely spread over regions with different climates. Determining the key life history traits is important for understanding how local conditions affect its successful establishment and to develop adaptive management strategies.
- 2 Field and laboratory studies were conducted from 2010 to 2012 on an *A. glabripennis* infestation in Northern Italy, to determine its seasonal phenology, adult beetle longevity, density of successful emergence, infestation age and overwintering life history.
- 3 Adult beetle emerged from infested trees from 22 May to 28 June. Ninety percent of emergence was reached around 20 July. The first 1% of emergence was accurately predicted by an accumulated degree-day model.
- 4 In the laboratory, the mean longevity of males and females developed under natural conditions was 27.8 ± 1.7 days and 24.9 ± 1.8 days, respectively. In northern Italy *A. glabripennis* largely overwinter as mature larvae in the xylem. The mean density of exit holes was 24.0 ± 2.7 / sq m of bark, with successful emergence from branches as small as 3.2 cm in diameter. Although the

infestation was discovered in June 2009, the oldest exit hole found in infested trees dated from 2005.

Introduction

The Asian longhorn beetle (ALB) *Anoplophora glabripennis* (Motschulsky), native to China and the Korean Peninsula (Lingafelter & Hoebeke, 2002), is a highly polyphagous wood borer (xylophagous) of deciduous trees. It attacks both young and old healthy and stressed trees, and recently cut logs (Peng & Liu, 1992; Gao *et al.*, 1993; He & Huang, 1993). Although ALB predominantly infests *Populus* spp., *Salix* spp., *Ulmus* spp. and *Acer* spp. in China (Wang, 2004), it is reported to damage 34 tree species belonging to 14 genera in 10 families (Lingafelter & Hoebeke, 2002; Haack *et al.*, 2010). However, reports of ALB in China are limited to plantations, windrows in agroforest landscapes, urban and other highly disturbed areas, notably with little or no species diversity (Li & Wu, 1993; Williams *et al.*, 2004). In Korea, however, ALB is found attacking only two native species, *Acer mono* Maxim and *Acer truncatum* Bunge, both growing in species rich natural areas (Williams *et al.*, 2004).

Inadvertently introduced via international trade, largely associated with the use of solid wood packing material, breeding populations of ALB have been reported since the beginning of 1990s in many states of USA (Haack *et al.*, 1996; Poland *et al.*, 1998; Haack, 2003; NAPPO, 2008), Canada (Hopkin *et al.*, 2004), and Europe (Tomiczek *et al.*, 2002; Cocquempot & Hérard, 2003; Benker *et al.*, 2004; Maspero *et al.* 2007; Hérard *et al.*, 2009; Hugel & Brua, 2009; EPPO, 2010; 2013; Faccoli *et al.*, 2011; Forster & Wermelinger, 2012). In addition, ALB continues to be intercepted at different ports worldwide. In its new range, *i.e.* outside its countries of origin, it has thus far been reported on at least 18 deciduous tree species belonging to 12 genera in North America, and on species belonging to 8 genera, overwhelmingly *Acer*, in Europe (Hu *et al.*, 2009; Haack *et al.*, 2010). Based on this information, ALB is now considered a dangerous quarantine pest in North America and the European Union, and one the world's top 100 worst invasive alien species (<http://www.issg.org/database/species/search.asp?st=100ss>).

In general, results from the few field studies on the biology of ALB show wide variations in key life history traits (see Hu *et al.*, 2009 and Haack *et al.*, 2010 and literature therein), which are often attributed to climatic conditions, host preference, host suitability and/or natural mortality. Moreover, the adaptability of ALB to these dynamic factors severely limits the predictability of its key life history traits in nature, which in turn significantly affect the development and integration of the science-based adaptive management strategies that are essential for the success of early detection and rapid response to new introductions and existing established populations.

To date, no detailed studies have reported the natural life history of ALB in southern Europe and Mediterranean regions. Furthermore, although the infestations in New York, Toronto and

northern Italy are at similar latitudes, 40°70'N, 43°60'N and 45°80'N, respectively, the climatic conditions differ significantly. New *et al.* (2002) and Hijmans *et al.* (2005) reported that North America is generally much colder than Europe at the same latitudes. This paper therefore reports investigations on the natural life history of ALB in northern Italy, with special attention to its seasonal phenology, adult longevity, infestation age and density, and overwintering ecology. In addition, field data concerning adult emergence were used to validate the existing degree-day model for predicting adult ALB emergence according to Smith *et al.* (2004).

Materials and methods

Study Area

Studies were conducted in the town of Cornuda (45°80'N, 12°01'E) and the neighbouring municipalities (province of Treviso, NE Italy), where a large ALB infestation was first discovered in June 2009 (Faccoli *et al.*, 2011). Under an eradication programme, more than 1,000 infested trees were found from 2010 through 2013. The study area is located at about 160 m a.s.l., in a hilly landscape. Cornuda is surrounded by mixed deciduous forests and riparian habitats along the Piave river. The natural forests are primarily composed of *Carpinus betulus* L., *Fagus sylvatica* L., *Acer pseudoplatanus* L., *Quercus robur* L., and *Fraxinus excelsior* L. on shady damp slopes, and *Fraxinus ornus* L., *Betula pendula* Roth., *Ostrya carpinifolia* Scopoli, and *Quercus pubescens* Willd. on sunny dry slopes. Despite the abundance of potential host tree species in forests and natural areas and along their edges, no infested trees were found during an intensive survey of these areas. Both native and exotic hardwood trees grow in the town parks and private gardens, and along the main roads, including susceptible (*e.g.*, *Ulmus*, *Acer*, *Betula*, *Aesculus* and *Salix*) and non-susceptible (*e.g.*, conifers) species. The study area is located along the southern border of the Italian Alps, in a North-South climatic transition from continental to Mediterranean conditions, and characterized by temperate summer and winter. The mean January temperature is approximately 2 °C to 4 °C. Annual precipitation ranges from 1,100 mm to 1,200 mm, and is concentrated in spring and autumn.

Phenology

A specific eradication protocol has been applied against the ALB population in Cornuda since June 2009. This includes the winter monitoring by ground visual checking of all trees belonging to the most common ALB host genera growing within 2 km of each infested tree (Faccoli *et al.*, 2011). Large trees or trees showing unclear symptoms are checked with the help of tree-climbers. Trees found to be infested, *i.e.* showing exit holes or oviposition pits, are referenced geographically and in

May they are cut down, logged, gathered into a safety area and chipped to kill the pupae just before adult emergence. In late May 2010, 2011 and 2012, more than 500 infested logs approximately 35 cm in length and 15-20 cm in diameter, chosen among the most infested branches of *A. pseudoplatanus*, *B. pendula*, *Ulmus pumila* L., *Aesculus hippocastanum* L. and *Salix* spp., were not chipped but placed singly or in pairs in aerated orange plastic tubes (50 cm long, 30 cm diameter) to await adult emergence. Trunks were not sampled because ALB mainly infests the upper part of the trees and the main branches in the canopy; moreover, logs and bolts obtained from trunks were too large to be stored in the emergence tubes. Both ends of the tubes were covered with fine metal mesh screen to retain the emerged insects until checking and collection. The tubes were stored outdoors, piled horizontally on an open iron shelf in 5 layers (each layer having 5 tubes), under a plastic roof to protect them from rain and direct sunshine, but ensure good ventilation and thermal exchange. Before being placed in the tubes, the cut surfaces of the logs were sealed with paraffin to reduce drying. Tubes were checked three times per week until the following November. Beetles found in the tubes were collected, counted and sexed according to Lingafelter & Hoebeke (2002). Air temperature within the tubes was recorded hourly using a data logger (HOBO Temp[®]) inserted in a tube to record a temperature as close as possible to that of the logs.

The degree-days (DD) required for ALB to emerge were computed with the “rectangle method” of Gilmore & Rogers (1958), where a base temperature (T_0) characterizing a developmental stage or the occurrence of some life history event, is subtracted from the daily mean temperature (\bar{T}). This method is reliable when the appropriate base temperatures and starting dates for computation are used (Legg *et al.*, 2000; Stevenson *et al.*, 2008). The base temperature threshold for ALB emergence is $T_0 = 10$ °C (Smith *et al.*, 2004), while the mean daily field temperature (\bar{T}) for 2010-2012 (3 years) was recorded at a weather station located within the infestation area and close to the rearing area (Maser, 45°81'N, 11°98'E, 147 m a.s.l.). Therefore, $DD = \bar{T} - 10$ if $\bar{T} - 10 > 0$, otherwise $DD = 0$. Accumulated degree-days (ADD) were calculated by summing DD from January 1 to the current sampling date within each year. Therefore, the predicted percentage of cumulative emergence = $EXP(-EXP(-0.004 * ADD + 3.5)) * 100$ (Smith *et al.*, 2004). The absolute values of the difference between predicted and actual weekly cumulative emergence were calculated and normality tested (SAS Proc Univariate). If the data were normally distributed, a t-test (SAS Proc t-test) was conducted on the data. Otherwise, the Wilcoxon signed rank test (SAS 9.3 Proc Univariate) was conducted on the absolute values of the differences to determine if the mean of the absolute values was significantly different from zero, *i.e.* if the predicted and actual cumulative emergence were significantly different. For each investigated year, air temperatures recorded from

May to November by the weather station and the data logger placed inside the tubes were compared statistically by linear regression.

Dating and density of exit holes

In 2009, the density ALB exit holes was calculated from logs sampled from 25 mature trees of sycamore (*A. pseudoplatanus*) that were felled in late autumn at the end of the ALB emergence period. Three logs (about 30 cm in length) were cut from infested branches of each tree, for a total of 75 logs. The trees and sample logs were selected randomly from trees and branches representative of the mean level of infestation in the area. Sections of trunks were not sampled because ALB mainly infests the upper parts of trees and main branches. Length and diameter of the logs were measured and number of emergence holes counted.

Host colonization by ALB is a multiyear process where an individual tree is repeatedly attacked over a period of years until it is no longer attractive and/or of suitable quality for successful development. Therefore, when based solely upon the density of exit holes, results from the evaluation of host preference and host suitability may be compromised. Although beyond the scope of the study, we used dendrochronological methods to determine the year in which individual exit holes were chewed by an emerging adult beetle, thereby accounting for the multiyear process of host colonization and death of the infested tree (Sawyer, 2007; Favaro *et al.*, 2013). The logs were cut orthogonally to the branch axis in the centre of each exit hole. The resulting surfaces were smoothed with sandpaper and the tissue layers around the hole were dated by analysis of the growth rings by stereoscope.

The surface area of each log was calculated according to the log sizes and density of exit holes reported as the number of holes per square metre of bark. Logs were arbitrarily partitioned into 4 diameter classes, specifically < 5 cm, 5 cm - 6.9 cm, 7 cm - 8.9 cm, ≥ 9 cm, and density of exit holes compared among the 4 diameter classes and the host trees (SAS 9.3 PROC GLM).

Adult longevity

In 2011, ALB males and females newly emerged from the infested logs stored in the plastic rearing tubes (see phenology section) were collected from 23 May to 5 September, recording the emergence day of each adult. The elytra of each beetle were marked with a permanent marker to denote the date of emergence. Beetles were then sexed, and males and females placed separately in metal mesh cages (100 cm x 100 cm x 200 cm), avoiding any mating and egg deposition that might affect adult longevity, especially of the males. Moreover, there was no oviposition substrate within the cages. As many adults stored together in the same cage often fight and mutilate each other, affecting

longevity, there were never more than 20 adults per cage. Because food quality may drastically affect longevity, the adults were fed upon freshly cut twigs of maple (*A. pseudoplatanus*) placed in a glass with fresh water and renewed twice a week. The cages were kept under laboratory conditions at 22 °C and 16h:8h L:D photoperiod, and the ambient air temperature recorded hourly. The cages were checked twice weekly and dead beetles removed and counted. Longevity of males and females was compared using SAS 9.3 PROC t-test.

Overwintering

The population of ALB occurring in four host tree species growing in Cornuda was investigated to determine which instars overwinter. At end of January 2010 (18th – 22nd), logs ca. 40 cm in length and 10 cm in diameter were collected from naturally infested trees of *A. pseudoplatanus* (n = 30), *B. pendula* (n = 41), *U. pumila* (n = 28) and *Salix alba L.* (n = 40). About 10 trees per species were sampled, collecting logs from dying branches showing infestation signs (*i.e.*, oviposition pits and exit holes). The logs were debarked and split, and the following data were recorded: 1) oviposition pits without eggs (empty pits), 2) unhatched eggs, 3) live young larvae in phloem galleries, 4) dead larvae in galleries, or empty galleries in phloem, 5) live mature larvae in galleries in xylem. The empty pits were reported as percentage of the total oviposition pits found in each tree species, whereas the developing instars (young, mature and dead larvae) were reported as percentages calculated on the fertile oviposition pits, *i.e.* pits with eggs. Data concerning overwintering stages were compared by a binomial generalized mixed model applied singly to overwintering stage and host tree. The analyses were performed using the SAS9.3 PROC GLIMMIX.

Results

Phenology

For each investigated year, air temperatures from a weather station and the data logger set up inside the tubes showed no statistical differences. The temperature inside tubes was slightly warmer than outside in late spring and beginning of summer but was cooler in summer, with a mean difference of only about ± 1 °C (see for instance Fig. 1 referring to 2011). In 2010 beetles emerged from 28 June to 9 August (Fig. 2a), in 2011 from 22 May to 23 August (Fig. 2b), and in 2012 from 21 June to 9 August (Fig. 2c). Emergence of adults peaked from 5 to 12 July 2010, 14 to 28 June 2011, and 5 to 12 July 2012, spanning approximately one, two and one week respectively. In 2010 and 2012, the initial emergence occurred 37 d and 31 d later than in 2011 respectively. In the three investigated years, 90% emergence was reached within 3 d of each other: 20 July, 21 July, and 19

July, respectively. The overall phenology of ALB emergence was consistent over the three years. The sex ratio (M:F) of emerging beetles was 1.02:1 (45:44), 1.24:1 (150:121), and 1.33:1 (168:126) in 2010, 2011 and 2012, respectively. The phenology of ALB males and females showed a similar temporal pattern in the three investigated years (Fig. 2a, 2b and 2c, respectively).

According to the recorded air temperature, 1% of cumulative emergence of ALB was predicted by the model on 11 June 2010 (491.3 ADD), 3 June 2011 (501 ADD), and 14 June 2012 (496.7 ADD) (Fig. 3a, 3b and 3c, respectively). A peak of emergence was predicted from 13 July (915.5ADD) to 17 July 2010 (988.8 ADD), 9 July (911.3 ADD) to 13 July 2011 (976.7 ADD), and 8 July (867.8 ADD) to 12 July 2012 (931.2 ADD). Ninety percent of actual emergence occurred by 24 August 2010 (1,448.2 ADD), 20 August 2011 (1,437.5 ADD) and 17 August 2012 (1,440.9 ADD). Lastly, 95% of cumulative emergence was predicted by 12 September 2010 (1,623 ADD) (Fig. 3a), 1 September 2011 (1,621.4 ADD) (Fig. 3b), and 29 August 2012 (1,623.3 ADD) (Figs 3a, 3b and 3c, respectively). The test for normality indicated that the absolute values of the difference between predicted and actual weekly cumulative emergence had normal distribution in 2010 (Shapiro-Wilk test, $n = 7$, $W = 0.87$, $P = 0.21$), and 2012 (Shapiro-Wilk test, $n = 7$, $W = 0.89$, $P = 0.31$), but not in 2011 (Shapiro-Wilk test, $n = 13$, $W = 0.84$, $P = 0.02$). Comparing the absolute values, statistical tests showed that the predicted and actual cumulative emergence were significantly different for each of the three years of the study (t-test, d.f. = 6, $T = 2.98$, $P = 0.02$ and $T = 3.71$, $P = 0.009$, for 2010 and for 2012, and Signed Rank test, $n=13$, $S = 45.5$, $P = 0.0002$ for 2011).

Dating and density of exit holes

The mean diameter, length and surface area of the 75 sampled logs was 7.1 ± 0.45 cm, 31.8 ± 0.92 cm, and 706 ± 46 sq cm, respectively. There were 139 ALB exit holes in the logs. About 85% (119) of them dated from 2009, whereas 10% (16) were from 2008. Two holes dated from 2007 and one 2006, while the oldest exit hole was from 2005. Considering only the most recent exit holes (119 from 2009), their mean number per log was 1.6 ± 0.24 , with no statistical differences among the 25 sampled trees (F test, $F = 0.43$, d.f. = 24, 45, $P = 0.32$). The mean density of emergence holes in 2009 was 24.0 ± 2.7 per sq m (Table 1), with successful emergence from branches as small as 3.2 cm in diameter. There was no significant difference in density of emergence holes among the four classes of branch diameter (F test, $F = 0.52$, d.f. = 3, 71, $P = 0.56$).

Adult longevity

In 2011, the longevity of 201 ALB adults (94 males and 107 females) was studied in relation to the emergence date, which occurred between 23 May and 5 September. The mean longevity of ALB males and females was 29.6 ± 1.6 days and 31.2 ± 1.5 days, respectively. T-test showed no significant difference in mean longevity between sexes (t-test, Method = pooled, d.f. = 199, $T = 0.73$, $P = 0.46$) (Fig. 4a). The association between adult longevity and emergence date was analysed by fitting a polynomial line to the data. The R^2 value for males ($n = 94$) and females ($n = 107$) was 0.023 and 0.104, respectively (Figs 4b and 4c), indicating the absence of a significant association between adult longevity and emergence date for both sexes.

Overwintering

Results showed that ALB largely overwinter as larvae, particularly as mature larvae in the xylem (Fig. 5). The number of empty pits and dead larvae in the phloem showed significant differences among host tree species (F test, $F = 6.6$, d.f. = 4, 135, $P < 0.05$). The percentage of empty pits, *i.e.* oviposition pits without eggs, approached 30% on *U. pumila* (25.7%), *A. pseudoplatanus* (29.2%) and *B. pendula* (32.1%), and was significantly lower (10%) on *S. alba* (F test, $F = 15.2$, d.f. = 4, 135, $P < 0.05$). According to the different host-trees, the percentages of unhatched eggs and live larvae overwintering in the phloem were 0 - 6% and $< 10\%$, respectively. The percentage of dead larvae in galleries in phloem was significantly lower (6.7%) on *A. pseudoplatanus* than on the other tree species, *U. pumila* (15.0%), *S. alba* (27.7%) and *B. pendula* (36.0%) (F test, $F = 21.4$, d.f. = 4, 135, $P < 0.05$). Clearly, most ALB individuals overwinter as mature larvae in galleries in xylem (60% on *S. alba* and *B. pendula*, 70% on *U. pumila* and ca. 80% on *A. pseudoplatanus*). No dead mature larvae, nor any pupae, were found in the xylem during the sampling in January.

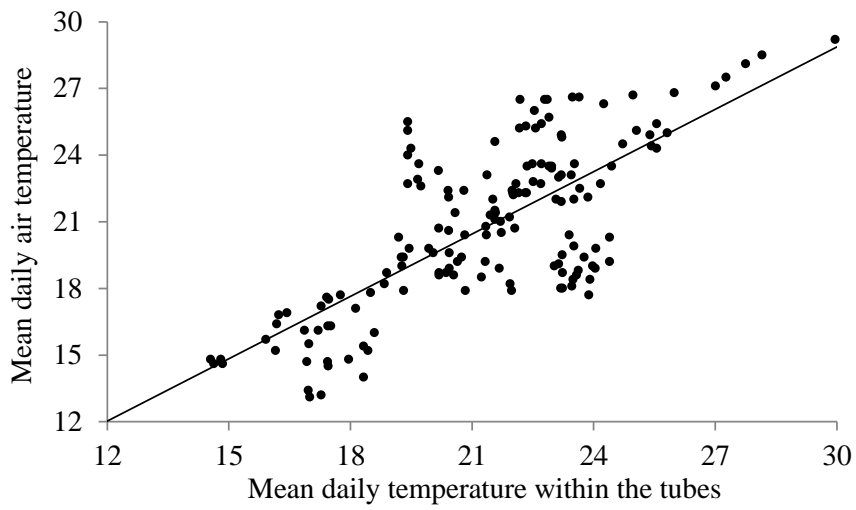


Figure 1 Correlation between mean daily temperature recorded inside and outside the tubes containing the logs infested by *A. glabripennis* ($y = 0.9315x + 0.9067$, $R^2 = 0.549$, $P < 0.01$).

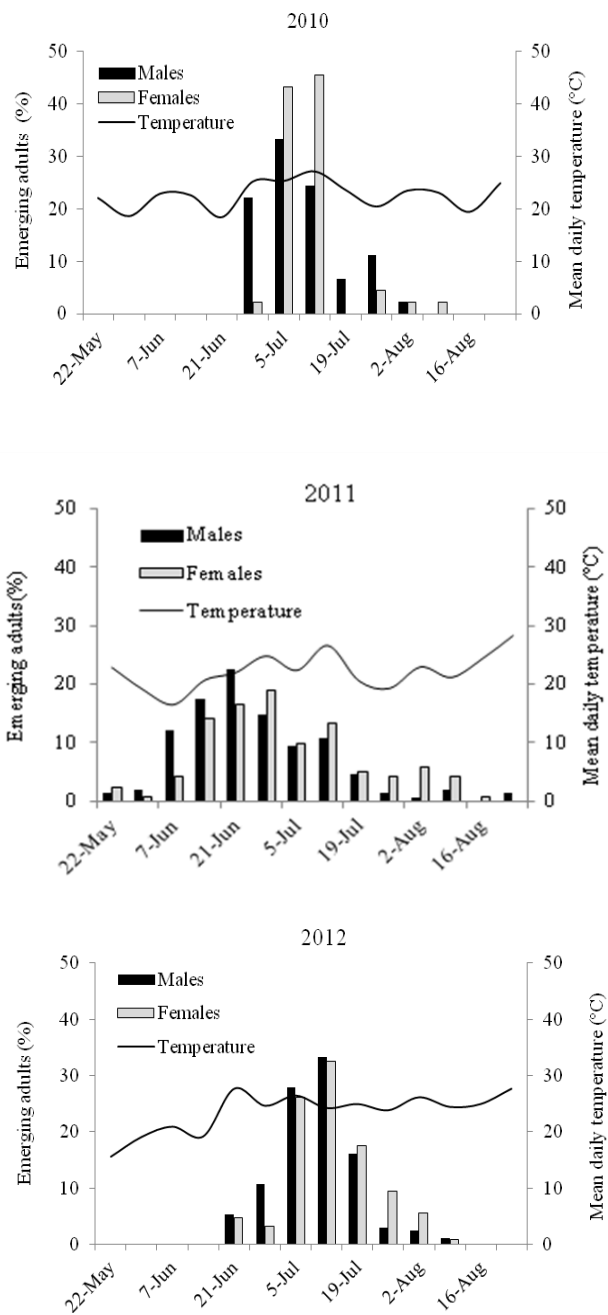


Figure 2 Emergence of *Anoplophora glabripennis* females and males at Cornuda (Italy) in 2010, 2011 and 2012. Temperatures are reported as the mean of the hourly temperatures.

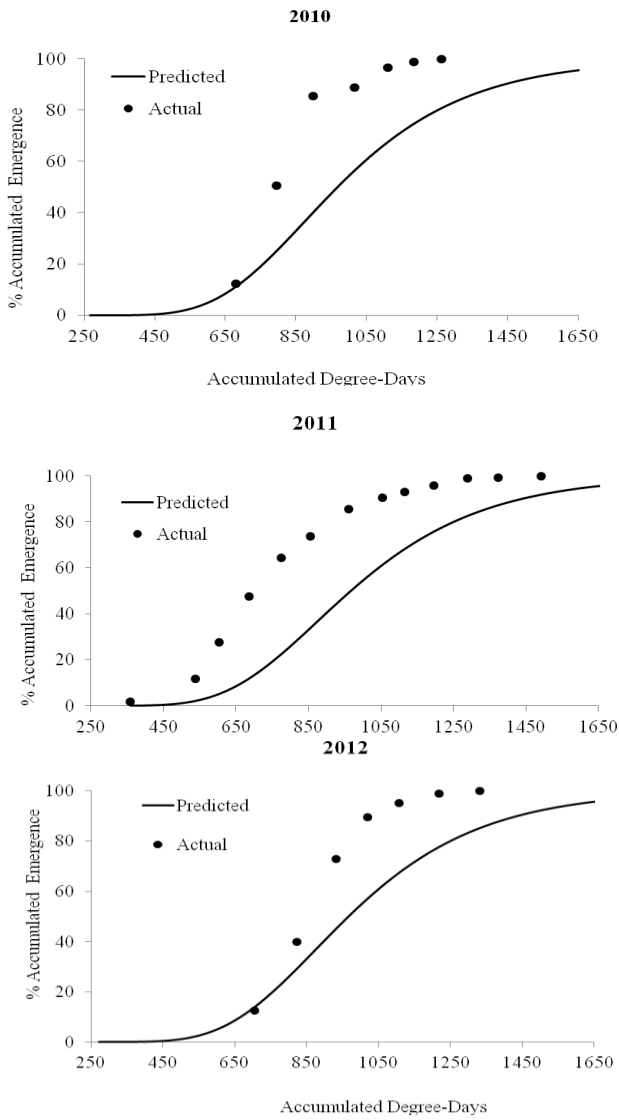


Figure 3 Predicted and actual emergence of *Anoplophora glabripennis* adults at Cornuda (Italy) in 2010, 2011 and 2012.

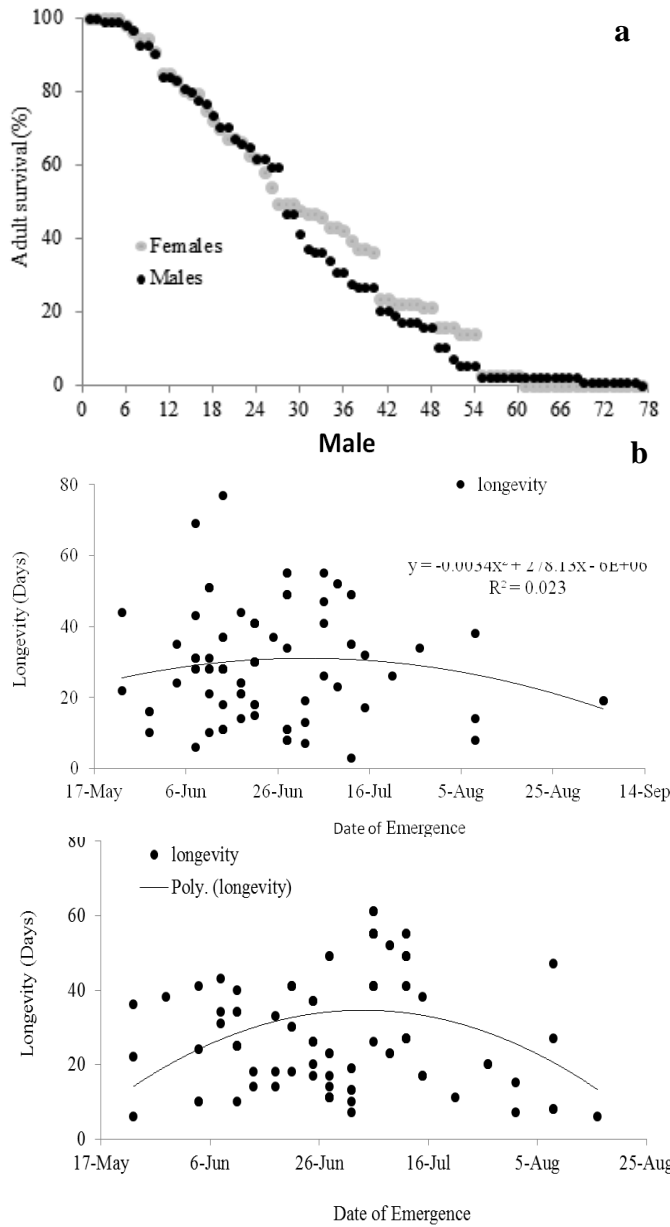


Fig.

males (females and males) in days (a), and males (b) ($y = -0.0034x^2 + 278.13x - 6E+07$, $R^2 = 0.023$) and females (c) ($y = -0.0116x^2 + 941.21x - 2E+07$, $R^2 = 0.104$).

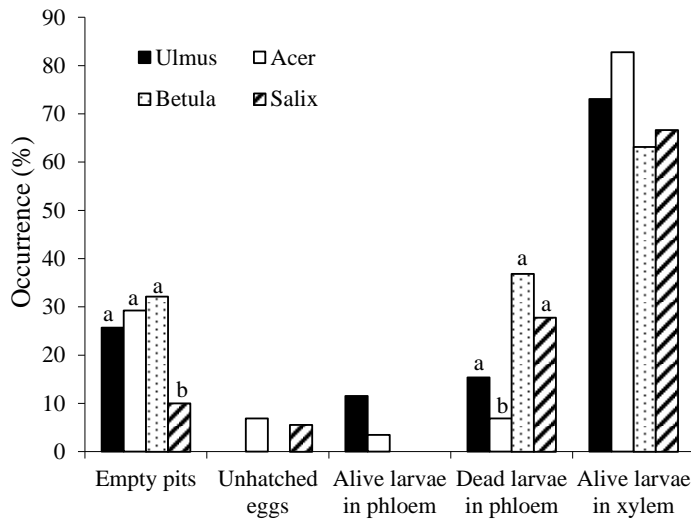


Figure 5 Overwintering life stages of *Anoplophora glabripennis* in four host tree species, *Salix alba*, *Ulmus pumila*, *Acer pseudoplatanus* and *Betula pendula*. Empty pits are reported as percentage of the total number of oviposition pits. Developmental life stages are reported as percentage of the total number of oviposition pits in which an egg was deposited. Different letters indicate a significant difference among host tree species within a given life stage (binomial generalized mixed model analysis).

Table 1 Log Characteristics and Density of Adult *Anoplophora glabripennis* Emergence Holes on *Acer pseudoplatanus*.

	Log Diameter (cm)	Log Length (cm)	Log Surface (cm ²)	Exit Holes (#)	Exit Holes per m ²
Mean ± SEM	7.2 ± 0.45	31.8 ± 0.92	706 ± 46	1.6 ± 0.24	24.0 ± 2.7
Min value	3.2	26.0	291.4	1	8.4
Max value	11.5	46.0	1,336.1	4	68.7

Discussion

Phenology

This part of the study evaluated ALB phenology in southern Europe and, by field observations, validated the model proposed by Smith *et al.* (2004). The seasonal phenology of ALB recorded shows the main emergence occurring at end-June – July. Also in previous field observations conducted in China 90% of emergence had occurred by 23 July 1995, 22 July 1996, and 22 July 1997 (Zhao & Naliaki, 1999), with a degree-day accumulation of about 1450ADD in mid-July (Smith *et al.*, 2001; Smith *et al.*, 2004).

The main results from our study were obtained in experimental conditions strictly comparable to natural conditions. In fact, based upon accumulated degree days, the beginning and the first 1% emergence in the study was accurately predicted by the ADD model reported by Smith *et al.* (2004). Although the adult emergence was in cut logs caged in plastic tubes kept outdoors, the whole insect development occurred in living trees naturally infested and exposed to natural climatic conditions, except for a few weeks – in some cases a few days as in 2011 – during pupation and just before emergence. Moreover, the plastic tubes were stored outdoors, piled horizontally, and kept under a plastic roof to protect them from rain and direct sunshine, but ensuring good ventilation and thermal exchange. Indeed, the comparison between air temperatures recorded inside and outside the tubes showed a mean difference of only about ± 1 °C. The natural log drying and within-tube temperature did not thus affect insect development, because they had already developed, or adult emergence. In fact, every year the beginning of emergence, *i.e.* end of development, was the same as that predicted by the ADD model.

Thereafter, Smith *et al.* (2004) estimated that the peak of adult emergence (corresponding to about 50% of the adults) would occur under natural conditions in China after 950 DD (when the accumulation started on 1 January and with a lower threshold of 10 °C). Similar values (924.6 DD) were found by Keena & Moore (2010) in laboratory conditions using the same temperature threshold of 10 °C. Our field estimates of the degree-days necessary for the emergence peak (ranging between 931.2 and 988.8 ADD, depending on the year) hence correspond fairly well with previous estimates made both in field and laboratory conditions.

Emergence data predicted by the model, however, do not fit very well with the collected field data, as the predicted incremental ADD and emergence of ALB slightly underestimated the rate of increase of the real emergence data observed in the field (Fig. 3). This may partly be explained by differences between the methods used in our study and those in which the degree day model was developed (Smith *et al.*, 2004), based only on data from rearing experiments carried out in laboratory conditions. More specifically, the temperature inside the tubes where the logs were

stored in our study was slightly warmer (about 1 °C) than the field temperature that led to the development of the degree day model. The field temperature in our study therefore slightly underestimated the xylem temperature of the logs in which the beetles were developing, thereby increasing the rate of the incremental accumulated degree days and emergence. Also Keena & Moore (2010) suggest that at least 2 °C should be added to air temperatures to adjust for the mediation of temperature by the wood.

After accounting for the small difference between the predicted and actual emergence, this is the first reported field study to validate the existing degree-day model for predicting adult ALB emergence. The impact of accurately predicting emergence of ALB cannot be overstated. In particular, accurate prediction of the initial emergence has been successfully used in China, Canada and the U.S. to time the implementation of management strategies, including visual surveys for infested trees and detection of adult beetles (*e.g.*, pheromone traps), and for control of adult beetles by contact insecticides and fungal bands, and systemic insecticide targeting adult beetles feeding on twigs and leaves (Ric *et al.*, 2006; Hu *et al.* 2009; Haack *et al.* 2010). Furthermore, prediction of adult peak emergence has been used to intensify and focus these strategies, and thereby significantly increase their effectiveness and econometric efficiency. Moreover, development and implementation of additional adaptive management strategies require phenology models for each of the life stages of ALB. The phenology models should be able to predict larval eclosion, larval development pupation, adult eclosion and emergence (Keena & Moore, 2010). While studies on incubation, larval development and pupation have already been reported – see for instance Keena (2006), Keena & Moore (2010) and literature therein –, they have largely been based on laboratory experiments where beetles were held under optimal artificial conditions, *e.g.* constant temperature and relative humidity, and reared on an artificial diet or cut logs. Therefore, given that the nutritional quality of its host is arguably the single most important factor that governs the development of the immature life stages of ALB living within live trees, development of reliable phenology models of immature ALB requires studies in live trees inhabited in nature, specifically accounting for the continuum of host stress levels and stages of decline associated with the multiyear process of host colonization, as well as host tree species.

Dating and density of exit holes

The oldest exit hole dated to 2005 suggests that ALB had been in the sampling site at least since 2004, and that the infestation was discovered about 5 years after the insect introduction (June 2009). Similar results were found by Favaro *et al.* (2013), who in the same site (Cornuda) performed the same experiment on a larger number of trees infested by ALB (46) belonging to 4 genera (*Acer*,

Ulmus, *Betula* and *Aesculus*), and by Sabbatini *et al.* (2012) on *A. chinensis* in Rome. The delay in discovery was probably due to the low population density in the first years of the infestation, with asymptomatic trees suffering a low level of damage. The first infested trees were observed when the colonisation density was high enough to induce visible tree decay.

Retrospective investigation of host colonization, specifically by assessing the density of adult emergence, has previously reported on the density of emergence holes (Yan, 1985; Peng & Liu, 1992; Haack *et al.*, 2006; Dodds & Orwig, 2011). The density of emergence holes has typically been calculated per tree (Haack *et al.*, 2006) or per diameter of trees measured at breast-height (DBH). In our study, however, density of emergence holes was measured as a function of the diameter of the sampled log, and then calculated per unit surface area of the log (*e.g.*, per sq cm and per sq m). The density of exit holes previously reported and those reported in this study are thus not directly comparable. Moreover, since density of exit holes is based upon the branch diameter and surface from which the adult beetle emerged, the results in this study significantly improve the precision of the correlation between the density of emergence and host diameter.

In field studies, Li & Wu (1993) noted that female ALB do not lay eggs under the bark of branches that are less than 5 cm in diameter, let alone complete development. To our knowledge this is the first study showing that adults can successfully emerge from branches as small as 3.2 cm in diameter, although we did not record where the oviposition pit was for this exit hole and if the larva tunneled upwards from a thicker portion of the branch, or – less probably – downwards from a thinner portion. Given that visual survey and removal of infested trees is the basis for successful eradication of ALB, these results will significantly increase the effectiveness of early detection of infested trees, particularly by tree climbers who might otherwise overlook branches or branch sections that were previously thought too small to be infested.

Adult longevity

Although observed in laboratory conditions, the longevity of adult male and female ALB reported in this study is consistent with previous field studies. Early field studies conducted in China showed that adult male and female ALB live for 3 d to 50 d and 14 d to 66 d, respectively (Li & Wu, 1993). More recently, Gao *et al.* (2009) evaluated adult longevity in field studies where wild male and female ALB were collected within an established infestation and caged on naturally occurring healthy *Betula platyphylla* trees. Twigs were freshly cut from *B. platyphylla* and provided to the caged beetles. Results showed that the mean survival of male and female beetles was 41.2 d and 39.1 d, respectively. Similar results were found in field studies where adult beetles were tracked

from emergence to death (M.T. Smith, unpublished). Collectively, the longevity of adult ALB reported in this study is hence consistent with previous field studies conducted in nature.

Conversely, the longevity of adult male and female ALB reported in this study differs greatly from previous laboratory studies. Under comparable temperature conditions, Keena (2006) reported that average survival of adult female and male beetles was 127.8 d and 85.3 d, respectively. This is approximately three to four times longer than the female and male beetle longevity found in our study. These differences can be attributed to at least two key factors. The studies reported by Keena (2006) were conducted under laboratory conditions and, more importantly, using beetles that had been in continuous culture on an artificial diet for three to seven generations and well adapted to the experimental conditions. Conversely, the beetles used in our study were collected upon emergence from natural infested trees and as such, had been exposed to the biotic and abiotic factors that typically limit fitness in nature, including adult survival. Therefore, the results reported by Keena (2006) significantly overestimated longevity of adult ALB found in nature. Similar findings are reported also by Smith *et al.* (2002).

While the longevity and date of emergence of adult ALB did not show a significant association in our study, adult longevity steadily increased until peak emergence and declined thereafter, particularly for female beetles (see Fig. 2b). Akbulut & Linit (1999) reported that the survival and reproductive performance of adult *Monochamus carolinensis* (Olivier), also a cerambycid species from the same tribe, is significantly higher for beetles emerging from trees cut in the spring than for those emerging from trees cut in the summer and autumn. They also reported that the nutritional quality of phloem and xylem of its host is highest in the spring and declines thereafter. The authors suggest that the decline in adult survival of adult beetles emerging later in the season may be associated with the simultaneous decline in nutritional quality of its host. It should be noted that the nutritional quality of its host is important to the fitness of larvae feeding within the host tree and adult beetles during their maturation feeding period.

In our study, however, the infested trees were not felled sequentially over the period of adult emergence, as in the studies reported by Akbulut & Linit (1999), but were all felled in the spring, prior to the initiation of adult emergence. Therefore, in the absence of the natural changes in the seasonal phenology of its host, the association between date of emergence and longevity was found to lack significance. However, the steady increase in adult longevity as emergence reached its peak and the subsequent decline thereafter may be associated with changes in host phenology, specifically the nutritional quality of the fresh twigs fed weekly to the adult beetles during the study. Clearly, given the complex life history and broad host range of ALB, further studies of adult longevity are needed, specifically addressing insect – host-plant interactions. This is particularly

important for ALB, where host colonization is a multiyear process involving repeated attack and slow death of host trees.

Overwintering

Previous investigations of the overwintering population of ALB also reported mature larvae as the predominant life stage (Li & Wu, 1993; Haack *et al.*, 2006). The eggs and early instar larvae found overwintering probably result from oviposition by female beetles very late in the season. Furthermore, the high proportion of larvae found dead in the phloem can largely be explained by a combination of the relatively poor insulating characteristics and resulting low winter temperature of the phloem, and the lower fat stores of larvae inhabiting the phloem than those overwintering as mature larvae in the xylem and parasitism. Zhao *et al.* (1991) reported that parasitoids account for 20.9% to 22.3% mortality of early stage larvae of ALB living in the phloem.

Collectively, integration of the predictive degree-day model of adult emergence and the estimated adult longevity reported here, with predictions of age specific fecundity (oviposition) (Smith *et al.*, 2002), incubation and larval development within the phloem, should provide valuable information for when to proceed with eradication protocols or biological control programmes, for instance by the release of egg and early larval parasitoids of ALB (Smith *et al.* 2003; Yao & Yang, 2008). A similar approach was also proposed for other *Anoplophora* species, such as *A. chinensis* in Italy (Delvare *et al.*, 2004; Franck Hérard, pers. comm.). Therefore, detailed information that accurately predicts different aspects of the life history of ALB under field conditions is of paramount importance in effective and successful exclusion, eradication and/or management of this and other invasive species (Damos & Savopoulou-Soultani, 2012). Such data may also be important in risk assessment of the potential for invasive species to establish (MacLeod *et al.*, 2002).

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Chapter 4

Host preference and breeding performance of the Asian Longhorn Beetle, *Anoplophora glabripennis* (Motschulsky) (Coleoptera Cerambycidae), in Southern Europe

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I collected the data, contributed to data analysis and drafted the paper.

Abstract

The Asian Longhorned Beetle, *Anoplophora glabripennis*, is a highly polyphagous invasive species having a broad range of host species, presenting relevant differences between infestation areas.

Host preference and breeding performance were assessed in a population in northern Italy. Choice and no-choice experiments were carried out in both field and laboratory conditions. In field, between 2009 and 2012 horse chestnut results the preferred host showing the highest percentage of infested trees (11.8% of the available trees), although maple is the main attacked host genus (426 infested trees but corresponding to only 4.4% of the available maples). This difference is probably due to the greater abundance of *Acer sp.* trees in the infestation area and the low ALB propensity for dispersal. Other susceptible species were birches (8.9%), elms (5.2%) and willows (3.3).

In laboratory, choice trials recorded differences among the tested trees concerning female fecundity, eggs survival and larval survival, resulting in the highest performance on maple trees. No-choice experiments confirmed the high suitability of maple, showing the highest beetle reproductive performance. It is also reported that birch, which from literature are known to be among the main host, resulted in the lowest beetle suitability, despite a notable number of felled birch trees during the eradication.

Differences regarding host tree preference and suitability between ALB populations are reported in literature and here confirmed. The reasons of these differences are still unclear and further investigations are required.

Introduction

The Asian Longhorn Beetle (ALB), *Anoplophora glabripennis* (Coleoptera: Cerambycidae), is a species native of China and Korea largely polyphagous on broadleaves. In the last decades, ALB has been recorded several times as invasive pest in USA (Haack et al., 1996; Poland et al., 1998; Haack, 2006), Canada (CFIA, Canadian Food Inspection Agency, 2003) and Europe (Tomiczek et al., 2002; Cocquempot et al., 2003; Benker et al., 2004; Eppo, 2008; 2010; 2011). In June 2009 a population of ALB was detected also in the municipality of Cornuda (NE Italy), where an eradication program is still in progress (Faccoli et al., 2011; 2014a). The pathways of introduction and spreading of ALB in new areas frequently follow the international trade of wood packing material (Hu et al., 2009). Everywhere introduced, ALB posed an enormous threat to broadleaves growing in urban and suburban parks and gardens (Haugen, 2000; Nowak et al., 2001).

Emergence of ALB adults begins in the second half of May and may continue until October, although the main swarming occurs in late June - early July (Li and Wu, 1993). Newly emerged adults usually remain on the same host-tree for about 7-10 days, where they carry out a maturation feeding on leaf petioles and young bark. In average, adults survive for about a month (Faccoli et al., 2014a), although survival longer than three months has been recorded in lab conditions (Keena, 2006). After mating, female lays the eggs singly under the bark of trunk and branches usually larger than 3-5 cm in diameter (Li and Wu, 1993; Faccoli et al., 2014a). Eggs are laid singly in characteristic oviposition pits chewed out by the female. About two weeks after oviposition the larva hatches and starts to feed on phloem. Once the third instar it reached, the larva bores into the wood where it completes the development. Pupation takes place in a pupal chamber bored in the sapwood, and after a couple of weeks the new adult emerge through a large circular hole (about 10 mm diameter). Unlike most cerambycids, ALB attacks mainly healthy, which remain asymptomatic also for many years. As several generations can develop year by year on the same tree, the insect can kill also originally vigorous trees (Hu et al., 2009; Haack et al., 2010).

In general, most aspects of ALB biology and ecology largely change according with climate and latitudes. For instance, host preference showed by adults during maturation feeding and oviposition is known to be different between populations (Haack et al., 2006; Hu et al., 2009). A great variation of the main host trees has been recorded comparing populations from the native Asian regions with populations from the north American and European areas of introduction (Hu et al., 2009; Haack et al., 2010). Different ALB populations show different host preference, and tree species heavily infested in a region are not colonised in other localities. For instance, European and North American populations of *A. glabripennis* show different host preferences (Hu et al., 2009). In Europe, ash trees (*Fraxinus* sp.) have never been reported as ALB host (Tomiczek & Hoyer-

Tomiczek, 2007), whereas in Chicago *F. pennsylvanica* (Marsh.) was one of the main attacked tree species.. Host suitability may affect insect breeding performance, change the mean development time and increase egg and larval mortality, introducing new aspects of uncertainty about the ALB life history. The large adaptability of ALB to different hosts, which strongly affect its phenology, voltinism and breeding performances, makes the biology of this species hardly predictable without specific studies. Moreover, an accurate knowledge of ALB host preference is a crucial point for the infestation management and the successfully application of appropriate eradication plans. Although the scientific literature concerning various aspects of biology and ecology of ALB is extremely rich (Hu et al., 2009; Haack et al., 2010), no detailed information is available about ALB host preference in southern Europe and Mediterranean regions.

The main aims of the present paper are to investigated the host preference and the breeding performance in different tree species of the ALB population occurring in southern Europe .

Materials and Methods

Study area

The study has been carried out in the village of Cornuda (45° 80' N, 12° 01' E) and in the neighbouring municipalities (province of Treviso, NE Italy), where a large ALB infestation was recorded since June 2009. Although a still running eradication plan was immediately applied, more than 1100 trees were found to be infested by ALB in the following two years (Faccoli et al., 2011; 2014). The study area is located at about 160 m a.s.l., in a hilly landscape. The village is closely surrounded by mixed broadleaf forests and riparian habitats, which follow a large river. Despite the large availability of potential host trees and the application of a specific monitoring program, no infested trees were found in the forests and natural areas neighbouring the village of Cornuda and the infestation area, not even along the forest edges. In the city parks, private gardens and along the main roads there are many trees of both native and exotic hardwood species, some susceptible to ALB, such as elms, maples, birches, horse chestnuts and willows, some less or not susceptible, such as conifers. The whole area is located along the lower edge of the NE Italian Alps, with a climate showing Mediterranean conditions characterized by temperate summer and winter. The mean January temperature is about 3°C; precipitations are concentrated in spring and fall, with an annual mean of about 1200 mm (Faccoli et al., 2014b).

Insect collection and rearing

ALB adults needed for the experiments were obtained from laboratory rearing of infested logs. In early spring 2012, several hundreds infested logs were collected from trees cut in the infestation area during the application of the eradication plan. The logs (about 40 cm long and 10 cm in diameter) belonged to different tree species, including mainly *A. pseudoplatanus*, *B. pendula*, *Ulmus pumila*, and *Salix* spp. The logs were then moved to a field laboratory, and kept at field temperature in plastic tubes (50 cm long, 30 cm diameter) closed with fine wire mesh. The rearing tubes were checked weekly until June for adult emergence. Then, during beetle emergence (June–August), the tubes were checked three times a week and all callow adults were collected. The newly emerged adults were sexed (Lingafelter & Hoebeke, 2002), placed in separated transparent plastic boxes (40x60x30 cm), and provided with fresh twigs of maple (Hu *et al.*, 2009; Haack *et al.*, 2010); the twigs were replaced twice a week. Before being tested in the following experiments, the adults were held in the boxes for at least 20 days to ensure their full sexual maturation (Li & Liu, 1997; Keena, 2002; Smith *et al.*, 2002). In July, healthy and fully matured adults were collected from the maturation boxes and randomly assigned to the experimental trials.

Choice experiment

Field trials. Since June 2009 a plan of eradication of the ALB population occurring in the village of Cornuda was activated in a joint project among the University of Padova, the Regional Plant Protection Organization, and the Regional Forest Service (Faccoli *et al.*, 2011). The eradication included the visual checking of the infestation symptoms (emerging holes and oviposition pits) occurring on all trees belonging to the main ALB host genera known from literature (Hu *et al.*, 2009; Haack *et al.*, 2010) growing within 2 km from each infested tree (Tab. 2). During the monitoring, carried out twice a year in spring and fall, the host-list was progressively updated as ALB was found also on other tree species, and the infestation area was progressively enlarged following the new findings. Trees were generally checked from the ground by a team of operators trained by the Regional Plant Protection Organization. Large trees or trees showing unclear symptoms were doubly checked by tree-climbers of the Regional Forest Service (Faccoli *et al.*, 2011). Data concerning naturally infested trees recorded during a 5 years long monitoring (summer 2009 – spring 2013) were used to simulate a field choice experiment assessing the ALB preference among different host trees naturally available in the landscape.

Laboratory trials. In July 2012, about 30 pairs of mature ALB adults (one male and one female) were placed within wire mesh boxes (100x100x150 cm) each containing a standing fresh log (about

40 cm long and 10 cm diameter) of each of the four main host genera known to be infested by ALB in field (Tab. 2). The species chosen are *Acer pseudoplatanus*, *Betula pendula*, *Ulmus glabra* and *Salix alba*. The experiment was carried out on six replicates, *i.e.* six boxes for a total of 24 logs. Every three days the logs were rotate within the boxes to reduce any possible position effect. Fresh maple twigs replaced twice a week allowed ALB adult feeding. Insects died within 2 days from the beginning of the experiment were replaced with new ones. Adults were held in the rearing boxes for two months (Faccoli et al., 2014b).

No-choice experiment

Field trials. In July 2012, a field experiment was conducted on breeding performance of ALB on reared on different host trees. Six trees belonging to the four ALB host species investigated in the previous experiment (*A. pseudoplatanus*, *B. pendula*, *U. glabra*, and *S. alba*) were tested, for a total of 24 trees (Tab. 1). A pair of mature ALB adults (one male and one female) was placed inside a cage of 0.5 mm wire mesh fixed at about 3 m from the ground on each of three canopy branches (about 10 cm in diameter) per experimental tree, for a total of 144 beetles (72 pairs) (Tab. 1). The cage was constructed by wrapping a sheet of wire mesh (100 x 50 cm) around the branch, stapling the ends to the branch and further sealing the ends with metal ribbon. The resulting cage was about 90 cm long and 30 cm in diameter, allowing enough room for the adults to move freely along the branch, feed, mate and lay eggs. Three cages were set up in different braches of each tested tree. Two days later each insect pair was checked and any dead individuals were replaced with new ones of the same age. The tested tree branches were as similar as possible in size and only reachable from the ground by ladder, to prevent tampering. Two months later, the cages were removed, the branches cut from the trees and the resulting logs taken to the laboratory.

Laboratory trials. In the same month (July 2012), a pair of mature ALB adults (one male and one female) was placed within a ventilated transparent plastic boxes (50x40x60 cm) each containing three fresh logs (about 40 cm long and 10 cm diameter) of *A. pseudoplatanus*, *B. pendula*, *U. glabra* or *S. alba*. Six replicates were set up for each tree species, for a total of 24 boxes and 48 ALB adults (24 males and 24 females). The insects were provided with fresh maple twigs replaced twice a week. Adults died within 2 days from the beginning of the experiment were replaced with new ones. Insects were held in the rearing boxes for two months.

In both field and laboratory experiments, at the end of the trials the tested logs were debarked and analysed to assess ALB breeding activity, measuring the realised female fecundity, *i.e.* number

of laid eggs, and the egg survival, *i.e.* percentage of eggs giving larvae. Lastly, the percentage of alive larvae found under bark in relation to the alive eggs gave the larval survival.

Statistical analysis

Every single tree or box was considered as a true replicate in the following statistical analysis. Thus, the mean of data measured from the 3 logs coming from the same tree or the same box was used as value for that tree. Number of laid eggs (female fecundity), egg survival and larval survival of the young larvae were the variables recorded according to the different host trees. The analysed variables, reported as mean values (\pm SEM) per tree or bark square meter according with the tested variable, were compared by single analysis of variance (ANOVA) (Zar, 1999) looking for significant differences among different host-tree. Homogeneity of variance was tested by Cochran's test (test C) and normality by the Kolmogorov–Smirnow test (test D). When necessary, data were log- [$X' = \log(x+1)$] or arcsin- ($X' = \arcsin\sqrt{x}$) transformed to obtain homogeneity of the variance and normality. Wherever significant differences occurred, Tukey's Honestly Significant Difference (HSD) multiple comparison test was applied for mean separation (Zar, 1999). Non-homogeneous, non-transformable data were analyzed with the χ^2 goodness-of-fit test using Yates' correction for continuity, or by the Kruskal-Wallis Anova. Wherever necessary, the recorded variables were each-other related using multiple regression. An R^2 value, adjusted for the number of parameters (Zar 1999), was used to assess the goodness-of-fit of all possible models. Differences at the 0.05 confidence level were considered significant. Analyses were performed using Statistica 3.11[®] for Windows[®] software (StatSoft, Inc., Tulsa, OK).

Results

Choice experiment

Field trials. A total of 29,564 host trees growing over an area of about 5,625 ha were checked in the 5 years of survey. Trees found to be infested by ALB were 1,140 (3.8% of the checked). With 426 trees, maples were the main infested species followed by elms (328), birches (208) and willows (150) (Tab. 2). Other tree species, such as Horse chestnut, plum trees and poplars, were infested only marginally with respectively 17, 9 and 2 attacked trees (Tab. 2). Considering the relations between infested and available trees, ALB showed, however, a clear preference for the horse chestnut, with about 12% of infested trees, followed by birches (8.9), elms (5.2), maples (4.4) and willows (3.3).

Laboratory trials. Realised female fecundity (mean number of laid eggs per female) was different among the tested tree species (Anova, $df = 3; 8$, $F = 5.45$, $P < 0.05$), with values higher on maple

(39.6) than elm (12) and birch (6.6). Willow showed middle values (18) (Tukey test, $P < 0.05$) (Fig. 1). Mean egg survival was also different among species (Anova, $df = 3; 8, F = 5.89, P < 0.05$), with birch showing values (no surviving eggs) lower than maple (68.8%), willow (54.4%) and elm (46%) (Tukey test, $P < 0.05$) (Fig. 1). Lastly, larval survival showed significant differences among tree species (Anova, $df = 3; 8, F = 1,125.14, P < 0.001$), as birch had values (no surviving larvae) lower than the other tree species (Tukey test, $P < 0.05$), with maple, willow and elm having respectively 95.5%, 100% and 100% of larval survival (Fig. 1).

Having the possibility to choose their host tree, in natural conditions females preferred to reproduce on horse chestnuts, although in laboratory the highest breeding performance – concerning both female fecundity and egg-larval survival – were recorded on maple.

No-choice experiment

Field trials. The ALB fecundity tested in a no-choice experiment carried out in field varied from 0 to 60 eggs per female, with a mean of about 17.4 eggs/female. Female fecundity was affected by the host tree (Anova, $F = 6.66, d.f. = 3; 22, P < 0.01$), with birch showing values (1.8) significantly lower than maple (35) and willow (20.7); elm (15.5) showed middle values (Tukey test, $P < 0.05$; Fig. 2). Egg survival showed the same trend of female fecundity with significant differences among tree species (Anova, $F = 5.01, d.f. = 3; 22, P < 0.01$). Egg survival recorded for maple and willow – 91.6% and 79.9%, respectively – was higher than birch (11.1%); again elm (67.2%) showed medium values (Tukey test, $P < 0.05$) (Fig. 2). Lastly, larval survival was lower in (Anova, $F = 1.98, d.f. = 3; 22, P < 0.05$) birch (no surviving larvae) than in maple (64.6%), willow (63.8%) and elm (53.7%) (Tukey test, $P < 0.05$; Fig. 2).

Laboratory trials. The ALB fecundity tested in the no-choice experiment carried out in laboratory conditions varied from 2 to 60 eggs per female, with a mean of about 25.4 eggs/female. Female fecundity was deeply affected by the host tree (Anova, $F = 4.69, d.f. = 3; 8, P < 0.01$). The lowest fecundity was recorded on birch with a mean of only about 4 egg per female, significantly lower than those observed on maple (23.3), willow (40.6) and elm (21.6) (Tukey test, $P < 0.05$) (Fig. 3). Also egg survival, reported as proportion of alive eggs, *i.e.* hatched, on the total number of laid eggs, showed significant differences among host-trees (Anova, $F = 8.95, d.f. = 3; 8, P < 0.001$), with maple and willow showing an egg survival – 82.5% and 57.7%, respectively – higher than birch (11.1%); elm (30.6%) differed only from maple (Tukey test, $P < 0.05$; Fig. 3). Similarly, larval survival was different among tree species (Anova, $F = 47, d.f. = 3; 8, P < 0.001$), with birch showing values (no surviving larvae) lower than maple (91.6%), willow (79%) and elm (100%) (Tukey test, $P < 0.05$; Fig. 3).

In conclusion, females forced to reproduce on birch had the lowest fecundity and suffered the highest egg mortality; differently, maples were the host trees allowing the highest ALB breeding performance, *i.e.* the lowest difference between egg and larval survival.

Table 1 Tree species, replicates and insect pairs tested in the no-choice field experiment.

Tree species	N of trees	N of replicates	N of pairs	N of insects
<i>Acer pseudoplatanus</i>	6	3	18	36
<i>Betula pendula</i>	6	3	18	36
<i>Ulmus glabra</i>	6	3	18	36
<i>Salix alba</i>	6	3	18	36
Total	24	12	72	144

Table 2 number of checked and infested trees per genera monitored in the infestation area between 2009 and 2012.

Monitored genera	Checked trees	Infested trees	%
<i>Acer</i> spp.*	9684	426	4.4
<i>Ulmus</i> spp.	6351	328	5.2
<i>Betula</i> spp.	2331	208	8.9
<i>Salix</i> spp.	4514	150	3.3
<i>Aesculus hyppocastanum</i>	144	17	11.8
<i>Prunus</i> spp.	3009	9	0.3
<i>Populus</i> spp.	1613	2	0.1
<i>Carpinus betulus</i>	1085	0	0
<i>Fagus sylvatica</i>	165	0	0
<i>Platanus</i> spp.	668	0	0
Total	29564	1140	3.8

* spp.: the monitored genus includes many different species.

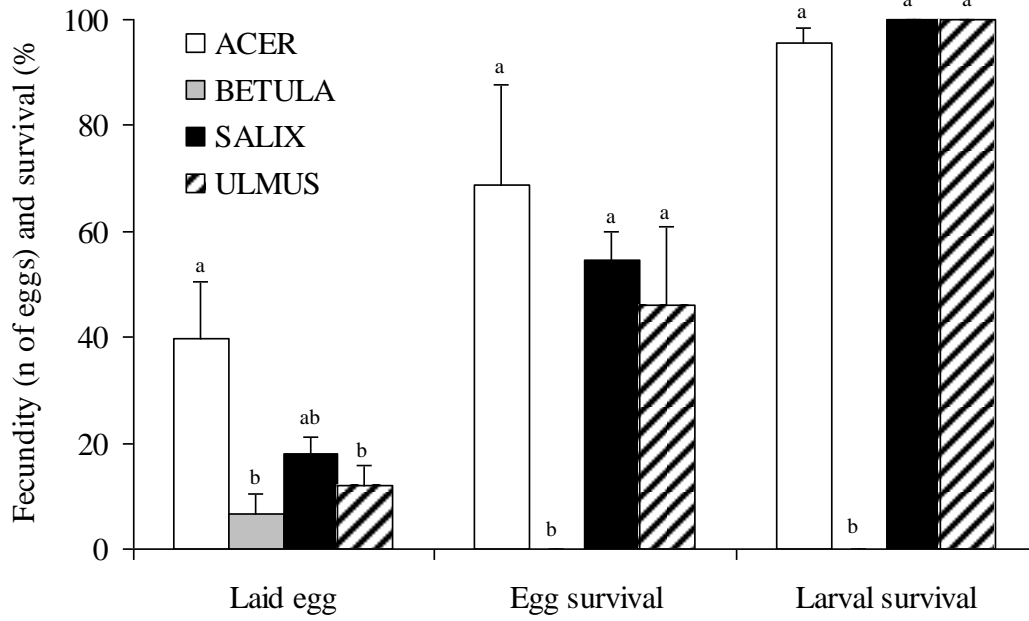


Fig. 1: Host preference and breeding performance of ALB in laboratory choice test.

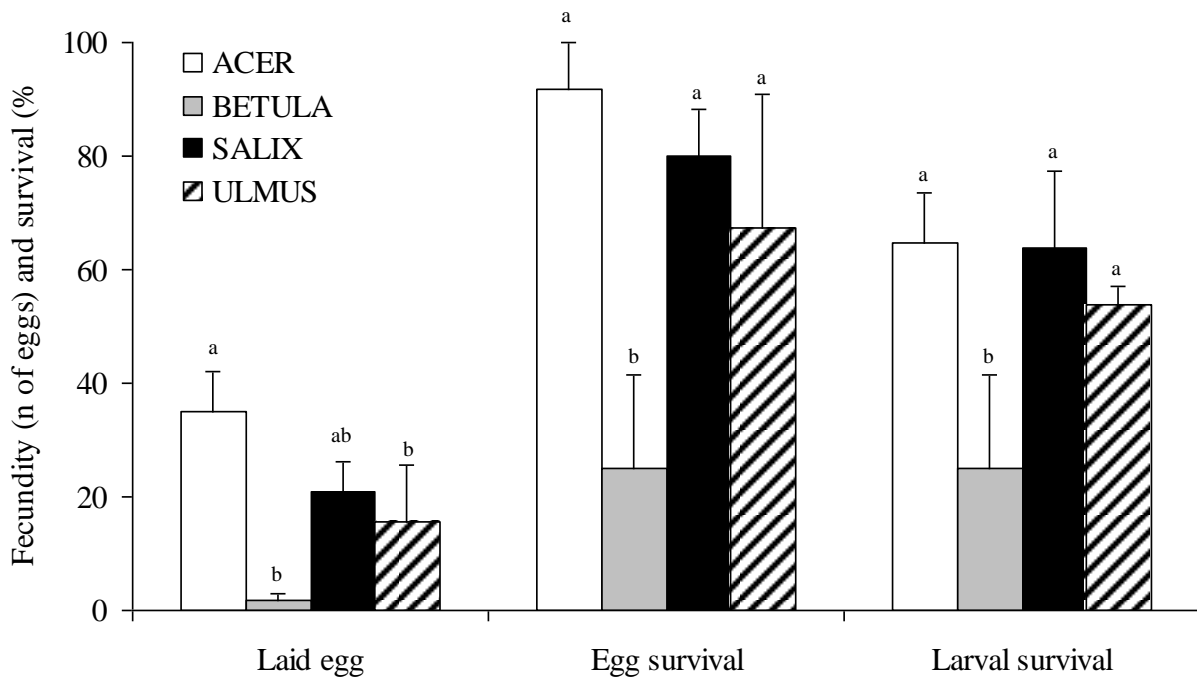


Fig. 2: Host preference and breeding performance of ALB in field no-choice tests.

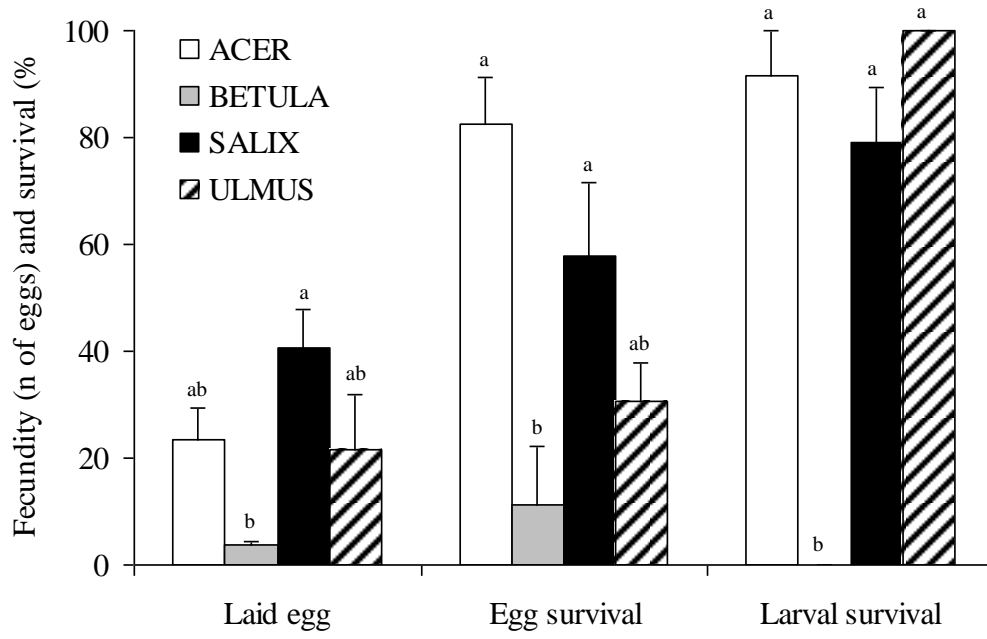


Fig. 3: Host preference and breeding performance of ALB in laboratory no-choice tests.

Discussion

This is the first study reporting detailed information concerning host preferences of ALB in southern Europe in natural conditions. Moreover, the same host preference were tested by specific laboratory and field choice and no-choice experiments. While the general results partially confirm data known from other ALB populations and infestation areas, the main outcomes of the present investigation suggest that the mechanisms of host selection and host acceptance of ALB are probably extremely complex and affected by both endogen and hexogen factors, varying between populations.

Infestations of *A. glabripennis* are reported in western countries since 1996 (Haack *et al.*, 1996), and many lists of host plants have been published often showing discrepancies among them (Hu *et al.*, 2009; Van der Gaag & Loomans, 2014), with the preferred hosts vary between infestation areas (Haack *et al.*, 2010). Also in this study ALB breeding performance results to be deeply affected by the host species. Differences among hosts are found both in field and laboratory conditions. Of the four genera tested in the present study and known to be susceptible to ALB colonization (*Acer*, *Ulmus*, *Salix* and *Betula*), maple always allows the highest ALB breeding performance. Our results confirm the observations carried out in other introduction areas, where maples are the greatly attacked hosts (Hu *et al.*, 2009; Haack *et al.*, 2010). Although maples are the most attacked species (426 of 1,140 infested trees), the number of infested trees was nevertheless only the 4.4% of the maples available in the infestation area (9,684 maples). In this respect, horse chestnut is the tree species mainly affected by ALB (11.8%) in northern Italy. The discrepancy between the most infested (maples) and the preferred host (horse chestnut) is presumably a result of both the large availability of maple trees over a lower density of other tree species, and the ALB spreading behaviour. Although ALB is a highly polyphagous species, it spreads hardly, slowly and over short distances (Smith *et al.*, 2002; Favaro *et al.*, 2015). Adults may simply infest the closest suitable trees occurring in the surroundings, however preferring – when available – horse chestnut rather than other species. Similar results were found also in other infestation areas, where horse chestnut is reported to be a very successful host of ALB (Hu *et al.*, 2009; Haack *et al.*, 2010), although with a relatively low number of infested trees (Tomiczek & Hoyer-Tomiczek, 2007).

Also birches are usually reported among the main ALB hosts (Hu *et al.*, 2009), supporting our results where infested birches (208 trees) represent 8.9% of the available ones. However, if compared with the infestation data collected by field monitoring, the low breeding performance observed on birches during the experimental trials is an unexpected result. When breeding on birches, ALB shows the lowest values in term of laid eggs, egg survival and larval survival in both laboratory and field essays. Although a possible explanation might be the quick log drying occurred

in laboratory conditions, the colonization failure even on alive trees makes this hypothesis unreliable. The high number of infested trees recorded in field may instead rely on an overestimation of ALB occurrence on birches during tree survey. In this respect, birches growing in urban parks and gardens are commonly infested by many wood borers, both longhorn beetles such as *Lamia textor* or *Cerambyx scopolii* (Bense, 1995), and cossids as the Leopard moth *Zeuzera pirina* or the Goat moth *Cossus cossus* (Heath & Emmet, 1985). Although *L. textor* and especially *Z. pirina* were found frequently on felled trees (authors observations), in most cases the tree survey carried out from the ground by the operators of the Regional Plant Protection Organization is unable to distinguish between infestation symptoms (such as exit holes) caused by different species of wood borers having ALB similar size. Moreover, following a precaution policy adopted during the application of the eradication protocol, all trees growing in the quarantine area and showing infestation symptoms potentially imputable to ALB are cut, chipped and listed as effectively ALB infested trees. Low breeding performance of ALB on birches was reported also in a previous study carried out on the same population (Faccoli *et al.*, 2014a), in which success colonization of birches resulted extremely low when compared to other tree species. Analysing the overwintering survival of ALB in four main hosts in natural conditions, *Betula* resulted to be the host genus with the highest percentage of empty oviposition pits and dead larvae in phloem, confirming the scarce ALB performance in birches.

ALB is reported to be able to fully develop in a host species, while in other populations the same host is reported to be suitable only for oviposition but not for larval development, or even recorded as unsuitable. For instance, it is reported that green ash trees are suitable for a complete development of ALB population from China and Chicago infestations, whereas in New York outbreak oviposition only is reported in green ash (Nowak *et al.*, 2001). Although for many insect species host plant quality is a key factor deciding about quantity and quality of the brood (White, 2014), for others does not. Sometimes, host choice made by insect females during oviposition can be erroneous in terms of host quality (Larsson & Ekblom, 1995), or they cannot differentiate between hosts on the basis of quality (Rauscher 1985). In a study on the susceptibility of four American tree species (*Acer saccharum*, *Acer rubrum*, *Fraxinus pennsylvanica*, *Quercus rubra*), Morewood *et al.* (2003) observed a relatively large ALB oviposition on green ash although only a few larvae survived in it. In this respect – even if not forced – ALB females accepts to oviposit also in suboptimal hosts deeply affecting brood survival and development. This reproductive behaviour seems to be a common trait in ALB, which presents different host preferences in different infestation areas (Hu *et al.*, 2009; Haack *et al.*, 2010). Comparing ALB host plants in China, Chicago and New York, Nowak *et al.* (2001) found that many of the infested tree species were

suitable for oviposition only but not for larval development, and that adult emergence was confirmed in only a part of them. Moreover, *Fraxinus*, *Malus*, *Platanus* and *Tilia* resulted to be accepted hosts in one site but not in others (Nowak *et al.* 2001). Even poplars, which are among the main preferred hosts in China (Zhao *et al.* 1997, Wen *et al.* 1998, Hu *et al.* 2009), were not fully accepted in Chicago and New York. Similarly, of the 1,613 poplar trees occurring in our infestation area, only two have been found attacked by ALB.

While some tree species may have effective defences against ALB (Morewood *et al.* 2004a, 2004b) and its symbionts (Geib *et al.* 2009, 2012, Schloss *et al.* 2006) affecting oviposition (i.e., host-acceptance) or larval development, the large variations in the host range shown by ALB may be regulated also by other factors. The preferences expressed by ALB for a specific host species in a particular infestation area, but the avoidance of the same tree species in another one, seems to be the result of specific traits involving both the origin of the founders – i.e., the genetic characteristics of the population – and their adaptation to the local conditions found during the colonisation of new areas. In a recent paper concerning the possible effect of habitat and tree suitability on ALB tree colonization, Faccoli *et al.* (2014b) demonstrated that the host-selection and the reproductive behaviour of ALB largely depend on habitat type and nutritional characteristics of the potential host-trees. Same host-tree species growing in habitats having different nitrogen availability allowed ALB colonization only following a mechanism of compensatory feeding of different intensity (White, 2014). Suitability of different trees may be instead due to other factors, such as secondary chemical compounds (Faccoli *et al.* 2014b). One plausible explanation of the extraordinary host adaptation of ALB is proposed also by Morewood (2003), which argued this behaviour as a strategy maintaining the beetle population in an area for a long time, avoiding long dispersal in favour of the exploitation of all available trees, changing the forest composition over time. A reduced breeding performance on sub-optimal hosts may then be the ecological cost of this broad polyphagia.

Although many studies were carried out about ALB host preference, a number of molecular, biological and ecological aspects are still unclear. Differences in host preferences, host choice and host suitability were largely reported in literature, but the underlying host-selection mechanisms remain scarcely understood. Further studies should be focused, for instance, on the molecular characterization of ALB populations from various infestation areas in relation to host preference, suggesting interesting findings on the host-selection mechanisms of this extremely invasive pest.

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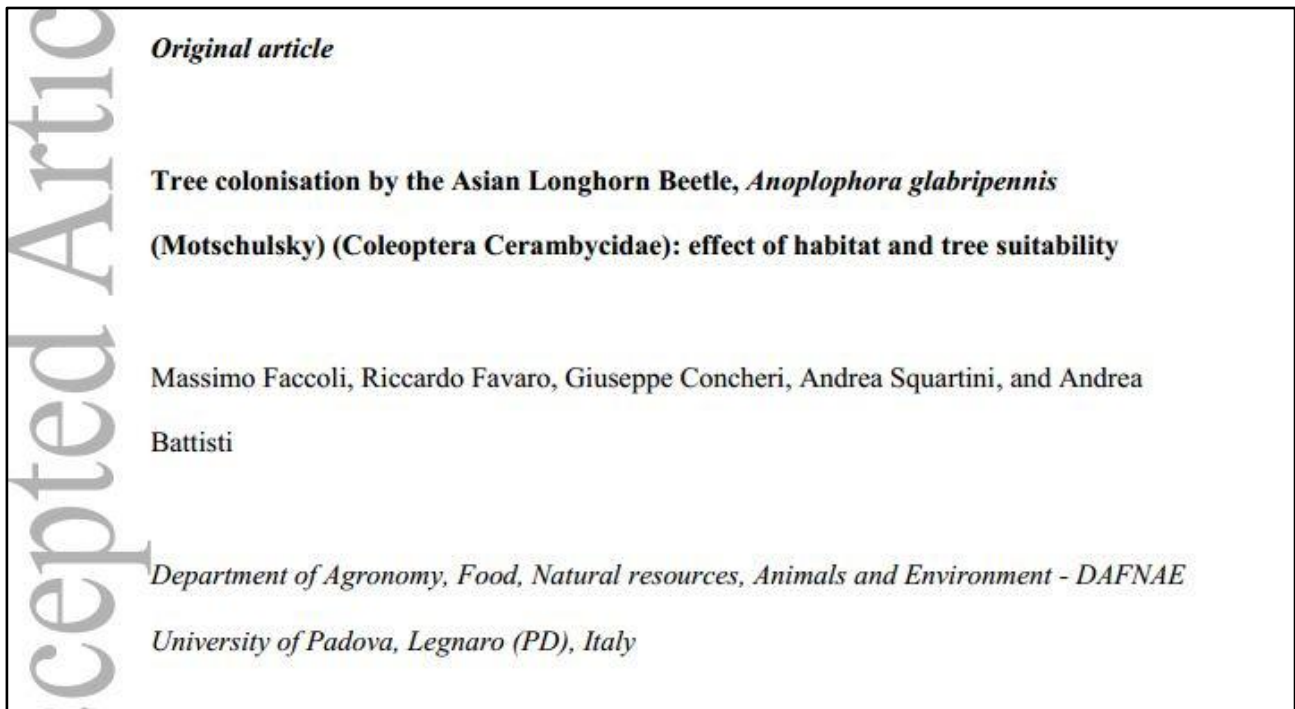
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Chapter 5

Tree colonization by the Asian Longhorn Beetle, *Anoplophora glabripennis* (Motschulsky) (Coleoptera Cerambycidae): effect of habitat and tree suitability

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I collected the data, contributed to data analysis and drafted the paper.



Abstract

Tree colonisation and feeding activity of the invasive wood-borer Asian Longhorn Beetle (*Anoplophora glabripennis*), an Asian pest introduced into North America and Europe, was studied in a newly invaded area in Italy. The hypothesis being tested was that the reproductive success of the insect depend on habitat type and tree suitability. Adult beetles were caged on branches of host and non-host species, in both urban and forest habitats. Two months later, number and size of feeding patches on plant tissues, eggs laid, and surviving larvae were assessed. Bark concentration of C and N was also measured from the same trees. Results indicated that the mean area of plant tissues consumed by adult feeding was significantly larger on trees growing in forest than in urban habitat, although within the same habitat there were no differences between susceptible and non-susceptible trees. ALB tree colonisation, in terms of number of eggs laid and young larvae survival, was not affected by habitat while it was higher on susceptible trees. Although trees growing in forests had a lower nitrogen concentration, they allowed colonisation rates similar to those of trees growing in the urban habitat. Hence, the amount of carbon and nitrogen did not fully explain tree suitability or habitat selection. We suggest compensatory feeding as a potential mechanism that might explain this peculiar situation, as supported by a more intensive feeding activity recorded on

trees in the forest. Suitability of different trees may be due to other factors, such as secondary chemical compounds.

Introduction

The Asian Longhorn Beetle (ALB), *Anoplophora glabripennis* (Motschulsky) (Coleoptera Cerambycidae), is a wood boring pest native to China and South Korea (Cavey *et al.*, 1998; Lingafelter & Hoebeke, 2002; Williams *et al.*, 2004). ALB is a highly polyphagous species that feeds on hardwood tree species from at least 15 families recorded in Asia, Europe and North America (Smith *et al.*, 2009). In both its native range and invaded countries, ALB infests species primarily in the genera *Acer*, *Betula*, *Populus*, *Salix* and *Ulmus* (Lingafelter & Hoebeke, 2002; Haack *et al.*, 2006; Hérard *et al.*, 2006; Turgeon *et al.*, 2007). However, tree susceptibility may differ greatly within the same genus (Gao *et al.*, 1997; Morewood *et al.*, 2004a), as reported for *Populus* species in China (Wang, 2004; Yin & Lu, 2005) and for maple species in North America (Dodds *et al.*, 2014), although the mechanisms driving tree susceptibility have not yet been reported. Host range of phytophagous insects is usually governed by adaptation to plant chemistry, which varies dramatically across plant species, especially if belonging to different families. While some compounds may serve as cues by which adult beetles locate and recognize host plants (Byers, 1995), avoidance of unsuitable trees may be due either to a lack of nutritional elements – for instance nitrogen (Mattson 1980, White 1993) – or to the detection of potentially toxic secondary metabolites (Agelopoulos *et al.*, 1999). Considering the wide polyphagy of ALB, the mechanisms affecting tree suitability for this species are, however, still unclear, especially if they are assumed to be based on characteristics common to such a large range of different host trees. In this respect, the nutritional characteristics of the tree tissues would be a possible factor affecting tree susceptibility to ALB. It is indeed very well known that the nutritional quality of plants may have an impact on the life processes of insects that feed on them (Dixon, 1971; Hosking & Hutcheson, 1979; Mattson, 1980; Leather, 1995; Akbulut & Linit, 1999). For instance, breeding performance of bark and wood boring beetles is often limited by low nitrogen concentrations of the tree tissues they feed on (Hodges & Lorio, 1969; Ayres *et al.*, 2000; Schloss *et al.*, 2006). Chemical composition of plant tissues may thus characterize ALB tree suitability (Morewood *et al.*, 2004b; Zhao *et al.*, 1994).

Depending on local temperatures and photoperiod, emergence of ALB adults lasts from April to November, with peak flights usually in June-July. While most cerambycids colonise dying or dead trees, ALB infests apparently healthy trees (Hu *et al.*, 2009; Haack *et al.*, 2010). After emergence and before mating and oviposition, ALB adults undergo a period of 8-15 days of obligatory maturation feeding (Li & Wu, 1993; Keena, 2006). They feed on petioles, veins of leaves, twigs or tender bark (including both periderm and phloem) of healthy host trees, where the sugars and nutrients needed to reach full sexual maturity and sustain their normal activity are found (Li & Liu, 1997; Keena, 2002; Smith *et al.*, 2002). After ovarian maturation and mating, the female chews an

oviposition niche through the bark and inserts a single egg beneath the bark of the upper trunk or main branches. Under natural conditions most females may lay more than 100 eggs, according to age and body size (Hu *et al.*, 2009). Larvae develop in the wood for 1-2 years depending on temperature and host species, and adults emerge in summer through a characteristic circular exit hole (Haack *et al.*, 2010 and literature therein). ALB reproduction may not depend only on tree species and characteristics, but also on habitat. In the countries of introduction, ALB 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; but see Dodds & Orwig, 2011). Studies conducted in South Korea (Williams *et al.*, 2004) – where ALB is native – suggest that it is not a true forest species but is adapted to riparian habitats characterized by long edges. This hypothesized specialization for edge habitats would explain the high adaptability of ALB to hedges and tree rows – such as along roads and in parks – typical of urban habitats (Williams *et al.*, 2004). According to this hypothesis, the lack of ALB in the forests of the invaded countries should therefore be expected. Two recent papers (Dodds & Orwig, 2011; Dodds *et al.*, 2014), nevertheless, report that in Massachusetts (USA) this beetle is invading two hardwood stands. The three infested areas (Bovenzi Conservation Area, Boylston and Delaval Tract) are actually three small mixed hardwood stands of about 40 ha, 10 ha and 5 ha respectively (for comparison, NY Central Park infested by ALB at the end of 90ies is about 340 ha), surrounded by city neighbourhoods and streets, making them comparable to urban parks or to small rural stands. In June 2009 a large ALB infestation was recorded at a small town in Northern Italy (Cornuda). Although a large part of the 2 km-wide buffer zone falls within a natural hardwood forest closely bordering the infested town, no infested trees were found in the forest but only along its edges (Faccoli *et al.*, 2011). The effect of habitat characteristics on tree suitability to ALB is thus an interesting question deserving attention.

The main aims of this study were to test host acceptance, feeding performance, and tree colonisation of ALB on host and non-host trees growing in urban and forest habitats. In this respect, we hypothesize that tree colonisation by ALB may vary according to habitat and host tree, because of evolutionary history and differences in tree quality. The hypothesis was tested by a specific field bioassay done on healthy mature trees. In parallel, the concentration of some bark chemical components (carbon and nitrogen) was measured in the trees looking for relationships with tree type (host or non-host) and habitat (forest or urban) that might explain any variation in insect performance observed in the bioassays.

Materials and Methods

Study area

The study was conducted in the small town of Cornuda (45° 83' N, 12° 01' E) and neighbouring municipalities (province of Treviso, NE Italy), where a large ALB infestation has been recorded since June 2009. The study area is located in a hilly landscape at about 160 m a.s.l. The town is closely surrounded by riparian habitats along a large river and a natural mixed hardwood forest (Asolo-Bosco del Fagaré, about 1,300 ha) composed mainly of *Carpinus betulus*, *Fagus sylvatica*, *Acer pseudoplatanus*, *Quercus robur* and *Fraxinus excelsior* on the more mesic slopes, and *Fraxinus ornus*, *Betula pendula*, *Ostrya carpinifolia* and *Quercus pubescens* on those more xeric. However, despite the ample availability of potential host trees and the application of a specific monitoring programme leading to the discovery of more than 1000 infested trees (Faccoli *et al.*, 2011), no infested trees were found in the forests and natural habitats neighbouring the infestation area. The whole area lies along the lower edge of the NE Italian Alps, with climate characterized by a South-North transition from Mediterranean conditions, with temperate summers and mild winters, to a more continental climate with hot summers and cold winters. The mean January temperature is about 2-4 °C; precipitation is concentrated in spring and autumn, with an annual mean of about 1,400 mm.

Insect collection and rearing

A specific eradication protocol is applied against the ALB population occurring in Cornuda. This includes the winter monitoring by ground visual checking of all trees belonging to the most common ALB host genera growing within 2 km of each infested tree (Faccoli *et al.*, 2011). Large trees or trees showing unclear symptoms are checked with the help of tree-climbers. Trees found to be infested, *i.e.* showing exit holes or oviposition pits, are referenced geographically and cut-down in May, logged, gathered in a safety area and chipped to kill the pupae just before adult emergence. In late May 2010 more than 500 infested boles approximately 35-40 cm in length and 15-20 cm in diameter, chosen among the most infested branches of *A. pseudoplatanus*, *B. pendula*, *Ulmus pumila* L., *Aesculus hippocastanum* L. and *Salix* spp., were not chipped but placed singly or in pairs in aerated orange plastic tubes (50 cm long, 30 cm diameter) to await adult emergence. Trunks were not sampled because ALB mainly infests the upper part of the trees and the main branches and because too large boles would not fit in the emergence tubes. Both ends of the tubes were covered with a fine metal mesh screen to retain the emerged insects until checking and collection. The tubes were stored outdoors, piled horizontally on open iron shelves in 5 layers (each layer having 5 tubes), under plastic roofs to protect them from rain and direct sunshine, but ensure good ventilation

and thermal exchange. Before being placed in the tubes, the cut surfaces of the boles were sealed with paraffin to reduce drying. Tubes were checked three times per week until the end of the emergence period. Beetles found in the tubes were collected, counted and sexed according to Lingafelter & Hoebeke (2002). Air temperature within the tubes was recorded hourly using a data logger (HOBO Temp[®]) inserted in a tube to record a temperature as close as possible to that of the boles. ALB males and females newly emerged from the infested boles were placed separately in metal mesh cages (100 cm x 100 cm x 200 cm), avoiding any mating and egg deposition. Moreover, there was no oviposition substrate within the cages. As many adults stored together in the same cage often fight and mutilate each other, affecting longevity and performance, there were never more than 20 adults per cage. The adults were fed upon freshly cut twigs of maple (*A. pseudoplatanus*) placed in a glass with fresh water and replaced twice a week. The cages were kept under laboratory conditions at 22 °C and 16h:8h = L:D photoperiod, and the ambient air temperature recorded hourly. Before being tested in field experiments, adults were held in the cages for 20 days to ensure their full sexual maturation (Hu *et al.*, 2009; Haack *et al.*, 2010). As polyphagous insects may have higher performance on the host tree on which they were reared (Mopper, 1996), we cannot exclude that this may have affected our experiment. However, the maturation feeding of adults carried out in the cages on the same host species (maple) should level out such an effect. In July, healthy and fully matured adults were collected from the cages and randomly assigned to the experimental trials.

Field tests

In July 2010, a field experiment on ALB tree colonisation was conducted on ALB host and non-host trees growing in both forest and urban habitats. Apparently healthy, *i.e.* with no visible signs or symptoms of infestation, mature trees were chosen randomly in both the town centre (from hedges, gardens and public parks) and in the forest surrounding the town (see description of the study area). As urban areas and forests are extremely heterogeneous, trees of the same species were chosen as similar as possible in age, health conditions, trunk and canopy size. In each habitat, five trees belonging to each of three hosts (*A. pseudoplatanus*, *F. excelsior* and *B. pendula*) and three non-host species (*C. betulus*, *O. carpinifolia* and *F. sylvatica*) were tested, for a total of 30 trees per habitat (urban Vs forest) and 30 trees per host category (host Vs non-hosts). Tested tree species were assigned to either host or non-host group according to Haack *et al.* (2010) and previous field observations (Faccoli *et al.*, 2011).

A pair of mature ALB adults was placed inside a cage of 0.5 mm wire mesh fixed at about 3 m from the ground on a canopy branch (about 10 cm in diameter) of each experimental tree, for a total

of 120 beetles (60 pairs). The cage was constructed by wrapping a sheet of wire mesh (100 x 50 cm) around the branch, stapling the ends to the branch and further sealing the ends with metal ribbon. Each caged branch had also a number of small fresh twigs providing leaves needed for adult feeding. The resulting cage was about 90 cm long and 50 cm in diameter, allowing enough room for the adults to move freely along the branch, feed, mate and lay eggs. Two days later each insect pair was checked and any dead individual was replaced with a new one of the same age and sex. The tested tree branches were as similar as possible in size and only reachable from the ground by ladder, to prevent tampering. Two months later, the cages were removed, the branches cut from the trees and the resulting boles taken to the laboratory. After collection, the boles were analysed for ALB feeding activity and tree colonisation, measuring the following parameters:

- adult feeding activity: number and size of the feeding patches occurring on branches, twigs, new shoots, leaf petioles and leaves (globally indicated as feeding patches) were recorded individually measuring by a calliper width, length, diameter and surface of plant tissues removed by adults during the feeding activity. These measurements are not particularly difficult because bark removal from branches is usually quite regular in shape (generally rectangular patches) and hence easy to measure. In the case of twig and shoot feeding, instead, the bark is completely removed and in that case the area was measured using the diameter and length of the attacked part. Finally, leaves showing signs of feeding activity were collected and the removed surface assessed by analysis of a scanned image.

- number of oviposition pits occurring on the bark: they were easily distinguishable from feeding patches by their characteristic shape and size, and by the possible occurrence of eggs or larval galleries in the phloem beneath the pit;

- number of sterile oviposition pits: the occurrence of eggs (or larvae) was checked in the phloem beneath each oviposition patch to identify sterile pits and give an indication of attractiveness of the tree for oviposition;

- female fecundity: number of eggs laid by the females assessed by dissection of each oviposition pit found in the boles (after debarking), and corresponding to the number of unhatched eggs (i.e., egg mortality) and the live larvae found in the phloem;

- larval survival: percentage of young larvae (first and second instar) found live in the phloem (after debarking) in relation to the number of eggs laid. As the boles were debarked only two months after the beginning of the field experiment most larvae were still in the phloem and not in sapwood. The few sapwood entrance holes were however counted and considered as live larvae. Because the ALB third instar larvae bore tunnels deeply in the wood the possibility of any accurate observations concerning their survival is strongly reduced. Moreover, larval development is very

slow, taking at least one year, making the full development from egg to adult unpredictable and unreliable in cut boles.

Because temperature may have a strong effect on ALB adult survival, reproduction, and egg hatching (Keena, 2006) the tested trees were selected in sites exposed to a similar mean temperature. The forest was usually cooler during the day and warmer at night but the mean daily temperature did not show significant differences between the two habitats (forest and urban) as they were located at the same elevation and latitude.

Bark sampling and elemental CN analysis

In July 2010, five bark plugs (5 mm diameter) were collected around each branch exposed to ALB feeding and colonisation, for a total of 60 trees and 300 samples. As different tree species may have a different phloem-periderm proportion, a preliminary analysis was performed on 20 trees of *A. pseudoplatanus* and *C. betulus* sampled in both urban and forest habitats. The results showed that periderm was always thicker than phloem and that their biomass ratio did not vary significantly between species (ANOVA, $df = 1; 32$, $F = 288.36$, $P < 0.001$ and $F = 0.25$, $P = 0.61$, for thickness and biomass ratio respectively). Thus, C and N concentration of bark samples (periderm + phloem) can be legitimately compared across different tree species.

All bark samples were collected on the same day and stored individually in Eppendorf tubes at -20 °C until analysis, which measured C and N concentrations expressed as *percentage of dry weight* (samples were oven dried overnight at 50 °C) upon combustion and differential sequential trapping of C and N oxides. Measurements were conducted on a “MACRO Vario elemental analyzer[®]” (Elementar Analysen Systeme GmbH[®], Hanau, Germany) following manufacturer’s protocols.

Statistical analysis

The values of C and N concentrations obtained from the 5 bark plugs sampled in each tree were averaged to obtain a single ($n = 1$) value for that tree, so that each tested tree was considered as a true replicate for both the CN chemical analysis and tree colonisation experiment. Number of oviposition pits, eggs laid, egg mortality, larval survival and feeding activity (i.e., surface of plant tissues removed by feeding adults) were the response variables.. Mean concentrations of C and N per tree, and the C/N ratio were considered as variables for the CN analysis, and reported as percentage of dry matter. The analysed variables, reported as mean values (\pm SEM) per tree, were compared by a two-way analysis of variance (ANOVA) (Zar, 1999) looking for significant differences and interactions among tree types (i.e., host and non-host) and habitats (i.e., urban and forest habitats). Homogeneity of variance was tested by Cochran’s test (test C) and normality by the

Kolmogorov–Smirnow test (test D). When necessary, data were log- [$X' = \log(x+1)$] or arcsin- ($X' = \arcsin\sqrt{x}$) transformed to satisfy requirements of homogeneity of variance and normality. Wherever significant differences occurred, Tukey's Honestly Significant Difference (HSD) multiple comparison test was applied for mean separation (Zar, 1999). Non-homogeneous, non-transformable data were analysed with the χ^2 goodness-of-fit test using Yates' correction for continuity. Differences were considered significant at $P < 0.05$. Analyses were performed using Statistica[®] for Windows[®] (StatSoft Inc., Tulsa, OK).

Results

Insect performance

The mean area of plant tissues removed by adults during feeding (feeding patches) was significantly larger on trees growing in forest (17.6 cm²) than in urban (6.6 cm²) habitats (ANOVA: d.f. = 3; 56, $F = 5.21$, $P < 0.05$) (Fig. 1). However, within the same habitat, there were no significant effects of tree suitability on feeding activity of ALB adults (Fig. 1).

The mean number of oviposition pits recorded on the branches (i.e., per female) was affected by tree type (host or non-host) (ANOVA: d.f. = 3; 56, $F = 5.35$, $P < 0.05$) but not by habitat, with values higher in host than non-host trees, in both urban (24.5 Vs 0.2) and forest (21.1 Vs 2.0) habitats. Instead, the mean number of sterile pits – i.e., oviposition pits with neither eggs nor larvae – was low (on average 14.6%) and with means similar between hosts and non-hosts, and between habitats. The highest recorded fecundity corresponded to 60 eggs laid on maple (host species) in urban habitats. Female fecundity was also affected by tree type, with a higher mean number of eggs laid per female on host (17.1 eggs) than non-host (1.2 eggs) trees (ANOVA: d.f. = 3; 56, $F = 8.71$, $P < 0.05$) (Fig. 1), but not by habitat, with fecundity values similar in both urban (10.6 eggs) and forest habitats (7.6 eggs) (Fig. 1).

Survival of the young larvae, reported as percentage of live larvae relative to eggs laid, was variable but showed a trend similar to that of eggs, with mean values higher on host (13.3%) than non-host (1.1%) trees (ANOVA: d.f. = 3; 56, $F = 7.53$, $P < 0.05$) (Fig. 1). No significant differences in the mean survival were found between forest (6.4%) and urban (7.9%) habitats (Fig. 1).

In all the tested variables (feeding patches, female fecundity and larval survival) tree type and habitats showed no significant interactions.

CN analysis

The CN analysis showed significant differences among tree types (host or non-host) and habitats (urban or forest) according to the tested variables (Fig. 2). The mean N concentration (range of N

concentration per tree: 0.43 - 0.84%) was higher in urban than in forest habitats, for both host and non-host trees (ANOVA: d.f. = 3; 56, $F = 32.65$, $P < 0.001$) (Fig. 2). As expected, the opposite trend was observed with the C/N ratio (range of C/N ratio per tree: 50.9 - 108.8), with higher values in forest than in urban habitats (ANOVA: d.f. = 3; 56, $F = 21.72$, $P < 0.001$) (Fig. 2); no significant differences between tree types occurred within the same habitat (Fig. 2).

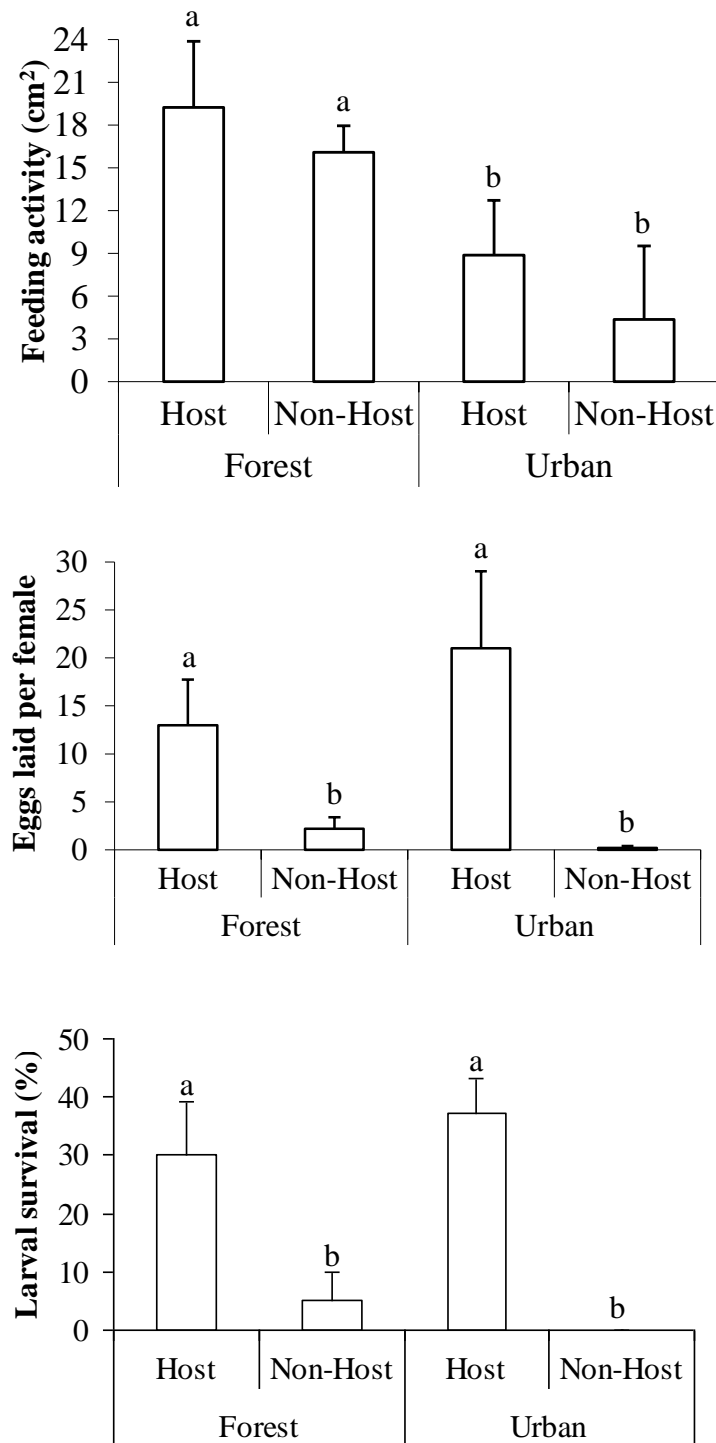


Fig. 1 Feeding activity (area of plant tissues removed by adults) and tree colonisation (fecundity and survival of young larvae) of *A. glabripennis* recorded in different groups of trees (host and non-host) and habitats (forest and urban). Different letters indicate significant differences at $P < 0.05$.

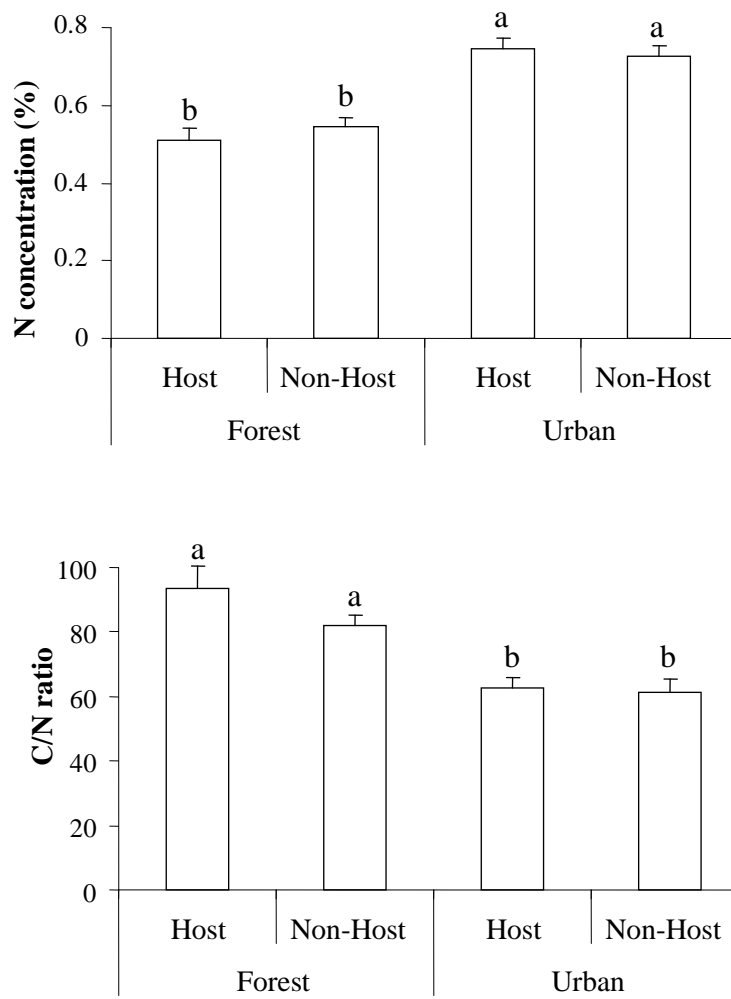


Fig. 2 Nitrogen concentration and C/N ratio of bark samples collected in different groups of trees (host and non-host) and habitats (forest and urban). Different letters indicate significant differences at the ANOVA test ($P < 0.05$).

Discussion

The main results emerging from this study show that ALB tree colonisation is mainly affected by tree type (host or non-host) rather than habitat, with the highest reproductive performance recorded on host trees in both forest and urban habitats. Although this is not a surprising result, the study shows that ALB can also successfully infest trees growing in natural forests, where both host and non-host trees have significantly lower nitrogen concentrations than in urban habitats. In this respect, bark nitrogen concentration does not seem to directly affect ALB tree colonisation, but only adult feeding.

Urban habitats and the managed status of ornamental trees result in higher nitrogen availability in plant tissues (Braman *et al.*, 1998), as found also in this study. The causes can be sought in a low tree density resulting in a lower competition for the environmental resources, in plant rearing practices (including fertilization from the nursery stages onwards), as well as in a higher and closer exposure to anthropogenic nitrogen deposition sources occurring in such habitats. For instance, the atmospheric nitrogen originating from the combustion of fossil fuels can be substantial, exceeding 30 kg/ha in many urban regions of Europe and North America (Taylor *et al.*, 1994; Bobbink, 1998), with an N flux 1.5-2.3 times higher than in rural and natural sites (Redling *et al.*, 2011). Increasing nutritional quality of plant tissues from the nitrogen enrichment occurring in urban habitats may influence the population dynamics of herbivore insects on a wide scale (Kytö *et al.*, 1996; Herms, 2002; Raupp *et al.*, 2010). Nitrogen limitations are particularly critical for wood boring insects (White, 1993), which face an extremely severe challenge because of the low nutritional value of wood (Hodges & Lorio, 1969; Scriber & Slansky, 1981; Slansky & Scriber, 1985). Nevertheless, nitrogen concentration only marginally affected the success of ALB tree colonisation and the possibility to infest trees growing in forest habitats. As low nitrogen concentration can limit growth and reproduction, herbivores can select for mechanisms or behaviours that increase nitrogen acquisition (Mattson, 1980), such as increasing the intake rate (Ayres *et al.*, 2000). ALB appears to deal with the lower N concentration in forest trees simply by increasing the feeding activity needed for adult survival and egg production. This is commonly known as *compensatory feeding*, and can be observed in various animal *taxa* (Karasov & Martínez del Rio, 2007). In ALB the compensatory feeding appears to negate the difference in host quality between forest and urban trees.

The scarce ALB reproduction in forests may therefore be due to ecological factors other than nitrogen concentration in tree tissues. A first prudent hypothesis could be that ALB establishes where it is introduced, which has historically been urban areas because of the role of infested wood arriving via international trade. Most of the recorded ALB invasions occurred in cities or towns often located far away from natural stands and forests, and ALB is known to have a scarce dispersal

capacity, usually lower than 2 km per year (Smith *et al.*, 2001; 2004; Bancroft & Smith, 2005; Sawyer, 2006; USDA-APHIS, 2008). Moreover, ALB tends to re-infest the same tree until the host quality is too poor (Haack *et al.*, 2006), which often takes several years. ALB dispersal is thus generally low when good hosts are plentiful and near the original infestation area. The adults would thus have little possibility of reaching natural forests, often located dozens of kilometres from infested urban areas. Nevertheless, in our experimental site the infestation area closely borders a natural hardwood forest where – after a 6-year long monitoring (2009-2014) – ALB infested trees were found only along the forest edge and never within the forest.

A second hypothesis suggests that the absence of ALB from forests may be due to the presence of limiting biotic factors in these habitats, such as generalist predators more closely associated with natural ecosystems than urban habitats. For instance, generic larval predators like woodpeckers or several insect families of predators and parasitoids of longhorn beetles may play a crucial role in limiting or preventing forest colonisation by ALB adults (Pan, 2005; Li *et al.*, 2007; Huang *et al.*, 2008). However, no specific data are available concerning these hypotheses.

While tree nitrogen concentration varies between habitats, its concentrations are similar in both host and non-host trees. The different suitability of tree type for egg laying and larval development would thus be due to additional factors, for instance secondary chemical compounds that may vary strongly among host and non-host trees but not among habitats. One example of this is the callery pear (*Pyrus calleryana*), native to China and resistant to both larvae and adults of ALB (Morewood *et al.*, 2004b). Morewood *et al.* (2004b) and Zhao *et al.* (1994) suggest that the resistance to ALB is probably caused by the chemical composition of the tree, which may negatively affect beetle development. Bark chemical composition may thus crucially affect ALB wood colonisation, as known in many other wood-boring beetles (Mattson *et al.*, 1988; Hanks *et al.*, 1995).

In conclusion, our data begin to cast light on the hitherto unexplained factors governing the spread of a major insect pest that is continuing to pose crucial threats to urban parks and landscapes in European and north American towns. Through compensatory feeding by ALB adults, the mean nitrogen concentration in trees does not seem to be a determining factor on oviposition and development of young larval instars in different hosts and habitats. Other hitherto undefined biological or ecological aspects must therefore play a significant role.

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Disclosure

The authors declare that they have no conflict of interests.

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Chapter 6

Spatial spread and infestation risk assessment in the

Asian Longhorned Beetle

Anoplophora glabripennis

(Coleoptera Cerambycidae)

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I drafted the paper and contributed to data analysis.

Abstract

The Asian Longhorned Beetle (ALB), *Anoplophora glabripennis* (Motschulsky) (Coleoptera Cerambycidae) is recognised as potentially one of the most damaging invasive insects in Europe and North America. International trade has increased the risk of accidental introduction of ALB. An eradication programme was initiated in north-east Italy in June 2009, when an ALB infestation was discovered. The infestation was monitored by annual surveys of all host-tree species growing in the eradication area. Infested trees were cut down and chipped. This study analyses the spatiotemporal distribution of infested trees for a 5 year period from 2008-2012 using a generalised linear model approach. The results show that spread and infestation risk were significantly affected by 1) distance of suitable hosts from the nearest infested trees, 2) number of infested trees in the surroundings and 3) annual variations. The significant differences in beetle dispersal between years to some extent reflect the onset of the eradication programme. The model allowed the estimation of arbitrary probability-based management boundaries surrounding ALB infested trees. For example the model estimates a 0.1% probability of attack on a suitable host tree 1,910 m from an existing attack.

Introduction

Increasing international trade has led to the introduction of many alien insects to both North America (Haack 2006, Langor et al. 2009, Haack et al. 2010) and Europe (Faccoli 2008, Roques et al. 2009, Kirkendall & Faccoli 2010, Rassati et al. 2013). After successful establishment, alien species begin to spread in their new environment with populations occurring outside their native range of distribution (Liebhold & Tobin 2008). Although the introduction and establishment of most alien species can be prevented through bio-security measures such as specific inspection and quarantine protocols, accidental introductions still occur. A reliable assessment of the dispersal rate of an invasive species therefore plays a crucial role in the success of eradication programmes applied after an invasion (Liebhold & Tobin, 2008). Indeed, spread prediction can allow management guidelines to be developed and quarantine boundaries determined (Tobin et al. 2007). According to Liebhold & Tobin (2008) there are two conceptual methods to predict dispersal of alien species. The first uses a dispersal model based on specific life-history traits of the target species. Biological and ecological parameters are used to build equations (*i.e.* models) describing a continuous dispersal of the alien species. However, the spread is usually a complex event resulting from the interaction of many different mechanisms, such as human-mediated long-distance dispersal, Allee effect and new introductions (Liebhold & Tobin 2008). This method was developed for the Asian Longhorned Beetle (ALB), *Anoplophora glabripennis* (Coleoptera Cerambycidae), with mark-recapture studies providing the rate of spread (Zhou et al. 1984, Wen et al. 1998, Smith et al. 2001, Smith et al. 2004, Williams et al. 2004, Bancroft & Smith 2005). The second method predicts the spread of a target species in relation to historical spread data. A well-known example is the spread of the gipsy moth, *Lymantria dispar* (Lepidoptera Lymantriidae), in North America, which was analysed using records available since 1900 (Tobin et al. 2007). This could be a more reliable approach but is usually limited by the large space-time dataset required, which recently discovered invasions may not provide (Hastings et al. 2005). However, Lu (2005) applied the second method to a short-time data series (a few years), creating a simulation model of ALB spread based on dendrochronological data recorded in the infestation area of New York, but differences between model predictions and real spread remained because of the role of other factors in the dispersal. Wichmann & Ravn (2001) also developed a study of the spread of *Ips typographus* following windfall in a spruce forest, based on a short series of historical data.

In the last two decades ALB has been recognised as one of the most harmful invasive insects in Europe and North America (Haugen 2000, Nowak et al. 2001, Haack et al. 2010). The species, originally from China and Korea (Cavey et al. 1998, Lingafelter & Hoebeke 2002, Williams et al. 2004a), was first found outside Asia in New York City in 1996 (Haack et al. 1996, Cavey et al.

1998). After this, the species was found in an increasing number of North American states (Hu et al. 2009, Shatz et al. 2013), and since 2001 also in Europe (Tomiczek et al. 2002, Hérard et al. 2006, Maspero et al. 2007, EPPO 2008a). As the insect arrives in new areas mainly via wooden packing materials used for international cargo (Hu et al., 2009), the increasing trade between Asia, Europe and North America results in a higher risk of new ALB introductions. Intensive field surveys have been carried out in the eradication areas in the US (Haack et al. 2010). Surveys require the establishment of eradication boundaries, which are usually defined by the outermost infested trees, and a safety buffer zone around them. Boundary identification depends on the insects spreading capacity. Higgins et al. (1996) report active dispersal as the most important factor contributing to the spread of exotic species in new areas. Knowledge of spreading capacity is, hence, of major importance in the management of current and future biological invasions. Although ALB is reported to be a “weak flyer” (Zhou et al. 1984), data referring to its active dispersal show wide variation among populations (Wen et al. 1998, Smith et al. 2001, Williams et al. 2004, Bancroft & Smith 2005), especially concerning mean daily movement, which ranges from 2.2 m/d (Zhou et al. 1984) to 32.4 m/d (Smith et al. 2004). Because of the contrasting values, these results are scarcely applicable to pest management protocols. Variability in results are due to many factors, including the experimental design adopted during field trials and surveys. For instance, Dingle and Holyoak (2001) suggested that a dispersal phase of newly emerged beetles may occur just after emergence, and hence beetle age can affect their spread. Williams et al. (2004) reported that the release of beetles from a single point during mass mark-recapture studies can lead to an overcrowding situation affecting the dispersal behaviour through a density-dependent mechanism. Schwartz & Arnason (1996) suggested that species movement varies in space and time, and that mark-recapture models give reliable predictions only for populations living in similar environmental conditions. Indeed, remarkable differences in total spreading distance and daily rates were even found in studies conducted in the same experimental area and in similar conditions (Smith et al. 2001, 2004). In this study we present a descriptive model assessing the spreading capacity of an ALB population in North-Eastern Italy. The model provides probability values of infestation of healthy host-trees using historical data from 5 years monitoring of the ALB infested trees. Whereas previous papers were based on mark-recapture studies (Zhou et al. 1984, Wen et al. 1998, Smith et al. 2001, 2004, Williams et al. 2004, Bancroft & Smith 2005), the aim of the present study is to investigate ALB dispersal through the analysis of the spatial distribution of ALB infested trees. We assume that the data from an annual field survey, providing spatial and temporal distribution of the infested trees, may be related to the dispersal of ALB adults in the infestation area and can thus be used as a proxy

for the spread assessment. A generalised linear model (GLM) is used to analyse the dataset and estimate the boundaries of probability-based management areas.

Materials and Methods

Investigation area and tree survey

The study was conducted in the municipality of Cornuda (45° 83' N, 12° 01' E; Treviso province, NE Italy), where a large infestation of *A. glabripennis* was discovered in June 2009 (Faccoli et al. 2011). The area is suburban with patches of agricultural land. The eradication programme, still in progress, started immediately. The eradication area was set to one kilometre around each tree found to be infested, with a buffer zone of another kilometre. Since summer 2009, a periodic survey has been performed twice a year (summer and winter) inspecting all potential host trees, as identified in the literature (Hu et al. 2009, Haack et al. 2010), occurring in both infestation and buffer areas. Tree inspection is done from the ground with binoculars, looking for symptoms of ALB colonisation, such as adult exit holes, larval frass and oviposition scars. If either is found, the tree is cut and chipped. Branches and canopy of large trees are carefully checked by trained tree climbers. Most of the checked trees are located in gardens, private and public parks, roadsides and crop fields, as isolated trees or in small groups. As decided by the plant protection organization, there was no preventive removal of potential host trees. The infestation area covered a total of about 5,600 ha.

Dataset

Tree monitoring conducted during 4 consecutive years (2009-2012) produced the dataset analysed in the study. A total of 12,732 trees were individually checked, of which 466 were found to be infested by ALB. Infested trees belonged mainly to four genera: *Acer* (36%), *Ulmus* (28%), *Betula* (18%), *Salix* (13%). A few trees of other genera such as *Aesculus* (1%), *Populus* (0.2%), *Prunus* (0.9%) and *Cercidiphyllum* (0.2%) were also found to be infested. However, only the four main genera (*Acer*, *Betula*, *Salix* and *Ulmus*), which account for 95% of the infested trees, were considered in the analysis.

Spatial coordinates (GPS), tree characteristics (genus, diameter, height), and symptoms of ALB colonisation, if any, were recorded for all the checked trees. For the infested trees, the infestation year was also assessed; trees with exit holes were considered infested during the previous year, as ALB is univoltine in northern Italy (Favaro, pers. observ.), whereas trees showing only oviposition scars on the bark were considered infested in the current year. In 2009, trees showing exit holes were considered as infested in 2008 or previously, thus extending the dataset by one year (2008-2012). Each year the dataset was updated with data on the newly-found infested trees. The GPS

device used in the study had an accuracy of about 5 metres. The spatial resolution of the analysis was therefore limited to tree-clusters, i.e. a group of trees with same ALB status (infested/not infested) growing within 5 m of each other and belonging to the same genus. The total number of tree-clusters are reported in Table 1. Given the proximity of the trees in a cluster, they are assumed as a single point.

Statistical analysis

The binomial individual tree-cluster data (tree-clusters attacked or not) was analysed using a generalised linear model (GLM) with a probit link-function (McCullagh and Nelder 1989). The dependent variable is the probability (P) that a tree-cluster will be attacked. The tested independent variables were: attack index (ati), distance to nearest attacked cluster (dna), available hosts index (ahi), cluster size (cs) (size of the tree in cm DBH), genus (g) (*Acer*, *Betula*, *Salix*, or *Ulmus*), and year (y). The first four variables were considered continuous, the last three were class variables.

Attack index (ati) for cluster i is an index of how many attacked trees are nearby, and is calculated as:

$$(1) \quad \text{ati}_i = \sum (\exp(-d_{ij} / c_a))$$

where c_a is a constant, and d_{ij} is the distance between cluster i and each cluster (j) attacked in the previous year.

Available hosts index (ahi) is an index of the number of hosts available in an area around tree-cluster i and it is calculated similar to the attack index:

$$(2) \quad \text{ahi}_i = \sum (\exp(-d_{ij} / c_h))$$

where c_h is a constant, and d_{ij} is the distance between the cluster i and each of the other not-attacked clusters (j) in the current year.

The constants c_a and c_h in equations 1 and 2 are estimated for all models (full as well as reduced) using an iterative approach. The indices (ati and ahi) are each calculated for all tree-clusters and years for a range of values of c, which represents a distance threshold within which 0.36 (1/e) of the attacks occur. Limiting c values to a range from 0 to 5000 m ensures that the final model is biologically plausible, as all known records of self-propelled travel by ALB is significantly less than 5000 m (Haack et al. 2010, Hu et al. 2009, Smith et al. 2001, Smith et al. 2004).

The genus variable (*g*) accounts for the host plant genus considered in this study. *Acer*, *Betula*, *Salix* and *Ulmus* are the main infested genera in the study area. The diameter at breast height (DBH) values of the trees in a cluster are considered in the cluster size variable (*cs*), as a parameter of host plants size.

All the independent variables and their interactions were examined. The non-significant variables and variable interactions were determined and iteratively eliminated based on their significance level ($P > 0.01$). The least significant variables/combinations were eliminated first. Non-significant class variables or combinations were not eliminated at first, if at least one class was significantly different from the other classes. Similarly, lower-level non-significant effects were not eliminated if there were significant combined effects of the same variable. The elimination was repeated until all parameters left were significant at 1% level ($P < 0.01$).

The data (including the indices) were fitted using the "GLM" routine of the free software "Project R" (R Core Team 2012). The fits were evaluated using Akaike's Information Criterion (AIC), and the best fit was identified minimising AIC. The procedure was repeated for a subset of *c* values, essentially narrowing in on the best possible values of *c*, until there was no or very little difference in AIC. This gives approximations of the constants that provide the model best fitting the data:

The minimum distance from an attacked tree-cluster to a tree-cluster attacked in the previous year was determined for all attacked tree-clusters, and the cumulative distribution of minimum distances plotted for each of the years 2009-2012.

Detection of the attacked trees during surveys is not perfect, and some tree is missed every year. The model considers that the percentage of infested trees missed during the annual surveys is constant. Because each new attack originates from an old attack (assuming that there are no new introductions), the beetles in a new attack should have travelled at least as far as the distance to the closest old attack. Hence, the distribution of distances from new attacks to nearest old attack reflects the dispersal capacity (maximum distance) of the beetle (Wichmann & Ravn 2001).

The result of the final GLM model was illustrated by plotting the probability of attack as a function of distance from a single attacked tree-cluster ("one-attacked-tree" scenario). This was repeated for each of the years 2009-2012. The illustrations of the "one-attacked-tree" scenario for each year, and the parameterised GLM model were used to determine a set of "risk boundaries" based on fixed probabilities of attack. Finally the model was applied to the data and the attack probability for each tree was shown in a series of annual maps of the infested area.

Results

The number of infested trees detected during the considered years (2008-2012) shows that the highest value was reached in 2010, the year after the infestation discovery, with 166 infested clusters (Tab. 1). While in 2009 there was an increase in infested trees, the number progressively decreased after 2010 due to the eradication programme, reaching a minimum in 2012 with only 28 infested clusters found in the entire area monitored. Over the course of five years a total of 466 infested clusters were analysed.

The variables genus (g), cluster size (cs), available hosts index (ahi), as well as their combined effects, were rejected by the model because there was no significant effect. Attack index (ati), distance to nearest previously infested tree-cluster (dna) and year (y) were significant variables. Attack index and distance to nearest attack ($ati*dna$), and attack index and year ($ati*y$) showed a significant interaction. The final GLM model therefore became the following:

$$(3) \quad P \sim ati + dna + y + ati*dna + ati*y$$

The constant c_a in equations 1 for the GLM model is approximately 337 m. The GLM fit summary statistic for the final model (equation 3) is reported in Table 2.

The cumulative distribution of minimum distances covered by ALB from each new infested tree-cluster (in the year y) to the nearest previously infested tree-cluster (in the year $y-1$) (Fig. 1) indicates a non-linear spreading trend, where the insect spreads mainly locally within and around the previously attacked tree-clusters. Despite differences between years, 80% of the dispersal ranges between 0 to 300 metres from the closest infested tree-cluster (Fig. 1). Some individuals, however, can also move farther than 2,000 metres.

The application of the final model (equation 3) in a "one-infested-cluster" scenario predicts the probability of attack of healthy trees as a function of distance from a single infested tree-cluster (Fig. 2). According to the probability values provided by the GLM model (equation 3 and Table 2), and by the "one-infested-cluster" scenario (Fig. 2), the boundaries of the eradication area are fixed in relation to the chosen infestation risk (*i.e.*, probability of infestation). The results show that, as expected, the probability of infestation of healthy trees decreases inversely to the distance, according to the model function (Table 3). The year effect (y) on the infestation probability is highest for 2010 (0.33167), and then decreases drastically in 2011 and 2012 (-0.12871 and -0.72664, respectively) (Table 2, Fig. 2). The spread estimates based on the combined effect of attack index and year (negative since 2010) shows a time-lag lower than the overall effect of year

(Table 2). In other words, not only are beetles removed by the eradication programme, their dispersal (*i.e.*, risk hereof) is also influenced.

Table 1: Number of tree-clusters and their status during the 5 years of survey.

Year	Not infested	Infested
2008 or previous	2754	83
2009	2721	116
2010	2471	166
2011	2407	73
2012	2379	28
Total	12732	466

Table 2: Summary of GLM fit for the final reduced Model (4).

Variable	Estimate	Std. Error	z-value	P-value
Intercept	-1.72814	0.12272	-15.110	2.0E-16
Ati	0.08120	0.01176	6.902	5.14E-12
Dna	-0.88626	0.13844	-6.402	1.54E-10
Year 2010	0.33167	0.11330	2.927	3.42E-03
Year 2011	-0.12871	0.13537	-0.951	3.42E-01
Year 2012	-0.72664	0.19562	-3.715	2.04E-04
ati*year 2010	-0.02040	0.01291	-1.581	1.14E-01
ati*year 2011	-0.05302	0.01194	-4.441	8.96E-06
ati*year 2012	-0.04935	0.01253	-3.939	8.17E-05
ati*dna	-0.23473	0.03735	-6.332	2.41E-10

Table 3: Safety distance (metres) around each infested tree-cluster related to the probability of infestation of new trees. High distance means low probability of infestations and *vice versa*.

Year	P<0.00001	P<0.0001	P<0.001	P<0.01	P<0.02	P<0.05	P<0.1
2008-2009	2,860	2,250	1,540	660	370	-	-
2009-2010	3,240	2,620	1,910	1,040	730	280	-
2010-2011	2,720	2,100	1,390	510	210	-	-
2011-2012	2,050	1,420	700	-	-	-	-

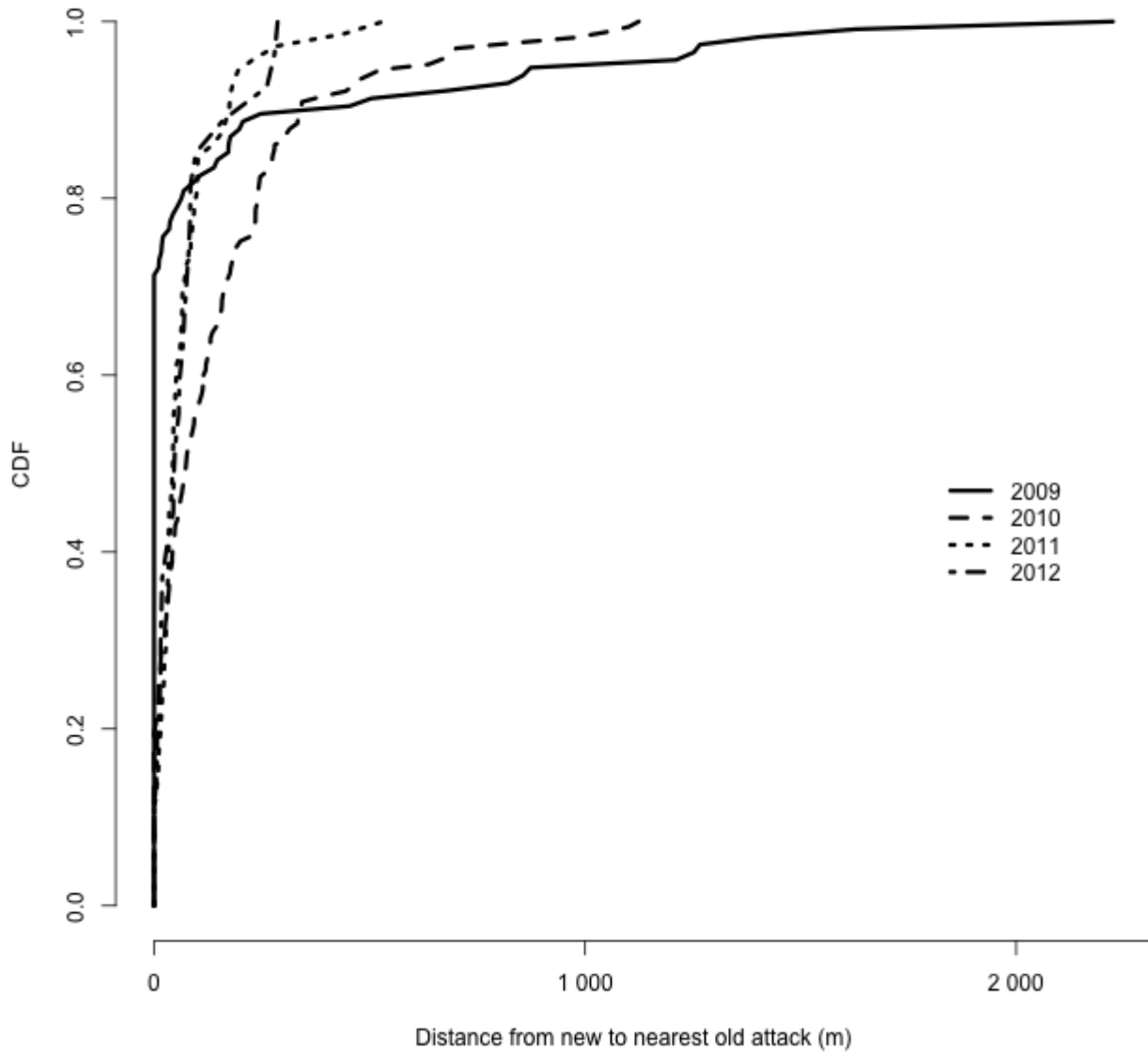


Figure 1: Cumulative distribution function of minimum distance from each new infested tree-cluster to the nearest infested tree-cluster recorded in the previous year.

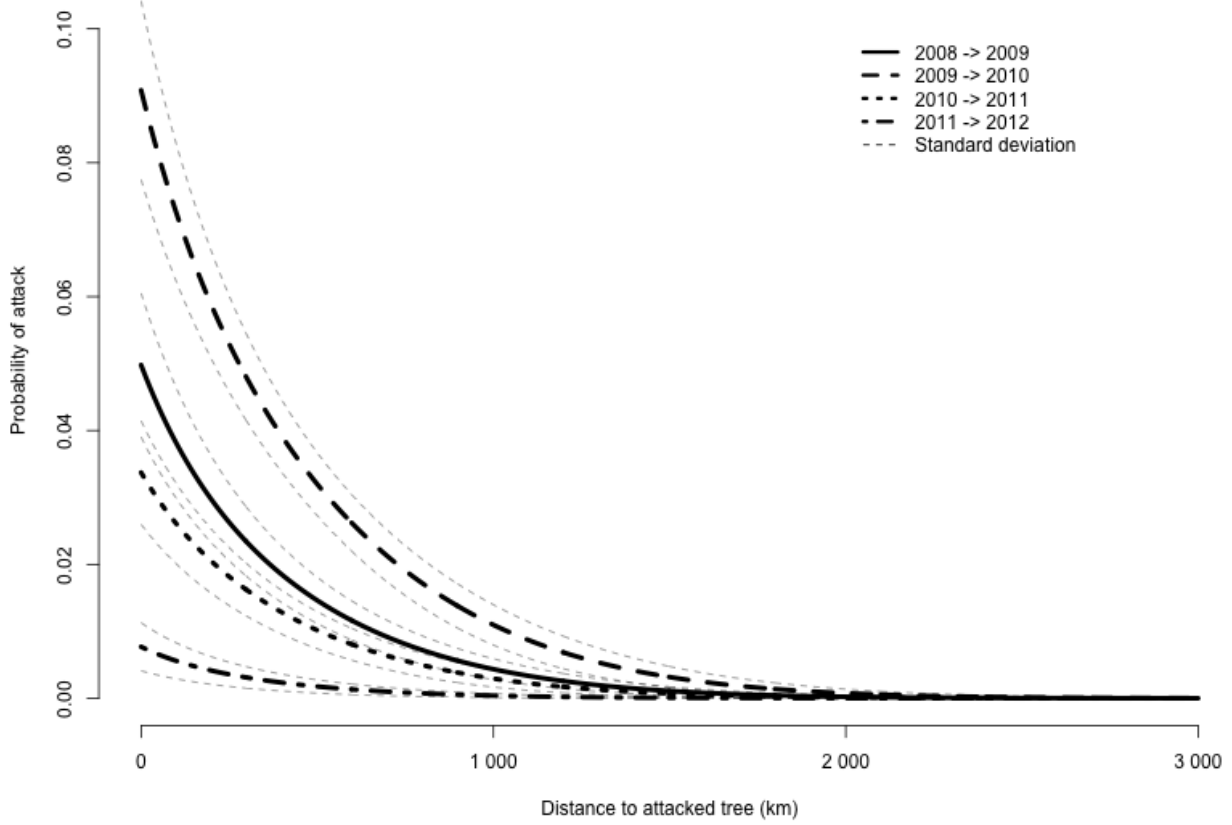


Figure 2: Probability of attack (from 0 to 1) as a function of distance from a single infested tree-cluster predicted by the model in Equation 3 with the parameter estimates in Table 2.

Discussion

The study analysed data concerning the spatial spread of an ALB population occurring in Northern Italy. The resulting model shows that the ALB potential dispersal is influenced by the number of infested clusters (*i.e.*, the attack index) around the healthy ones, by the distance from the nearest clusters infested in the previous year, and by the considered year. The number of healthy tree-clusters occurring in the infestation area as well as host plant genus and size are not significant factors for predicting ALB dispersal. According to the three significant variables the model estimates attack probabilities of healthy trees in relation to the distance from a release point (*i.e.*, previously infested trees).

At similar distances from the infested trees, the probability of infestation of healthy trees varies between years, being for instance much higher in 2010 than in 2009. This might be due to the increase in population size observed in the early years of the invasion. The effect of year (y) shows a significant interaction with the attack index (ati), which accounts for the number of infested clusters occurring in the monitored area. Hence, population density affects the spread of ALB in accordance with the density-dependent dispersal reported by Bancroft & Smith (2005). Our study clearly shows a risk of ALB spread increasing with population size. Similarly, the decrease of population size observed in 2011 and 2012 corresponds to a decrease in ALB dispersal and a reduced probability of healthy trees being infested. Data from 2008, and partially from 2009, refer to years with no eradication activities, and this probably affected the annual variations of the parameters observed at the beginning of the infestation. On the discovery of the infestation in June 2009, the eradication programme was applied only partially as just some of the infested trees were removed during summer and the majority were cut and chipped in winter 2009-2010. During spring and summer 2009, beetles were thus able to emerge and spread in huge numbers. Once eradication measures were working in full, the frequency and distribution of infested clusters reduced markedly (Table 1). This reduction is reflected in the dispersal parameters assessed by the model, where the highest attack probability occurred in 2010 and then decreased in the following years. In this respect, our model highlights the importance of managing a new infestation as soon as possible, to reduce the probability of spreading in the field.

The probability that ALB adults actively disperse farther than 1,900 m is extremely low (< 0.001). This is in agreement with previous studies reporting maximum spreading distances of ALB ranging from 1,442 m to 2,600 m per year (Smith et al. 2001, 2004). The distribution of the minimum distances shows, however, that, even if the probability is very low, new attacks may occur farther than 2 km away from the nearest trees infested during the previous year (in our dataset up to 2,224 m). The eradication programme applied in the study area adopted two boundaries for eradication

and buffer zones of 1 and 2 km around each infested tree respectively. According to the model, these distances correspond to an infestation risk (based on 2010 data) of 1.1% and 0.07% respectively, suggesting that, even if the probability of new infestations is low, there is still a possibility that beetles may infest trees growing outside the eradication zone. USDAAPHIS carried out an intensive survey in ALB-infested areas. A core area of 800 m radius around each infested tree and in a buffer zone of 800-1,600 m, while they performed an extensive survey in a larger area of 40 km (Haack et al. 2010). According to our model (based on 2010 data), these boundaries correspond to infestation probabilities of 1.7%, 0.2% and 2EXP-14%, respectively. As the ALB infestation was declared eradicated in 2009 (Haack et al. 2010), this suggests that the survey distances applied by USDA-APHIS were effective for pest eradication although a very low risk of infestation still existed. In this respect, limits for management zones are somewhat arbitrary. It is up to each government (or inter-governmental) agency to decide how much is at risk (natural resources, values, amenity values etc.), and relate this to how risk-averse their actions need to be. Some arbitrary boundaries are presented in Table 3 and may serve as broad guidelines. It is recommended to use the worst case scenario (in our study parameter estimates based on data from 2009 to 2010). The maximum dispersal found was from a year with partial management (2009 to 2010), and this may have underestimated the real dispersal. Hence, the maximum probabilities presented in this study may be inherently underestimated.

Furthermore, the probability based distances proposed in this paper can be chosen also for defining the radius of preventive removal of potential host trees, according to the risk assumption.

The present study reports data and analysis of a single ALB invasion in Northern Italy. Because the spread rates may vary over space and time, the results presented are valid only in the current situation. With some caution it would be possible to generalise the conclusions, and apply them elsewhere in Europe and North America. Prior to application, however, a range of variables should always be considered, such as climate and available tree species in the area etc., which may impact on the dispersal of ALB. Comparing results from this dataset to other infestations, it could be possible to derive similarities and differences in the beetle dispersal behaviour.

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Conclusions

The Asian Longhorn Beetle has shown to be a threatening pest in the infestation area considered in this study. After five years since the first detection (2009), more than one thousand trees have been attacked by the beetle. As in other outbreaks, the eradication efforts require many years before to affect the population density.

A new home

The exit holes dating analyses presented in chapter 2 revealed the wood packaging materials as the most probable introduction pathway and that the infestation takes many years to be recognized. Future detection efforts must consider a monitoring activity in the vicinity of those incoming points whose are involved in international shipping.

Once an introduction occurs, establishment may take place. This requires insect adaptation to a new environment. Chapter 3 reports some life-history traits of *Anoplophora glabripennis* in the new infestation area, confirming the thesis that the beetle phenology is strictly linked to temperature and that can be narrowly predicted using climate-based models (MacLeod *et al.* 2002, Smith *et al.* 2004). The obtained data provide valuable information for when to proceed with eradication protocols or control programs, such as adult emergence period and overwintering stages.

A considerable contribution to current and future infestation management is also provided by the dispersal study developed on chapter 6. The model connects the probability of attack with the distance. Which is a very easy, understandable and applicable tool. If until now the eradication measures have been taken in a way somewhat arbitrary, now the concept of risk is well defined and can be assumed depending on the needs. Not only, the model provides other three main outcomes. First, the dispersal has been influenced by population density, decreasing in time with the ongoing eradication. Which is, however, a well-known concept. Another confirmation of previous studies (Smith *et al.* 2001, 2004) is the scarce tendency of *A. glabripennis* to disperse, branding the beetle as a weak flyer, that tends to spread not farther than 2 km per year (probability < 0.01). Most surprising, the model does not recognize any significant difference in host genus, concerning the spread. This point appears to be in contrast with the renowned preference of ALB for some host plants.

Dealing with new hosts

A fundamental aspect provided in the study is the host preference in the study area. This, in fact, contributes to the list of susceptible plant species in a worldwide knowledge. Despite many common host (see Gaag & Loomans 2014), differences are always present between infestation areas. Probably, the main interesting trait of ALB is the adaptation to host plants. Considering the study site, it has been reported (Chapter 4) that the main attacked host (as total amount) is maple, as confirmed from many other outbreaks, while birch, which is reported as well as main host, affects very bad the reproductive performance of ALB. Nevertheless, the insect continues to reproduce on it, even with a low larval survival. Moreover, as results from the dispersal model of chapter 6, there seems to be no host preference influence when beetle spreads, raising the doubt about an efficient host choice.

This behavior opens to fascinating questions about the beetle adaptation strategy. Are ALB females bad mothers which do not recognize the host quality? Or does the preference involves genetic traits linked to the original population? Similar discrepancies have been reported also in other publications, but the point is still unclear and worth to be investigated.

Another open question about ALB is the low tendency to colonize natural forest stands (Hu *et al.*, 2009; Haack *et al.*, 2010). One of the plausible answer is that the habitat type may affect host trees belonging to the same species. In chapter 5, urban and forest habitat have been tested against susceptible or not tree species. Beetle preference resulted to be related to the host type, rather than the habitat. Host quality, measured as nitrogen content, is higher in urban areas, and beetles in forest hosts deal with it through a compensatory feeding mechanism. After all, what really affects the reproductive performance is, once again, the tree species, confirming a preference for some host, even with substantial differences among them.

In conclusion, this thesis analyses an infestation of the longhorn borer *Anoplophora glabripennis* in southern Europe, providing detailed studies concerning insect arrival and establishment in a new introduction site, adaptation to local habitats and host plants, and a reliable estimation of adult dispersal.

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