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**DETECTION AND MONITORING OF INVASIVE AND NATIVE SPECIES OF
WOOD-BORING BEETLES IN A CHANGING ENVIRONMENT**

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Padova, 17 January 2014

Fabio Chinellato

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Riassunto

Gli insetti del legno sono organismi particolarmente importanti per il loro impatto sugli ecosistemi forestali e sul valore economico dei prodotti di tali ecosistemi. I recenti cambiamenti ambientali, collegati al cambiamento climatico globale, hanno portato a variazioni nella distribuzione e nell'impatto delle specie di insetti del legno in molte aree forestali. A questo scenario si aggiunge un incremento delle merci trasportate in tutto il mondo e dei loro imballaggi, spesso di natura legnosa, e con essi aumenta il rischio di importazione di specie di insetti del legno in nuovi ecosistemi.

Questo lavoro si basa sul monitoraggio tramite trappole di coleotteri del legno, principalmente Buprestidi (Coleoptera: Buprestidae), Cerambicidi (Coleoptera: Cerambycidae), Curculionidi (Coleoptera: Curculionidae) e Scolitidi (Coleoptera: Curculionidae: Scolytinae), al fine di valutare la presenza di specie invasive esotiche e l'effetto del clima, in particolare della temperatura, sulla distribuzione e sulla performance di alcune tra le principali specie più comuni dell'area Alpina. A questo si aggiunge uno studio sull'applicazione di nuove metodologie per il monitoraggio basate sull'utilizzo di telecamere digitali controllabili a distanza via web.

Un primo studio richiama le principali specie di insetti del legno invasive per l'Europa e le tecniche per il loro monitoraggio e controllo, a cui segue la sperimentazione di un dispositivo per il monitoraggio remoto di coleotteri del legno.

Il secondo studio riguarda la distribuzione degli insetti del legno lungo un gradiente altitudinale, utilizzato come analogo spaziale del cambiamento climatico, e mostra l'effetto positivo della temperatura sull'abbondanza delle specie maggiormente aggressive a carico dell'abete rosso (*Picea abies*).

Il terzo studio indaga l'effetto delle alte temperature estive sulla performance di *Ips acuminatus* all'interno della stessa stagione e tra anni successivi, dimostrando che temperature particolarmente alte sembrano influenzare positivamente la crescita della popolazione nello stesso anno, ma negativamente quella dell'anno successivo.

Il quarto studio presenta un'applicazione delle trappole fotografiche controllate in remoto per la early detection dei Cerambicidi del genere *Monochamus* spp., considerati pericolosi in quanto vettori del nematode del pino *Bursaphelenchus xylophilus*. Alle trappole fotografiche è stato associato un sistema di identificazione molecolare sul campo basato

sulla tecnica denominata LAMP-PCR. Il risultato è un sistema integrato in grado di concentrare gli sforzi di controllo delle trappole e analisi molecolare in campo alle sole trappole che mostrano la presenza delle specie target.

A dimostrazione dei diversi ambiti di applicabilità delle nuove tecnologie proposte, al termine di questo lavoro viene aggiunto uno studio svolto in Nuova Zelanda nel quale la tecnologia delle foto trappole fotografiche vengono utilizzate per il monitoraggio di una specie dannosa per i frutteti.

Summary

Wood-boring insects are extremely important organisms because of their impact on forest ecosystems and on the economic value of forest products, wood in particular. Recent environmental modifications, linked to global climate change, lead to a variation in both distribution and impact of wood insects species in many forest areas. New trade patterns are added to this scenario, with an increase in exchange of goods and their packaging, often wood-made, and with them an increase in risk of spreading wood insect species in new ecosystems.

This work is based on trap monitoring of wood-boring beetles, mainly jewel beetles (Coleoptera: Buprestidae), Longhorn beetles (Coleoptera: Cerambycidae), weevils (Coleoptera: Curculionidae) and bark beetles (Coleoptera: Curculionidae: Scolytinae), in order to evaluate the effect of climate, and in particular of temperature, on distribution and performance of some of the main species in the Alpine area. Furthermore, this work includes a study on the application of new monitoring tools based on the application of digital cameras remotely checked through the web, able to improve the early detection strategies for invasive species.

A first study recalls the main wood-boring insect species invasive for Europe and their detection and monitoring techniques, and it is followed by the experimental trial of a new device for remote monitoring of wood-boring beetles.

The second study concerns the distribution of wood-boring insects along an elevational gradient, considered as a spatial analogue of climate change, and it shows the positive effect of temperature on the abundance of most aggressive species against Norway spruce (*Picea abies*).

The third study evaluates the effect of warm summer temperatures on the performance of *Ips acuminatus*, a bark beetle associated with Scots pine in the Alps, in the same season and between consecutive years. It shows that particularly high temperatures are likely to affect positively the population growth in the same season, but negatively the population growth of the following year.

The fourth study presents an application of remote controlled photographic traps to the early detection of longhorn beetles belonging to the genus *Monochamus* spp., considered dangerous for being the vector of the pine wood nematode *Bursaphelenchus xylophilus*.

Camera traps have been associated to a molecular analysis device for the species identification based on a technique named LAMP-PCR. The result is an integrated system able to focus the efforts of trap checking and field molecular analysis only to those traps showing the presence of the target species.

To show the wide applicability of the new proposed technologies, at the end of this work is added a study carried on in New Zealand in which remote camera traps are applied to study the phenology of a stone fruit pest.

Chapter 1

Introduction

The impact of both native and invasive insect species in forest ecosystems is extremely important to assess and predict in order to protect the multiple services provided by these complex ecosystems, such as timber production, soil protection and landscape and biodiversity conservation (Alfaro *et al.*, 2010). Changing environmental conditions, such as global warming, may significantly modify the activity of plant-feeding insects both directly (*e.g.*, offspring abundance, phenology, voltinism, winter survival), as well as indirectly by acting on the host plants (*e.g.*, water stress) (Hodkinson, 2005) and natural enemies (Berggren *et al.*, 2009). Modifications in environmental conditions (both biotic and abiotic) can lead to optimal colonization conditions for alien species (also called non-native, non-indigenous, foreign, exotic) in new environments.

Invasions by alien species are considered an important component of global environmental change, affecting – mainly negatively – economic value, biological diversity and function of invaded ecosystems (Wittenberg & Cock, 2001), and biological invasions of alien species are forecasted to increase with changing global conditions (Sala *et al.*, 2000).

Alien terrestrial invertebrates represent one of the most numerous groups of introduced organisms in Europe, and insects are the taxonomic category far more represented, being the 80% of the total number of invertebrate species established in Europe (Roques *et al.*, 2009) and being able to adapt to almost all ecosystems. Invasive forest insects are considered a major threat to forest health (Gandhi & Herms, 2010), in particular those species related to wood, like ambrosia and bark beetles (Coleoptera: Curculionidae: Scolytinae), can cause important economic damage to trees all around the world (Marini *et al.*, 2011).

Invasion of alien species has to pass through tree stages, defined as ecological barriers or filters: arrival, establishment, and spread (Liebhold & Tobin, 2008), often the first stage is human-induced and is expected to grow – in the case of wood-related insects – with increasing in global trade (*e.g.* Haack, 2006), especially along the pathways associated with the commerce of live plants, plant products, wood packaging materials in cargo containers, where it is hard to detect them (Work *et al.*, 2005, Brockerhoff *et al.*, 2006; Colunga-Garcia *et al.*, 2009). Furthermore, an inventory on the phytosanitary interceptions in wood and wood products during the period 1995-2004 revealed that wood boring beetles largely

dominated the forest community associated to this pathway (Roques & Auger-Rozenberg, 2006).

The establishment, and the consequent spread, of an alien species in a new environment depends on many abiotic and biotic factors (Holmes *et al.*, 2009; Roura-Pascual, 2011), including climatic features of the new environment, host species, habitat area, and interaction with native species. For bark and ambrosia beetles, climate and import values seems to be strong predictors for the establishment of new species in both Europe and USA (Marini *et al.*, 2011).

Early detection of alien species is extremely important in order to enhance the probability of interception and the chance of eradication. Early detection activities are usually developed by national plant health authorities under the framework set by international organizations (IPPC and EPPO), which develop standards aimed at intercepting and identifying incursions of alien pests (FAO, 2011). Coordination of data obtained from high-risk entry sites, such as ports and airports, is crucial to implement monitoring and prevention strategies; a recent study in Italian ports (Rassati *et al.*, 2013) shows that a monitoring protocol including the forest areas surrounding the potential entry site is a useful complement to port surveillance, giving information on the port surveillance itself and on the effective possibility of establishment of alien species.

Climate change is known to be an important factor able to influence insect distribution and impact in many ecosystem, especially mountain forests (Hodkinson, 2005), but it is also recognized to be – together with increasing globalization of economic activities – one of the main drivers accelerating biological invasions (Perrings *et al.*, 2005; Pautasso *et al.*, 2010). Climate change can affect invasion processes from the uptake of the alien organism in its native area to the establishment and spreading in a new environment, affecting both the adaptive capability of the invasive organism and the resistance capacity of the invaded environment (Pautasso *et al.*, 2010) The effect of climate change on forest insects communities affects both directly and indirectly plant-feeding insects (Klapwijk *et al.*, 2012). Direct effects involve the insect life cycle, affecting development time, overwintering survival voltinism and diapauses; development rate increases with temperature especially in mid to high latitudes (Hodkinson, 1999), and an increased

development rate can affect voltinism in facultative multivoltine species (Tobin *et al.*, 2008). Winter mortality is likely to decrease with increasing temperature (Ayres & Lombardero, 2000; Tran *et al.*, 2007; Jepsen *et al.*, 2008) while non-diapausing species can feed and develop in periods that were previously too cold (Bale *et al.*, 2002).

Indirect effects of climate change involve host plants and natural enemies of plant-feeding insects. Plants response to climatic variations involve both phenological development and defensive strategies, and insect response to such variation can vary according to biological traits of the herbivorous species, mainly those affecting phenology, and its ecological guild (Klapwijk *et al.*, 2012). In the case of bark beetles and other wood-related species, which normally reproduce on dead or deadly wood but in some case may cause epidemic tree mortality (Bentz *et al.*, 2010), warmer conditions are often associated to stress conditions for host plants and consequently lower attack densities are needed to overcome defense reactions in host plants and trigger high beetle reproduction rates (Waring & Pitman, 1985; Raffa *et al.*, 2008; Jactel *et al.*, 2012). Natural enemies of phytophagous insects (*i.e.* predators and parasitoids) are affected by climate change and usually respond in a prey density-dependent way to environmental changes (Hoekman, 2010). Of course climate changes influences herbivores and their enemies in a similar way, affecting overwintering survival, development rate and biological activity (Humble, 2006; Hance *et al.*, 2007), so the matching of response to climate variation between herbivores and their enemies is crucial in order to control possible outbreaks of phytophagous insects (Klapwijk *et al.*, 2012), considering that temperature sensitivity might increase with trophic level (Berggren *et al.*, 2009).

The aim of this three-years study is twofold. First, to experiment and improve new tools for monitoring invasive species of wood-related insects in order to propose new early detection strategies. Wood infesting insects are the most likely to be transported to new countries when trees are cut and processed into wood packaging material such as pallets, crating and dunnage (Haack & Brockerhoff, 2011), and considering the impact of bark beetles and wood borers in forest ecosystems it is crucial to understand the effect on environmental conditions on both native and invasive species; in the same way, it is important to study and to develop new strategies for monitoring and early detection of potentially threatening

species. Second, to investigate the effect of temperature – as the main component of climate change – on native populations of wood-related insects, in particular bark beetles (Coleoptera: Curculionidae: Scolytinae), Longhorn beetles (Coleoptera: Cerambycidae), weevils (Coleoptera: Curculionidae) and jewel beetles (Coleoptera: Buprestidae). The results can be taken as a model to predict the effects of climate change to native and alien species.

The activities necessary to get the aim have been included in the European projects BACCARA (Biodiversity And Climate Change A Risk Analysis) and Q-DETECT (Developing quarantine pest detection methods for use by national plant protection organizations (NPPO) and inspection services) and have been developed with the collaboration of the New Zealand Institute for Plant and Food Research supervised by Dr. D.M. Suckling and the precious collaboration of the SCION research institute (Christchurch, New Zealand) under the coordination of Dr. E.G. Brockerhoff.

Chapter 2

Wood-boring beetles invasive to Europe – Available surveillance methods and new technological tools

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I contributed to field tests, data analysis and paper writing

Abstract

International trade of timber and woody plants, and the large and increasing use of wood packaging materials, call for new and more efficient surveillance methods of wood-boring insects. We review here the information available for the most important invasive alien species of wood-boring beetles in Europe, namely jewel beetles (Coleoptera: Buprestidae), longhorn beetles (Coleoptera: Cerambycidae), weevils and bark beetles (Coleoptera: Curculionidae and Scolytinae). Methods used for early detection as well as for monitoring of established species are scrutinized and discussed in relation to their possible application at local, national, and international scale. The increasing availability of devices for the remote detection of insects in traps has solicited the development of a tool specific for wood-boring insects. This has been developed thanks to EU-funded research and has brought to a prototype which is presented here. It consists of a camera-trap taking pictures of the caught items and sending them to a safe repository accessible through the web. In a pilot application of the technology we were able to detect a number of alien and native species in Italian harbors. The method could be exploited at best in surveillance networks targeted at containing the risk of introduction of alien invasive species of wood-boring insects.

2.1 Introduction

Economic and environmental implications of alien invasive species are increasing worldwide year by year because of the large amount of commodities quickly exchanged all over the world (Baker *et al.*, 2005; Roques, 2010). Cultivation of alien plant species as ornamental trees or new crops, the expansion of the European Union borders, and climate change are additional factors triggering biological invasions (Augustin *et al.*, 2012). Because living inside fresh timber, often used as packaging material without specific countermeasures (e.g. those indicated in ISPM 15, 2009), wood boring beetles, are among the best colonizers of new environments through human-mediated dispersion (Kirkendall & Faccoli, 2010; Sauvard *et al.*, 2011). For the scope of this review, they include jewel beetles (Coleoptera: Buprestidae), longhorn beetles (Coleoptera: Cerambycidae), weevils and bark beetles (Coleoptera: Curculionidae and Scolytinae).

Early detection of pests entering new areas is a crucial task in order to prevent ecological and economical damage. International trade of timber and woody plants, and the large and increasing use of wood packaging materials (WPM) often concentrated and stored in specific key-points, such as ports and airports (Haack, 2001; Liebhold *et al.*, 2006), call for new and more efficient surveillance methods (Brockerhoff *et al.*, 2006; Bashford, 2008; Wylie *et al.*, 2008).

The aim of the present paper is to briefly summarize the knowledge concerning the occurrence and spread of the main wood boring beetles invasive to Europe. Then the different methodologies and tools available for detection of arrival of new species at the entrance points and for monitoring the dispersal of species already introduced are presented. Finally, a new tool for pest surveillance using web-based technology is also presented.

2.2 Main wood-boring beetles invasive to Europe

2.2.1 Jewel beetles

The number of jewel beetles alien to Europe is low, with only three species (Denux & Zagatti, 2010). While two of them, *Buprestis decora* and *Chrysobothris dorsata* respectively from North America and Africa, have minor economic importance (Denux & Zagatti, 2010; Augustin *et al.*, 2012), the emerald ash borer (*Agrilus planipennis*), a particularly threatening Asian species, was introduced close to Moscow (Baranchikov *et al.*, 2008; Straw *et al.*, 2013). This species attacks many ash species (*Fraxinus* spp.), although in the native area is reported also on elms (*Ulmus* spp.) and walnuts (*Juglans* spp.) (Haack *et al.*, 2002; Baranchikov *et al.*, 2008). The species, native from Japan, north-eastern China, Korean peninsula, Taiwan and Russian Far East (Jendek, 1994; Haack *et al.*, 2002), was introduced in north America in 2002 (Haack *et al.*, 2002) where is responsible of huge damage in both forests and urban areas (EPPO, 2013). Even if the majority of adults disperse only for a few hundreds of meters from the source (McCullough *et al.*, 2005; Mercader *et al.*, 2009), a single insect can potentially fly for several kilometers increasing the potential spreading (Taylor *et al.*, 2007; 2010; Siegert *et al.*, 2008; 2009). A standard protocol is available for the species control (EPPO, 2013).

2.2.2 Longhorn beetles

Similarly to jewel beetles, longhorn beetle species are generally associated to weakened trees or fresh timber leading to tree death and wood degradation (Evans *et al.*, 2004). Some species may, however, infest also manufactured wood or even healthy trees.

Recently, a couple of Asian cerambycids, the Asian longhorn beetle *Anoplophora glabripennis* and the citrus longhorn beetle *A. chinensis*, deserved special attention worldwide as potential invasive species in Europe and North America (Hu *et al.*, 2009; Haack *et al.*, 2010) where are listed as quarantine pests (USDA-APHIS, 1998; EU Council Directive 2000/29).

On one hand, *A. glabripennis* infests healthy trees growing in urban and suburban areas (Haugen, 2000; Nowak et al., 2001), with deep impact especially on maples (*Acer* spp.), elms (*Ulmus* spp.), poplars (*Populus* spp.), and willows (*Salix* spp.). On the other hand, *A. chinensis* is more threatening on various fruit and ornamental trees (Lingafelter & Hoebeke, 2002).

Native species of pine sawyer beetles of the genus *Monochamus* have been shown or are suspected to vector the alien pine wood nematode *Bursaphelenchus xylophilus*, one of the most dangerous threats to European forestry

2.2.3 Weevils

Weevils are an extremely heterogeneous group of phytophagous beetles (Hill, 1997; Sauvard *et al.*, 2010), many of them harmful to woody plants in orchards, plantations and forests (Augustin *et al.*, 2012).

The two Australian eucalyptus weevils, *Gonipterus gibberus* and *Gonipterus scutellatus*, are among the most important alien species associated to trees in Europe (Sauvard *et al.*, 2010). They had a large negative impact on eucalyptus plantations everywhere they were introduced worldwide (EPPO, 2005).

Another important tree-related species recorded in the last years in the European countries of the Mediterranean Basin is the red palm weevil *Rhynchophorus ferrugineus*, native of south-eastern Asia and affecting as a pest more than 20 species of palms (Kehat, 1999; Faleiro, 2006). The pest rapidly spread in middle-east, Africa and Europe (Murphy & Briscoe, 1999), killing palms by the larval feeding activity carried out in the host stem, with important economic damage (Faleiro, 2006).

2.2.4 Bark and Ambrosia beetles

Bark and Ambrosia beetles (Coleoptera Curculionidae Scolytinae) are here considered separately from weevils because of their special life history.

Invasive scolytids are a threat for the worldwide forests (Brockerhoff *et al.*, 2006; Marini *et al.*, 2011). All non-European scolytinae are included in quarantine lists, alert lists, and national or regional lists of harmful organisms (Augustin *et al.*, 2012). Some alien species can have an extremely negative economic damage at both local and national scale, associated with ecological impacts such as displacement of indigenous species, disrupting of food webs and modification of functional traits of ecosystems (Kenis *et al.*, 2009). At least 20 alien species of scolytids are considered established in Europe (Kirkendall & Faccoli, 2010; Faccoli *et al.*, 2012; Montecchio & Faccoli, 2013).

Besides physical damage due to insect boring activities, bark and ambrosia beetles are known to be often associated to pathogenic fungi (Kirisits, 2004). The introduction of invasive species in new environments can thus lead also to serious forest diseases. For instance, the recent introduction of *Pityophthorus juglandis* in Europe allowed the introduction also of *Geosmithia morbida*, the pathogen causing the thousand cankers disease on walnut (Montecchio & Faccoli, 2013).

2.3 Surveillance of alien invasive species of wood-boring beetles

The International Plant Protection Convention (IPPC) define surveillance as an official process which collects and records data on pest occurrence or absence by survey monitoring or other procedures (Augustin *et al.*, 2012). Detection, monitoring and control of invasive pest populations is a fundamental point in order to avoid the spreading of newly introduced alien species and to understand the ecological patterns of a species introduced to a new environment. While the first detection of alien species is often a fortuitous event, monitoring and control efforts invested after detection are usually calibrated proportionally to the potential impact of the alien species on the invaded ecosystem and to the spreading risk. Monitoring of invasive alien species recently introduced in new habitats is usually difficult to perform because of the low population density (Augustin *et al.*, 2012). The most recent field techniques which can be used for both early detection and monitoring are briefly illustrated according to the main considered groups of wood-boring beetles.

2.3.1 Jewel beetles

Because symptoms of larval activity are not visible in the first year of infestation, detection and monitoring of invasive buprestids are usually based on adult trapping (Augustin *et al.*, 2012). Most of the information available has been developed recently in North America, after the invasion of the emerald ash borer. It is possible that the suggested methods are considered effective also for other jewel beetle species but this requires further testing.

Trapping adults of *A. planipennis* is possible by sex and aggregation pheromones (Bartelt *et al.*, 2007; Leito *et al.*, 2009; Silk *et al.*, 2009), but their effect on early stages of infestation is considered limited (Augustin *et al.*, 2012). Early infestations are thus monitored by purple prism-shaped sticky traps (Crook *et al.*, 2008, Francese *et al.*, 2008) baited with a blend composed by 80% of Manuka oil and 20% of phoebe oil (distilled respectively from the New Zealand Manuka tea tree, *Leptospermum scoparium*, and Brazilian walnut, *Phoebe porosa*), added with volatiles of green ash (Crook *et al.*, 2008; Marshall *et al.*, 2010). Trap location should be along woodland edges, open areas and parks, but the method is effective also for sites exposed to a risk of infestation because of wood transport (especially firewood). Traps should be placed as high as possible (at least 6 meters from the ground), in the canopy of dominant trees (Francese *et al.*, 2006).

Other methods for monitoring the emerald ash borer include detection of stressed trees, in which the presence of larvae and emerging holes is checked (McCullough & Siegert, 2006), and the use of sticky bands set up on trunks and logs of the host trees to trap landing adults (Lyons *et al.*, 2009). All these methods are, however, far less effective than traps (Augustin *et al.*, 2012).

2.3.2 Longhorn beetles

Similarly to buprestids, monitoring of longhorn beetles is based mainly on adult trapping because of the impossibility of visual assessment of larval activity, especially during the first larval development stages. Adults can be attracted using kairomones, including plant volatiles and bark beetle pheromones, and both long- and short-range sex pheromones.

Repellents and oviposition stimulants and plant defensive substances can be used as well (Allison *et al.*, 2004); Specific sex and aggregation pheromones produced by males are known only for five species (Ray *et al.*, 2006).

Multi-funnel and cross-vane traps are the most effective traps to catch longhorn adults. Trap color is important in trapping effectiveness, with a higher catching rate with black traps (Rassati *et al.*, 2012).

During last years many efforts were spent in order to identify volatiles attractive to *Anoplophora* species with the aim to facilitate their early detection and monitoring (Haack *et al.*, 2010). Although many studies show the potential attractiveness of volatiles released both by females (Li *et al.*, 1999) and males (Zhang *et al.*, 2002), and combinations with other chemical blends for *A. glabripennis* (Nehme *et al.*, 2009), the use of generic kairomones is an available option (Allen & Humble, 2002); recently, a contact sex pheromone was discovered for *A. chinensis* as well (Mori, 2007; Yasui *et al.*, 2007). Studies on dispersion of *A. glabripennis* were conducted using capture-mark-releasing techniques, showing that in average this species can actively disperse 20m per day and that colonization of nearby plants is related to beetle density, weather conditions, beetle size and tree size (Bancroft & Smith, 2005).

Differently from *Anoplophora* spp., a blend of generic host volatiles (including alpha-pinene, ethanol and 3-carene) is very attractive for *Monochamus* spp. (Fan *et al.*, 2007; Costello *et al.*, 2008). The addition of bark beetles pheromones, such as ipsenol, seems to increase trap efficiency (Allison *et al.*, 2001), with some exception probably due to low population density (Miller, 2006; Fan *et al.*, 2010). The combination of generic host volatiles with the male-produced specific aggregation pheromone – allowing high captures of both males and females (Pajares *et al.*, 2010) – is so far the most efficient combination known to catch adults of *Monochamus galloprovincialis* (Rassati *et al.*, 2012).

2.3.3 Weevils

Weevils normally show good responses to both general baits, such as host volatiles, and specific long- and short-range pheromones (Augustin *et al.*, 2012). Differences between

species in physiology and behavior make, however, impossible to find a generic blend effective for the whole group.

A male aggregation pheromone was identified for *R. palmarum* (Rochat *et al.*, 1991), although higher trapping performances are obtained adding to the pheromone blend either plant material (palm fronds and sugarcane) (Oehlschlager *et al.*, 1993) or a mixture of 5-10% acetoin on ethyl acetate (Said *et al.*, 2005).

R. ferrugineus can be trapped as well with a mixture of food bait (plant material) and pheromones (Faleiro, 2006). A key aspect for detection is to check damaged plants presenting attack symptoms; new technologies based on acoustic sensor and image processing softwares are developed in order to detect larval activity inside palm trunks (Potamitis *et al.*, 2009; Hussein *et al.*, 2010; Al-Saquer & Hassan, 2011).

2.3.4 Bark and Ambrosia beetles

Differently from other groups, surveillance of bark and ambrosia beetle populations is commonly performed by checking infested wood material or by capturing flying adults with baited traps. Monitoring of infested wood is mainly based on the identification of the species-specific gallery patterns occurring in tree phloem, in case of bark beetles, or in sapwood in case of ambrosia beetles. Species identification by morphological analysis of larvae is instead extremely difficult (Augustin *et al.*, 2012). The easiest way to survey bark and ambrosia beetle populations is using black multi-funnel, cross-vane or panel traps. Trap efficiency can vary according to the different target species and the environment in which the monitoring is carried out, suggesting to use species-specific trap designs (Nageleisen & Bouget, 2009). Scolytids can be attracted using specific aggregation pheromones or more generic attractant lures, such as host volatiles and alcohol (Byers, 2004). An example is provided by Rassati *et al.* (2013) who successfully used generic traps to detect wood-boring insects, and especially bark beetles, at ports of entry in Italy.

2.4 Pest surveillance using web-based technology

The demand of automatic devices for remote monitoring of ecological variables is increasing because the quick improvement of technical facilities and the need of saving time, reducing or avoiding direct field observations, solving logistic problems, and improving data consistency (Richardson *et al.*, 2007; Garrity *et al.*, 2010; Ryu *et al.*, 2010). In the past, remote cameras were used mainly in plant studies (Ahrends *et al.*, 2009; Richardson *et al.*, 2009; Graham *et al.*, 2010; Ide & Oguma, 2010; Migliavacca *et al.*, 2011; Sonnentag *et al.*, 2011). Nonetheless, few recent studies focused on the possible use of cameras for detection and monitoring of insect occurrence and activity, with various prototypes under development. For instance, the system proposed by Jian *et al.* (2008) and tested on the oriental fruit fly, *B. dorsalis*, consists of a high precision automated trapping and counting device. The device counts the number of trapped flies and then sends the information to a remote monitoring platform in the form of a short cell phone message through a wireless Global System of Mobile Communication (GSM). Because the trapped flies are counted as they cross an infrared interruption sensor of a double-counting optical mechanism (Lin *et al.*, 2006), the monitoring system gives only the number of trapped insects assuming that all these are really and only oriental fruit flies. This tool is, hence, suitable only for monitoring species provided of specific attractants and for which is not required the identification but only the assessment of the population density, *i.e.* number of insects. In case of traps catching many species belonging to different families, as recorded in the present study, the need to identify the trapped species is the main purpose. In this respect, Lopez *et al.* (2012) proposed a monitoring system able to capture and send images of the trap content to a remote control station. Nevertheless, to increase the battery life-span, the device was based on a low-cost sensor producing low-resolution images allowing the prompt identification only of very large species, such as the Red Palm Weevil (*Rhynchophorus ferrugineus*), on which the device was tested. A large improvement of the image quality was obtained with the automatic electronic traps designed by Guarnieri *et al.* (2011) to monitor the codling moth, *C. pomonella*. The traps were developed to survey the flight of moth males attracted to sexual pheromone, for a better definition of both pest density and spraying time.

Wood-boring insects are among the best candidates for such a technology because of their ability to move inside timber and untreated wood packaging material carried worldwide through international trade (Kirkendall & Faccoli, 2010; Sauvard *et al.*, 2011). Traps set up for the detection of invasive alien species must be frequently inspected and specific actions can be immediately taken in case of interception of a quarantine species. Hence, any technology which concurs to optimize a surveillance program, reducing checking time and improving the precision of the method, warrants specific investigations.

A prototype camera-trap specifically designed for the remote detection of wood-boring beetles has been developed in two EU-funded projects, PRATIQUE and Q-DETECT. It is based on a specifically modified security camera (BioCam, Mi5 Security, Auckland, New Zealand) (Fig. 1), composed by a wide-angle lens, 1 MegaPixels sensor, rechargeable battery pack and internal modem for General Packet Radio Service (GPRS) connection was used. The interval between images taken by the camera can be programmed and saved in a Secure Digital (SD) memory card. The images can be stored in the same SD card and simultaneously sent to a safe repository accessible through the web, from which they are downloadable. On the same repository it is possible to check the level of battery charge of each camera and the GPRS coverage as well.

The camera was coupled with a multi-funnel trap composed by 12 black overlapped funnels connected with a drained collector (Econex Multi-Embudos[®], Spain). The trap was baited with a blend of the generic lures (-) α -pinene and ethanol (Serbios[®], Italy), known to be attractive to many wood boring beetle species (Moeck, 1981; Joseph *et al.*, 2000; Kelsey & Joseph, 2000; Brockerhoff *et al.*, 2006; Rassati *et al.*, 2012), and a commercial kit specific for *Monochamus galloprovincialis* (Galloprotect 2D[®] - SEDQ[®]). The trap collector was modified adding a transparent plastic pipe ending with a tiny net bottom to drain water (Fig. 2). The new collector length was then optimized in order to reach the ideal focus distance between the camera and the collector bottom (about 20 cm).

After some preliminary tests carried out in laboratory (November 2011 to January 2012) taking pictures from sample insects put inside the trap collector, a camera-trap was set up in two experimental field tests. The first test was conducted from March 26th to May 11th 2012 (39 days in total) placing the trap along the edge of a bush in the campus of the University

of Padova (Agripolis, Legnaro, Italy). The second field test was carried out in the Monfalcone port (NE Italy) from July 10th to August 10th 2012 (30 days). In both the experiments the camera was set up to take 4 images per day every 6 hours (at 6 am, 12 am, 6 pm and 12 pm) in order to test different light conditions. Trapped insects were first identified from the pictures sent by the camera to the repository accessible through the web. Every second week, the trap was checked on-site and the trapped insects taken to the laboratory for identification at species level by morphological traits. Insect identifications performed from pictures were then compared with those done on collected specimens to give the level of accuracy of the off-site detection.

A total of 50 beetles belonging to 4 species of longhorn and 6 species of bark beetles were analyzed during both lab and field tests (Tab. 1, Fig. 2). As expected, the image analysis was less reliable than the direct morphological identification, which was possible for all trapped insects. While all trapped beetles were correctly identified at family level in the image analysis (Tab. 1), the correct identification at genus level was possible only for longhorn beetles and one genus of bark beetles (*Ips*) (Tab. 1). The image quality was not high enough to allow a correct species identification for any of the specimens collected. The use of 4 pictures per day gave at least one high quality image (normally at 6 am and/or 6 pm), avoiding problems associated with darkness or overly direct light. In both field tests the camera-trap had a battery life of about 22 days, with four shots a day.

The device is a promising tool for the remote check of traps deployed for the detection of alien wood-associated beetles. Many camera-traps can be used simultaneously and independently in as many sites as needed, avoiding complex and expensive logistic organization. On-site checking of the trap is needed whenever the change of battery pack or lure is necessary, or when the collection of the insects is required.

The effectiveness of the off-site detection would largely increase in case of single target species (or group of similar species) responding to a specific aggregation lure, as in the case of *Monochamus galloprovincialis*, the vector of the pine wood nematode *Bursaphelenchus xylophilus* (Pajares *et al.*, 2004; 2010). Here, the morphological identification of the trapped individuals is usually not required, because the use of specific lure usually excludes the capture of non-target species. Moreover, large target species (such as many longhorn beetles) can be identified directly from pictures, at least at genus level. In case of small and

hardly recognizable target species (such as bark beetles), the device allows the user to focus the on-site checking only on those traps showing the presence of specimens with morphological features similar to those of the target species, reducing the general costs of the surveillance. In this respect, if target species are particularly rare, such as alien species included in the lists of the quarantine organisms, then lower costs should result from avoiding the checking of empty traps (Augustin *et al.*, 2012). Although only one alien species was caught during the trial for the testing of the device (the ambrosia beetle *Xyleborus atratus*), some of the species were clearly coming with commodities or wood packing material. This is the case of the spruce bark beetle *Ips typographus* at University campus and the European fir engraver beetle *Pityokteines vorontzovi* found in Monfalcone port, which can be considered as not occurring in the study areas, and likely move with the trade of coniferous wood.

Beside to be suitable for both large and small species, provided or not with specific attractants, the device is immediately ready for application without the need for other components to be connected (except for the SIM card for GPRS connection), and requires only a very low maintenance effort, limited to the periodical recharge of the batteries and the removal of insects from the trap collector. In addition, the picture repository accessible through the web allows a safe, shared, quick and simultaneous access of the users to the stored pictures. User-friendly software's for web setup and managing require a few skills, with no need for long and complex training activities. Although the camera trap presented here is already a promising tool for off-site detection of wood boring insects, further development of the technology is desirable. This may consist of a better integration of the camera in the trap, *i.e.*, miniaturizing the camera components, and an increasing of image quality and battery life.

The large-scale availability of web-based surveillance, combined with the development of efficient trapping devices and other technologies (acoustic detection, electronic noses, radiography, dogs, on-site DNA probing) may pave a new way for a successful detection and monitoring of invasive alien species of wood-boring beetles as well as of other insects. They can be deployed at local, national, and international scale and must be incorporated in collaborative networks in order to be exploited at best. Due to the high unpredictability of

the nature of invasive alien species, the development of generic methods should be given priority, in order to maximize the detection efficiency by keeping costs as low as possible. The adoption of an efficient surveillance network together with a strict application of the existing international measures (ISPM 2009) and with eradication plans may hopefully contribute to reduce the threat of invasive wood-boring insects.

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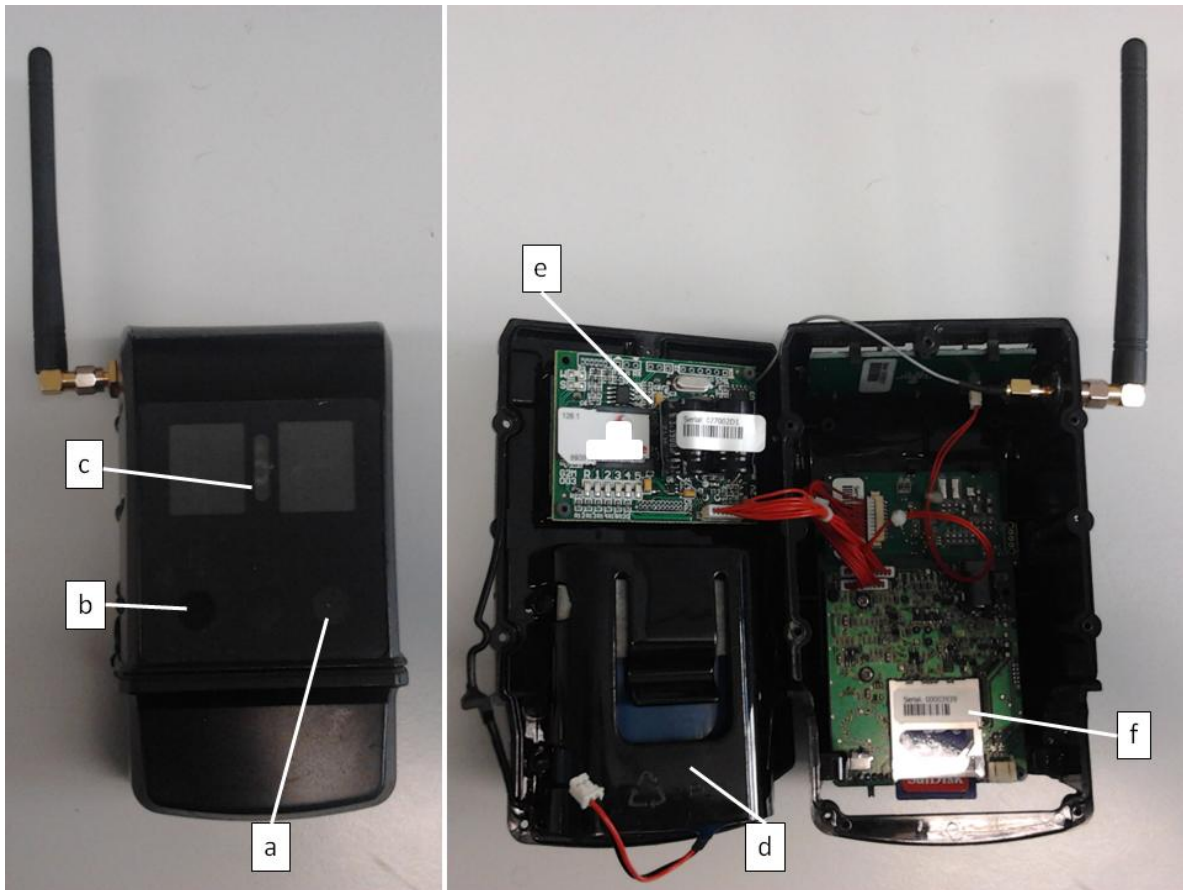


Figure 1: Structure of the automatic camera: front (left) and inside (right). Main components: a. 3.7 mm lens. b: remote controller sensor (to arm/disarm the camera). c: motion detector (not used in this study) and status light signs. d: Rechargeable battery pack. e: modem for GPRS connection and SIM card. f: SD memory card (contains settings and pictures).



Figure 2: Sample pictures taken by the camera in laboratory conditions showing longhorn beetles of the genus *Arhopalus* (5 individuals) and *Spondylis* (4 individuals) (left). Picture taken in the field (Monfalcone) with catch of several small bark beetles, four longhorn beetles, and one large non-target beetle (right). Insects have been later identified as in table 1.

<i>Location</i>	<i>Q.ty</i>	<i>Image identification</i>		<i>Direct identification</i>		
		<i>Family</i>	<i>Genus</i>	<i>Family</i>	<i>Genus</i>	<i>Species</i>
Laboratory	7	Cerambycidae	<i>Arhopalus</i>	Cerambycidae	<i>Arhopalus</i>	<i>A. ferus</i>
	2	Cerambycidae	<i>Monochamus</i>	Cerambycidae	<i>Monochamus</i>	<i>M. galloprovincialis</i>
	4	Cerambycidae	<i>Spondylis</i>	Cerambycidae	<i>Spondylis</i>	<i>S. buprestoides</i>
	9	Scolytinae	<i>Ips</i>	Scolytinae	<i>Ips</i>	<i>I. typographus</i>
University campus	2	Scolytinae	Unidentified	Scolytinae	<i>Hylurgus</i>	<i>H. ligniperda</i>
	2	Scolytinae	<i>Ips</i>	Scolytinae	<i>Ips</i>	<i>I. typographus</i>
	3	Scolytinae	Unidentified	Scolytinae	<i>Orthotomicus</i>	<i>O. erosus</i>
	2	Scolytinae	Unidentified	Scolytinae	<i>Xyleborus</i>	<i>X. atratus</i>
	6	Scolytinae	Unidentified	Scolytinae	<i>Xyleborinus</i>	<i>X. saxesenii</i>
Monfalcone port	1	Cerambycidae	<i>Chlorophorus</i>	Cerambycidae	<i>Chlorophorus</i>	<i>C. varius</i>
	5	Cerambycidae	<i>Spondylis</i>	Cerambycidae	<i>Spondylis</i>	<i>S. buprestoides</i>
	4	Scolytinae	Unidentified	Scolytinae	<i>Hylurgus</i>	<i>H. ligniperda</i>
	3	Scolytinae	<i>Ips</i>	Scolytinae	<i>Ips</i>	<i>I. typographus</i>
	1	Scolytinae	Unidentified	Scolytinae	<i>Pityokteines</i>	<i>P. vorontzovi</i>

Table 1: List of scolytid and cerambycid species identified by visual analysis of the pictures and corresponding identification by direct observation.

Chapter 3

Distribution of Norway spruce bark and wood boring beetles along Alpine elevational gradients

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I contributed to data collection, data analysis and paper writing



Distribution of Norway spruce bark and wood-boring beetles along Alpine elevational gradients

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- Abstract**
- 1 Temperature is probably the most important driver of insect response to climate change and has many implications at both individual and population levels. The present study explored how elevation, as a proxy for temperature, affects the abundance and diversity of bark and wood-boring beetles associated with Norway spruce (*Picea abies*) along its southern range.
 - 2 We selected three elevational gradients (approximately 900–1500 m) in spruce stands in the south-eastern Alps, each consisting of four locations. From April to September 2011, four traps of different types were installed at each location: three baited with generic lures (α -pinene and ethanol) and one baited with a pheromone specific for *Ips typographus*. In addition, three fresh spruce logs were exposed on the same locations.
 - 3 Species richness did not vary significantly with elevation, whereas the abundance of most individual species did. Generally, aggressive species responded positively to higher temperature, whereas most non-aggressive species responded negatively.
 - 4 In a warming scenario, it is likely that spruce forests will face increasing damage from aggressive species. This will threaten the growth and survival of Norway spruce at low elevation, especially at southern latitudes.

Keywords Alps, climate change, Coleoptera, *Ips typographus*, pests, *Picea*, temperature, trap.

Introduction

Climate change is considered as an important factor with respect to modifying natural ecosystems, directly or indirectly affecting all trophic levels (Bale *et al.*, 2002). Temperature is probably the most important driver of insect response and has a number of implications at both individual and population levels (Klapwijk *et al.*, 2012). Global warming may significantly modify herbivores both directly (e.g. offspring abundance, phenology, voltinism, winter survival), as well as indirectly by acting on the host plants (e.g. water stress) (Hodkinson, 2005) and natural enemies (Berggren *et al.*, 2009).

Forests are of crucial importance when assessing the consequences of global warming because they provide multiple ecosystem services, such as timber production, soil protection and landscape conservation (Alfaro *et al.*, 2010). Climate can also affect the abundance, composition and phenology of insect communities, as shown for stable flies (Gilles *et al.*, 2008), with

a more evident effect on generalist species than on specialist ones (Scheidel *et al.*, 2003). In some cases, environmental and climatic changes can lead to dramatic ecosystem and economic damage, as recorded in western North America with outbreaks of the mountain pine beetle *Dendroctonus ponderosae* (Kurz *et al.*, 2008) and in Europe with the expansion of the outbreak range of the pine processionary moth *Thaumetopoea pityocampa* (Battisti *et al.*, 2005) and the spruce bark beetle *Ips typographus* (Marini *et al.*, 2012).

Geographical gradients are often used as a proxy for simulating climate change effects (Hodkinson, 2005). Latitudinal and elevational gradients have been commonly used for testing the effects of temperature at different spatial scales (Andrew & Hughes, 2005; Adams & Zhang, 2009; Röder *et al.*, 2010). Because latitudinal gradients are often distributed over very large areas where many environmental factors may change along the gradient (e.g. day length, mean precipitation, inclination of solar radiation, forest composition and tree phenology), steep elevational gradients are generally recommended to separate the effect of temperature from other factors as efficiently as possible (Hodkinson, 2005).

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Norway spruce *Picea abies* (Karsten) is one of the most important forest tree species in Europe (Schmidt-Vogt, 1977), in terms of both timber production and the large area covered. Insect communities related to Norway spruce are expected to respond to global warming as this species grows in temperate-cold and boreal regions. Responses to the increase of temperature along elevational gradients can be investigated with respect to both diversity and the abundance of organisms. Diversity of species has been addressed in two studies conducted in central Europe for wood-boring beetles and their enemies (Tykarski, 2006; Röder *et al.*, 2010). In both studies, species richness decreased slightly with increasing elevation, although there was no consistency among major guilds. Insect abundance and impact have been shown to generally decrease with elevation (Garibaldi *et al.*, 2011). The effect of temperature on spruce pests has been studied especially for *I. typographus*, which is considered to be the most dangerous spruce pest in Europe (Schelhaas *et al.*, 2003). An increase in temperature can lead to a higher occurrence of a second or even third generation (Jönsson *et al.*, 2007, 2011; Faccoli, 2009) and it has been generally associated with greater forest damage (Marini *et al.*, 2012). *Hylobius abietis*, another important pest on spruce seedlings, has been shown to respond positively to temperature, with the prediction of a larger impact with global warming (Inward *et al.*, 2012).

Because an understanding of the dynamics of the individual species and communities in response to climate change is crucial for enabling the forecast of future hazards and optimizing forest management, the present study aimed to assess the possible effects of temperature on species richness (i.e. number of species) and the abundance of bark and wood-boring beetles associated with Norway spruce. Species were classified as aggressive and non-aggressive in accordance with the ranking proposed by Grégoire and Evans (2004), based on the frequency of damage detection in European countries. We selected steep elevation gradients in the Southern Alps where Norway spruce is at the southern edge of its distribution range. A recent study carried out on damage by *I. typographus* in the same area has shown that spring drought events elicit outbreaks (Faccoli, 2009) and that these occur mainly in low elevation stands because of more frequent drought events (Marini *et al.*, 2012). Based on the potential higher frequency

of aggressive species of bark and wood-boring beetles at low elevations, we expect that species such as *I. typographus* and the weevil *H. abietis* are positively affected by warmer temperatures.

Materials and methods

Study areas

Three spruce forests were selected in the south-eastern Alps (Veneto and Friuli Venezia Giulia regions, north-east Italy): Mauria, Pramasio and Tualis (Table 1). The investigated stands, chosen to be as similar as possible in age, composition and slope facing, were characterized by a dominance of Norway spruce, with other scattered woody species (mainly *Fagus sylvatica* L. and *Larix decidua* Miller) occurring at low density. To avoid confounding effects associated with local outbreaks, we selected sites under non-outbreak conditions (i.e. lack of tree mortality close to the experimental sites). An elevation gradient of a minimum of 600 m was selected at each site to obtain a substantial temperature gradient according to the generally accepted lapse rate of 0.64 °C every 100 m. All gradients were located in mature stands (>90 years old) with a density of 300–450 trees per ha and served by a logging road. Along each gradient, four locations separated from each other by approximately 200 m of elevation were selected (Table 1). At each location, air temperature was recorded hourly at 1 m above soil level using a data-logger (HOBO® U23 Pro v2-, resolution 0.02 °C with a RS1 solar radiation shield; Onset Corp., Bourne, Massachusetts). Hourly temperature was used to calculate the daily mean temperature from 20 April to 1 September 2011 (Table 1).

Target species and trap types

Our goal was to sample all species of bark and wood-boring insects within the Norway spruce sample stands. Insect trapping was performed using four types of traps (i.e. cross-vane, pitfall, trunk, and multi-funnel) to obtain the maximum information about the community of insects associated with bark and wood. The cross-vane traps (Polytrap™ PET, Model

Table 1 Description of the geographical and climatic characteristics of the investigated gradients

Gradient	Location	Coordinates		Elevation (m a.s.l.)	Average daily temperature (°C) (20 April to 1 September)
Mauria	M1	46°28'30"N	12°27'49"E	922	14.51
	M2	46°27'40"N	12°29'41"E	1122	13.06
	M3	46°27'14"N	12°30'47"E	1308	12.14
	M4	46°27'10"N	12°30'12"E	1550	11.81
Pramasio	P1	46°34'05"N	13°00'49"E	868	14.70
	P2	46°34'23"N	13°01'26"E	1181	13.20
	P3	46°34'37"N	13°01'49"E	1344	12.14
	P4	46°35'03"N	13°01'29"E	1470	11.46
Tualis	T1	46°32'23"N	12°52'34"E	998	14.92
	T2	46°32'30"N	12°53'03"E	1286	13.15
	T3	46°32'55"N	12°53'33"E	1432	12.08
	T4	46°33'14"N	12°53'16"E	1769	10.31

2010; Ecole d'Ingénieurs de Purpan, France) were composed of two transparent perpendicularly crossed panels, a funnel and a drained collector with insecticide added (Sed-Q, Spain). The trap was baited with the generic lures (–) α -pinene and ethanol (Serbios, Italy) to catch bark and wood-boring beetles attracted by stressed plants (Gandhi *et al.*, 2009). Bait and insecticide were changed every 6 weeks. Pitfall traps (Econex Eostrap, Spain) were a variant of the pine weevil trap (Nordlander, 1987) with four holes at ground level to intercept walking insects, lured by a dispenser of ethanol (Serbios, Italy), as already tested by Moeck (1981). Dry insecticide (Sed-Q, Spain) was added to the collector; bait and insecticide were changed every 6 weeks. Trunk traps (Stammfallen; http://www.pulapkibezobslugowe.com/index/html/index_DE.html) consisted of a collar placed around a tree stem at approximately 1.5 m from the ground to intercept insects climbing the stem, such as weevils. A collector with dry insecticide (Sed-Q, Spain), changed every 6 weeks, was located at the top of the collar. No bait was used with this type of trap. The multi-funnel traps (Econex Multi-Embudos, Spain) were composed of 12 black overlapped funnels and a drained collector with a dry insecticide added (Sed-Q, Spain), and baited with a commercial pheromone specific for *I. typographus* (Superwood; Serbios, Italy). This bait generally also attracts other species of wood-boring beetles (Valkama *et al.*, 1997). Bait and insecticide were changed every 6 weeks. The whole trap set has been studied to collect the highest number of species, considering that different species can be attracted by different lures and different traps; data obtained by all the trap set have been considered as an unique value of abundance for the location.

On 15 April 2011, one trap of each type was set up in each location of the three selected gradients. Each trap was checked and emptied every 2 weeks until end of September 2011. Trapped insects were stored in alcohol and then identified to species level.

Logs

Three fresh spruce logs of (diameter 20 cm, length 50 cm) were exposed in each location to study the effective wood and bark colonization by the species caught by traps, and to include those species that do not respond to traps. Logs were cut in March from a tree growing at the bottom of each transect. Each log was baited with one-third of the commercial dispenser of *I. typographus* pheromone (Superwood) to help initial colonization but, at the same time, avoid overcrowding as a result of the attractiveness of a full pheromone dispenser (Schlyter *et al.*, 1987). Logs were set out in each location on April 2011, laying them close to each other on a mulch mat. One of the three logs per location was randomly selected and removed on April 2012 to measure the overall performance of both univoltine and multivoltine species. The logs were stored in pipe-cages for drying, and emerging insects were collected until July 2012. Each log was then debarked to identify and count maternal (for bark beetles) or larval (for wood-boring insects) galleries. Attack density was reported as number of galleries per m² of bark.

Statistical analysis

To test the effect of hourly mean temperature calculated across the whole period on both species richness (considered as number of species) and insect abundance in traps, we used a generalized linear mixed model (GLMM), with temperature of the location as fixed factor and gradient as random factor. We used a Poisson distribution with a log-link function for count data of species richness and individual abundance in traps. The total number of species and the abundance of each species were pooled for the whole trapping season (April to September) and for all types of traps used in the experiment. The effect of temperature on attack density of each species colonizing logs was tested using general linear mixed models because the response variable was not a count. Significance of effects was based on $\alpha = 0.05$. GLMMs were performed using the package lmer in R (R Development Core Team, 2012).

Results

Traps

A total of 51 672 insects were trapped, distributed in 15 species and two families (Curculionidae and Cerambycidae) of bark and wood-boring beetles (a complete list of captured insects per trapping device is provided in Table 2). Two bark beetles (*I. typographus* and *Cryphalus abietis*) and one ambrosia beetle (*Xyloterus lineatus*) were the most common trapped species, with 46 628, 363 and 4030 trapped adults, respectively. Four other bark beetle species (*Hylurgops palliatus*, *Hylastes cunicularius*, *Dryocoetes autographus* and *Xylechinus pilosus*) were trapped in lower numbers (284, 182, 69 and 47, respectively), whereas two other bark beetle species were only seldom recorded (*Pityogenes chalcographus* and *Phthorophloeus spinulosus*). *Hylobius abietis* was the only weevil observed (37 individuals) and, together with *I. typographus*, *H. cunicularius* and *X. lineatus*, is part of the collected species considered aggressive (Grégoire & Evans, 2004). Longhorn beetles included five species (*Rhagium bifasciatum*, *Pogonocherus fasciculatus*, *Rhagium sycophanta*, *Oxymirus cursor* and *Ropalopus hungaricus*), with only few individuals caught.

The number of bark and wood-boring insect species marginally increased as mean hourly temperature increased (slope = 0.14, $t = 3.597$, $P = 0.078$). All species of bark beetles responded to temperature, either positively or negatively (Figs 1 and 2 and Table 3). A strong positive trend was shown for *I. typographus* and a less strong one for both *H. palliatus* and *H. cunicularius*; the weevil *H. abietis* also responded positively to increased temperature. Negative responses to temperature of similar magnitude were shown for *C. abietis*, *D. autographus* and *X. pilosus*. The only ambrosia beetle found, *X. lineatus*, also showed a negative response to increased temperature.

Logs

The galleries found in the exposed spruce logs belonged to six beetle species (Table 4): three bark beetles (*D. autographus*, *I. typographus* and *H. palliatus*), with a mean number of

Table 2 List of insect species caught in traps according to family/subfamily, decreasing abundance and trap type

Family	Subfamily	Species	Abundance				
			Total	CV	MF	PF	TT
Cerambycidae	Cerambycinae	<i>Ropalopus hungaricus</i>	1	1	0	0	0
Cerambycidae	Lamiinae	<i>Pogonocherus fasciculatus</i>	6	0	4	0	2
Cerambycidae	Lepturinae	<i>Rhagium bifasciatum</i>	11	3	6	2	2
Cerambycidae	Lepturinae	<i>Rhagium sycophanta</i>	3	3	0	0	0
Cerambycidae	Lepturinae	<i>Oxymirus cursor</i>	3	3	0	0	0
Curculionidae	Molytinae	<i>Hylobius abietis</i> *	37	1	0	17	19
Curculionidae	Scolytinae	<i>Ips typographus</i> *	46 628	120	46 508	0	0
Curculionidae	Scolytinae	<i>Xyloterus lineatus</i> *	4030	3994	36	0	0
Curculionidae	Scolytinae	<i>Cryphalus abietis</i>	363	355	8	0	0
Curculionidae	Scolytinae	<i>Hylurgops palliatus</i>	284	276	8	0	0
Curculionidae	Scolytinae	<i>Hylastes cunicularius</i> *	182	180	2	0	0
Curculionidae	Scolytinae	<i>Dryocoetes autographus</i>	69	63	6	0	0
Curculionidae	Scolytinae	<i>Xylechinus pilosus</i>	47	46	1	0	0
Curculionidae	Scolytinae	<i>Pityogenes chalcographus</i>	7	7	0	0	0
Curculionidae	Scolytinae	<i>Phthorophloeus spinulosus</i>	1	0	1	0	0

Species marked with an asterisk are considered aggressive (Grégoire & Evans, 2004).

CV, cross-vane; MF, multifunnel; PF, pitfall; TT, trunk trap.

Table 3 Results of the generalized linear mixed model applied considering the pooled number of individuals caught in different traps and the density of galleries found in the logs for each species of wood insects with respect to the temperature gradient

Family	Subfamily	Species	Traps			Logs		
			Slope	SE	P	Slope	SE	P
Cerambycidae	Lepturinae	<i>Rhagium bifasciatum</i>	NS			0.410	0.158	< 0.05
Curculionidae	Molytinae	<i>Pissodes hercyniae</i>	–			NS		
Curculionidae	Molytinae	<i>Hylobius abietis</i>	1.399	0.462	< 0.01	–		
Curculionidae	Scolytinae	<i>Cryphalus abietis</i>	–0.413	0.049	< 0.01	–		
Curculionidae	Scolytinae	<i>Dryocoetes autographus</i>	–0.489	0.146	< 0.01	–0.438	0.150	< 0.05
Curculionidae	Scolytinae	<i>Hylastes cunicularius</i>	0.305	0.059	< 0.01	–		
Curculionidae	Scolytinae	<i>Hylurgops palliatus</i>	0.342	0.045	< 0.01	NS		
Curculionidae	Scolytinae	<i>Ips typographus</i>	0.980	0.005	< 0.01	0.570	0.135	< 0.01
Curculionidae	Scolytinae	<i>Xylechinus pilosus</i>	–0.350	0.161	0.029	–		
Curculionidae	Scolytinae	<i>Xyloterus lineatus</i>	–0.334	0.013	< 0.01	NS		

NS, slope not significantly different from 0; –, data not available.

galleries per m² of 33, 26.9 and 15.2, respectively), one ambrosia beetle (*X. lineatus*; 0.83 galleries/m²), one longhorn beetle (*Rhagium bifasciatum*; 9.8 galleries/m²) and one weevil species (*Pissodes hercyniae*; 2.3 galleries/m²) (Table 4). All of these species, with the exception of *P. hercyniae*, were also found in traps. Log infestation density increased with increased temperature for *I. typographus*, whereas it decreased for *D. autographus* (slope = 0.57, $t = 4.230$, $P < 0.01$ and slope = –0.44, $t = -2.926$, $P < 0.05$, respectively), showing the same trend observed in traps (Fig. 1). The longhorn beetle *R. bifasciatum* responded positively to increased temperature (slope = 0.41, $t = 1.305$, $P < 0.05$). *X. lineatus*, *H. palliatus* and *P. hercyniae* did not show temperature dependent trends of log colonization (slope = 0.01, $P = 0.731$; slope = 0.03, $P = 0.564$; and slope = –0.114, $P = 0.38$, respectively).

Discussion

Temperature affected the abundance of bark and wood-boring beetle species associated with Norway spruce at the southern

edge of its range in the south-eastern Alps, whereas the number of species was not significantly affected. In particular, higher temperatures recorded at low elevation positively affected the abundance of some of the most aggressive species of wood beetles in Europe (*I. typographus*, *H. cunicularius* and *H. abietis*) (Grégoire & Evans, 2004), whereas only one of the aggressive species (*X. lineatus*) showed a decreasing trend with increased temperature. Conversely, non-aggressive species showed a general decreasing trend in three cases (*D. autographus*, *C. abietis* and *X. pilosus*), whereas only *H. palliatus* responded positively to increased temperature. Because temperature is the most biologically relevant factor changing along steep elevational gradients, we consider that it mainly explains the variation observed (Hodkinson, 2005). The low number of non-aggressive species at low elevation sites can be explained by an increased competition with aggressive species (especially *I. typographus*), as well as by a niche shifting to cooler sites; because plant resources tend to be also limited at cooler sites, this would lead to a shrinking of the range, with a potential loss of biodiversity (Parmesan,

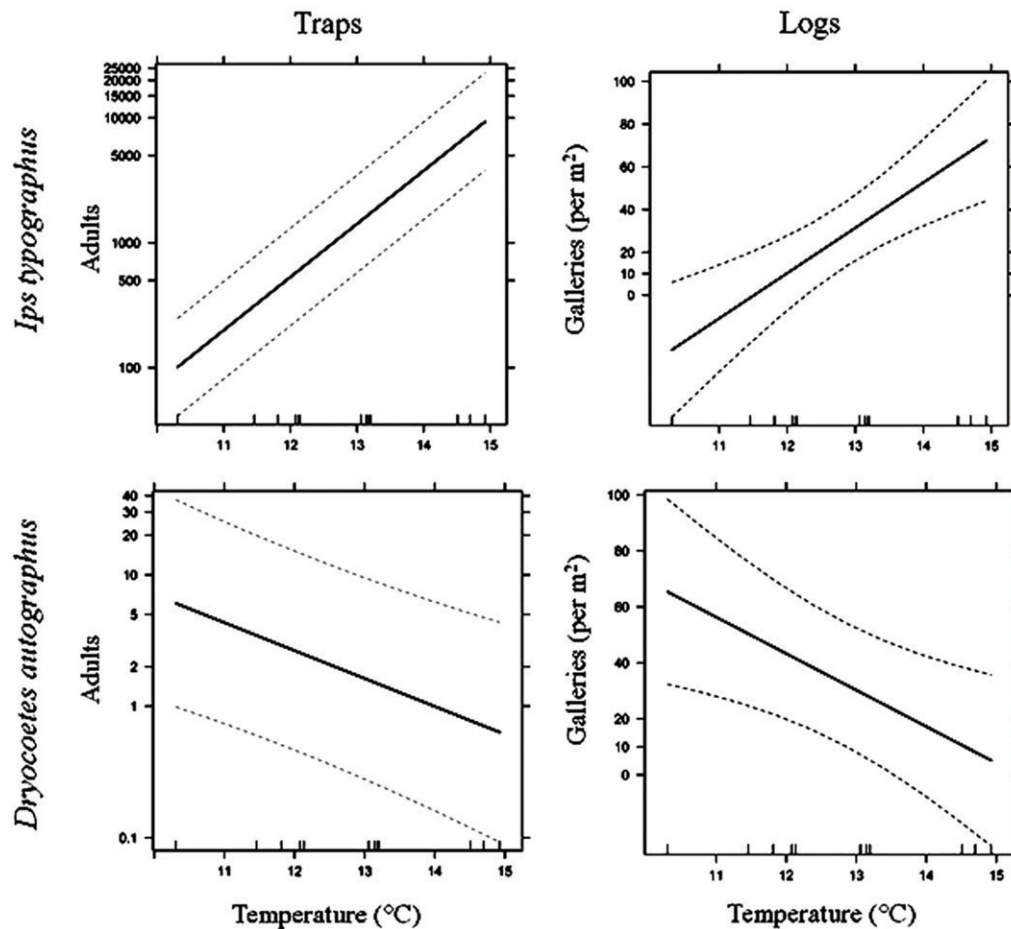


Figure 1 Results of the generalized linear mixed model (GLMM) for the two species showing a significant response to temperature both for captured adults (left column) and density of maternal galleries in logs (right column). Solid lines represent the fitted values obtained from the GLMM and dotted lines represent the 95% confidence intervals. Tickmarks on the x-axis show the mean temperature of each location (M3 and P3 overlapping).

Table 4 List of insect species that colonized wood logs according to family/subfamily, decreasing abundance and elevational locations

Family	Subfamily	Species	Gradients and Locations											
			Mauria				Pramosio				Tualis			
			M1	M2	M3	M4	P1	P2	P3	P4	T1	T2	T3	T4
Cerambycidae	Lepturinae	<i>Rhagium bifasciatum</i>	7.6	14.5	0.0	0.0	18.6	15.5	22.7	5.3	19.4	11.9	0.0	3.2
Curculionidae	Molytinae	<i>Pissodes herciniae</i>	0.0	0.0	0.0	0.0	0.0	9.3	0.0	0.0	0.0	11.9	0.0	6.4
Curculionidae	Scolytinae	<i>Dryocoetes autographus</i>	7.6	2.9	79.1	55.4	5.3	3.1	6.5	37.2	11.1	59.5	68.2	60.5
Curculionidae	Scolytinae	<i>Hylurgops palliatus</i>	25.5	34.7	0.0	0.0	5.3	37.1	6.5	5.3	8.3	8.9	13.0	38.2
Curculionidae	Scolytinae	<i>Ips typographus</i> *	76.4	26.1	0.0	0.0	45.3	6.2	16.2	10.6	121.6	11.9	3.2	6.4
Curculionidae	Scolytinae	<i>Xyloterus lineatus</i> *	0.0	2.2	1.7	0.0	2.2	0.8	0.0	0.0	1.3	0.0	0.7	1.1

Species marked with an asterisk are considered aggressive (Grégoire & Evans, 2004). Values represent the number of maternal (for bark beetles) or larval (for other wood-boring insects) galleries per m² of bark.

2005). The low number of species trapped, however, did not allow further analyses on species diversity along the gradients, and a new experimental setting targeted at maximizing species number would be needed.

Although latitudinal range expansions of bark beetle outbreaks have been reported for various species (Logan & Powell, 2001; Jönsson *et al.*, 2009; Safranyik *et al.*, 2010), the response

of bark beetles to elevational gradients is poorly known and with contrasting results. Williams *et al.* (2008) did not find the responses of the bark beetle community to be associated with ponderosa pine forests in Arizona, whereas Tykarski (2006) and Röder *et al.* (2010) found a slight decrease of species richness and abundance with increasing elevation in Norway spruce forests of central Europe. Models based on *I. typographus*

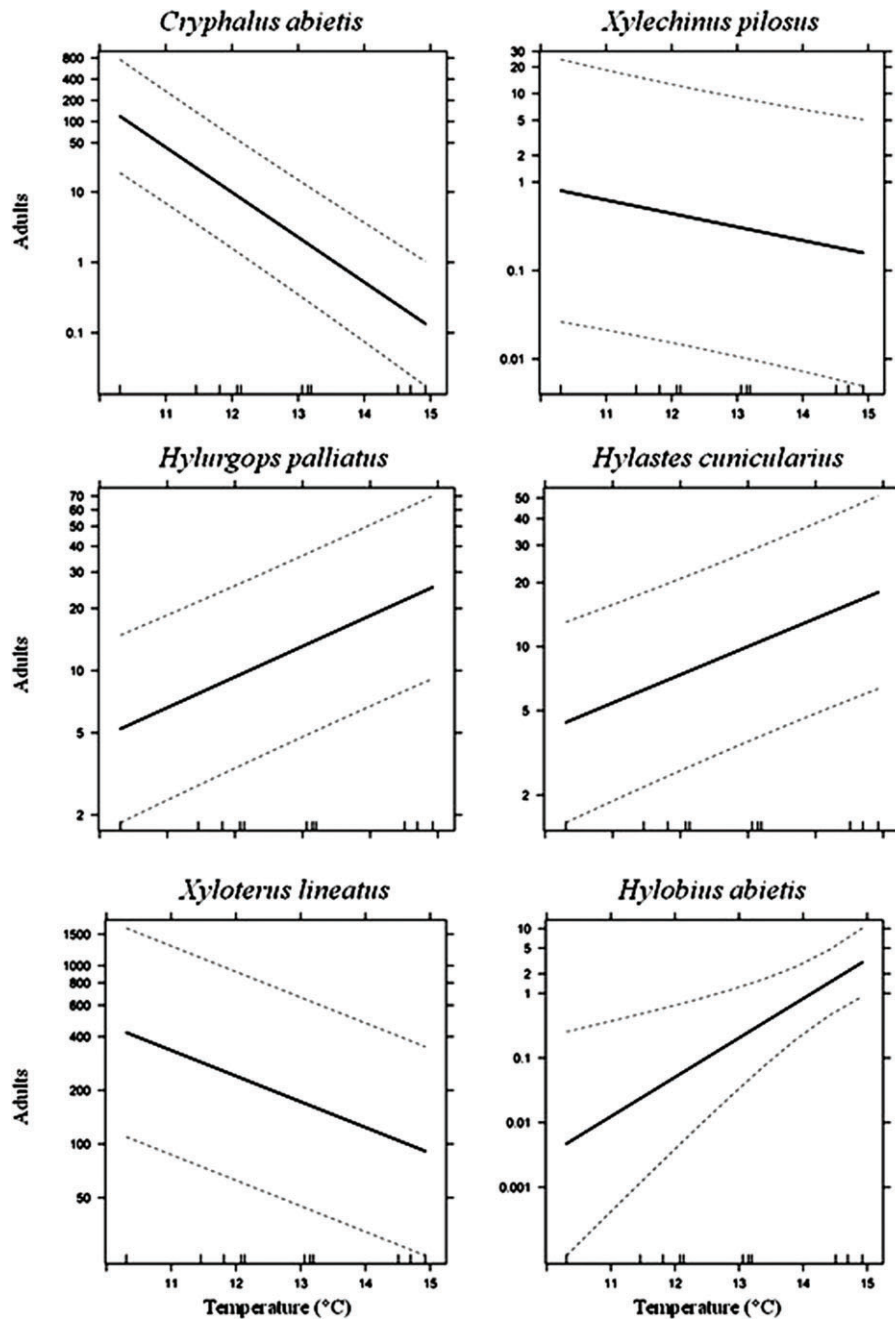


Figure 2 Results of the generalized linear mixed model (GLMM) for the six beetle species showing a significant response to temperature only for captured adults. Solid lines represent the fitted values obtained from the GLMM and dotted lines represent the 95% confidence intervals. Tickmarks on the x-axis show the mean temperature of each location (M3 and P3 overlapping).

life-history traits predict an increase of outbreak range and damage with decreasing elevation as a result of temperature warming (Baier *et al.*, 2007; Seidl *et al.*, 2009; Marini *et al.*, 2012). The contrasting results may be a result of the fact that some studies used elevational gradients over a large latitudinal span, probably sampling geographically separated populations that may respond numerically more to the history of local dynamics than to temperature (Williams *et al.*, 2008; Röder *et al.*, 2010). In addition, it is not always clear whether or not

the sampling areas were close to outbreaks, which may affect the abundance pattern, as in the study of Tykarski (2006). Conversely, the present study was conducted on steep gradients distributed over a very short topographical distance (only a few kilometres between the lowest and highest location), allowing minimization of the effect of local variables affecting insect populations, with the exception of temperature. In addition, the lack of outbreak areas within the chosen gradients reduces the noise that could affect the local catch pattern.

The mechanisms involved in the response of wood beetles to temperature can be both direct and indirect. Direct mechanisms are possibly associated with a better performance and faster development, eventually leading to multiple generations per year (Jönsson *et al.*, 2009). Indirect mechanisms are mainly driven by changes in host plant quality because trees may become more susceptible to beetle colonization when they grow under conditions of water stress (Jactel *et al.*, 2012), as is typical of the low elevation forests of the south-eastern Alps (Marini *et al.*, 2012). The different thermal thresholds required for emergence by the aggressive species found in the present study may explain the differences observed along the temperature gradient. *Xyloterus lineatus* emerges early in spring with a mean air temperature of approximately 12 °C (Chararas, 1962; Annala *et al.*, 1972), whereas the other aggressive species have an emerging threshold of approximately 18 °C (Chararas, 1962; Christiansen & Bakke, 1988). The low *X. lineatus* density recorded at low elevation (i.e. higher temperature) suggests that traps were probably deployed too late (mid-April), when most of the adults had already emerged and moved into new host-trees. Instead, the abundance of secondary, non-aggressive species in many cases showed a positive trend related to elevation, with the only exception of *H. palliatus*.

The analysis of the log infestation confirms the general trend observed in the traps, with aggressive species (*I. typographus*) showing a positive response to the thermal gradient, whereas non-aggressive species (*D. autographus*) have a negative trend. Although *I. typographus* and *D. autographus* were the only two species showing a significant response to elevation in both traps and logs, some species were collected in only one of the two. In the case of *H. abietis*, which was found in high numbers only in traps, this result is not unexpected considering that the species colonizes tree stumps more actively than trunks and logs (Von Sydow & Birgersson, 1997). The opposite trend was observed for the weevil *P. hercyniae*, which is found only in logs and never in traps baited with generic lures or bark beetle pheromones; this can be explained by the high selectivity of the genus *Pissodes* to its host trees and the lower attractiveness of traps (Bratti *et al.*, 1998). Lastly, some species, such as *H. palliatus*, *X. lineatus* and *P. hercyniae*, occurred only in a few logs or with a few individuals, making trend estimation or statistical comparison with traps unfeasible. Overall, it may appear that aggressive species outcompete the non-aggressive ones at low elevation, whereas the latter thrive at higher elevation, although often with low population density.

In conclusion, the results of the present study suggest that, in a warming environment scenario, spruce forests may face increasing damage as a result of aggressive species, whereas the abundance of non-aggressive species will probably decline because of host limitation. This could be exacerbated especially at the southern edge of spruce distribution, as in the study area. Attacks by aggressive species, especially *I. typographus*, could become more frequent as a result of both increasing insect populations and increasing spruce susceptibility. In this respect, the predicted global warming (Solomon *et al.*, 2007) will probably expose Norway spruce to new outbreaks and infestation of wood-boring beetles, increasing the rates of timber loss and inducing an upward shift in their attacks. Considering the increased susceptibility of spruce forests to

wood-related beetles, especially bark beetles, forest managers should promote silvicultural strategies aiming to reduce these negative effects. The most reliable and ecologically sound measure is probably to avoid new spruce plantations at low elevation and outside the natural climatic range of Norway spruce (Marini *et al.*, 2012). Further studies should be conducted to determine whether the same trend is shared by other aggressive species, belonging to different feeding guilds and feeding on different host plants.

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Chapter 4

Better today but worse tomorrow: how warm summers affect
breeding performance of a Scots pine pest

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Better today but worse tomorrow: how warm summers affect breeding performance of a
Scots pine pest

I contributed to data analysis and paper writing

Abstract

Recent climate change are known to affect many insect populations, including bark beetles.

In this paper we explore how warmer temperature may affect the performance of southern European populations of the pine bark beetle *Ips acuminatus*. During a seven-year long study (2007-2013) we analysed: (a) insect voltinism and phenology, (b) annual trend of the mean population density, and (c) their correlations with temperature. The mean number of adults per trap captured during the flying season (May-August) showed a bivoltine phenological pattern with two flight periods, in May after hibernation and in July, when a second generation begin. The breeding performance of the first generation was positively correlated with temperature. In the warmer years, the amount of summer captures resulted higher than the spring ones, suggesting a positive breeding performance of the first generation and the beginning of a large second generation. The population density was instead negatively correlated with temperature, and insect populations decreased following warmer years with a negative effect on the population trend. Results from this study suggest that warm spring-summer temperature can produce a within-year increase of breeding performance and voltinism of *I. acuminatus*, with a higher reproductive success of the first generation and the beginning of a large second one. In these cases there is, however, a between-years reduction of the population density probably due to a high winter mortality of the overwintering immature instars coming from an incomplete second generation.

Keywords: *Ips acuminatus*, *Pinus sylvestris*, global warming, population dynamic, bark beetle.

4.1 Introduction

Recent climate change are known to affect many insect populations, including bark beetles (Coleoptera: Curculionidae, Scolytinae) (DOBBERTIN *et al.*, 2005, 2007; BERG *et al.*, 2006; BIGLER *et al.*, 2006; LANGE *et al.*, 2006; JÖNSSON *et al.*, 2007; RAFFA *et al.*, 2008; FACCOLI, 2009). In the last decades, high summer temperature associated with long periods of drought has promoted a progressive forest decline especially in southern Europe and circum-Mediterranean countries. Besides the direct effect on trees, warmer climatic conditions may reduce insect developmental time (WERMELINGER and SEIFERT, 1998), increase voltinism (COLOMBARI *et al.*, 2012), and affect the diapause mechanisms (GEHRKEN, 1985; BAIER *et al.*, 2007), resulting in an increase of the infestation pressure on the host trees.

For many decades *Ips acuminatus* (Gyllenhal), a polygamous bark beetle attacking the upper part of stem and branches of Scots pine (*Pinus sylvestris* L.) (CHARARAS, 1962; BAKKE, 1968), has been considered of minor importance (BAKKE, 1968), causing only sporadic damage following infestations of other pine pests primary agents of tree mortality (CHARARAS, 1962). Nevertheless, in recent years many outbreaks have been reported in a number of Scots pine forests of central Europe (MRACEK, 1995; SVESTKA, and WIESNER, 1997) and south-western Alps (FORSTER and ZUBER, 2001; Wermelinger *et al.*, 2008). Large infestations recently affected also the eastern part of the Italian Alps (Dolomites, NE Italy) causing considerable economic (FACCOLI *et al.*, 2011) and ecological damage (COLOMBARI *et al.*, 2012, 2013). Besides the reduction in timber quality of the infested trees, there are other important non-timber concerns, such as soil protection, biodiversity conservation and the general landscape quality of the Dolomite valleys (COLOMBARI *et al.*, 2008, 2012).

Although *I. acuminatus* has recently been listed among the most damaging of European scolytids (GRÉGOIRE and EVANS, 2004), precise information concerning biology, population dynamics and their variations according with climate and climatic change are still largely unknown (COLOMBARI *et al.*, 2012). Along the southern Alps, where this pest has recently caused extensive damage (WERMELINGER *et al.*, 2008; COLOMBARI *et al.*, 2012, 2013), both *I. acuminatus* and Scots pine occur at the southern edge of their natural distribution range and in climatic conditions very different from those occurring in central

and northern Europe. As temperature is the primary driver of insect development, and is strongly affected by climate change, these populations may modify flight behaviour, developmental rate and voltinism. Increases in temperature could permit more rapid rates of development, with a possible increasing of the number of generations per year or a better breeding performance. In this respect the recent outbreaks of *I. acuminatus* recorded in southern Europe are apparently indirectly correlated to pine decline caused by high summer temperatures and drought, which have strongly contributed to Scots pine mortality observed in the Alps in the last decades (WERMELINGER *et al.*, 2008). Unfortunately, no studies have investigated how weather conditions and their variations may directly affect the reproductive performance of *I. acuminatus* and the dynamics of its populations.

In this paper we explore the possible relationships between climate change and breeding performance of *I. acuminatus* populations infesting Scots pine forests in an outbreak area of the north-eastern Italian Alps. We hypothesize that warmer climatic conditions may positively affect the reproduction of the southern European populations of *I. acuminatus*, increasing the breeding performance of the first generation and allowing the development of a second generation. With the aim of contributing to a better understanding of the mechanisms driving the population dynamic of a forest pest exposed to climate change, during a seven-year long study (2007-2013) we analysed: (a) insect voltinism and phenology, (b) annual trend of the mean population density, and (c) their correlations with temperature and its variations in order to find a close relationships between climate and insect breeding performance.

4.2 Materials and methods

4.2.1 Study site

The Scots pine forest we used for our study extends over three municipalities of the Cadore Valley (Borca, San Vito and Cortina) (46°40'N; 12°20'E), Province of Belluno, North-Eastern Italian Alps. The forest (about 22.3 km² in area) grows on S-SW facing slopes, 1,000-1,600 m a.s.l. The stands are older than 100 years, with a mean density of about 300 trees per hectare, and show poor growth because of limitation of nutrients and water (COLOMBARI *et al.*, 2012; 2013; FACCOLI *et al.*, 2012). The forest has natural regeneration

and no silvicultural management for timber production, although small phytosanitary clearcuts have been occasionally carried out to preserve the general health of the forest, which is very important for soil protection against erosion and avalanches (COLOMBARI *et al.*, 2012; 2013; FACCOLI *et al.*, 2012). Because of the increasing bark beetle infestations recorded in the last decade, since 2007 the Regional Forest Service has applied a specific sanitation felling programme in autumn of each year by cutting and removing all *I. acuminatus* infested trees from the valley (FACCOLI *et al.*, 2011).

4.2.2 Phenology and voltinism of the model species

As reported by COLOMBARI *et al.* (2012), in the study area *I. acuminatus* usually has two distinct attack periods (i.e., generations). The first attack is conducted in spring by adults of the parent generation (hereafter referred to as “spring adults”) that in middle-end May emerge from the overwintering sites. They colonize suitable trees and in summer, approximately 8 weeks later, the beetles of the offspring generation (hereafter referred to as “summer adults”) emerge from the infested trees looking for new suitable hosts where a second generation will develop in the latest part of the summer. The two groups of insects (“spring” and “summer” adults) are usually well separated along the seasons, indicating the end of one generation and the beginning of the following (Figure 1 and 2). In case of short and cold spring or summers, at the end of the first generation the “summer adults” stay in the bark and do not reproduce before hibernation (i.e., following spring).

4.2.3 Population monitoring by pheromone-baited traps

Between 2007 and 2013 the population of *I. acuminatus* occurring in the investigated forest was monitored by pheromone-baited traps (FACCOLI *et al.*, 2012). In early spring, dry black 7-funnel traps (Witasek[®]) were set up in recent clearcuts (less than 1 year old) located no closer than 30 m each another. The total number of traps set up annually slightly varied among years (Table 1).

Traps were baited with species-specific pheromone lures composed of Ipsenol, Ipdienol and (S)-(+)-cis-verbenol, and supplied by the Spanish chemical company SEDQ[®]-Sociedad Española de Desarrollos Químicos (Table 1). Every year, traps were checked twice per

month from May to end of August. All pheromone dispensers were replaced once, in June, two months from the beginning of the trial. All caught insects were identified at species level and counted. Population monitoring was performed by a team of foresters working for the Regional Forest Service, in collaboration with entomologists of the University of Padova.

4.2.4 Weather monitoring

Climatic data consisting of air temperature and precipitations were collected daily from 1996 to 2013 from a weather station installed within a field laboratory of the Padova University in an experimental sites within to the study area (San Vito di Cadore (BL), 46°27'11" N, 12°12'47" E, 1,107 m a.s.l.). Air temperature was recorded every 15 minutes. Elevation and slope facing of the weather station were similar to those of the pheromone traps.

4.2.5 Data analysis

The mean of the maximum daily temperatures recorded from May to August, the months of *I. acuminatus* breeding, was calculated for each of the last 18 years (1996-2013). The deviance of the mean temperature of each year (ΔT_x) was then calculated on the mean temperature of the whole investigated period.

According to FACCOLI and STERGULC (2006), the breeding performance of the first generation of *I. acuminatus* and the beginning of the second was assessed as percentage variation (ΔG_x) of summer (GSu_x) and spring (GSp_x) adult captures, and calculated year by year as follows:

$$\Delta G_x = [(GSu_x - GSp_x) / GSp_x] * 100$$

Similarly, the annual variation (ΔY) of *I. acuminatus* population passing from one year (Y_x) to the following one ($Y_{(x+1)}$) was calculated as follow:

$$\Delta Y_x = [(Y_{(x+1)} - Y_x) / Y_x] * 100$$

To describe the possible relationship between air temperature and population trend, the ΔY_x recorded year by year over a 7-yr period (2007-2013) was correlated to ΔT_x using a 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. As some of the analysed time-series showed autocorrelation among the available data, the correlation was corrected by the autocorrelation function (ACF) of Statistica per Windows (LEE *et al.*, 2002). Significance of effects was based on $\alpha = 0.05$, and statistic analysis was performed in Statistica 3.1 for Windows (Statistica, Tulsa, OK).

4.3 Results

4.3.1 *Species voltinism and population trend*

The mean number of adults per trap captured during the flying season (May-August) showed a bivoltine phenological pattern with two flight periods, the first (GSp) composed by parent adults emerging in May after hibernation, and the second (GSu) composed by offspring of the first generation emerging in July and beginning the second generation. This pattern was observed in all the monitored years excepted for 2010, when only the parent beetles were trapped. On one hand, in the bivoltine years 2007-2009 and 2011 the amount of spring captures (GSp) resulted higher than the summer ones (GSu), with a negative breeding performance of the first generation (ΔG_x) respectively of -71.8%, -78.9%, -86.4% and -55.0% (Figure 3a). In 2012 and 2013 such ratio (ΔG_{2012} and ΔG_{2013}) was instead reversed, with GSu much higher than GSp, and a positive performance of the first generation (89.3% and 72.7% respectively), i.e. offspring adults more than parent adults (Figure 3a). For 2010, having a single generation, we considered ΔG_{2010} equal to -100% (Figure 3a). On the other hand, the variation in performance between consecutive years (ΔY_x) show an opposite pattern, with positive values in 2008-2010 and 2011 (87.6%, 12.5%, 63.6% and 23.3%, respectively), and negative only in 2012 and 2013 (-41.9% and -82.9% respectively) (Figure 3b).

4.3.2 Temperature variation

On average, the last 10 years have been the warmest of the last 18, with a general trend indicating a progressive increase of the mean temperatures (Figure 4). The years 2003, 2005, 2012 and 2013 have been the warmest of the study period. All the years in which the *I. acuminatus* population was monitored (2007-2013) showed a mean of the May-August daily maximum temperature higher than the mean of the last 18 years (1996-2013) with the exception for the cool 2008 (-0.95°C) and for 2010 on the mean (-0.002°C); 2012 and 2013 have been the warmest year with a positive deviation of +1.03 and +1.04°C respectively (Figure 4).

4.3.3 Correlation performance vs. temperature

The breeding performance of the first generation (ΔG_x) was positively correlated with temperature deviation (ΔT_x) from the mean ($R^2 = 0.967$; $F = 43.3795$; $p = 0.0061$) (Figure 5). Performance of the first generation greatly increase in warmer summers (Figure 5).

The population trend between two consecutive years (ΔY_x) was instead negatively correlated with temperature deviation (ΔT_x) from the mean ($R^2 = 0.84$; $F = 21.76$; $p = 0.00956$), and insect populations decreased after warmer years (Figure 6).

4.4 Discussion

Weather conditions can affect population growth, distribution and voltinism of many forest insect species. In particular warm temperatures affect the distribution range and performance of pine processionary moth *Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae) in southern Europe (BATTISTI *et al.*, 2005), winter moth *Operophtera brumata* (Lepidoptera: Geometridae) in northern Europe (HAGEN *et al.*, 2007) and many species of bark beetles, as the mountain pine beetle *Dendroctonus ponderosae* in British Columbia (LOGAN *et al.*, 2003; CARROL *et al.*, 2004; KURZ *et al.*, 2008). Warm temperatures can furthermore affect fauna composition, favouring more aggressive against less aggressive species (CHINELLATO *et al.*, 2013). The main results from our study support these hypotheses also for *I. acuminatus*. In the investigated area, spring-summer temperature of the last years was generally warmer than in the past. This phenomenon

affected positively the generation performance of *I. acuminatus* within the same year, allowing the high reproduction of the first generation and the beginning of the second, but negatively the population trend between consecutive years, with a general decreasing of the population density following particularly warm years.

Breeding performance of the first generation of *I. acuminatus* was as much higher as the maximum temperature of the season was 0.5-1°C warmer than the mean of the last 18 years. Warmer temperatures provide optimal environmental conditions for larval development and allow a fast development of larvae feeding under tree barks resulting in a very high number of new adults quickly emerging in early summer with the possibility, i.e. time, to begin a second generation in the same year. *Ips acuminatus* has a large distribution area, from southern Alps to Northern Europe (PFEFFER, 1995). Because of the short and cool summers of central and northern Europe, in most European countries *I. acuminatus* is an univoltine species, producing only one generation per year and overwintering as adults in the bark of the infested Scots pines (BAKKE, 1968). Spring and summer temperature is thus a crucial factor in order to fully complete the first generation. Adults generally emerge in spring with a mean air temperature of about 18°C (BAKKE, 1968), although Alpine populations seem to be able to fly with lower temperature (COLOMBARI *et al.*, 2012). In this respect, an early spring emergence associated to warmer temperature makes the breeding season longer, giving more time to begin also a second generation. In addition, warmer spring and summer reduce the mean developing time, passing from 8-9 to 6-7 weeks per generation (COLOMBARI *et al.*, 2012), increasing voltinism and reducing larval exposition to natural enemies, with a general improvement of the breeding performance. Lastly, high temperatures and associated droughts deeply stress host trees increasing the attacking rate of *I. acuminatus* (WERMELINGER *et al.*, 2008) and the number of adults reproducing before winter (COLOMBARI *et al.*, 2012). Effect of high temperatures on bark beetle voltinism was studied in the same area also for the most aggressive species in Europe, *Ips typographus* (FACCOLI and STERGULC, 2006; FACCOLI, 2009). These studies show that the beginning of the second generation is directly related to the weather conditions occurring at the beginning of the season.

The population trend between consecutive years shows instead an opposite response to temperature, with warmer spring and summer affecting negatively the population density of

the following year. As previously reported, long and warm summers allow the complete development of the first generation and elicit the beginning of the second one. Time is however not enough for the full development also of the second generation, which face the winter usually as larvae or callow adults in the phloem (COLOMBARI *et al.*, 2012). While *I. acuminatus* adults may survive winter temperature lower than -25°C in the bark of the infested trees (BAKKE, 1968), younger developing instars are unsuitable to survive the long and cold alpine winter, resulting in a large insect mortality and a population decrease in the following year. A similar mechanism was described also in other bark beetle species living in the same region, where bivoltine populations of *Ips typographus* suffer a winter mortality of about 50%, which becomes close to 100% considering only larval instars (FACCOLI, 2002).

Although weather conditions play a key role for insect development and breeding performance of bark beetles, there are many other environmental variables involved, and spring-summer temperature alone is clearly not sufficient to propose a reliable model of population dynamic. Beside temperature, also humidity and precipitation, quantity and quality of the host-trees, amount of natural enemies may affect directly or indirectly beetle performance and reproduction. Spring and summer temperature can be however considered among the most crucial factors affecting *I. acuminatus* phenology and voltinism, and definitely population dynamic (COLOMBARI *et al.*, 2012). Warm summer temperature can increase flight activity of the first generation offspring eliciting the beginning of a second generation, but negatively affects the population growth in the following year, especially if associated with a cold winter. Warmer climatic conditions, hence, have only a quick short-time positive effect on the breeding performance of *I. acuminatus*, but they do not improve the general population trend in a middle-long temporal scale.

In conclusion, data presented in this study suggest that warm spring-summer temperature can produce a within-year increase of breeding performance and voltinism of *I. acuminatus*, but a between-years reduction of the population density probably due to a high winter mortality of the overwintering immature instars coming from an incomplete second generation. Further studies focused on this topic but carried out on larger datasets, also concerning other species, could be very helpful to better understand the effect of

temperature and its increasing on bark beetle populations, and their impact on forest ecosystems.

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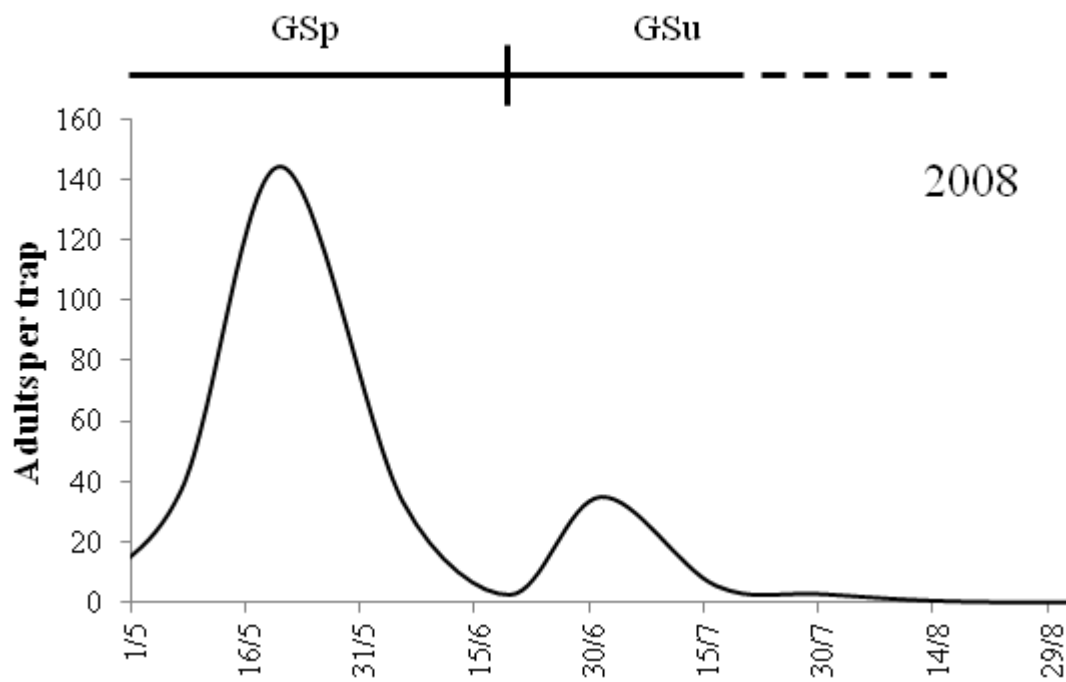


Figure 1: seasonal variation of *Ips scuminatus* adults (mean adults per trap) during the flight period in 2008 (May – August). The line above the curve shows the separation between spring (GSp) and summer generation (GSu).

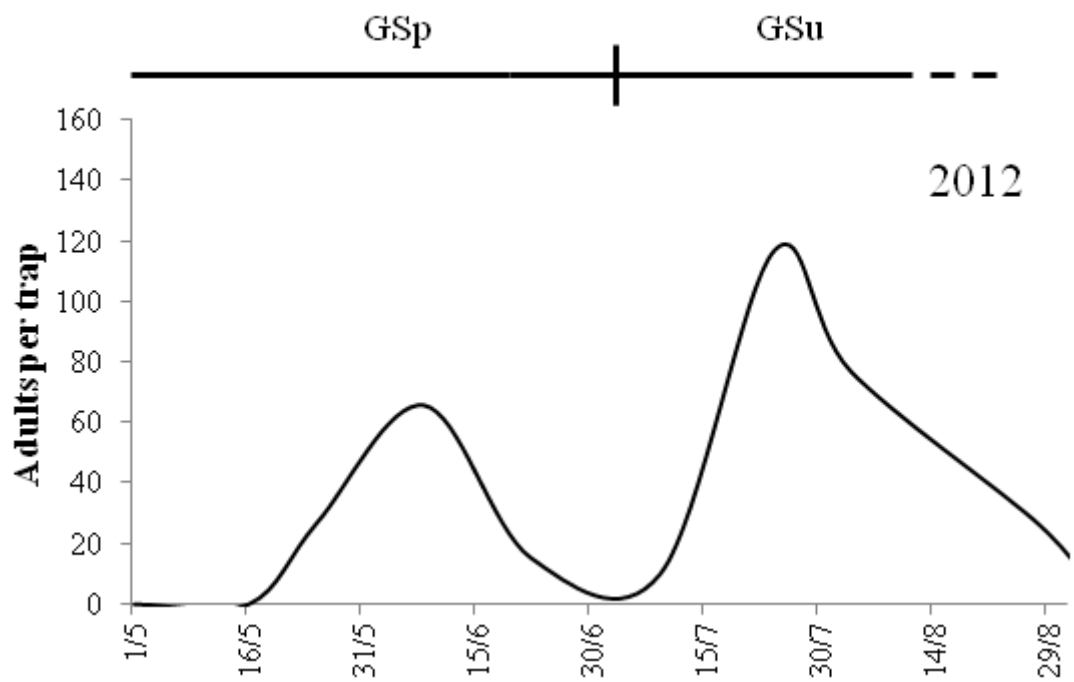


Figure 2: seasonal variation of *Ips scuminatus* adults (mean adults per trap) during the flight period in 2012 (May – August). The line above the curve shows the separation between spring (GSp) and summer generation (GSu).

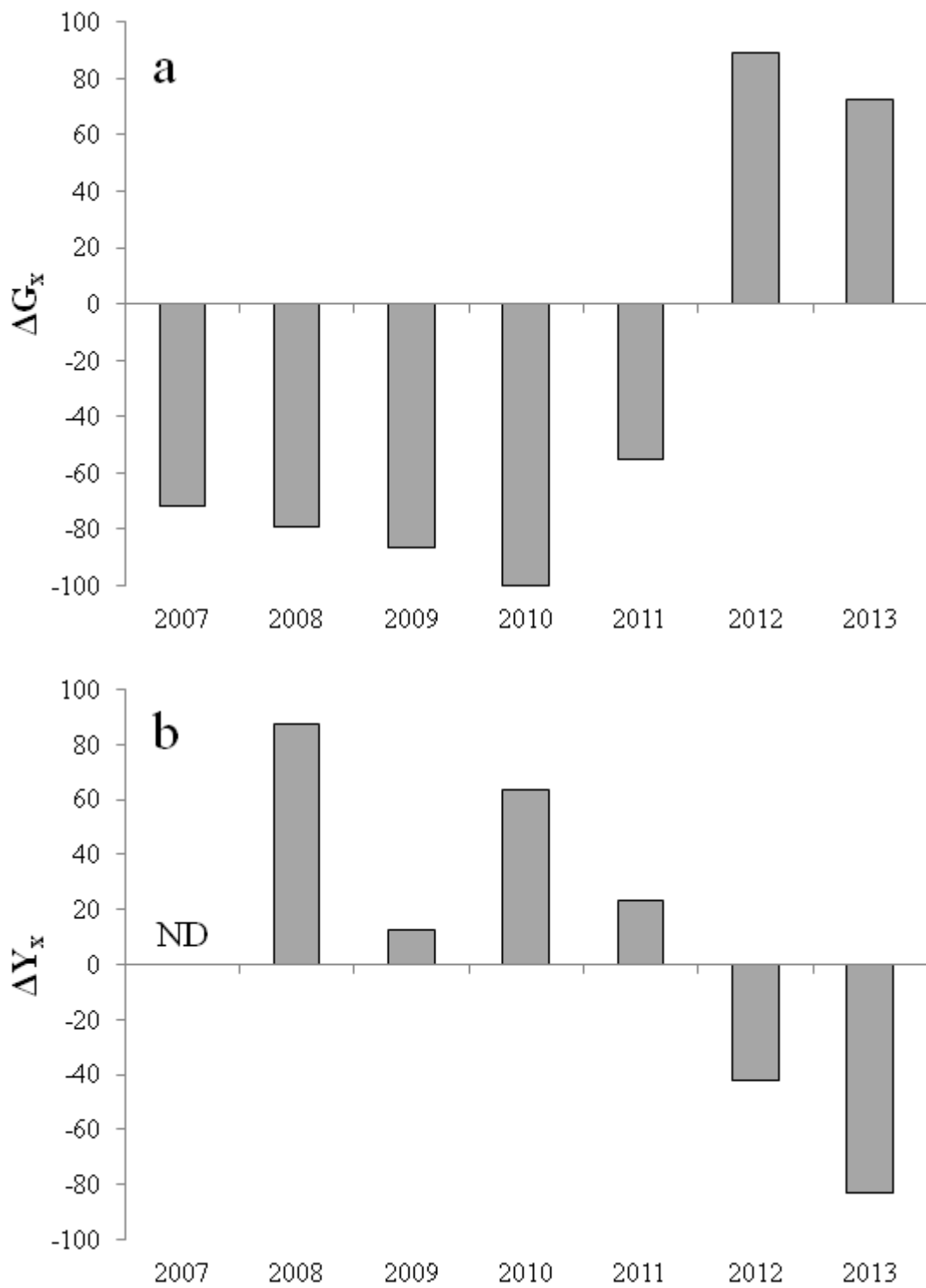


Figure 3: a) Summer captures compared with spring captures of the same year (ΔG) and b) total captures of one year compared with those of the previous one (ΔY) (ND = no data available).

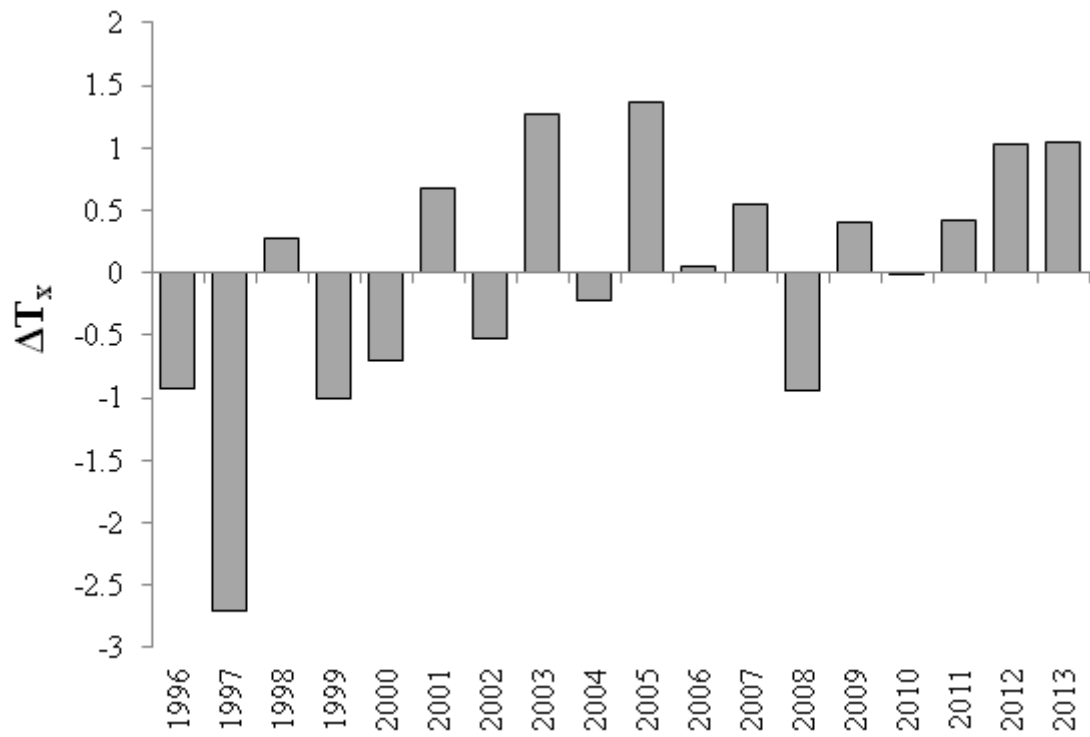


Figure 4: deviation of May – August daily maximum temperatures from the mean calculated for the same period in the last 18 years (1996 -2013).

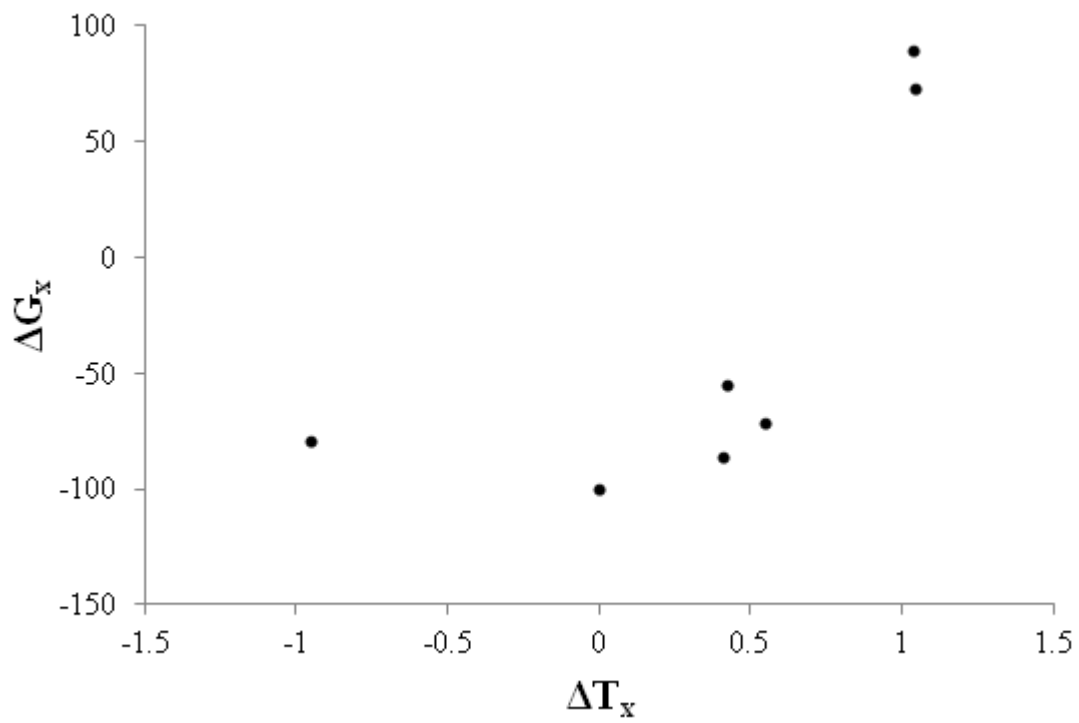


Figure 5: relationship between deviation of the May-August daily maximum temperatures from the mean calculated for the same period in the last 18 years (1996-2013) (ΔT_x) and variation between summer and spring captures of the same year (ΔG_x).

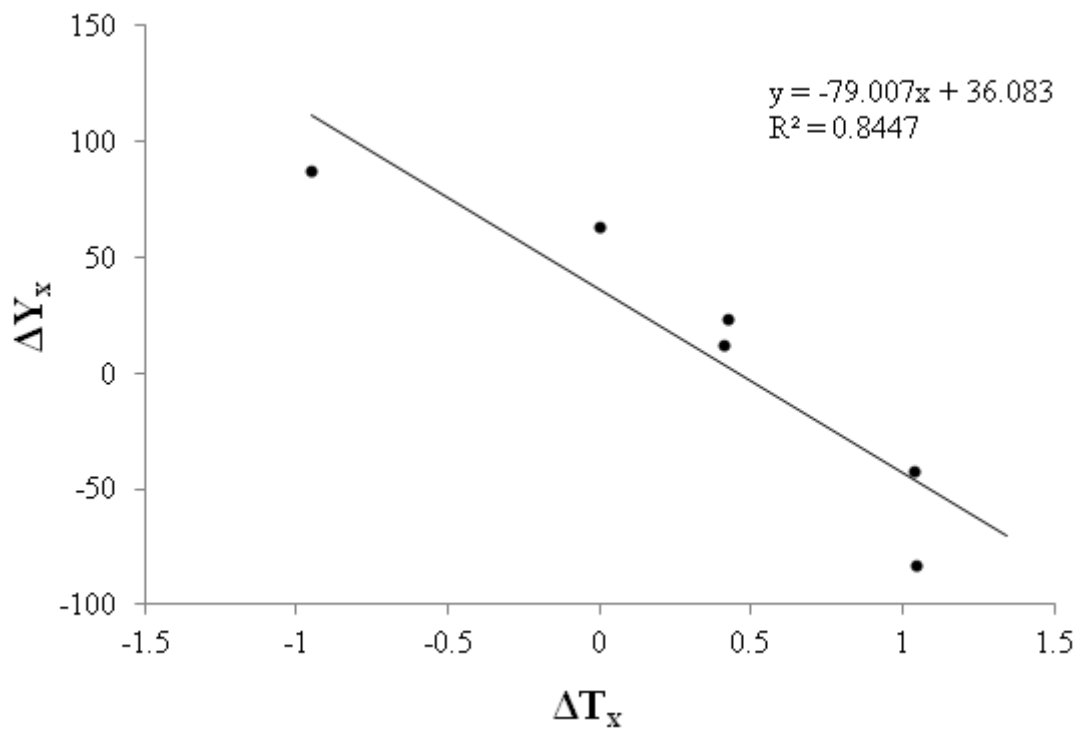


Figure 6: relationship between deviation of the May-August daily maximum temperatures from the mean calculated for the same period in the last 18 years (1996-2012) (ΔT_x) and variation between total captures of one year ($Y_{(x+1)}$) and the previous one (Y_x) (ΔY_x) ($F = 21.76$; $p < 0.01$).

Chapter 5

Smart-traps combined with molecular on-site detection to
monitor *Monochamus* spp. and associated pine wood
nematode

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Smart-traps combined with molecular on-site detection to monitor *Monochamus* spp. and associated pine wood nematode

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INTRODUCTION

The pine sawyer beetles *Monochamus* spp. (Coleoptera Cerambycidae) are the main vectors of the Pine Wood Nematode (PWN), *Bursaphelenchus xylophilus*, the agent of pine wilt disease in various parts of the world (Mamiya 1983). In Europe, *M. galloprovincialis* (Olivier) gained importance as a vector after the finding of the PWN in Portugal in 1999 (Sousa et al. 2001). An effective monitoring method based on early detection of both vector insects and associated nematode is needed in order to adopt appropriate phytosanitary measures (Rassati et al. 2012 and 2013).

MATERIALS AND METHODS

The present study shows a new technology for the remote detection of beetle catch combined with on-site molecular detection of both vector and nematode identity. A multi-funnel trap, baited with either specific or generic blend, and equipped with a specifically modified security camera (BioCam, Mi5 Security, Auckland, New Zealand), composed by a wide-angle lens, 1 or 3 MegaPixel sensor, rechargeable battery pack and internal modem for General Packet Radio Service (GPRS) connection was used. The interval between images taken by the camera can be programmed and saved in a Secure Digital (SD) memory card. The images can be stored in the same SD card and simultaneously sent to a safe repository accessible through the web, from which they are downloadable. On the same repository it is possible to check the level of battery charge of each camera and the GPRS coverage as well.

When a target beetle is detected, an on-site visit is planned, during which a fragment of the thorax is analyzed using a Loop Mediated Isothermal Amplification (LAMP) portable

device (Genie II, Optigene, UK) to identify the trapped species of *Monochamus* spp. and to detect the PWN possibly vectored by the beetles. Currently, primers were developed for the endemic *M. galloprovincialis* and *M. sutor*, and for the exotic *M. alternatus* and *M. carolinensis*. For the beetles identified as *Monochamus*, presence of PWN is also tested with the same device using slightly modified LAMP primers from Kikuchi *et al.* (2009), specific for the nematode ITS1 region. A positive control for the nematode is included in the test. The technique allows amplifying target DNA in a few minutes visualizing the results immediately.

RESULTS

Images obtained by cameras are definitely adequate to visually recognize large longhorn beetles such as *Monochamus* spp.. All the main morphological traits of the species are detectable (Fig. 1). The system works also under sub-optimal light conditions. LAMP primers designed to amplify the ITS2 region of *M. galloprovincialis*, *M. sutor*, *M. alternatus* and *M. carolinensis* show to be specific, giving a positive result only for these species after 10-15 minutes after the test start (Fig. 2). On the other hand, no positive insects for PWN have been detected until now.

CONCLUSIONS

Both technologies are designed for quick and cheap on-site analyses, and can be used by non-expert staff with a short training. In case of positive samples, they must be taken to the laboratory and analyzed more accurately with standard protocols for official confirmation.

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Figure 1 Picture taken by 3MP trap camera. One individual of *Monochamus* spp. is clearly recognizable on the left, together with several individuals of the longhorn beetle *Acanthocinus griseus*, one of the western seed bug *Leptoglossus occidentalis* (above) and several small bark beetles (right).

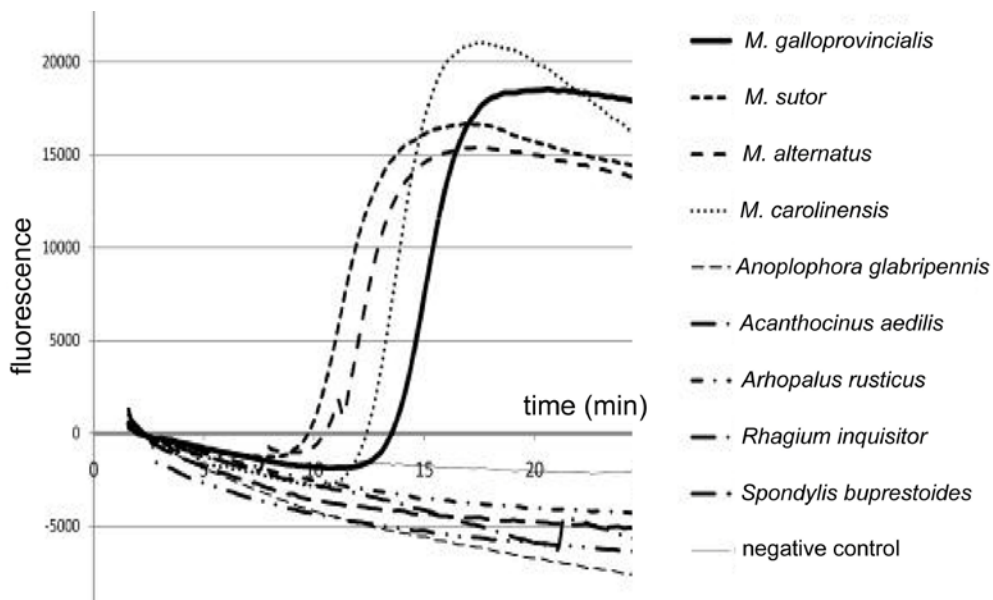


Figure 2. Amplification profile for the LAMP assay carried on *Monochamus* spp. and other cerambycid beetles. Positive curves are obtained in 10-15 minutes only for *M. galloprovincialis*, *M. sutor*, *M. alternatus* and *M. carolinensis*.

Chapter 6

Conclusions

Conclusions

The results of this three-year work can be considered an improvement in both developing new tools for early detection and understanding the effects of climate on wood-related beetle species.

The application of devices able to get images of the content of a trap and to send the picture to an accessible web server in real-time can be of crucial importance in implementing early detection of quarantine or potentially threatening insects. The advantage of having remote, real-time, images of the content of a trap can be extremely helpful in many cases:

- The number of traps installed for monitoring is very high.
- The position of installed traps is difficult to reach (lack of roads and infrastructures, long distances between traps).
- The traps need to be checked at uncomfortable moments of day and/or night, in the case of target species with peculiar phenological activity.
- The traps need to be checked very frequently, or even daily in cases of specific monitoring (Stanbury *et al.*, 2013).
- The non unusual combination of more than one of the aforementioned conditions.

The development of such tools has to be coupled with easy functioning and setting features in order to be used by technical operators (plant health officers, forest officers and managers) with little training, and simple enough to be easily set and fixed in case of malfunctioning.

The combined application of remote pictures and other on-field detection technologies, such as LAMP-PCR – as explained in chapter 6 – can lead to increased precision in field diagnostics of potentially threatening organisms and to money saving, due to the analysis focusing only on those samplings showing the probable presence of the target monitored species.

The proposed monitoring methodology can be applied for research on native species as well: all the conditions listed above can be found in common field studies, especially those investigating the flight phenology of species flying for short period or with peculiar population curves, as in the case of the New Zealand flowers thrip *Thrips obscuratus*, or to

monitor the presence of particularly rare species, such as the early establishment period of quarantine pests like the Asian longhorn beetles *Anoplophora* spp., avoiding the frequent check of empty traps.

Furthermore, the increasing web-based communication technology, coupled with miniaturizing of device size and prize reduction as in the case of smartphones, seems to provide cheaper and more efficient components able to adapt to the different monitoring needs. New studies and tests could lead to an use of different and more precise tools for remote image analysis, such as infrared, ultra-violet and motion sensors.

The effect of climate – in particular of temperature – on wood-related species, results to be an important driver in biology and phenology of insect populations. The studies carried on in these three years in alpine populations show further experimental on both indirect and direct effects of warm temperatures on potential damages on mountain host trees, and this is of crucial importance in order to understand the potential effect of a warming scenario as forecasted for the next years (IPCC, 2013).

In the case of Norway spruce *Picea abies* warm temperatures are likely to increase stress conditions for plants, especially at the southern edge of the species' distributional range, and consequently increase the attack rate of those wood-species considered aggressive and most damaging (Grégoire & Evans, 2004). Beside the effect on plants, such condition affects the guild's species diversity, causing an increased abundance of aggressive species – such as *Ips typographus* – and a consequent niche loss for less aggressive species in warmer areas and their shift at higher, and cooler, altitudes.

In the case of *Ips acuminatus*, warm temperature seem to play an important role on breeding and development success, affecting the abundance of generations in this typically bivoltine species: warm temperatures seem to increase the abundance of summer generation and consequently increase the number of adults flying in summer but, on the other hand, this seems to lead to a non-optimal development for winter survival and a consequent population decrease on the following year.

Of course, the effect of temperature is linked to other parameters fundamental for the development of the species, in particular the length of warm season can influence both the flight of the spring generation and the development of summer generation, directly

affecting the reproductive rate; in general, anyway, we can conclude that the effect of warm temperature can be different if analyzed on short or mid-long term.

At the very end we can conclude that monitoring wood-related species is a fundamental task in a continuously-changing environment. Global trade patterns and modified environmental features need to be faced with increased efforts in early detection and biological monitoring, including the development of new and efficient tools able to help both researcher and professionals to have efficient results in short time, possibly having the remote control of many study areas at the same time.

Monitoring the effect of climate conditions on native and already established population can be of crucial importance in order to understand the effects of environmental conditions in both host plants and insect biological traits and can be used to predict and model future patterns for species that are not yet introduced or established or to be extended to other, more difficult to monitor, native species. Furthermore, understanding the effects of temperature on ecologically important components – such as insects – can help in understanding the biological effect of a global warming scenario and help to choose and implement mitigation solutions.

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