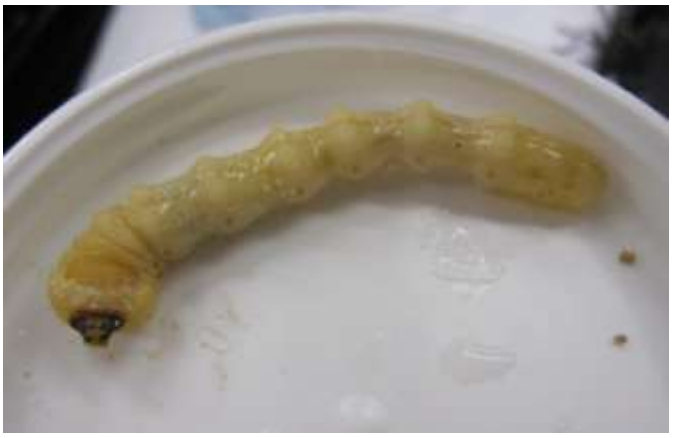
Risk Management for the EC listed Anoplophora species, A. chinensis and A. glabripennis. (ANOPLORISK)

ANOPLORISK

Final Draft Report

14/03/2014



Executive Summary

WP1: Project Management and Co-ordination

Introduction

Alongside the management of this project, the key role was to encourage interaction between the partners within the project and between the project partners and other projects and organisations that have an interest and involvement in understanding *Anoplophora spp*.

Meetings

There were three project meetings during the lifetime of this project:

1. Kick off meeting at Fera, York, UK on the 31st January and the 1st February 2011
2. Mid-project meeting at CRA-ABP, Florence, Italy on the 14th and 15th of December 2011
3. Final meeting at JKI, Braunsweig, Germany on the 4th and 5th of December 2012

Interactions

Two visits to significant outbreak sites were organised to help members of the consortium experience and understand the impact of long term outbreaks in different situations.

1. Worcester, Massachusetts, 22-25 march 2011. ALB outbreak reported in 2008 but had likely been there since the mid-late 1990's.
2. Lombardy and Veneto, Italy 12 - 13 Oct 2011. CLB and ALB outbreaks respectively.

Also, the Austrian team and their Anoplophora dogs visited outbreak sites in Austria, Croatia, Germany, Switzerland and the UK.

Outcome

These visits to the outbreak sites were of enormous value in helping consortium members understand the impact and scale of the task facing those with responsibility for dealing with the attempted management and eradication of these organisms. This was particularly relevant for the UK visitors to these sites as they were able to implement some of the lessons learned on the visits to the outbreak at Paddock Wood in Kent, UK which was found during the lifetime of this project. The response and subsequent probable eradication of the outbreak was influenced by knowledge gained during these visits and by other aspects of the projects work.

WP2: Development and testing of non-destructive detection methods

There are three main topics investigated:

1) Assessment of different image guided methods for their ability to detect an *Anoplophora* infestation in young host plants and in cut wood (P2 and P6)

2) Assessment of acoustic based detection system to detect and record noises made by feeding of xylophagous larvae in wood. (P1 with contribution of P5 and P9)
3) Use of special trained dogs for scenting Anoplophora (ABL/CLB) stages in infested plants and wood (P3)

The import requirements of the European Union concerning host plants of *Anoplophora chinensis* (CLB) include as one of the inspection methods the obligatory destruction of a specified number of plants of each consignment. Destructive sampling of plants became necessary because visual inspections led to false negative decisions on several occasions and therefore infested plants were released. However, destructive sampling is not ideal as it is costly to the importer and time consuming for the inspector. Effective non-destructive techniques could ensure that the inspection process remains as effective but provides significant efficiencies to both the inspectors and the importers. In addition visual inspection of mature trees in demarcated areas sometimes is difficult and infested trees have been missed during human based inspection. Aim of the current work package was to assess a range of new potential techniques which are already in use for other purpose e.g. x-rays, computed tomography, ultrasound and to further develop a range of promising techniques that are already partially developed e.g. acoustics and detection dogs.

Work of P1: The work undertaken by Fera and the University of York has further developed the acoustic detection system which has been tested in various situations from laboratory (York and FERA) through tests on native trees to use on imported Bonsai and recordings taken in Italy. Various sensors have been designed and tested with different resonant frequencies, housing design (including custom housings) and amplification factors. 8-channel and 16-channel multiplexed systems were developed for use by PSHI on imported Bonsai and a protocol for their use has been drawn up. The systems have been found to be successful and have additionally been employed in the laboratory at FERA for long term recording. Testing of the bite detection software has highlighted that there is still more work required to make this an effective, efficient system. A sound library for feeding sounds has been created for 11 species of wood boring beetle including *Anoplophora glabripennis*, *A. chinensis, Agrilus planipennis*, several bark beetles and one Lepidopteran larva (*C. cossus*). Discrimination between species has been shown to be feasible during a previous research project, but the software to enable this is no longer available. Discovery of the presence of *Otiorhynchus sulcatu*s larvae in the plants at FERA initially suggested that their feeding sounds are very similar to ALB; subsequent analysis has shown that this is sometimes the case highlighting the need for discrimination software if all the benefits of an acoustic detection approach are to be maximised. Two types of artificial larva have been designed and tested for use with the sensing systems. It is possible for a two sensor system to locate the approximate position of a larva to within 8-9cm if between the sensors, or whether it is on the distal side of either sensor. Higher resolution can only be attained if a much higher sampling rate than 44.1kHz is used. A forward-looking analysis of new systems and applications has been carried out indicating that future systems can be in two forms – stand-alone and wireless networks, Each system has application in different but overlapping scenarios.

Work of P 2: Research performed by NVWA & associate partners - Wageningen University and Philips Research - was based on analysis of X-ray images recorded on a system dedicated to luggage inspection (2D) and human health care (3D) respectively. Results show an increase in accuracy of inspection potential using X-ray instruments. Using the images of

a 2D luggage inspection scanner optimization of the automated borehole detection method resulted in an accuracy of 67%, Combining machine vision and human input resulted in a further increase in accuracy up to 83%. Scanning infested wood with a (high-end) Philips Brilliance iCT scanner for 3D imaging showed (Mol & Wolf, 2011) that: 1) air-filled bore holes down to 2mm in wooden samples can easily be visualized by CT imaging, 2) the shapes and sizes of bore holes in infested wood can be visualized in various 3D-display modes, 3) (dried) larvae of long horned beetles can be identified and visualized, 4) pseudo *Anoplophora* bore holes in large (40 unit) bunches of small trees can be spotted visually in the CT cross-sectional images. Successful application of 2D and CT scanners for the inspection task at hand seems technically feasible, but current scanners are not yet for feasible for regular phytosanitary inspections. To cover any future implementation, it will be necessary to identify further (inspection) applications for CT imaging and to optimize equipment for the phytosanitary environment.

Work of P 3: The detection of the scent of *A. glabripennis* and *A. chinensis* by detection dogs is independent from the development stage and the activity of the pest. The four Austrian Anoplophora detection dogs were successfully used for investigation of wood packaging material in ports and at stone importers, for the checking of imported plants, also Bonsai, in nurseries, garden centers and importers as well as for monitoring in infestation areas of *A. glabripennis* and *A. chinensis* in the European states Austria, Netherlands, Italy, Croatia, Switzerland, Germany and United Kingdom. A training program was developed and already 13 additional dogs from Austria, Germany and Switzerland were educated for the detection of *Anoplophora*.

Work of P 6: A CT-scanner developed for assessment of wood properties, several infrared thermography cameras, radar, electrical resistivity tomography and ultrasonic were investigated on their potential to detect insect stages as well as boreholes in young plants. For the trails a model system for host/insect was developed using standardized small logs cut from young trees and the goat moth (*Cossus cossus*) larvae. Each method has been tested on its ability to detect bore holes (with and without larvae) of different diameter and location in the stem. Infrared thermography could not detect neither larvae nor boreholes. Radar is dependent on the movement of the test objects; therefore boreholes could not be detected, but larvae motion even with short mandible movement was detectable. Electrical resistivity tomography showed some promising results but needs further development. Boreholes of small sizes (<5 mm) limited the accuracy of this method and it was not possible in all cases to distinguish larvae from surrounding wood. With the used ultrasonic equipment borehole size also limited the accuracy of the method. As already stated by P2 above the CT scanner gave the best results and ended up in a100% accuracy for borehole detection down to 2 mm, larval identification as well as the identification of frass.

In conclusion X-Rays and CT-Scanner have a high potential to be used for routine inspection of young plants. The rays (absorbed energy) do not harm the plants and the technology is independent of the physiological stage of the organisms in the trees. Acoustic detection is also far developed. Its application depends on the ability of the software to exclude ambient noise and on the activity of the insect. Detection dogs showed their potential to be used to detect ALB and CLB as well and became already a routine method either for inspection of import consignments (plants and wood packaging) or for analysis of infested trees in the wild.

Within this WP, the following papers were published:

1. Hoffmann, N.; Schröder, T. (2012): Potential of infrared thermography to detect insect stages and defects in young trees. Julius-Kühn-Archiv, 438: 166-167.
2. Hoffmann, N; Schröder, T. (in press): Potential of infrared thermography to detect insect stages and defects in young trees. Journal für Kulturpflanzen.
3. Hoyer-Tomiczek, U (2011).: Früh erkennen und wenig zerstören: Spürhunde erschnüffeln Baumschädlinge. Risikomanagement für die in der EG gelisteten Anoplophora-Arten 2011 (ANOPLORISK). Jahresbericht 2011, Bundesforschungs- und Ausbildungszentrum für Wald, Naturgefahren und Landschaft (BFW), Wien, S.27.
4. Hoyer-Tomiczek, U., Sauseng, G.: 2012. "Alternative Detection Methode for ALB and CLB". 2012. Fortschutz Aktuell 55, Bundesforschungs- & Ausbildungszentrum für Wald, Naturgefahren und Landschaft (BFW), Wien, 2012, S 43-45.
5. Hoyer-Tomiczek, U., Krehan, H., Hoch, G.: (in press). Wood boring insects in wood packing material: Recent interceptions and a new ALB outbreak in Austria. Abstract USDA, 2013.
6. Jansen, R.M.C. & Hemming, J. (2011). “Validation of X-ray for borehole detection in intact trees” Draft Wageningen UR Greenhouse Horticulture, Wageningen / Bleiswijk, December 2011, 26 pp.
7. Mol, C.R. & Wolf, R.M. (2011) “3D imaging for the detection of Anoplophora in wood” Report Philips Research, Eindhoven, August 2011, 30pp.

WP3: Development and testing of diagnostic techniques in the absence of the pest

There were five main topics investigated

1. Dissection of symptomatic wood in the laboratory to obtain insect frass and investigation of the collected frass for the presence of body parts of insects using stereomicroscope
2. Diagnosis/confirmation of *Anoplophora* spp. based on molecular analysis of insect body parts
3. Analysis of other *Anoplophora* species
4. Non destructive diagnosis of *Anoplophora* spp. tree colonization based on analysis of insect frass
5. Dissection of symptomatic plants and analysis of the annual ring in laboratory to date exit holes

The first two topics were performed by P7 (ILVO) and permit to define a correct way to extract, from the wooden parts of plants infested by woodboring insects, remnants of the body of the insect (head capsule and larval skins), using tools and methods to split the wood and by the use of laboratory instruments to detect and sample the insect remnants (stereomicroscopes and the most appropriate magnification, forceps and containers). On these insect remnants, it was defined an appropriate molecular analysis protocol for species identification.

Regarding the third topic, the partner P3 (BFW) performed with success molecular analysis on insects belonging to the genus *Anoplophora*, but different from *A. chinensis* and *A. glabripennis.* Thanks the possibility to obtain several specimens of exotic species of the genus *Anoplophora* from their native oriental region, molecular analysis was performed, and for the following *Anoplophora* species species-specific finger prints are now available:

Anoplophora chinensis, Anoplophora chinensis form *malasiaca, Anoplophora glabripennis, Anoplophora beryllina, Anoplophora davidis, Anoplophora elegans, Anoplophora granata, Anoplophora macularia, Anoplophora sollii.*

The fourth topic was performed by P5 (CRA-ABP) and permit to define a correct protocol for molecular analysis for species identification of woodboring insects (potentially applicable to a wide range of phytophagous insects) starting from the frass produced by the larvae during the feeding activity. This procedure poses interesting perspectives for pest identification in the absence of the insect (absence of adults, larvae or pupae for different reasons, or when in a first step, the plant destruction presents several impediments); this method is presumably applicable for many time after insect emergence from the infested plant, *i.e.* the frass contained in the beetle galleries into the wood of the infested plant can be suitable for molecular analysis for a long period of time (in appropriate environmental conditions), also for years.

The fifth topic was performed by P5 (CRA-ABP) and investigated a method for dating exit holes produced by the adults of *Anoplophora* during their emergence from the infested plants. The stepwise procedure described permit a correct sample processing, and analysis procedure necessary to dating the time of exit hole excavation, taking into account that trees must be alive at time of felling. In this way it is possible to count backwards from the exit hole, the number of annual growth rings and to define the timing of beetle occurrence. These information can be useful in infestation dynamics studies.

An additional topic was performed by P5 (CRA-ABP) which was not included originally in the topics proposed in the project. Within this WP, it was investigated the possibility to prepare a taxonomic key based on larval morphology aimed to identify, or at least to separate the two exotic species of the genus *Anoplophora* present currently in Europe (together with the recent exotic species *Psachotea hilaris*), from the other woodboring longhorned beetle of the native European fauna. This taxonomic key is provided with many detailed morphological pictures, which aid in the interpretation of the characters mentioned in the key. This key can be a rapid and useful tool in species identification during phytosanitary surveys (taking into account that most cited details can be observed in the field using a pocket magnifying glass), before eventually proceed with molecular analysis in doubtful cases.

Within this WP, the following papers were published:

1) Strangi A., Sabbatini Peverieri G., Rovrsi P.F., (2012). Managing outbreaks of the citrus long-horned beetle *Anoplophora chinensis* (Forster) in Europe: Molecular diagnosis of plant infestation. Pest Management Science, DOI 10.1002/ps.3416.

2) Sabbatini Peverieri G., Bertini G., Furlan P., Cortini G., Roversi P.F., 2012. *Anoplophora chinensis* (Forster) (Coleoptera Cerambycidae) in the outbreak site in Rome (Italy): experiences in dating exit holes. REDIA, XCV: 89-92.

3) Pennacchio F., Sabbatini Peverieri G., Jucker C., Allegro G., Roversi P.F., 2012. A key for the identification of larvae of *Anoplophora chinensis*, *Anoplophora glabripennis* and *Psacothea hilaris* (Coleoptera Cerambycidae Lamiinae) in Europe. REDIA XCV: 57-65.

WP4: Understanding the potential for dispersal at outbreak sites

There were 4 main topics investigated:

1. Investigate if *A. glabripennis* is able to complete its development on fruit trees in Europe
2. Establish whether conifers are suitable host trees for *A. chinensis*
3. Consider the impact of host density and other environmental variables on the dispersal and potential spread of *Anoplophora* species
4. Determine the susceptibility of the most important *Citrus* spp. to CLB

Monitoring activities in European areas with *Anoplophora*-infestation and specific host plants-tests with both *Anoplophora* species revealed interesting new results about additional new host plants.

In Austria the deciduous tree species *Fagus* sp., *Fraxinus* sp. and *Alnus* sp. are new hosts of *A. glabripennis* which had not been known as host plants so far in Europe and partially also in the rest of the world. Alder (*Alnus* sp.) could also be an additional host where only oviposition scars and young larvae of *A. glabripennis* have been observed but no further development of them due to cutting of these trees (further development of these young larvae in the cut branches failed under quarantine laboratory conditions of BFW). An ALB-infestation of fruit trees has never been observed during a monitoring period of 11 years in Upper Austria with exception of one finding in a new infestation area in 2012 where one *Prunus avium* tree showed oviposition scars and starting larval galleries, but no larvae.

Specific testing of apple trees (*Malus domestica*, cultivar: Golden Delicious) showed that this fruit tree is a suitable host-plant for complete development of *Anoplophora glabripennis* (ALB) under controlled conditions, but it is not proved for natural field conditions. The complete development of ALB is even possible in trees with stems of only 3 cm diameter. There is a potential risk in apple production (Golden Delicious) in Europe in case of ALB-infestation - especially if there is a „lack“ of „better“ hosts.

The Italian testing of conifers and deciduous trees including *Citrus* species as suitable hosts for *A. chinensis* (CLB) showed clearly its polyphagy and adaptation to many tree species. In fact, it was possible to observe within the field trial that adults fed on all tested *Citrus* species (*C.* x *sinensis*, *C. reticulata* and *C.* x *limon*), on *Populus x euramericana* and *Acer saccharinum* and also on conifers (*Taxus baccata*, *Cryptomeria japonica aritaki*, but not on *Pinus sylvestris*), oviposition scars only were not present on *Citrus x limon* and larval activity was present on 5 of 7 tested species with signs of oviposition (*Populus x euramericana, Cryptomeria japonica aritaki*, *Citrus x sinensis*, *Citrus reticulata* and *Acer saccharinum*). Due to the start of the trials in 2012 it is only possible to verify the full development of CLB on these host plants in the next future, maybe in summer 2013 or 2014.

It can be concluded that deliverable **D4.1 (Information about additional unknown host plants of *A. glabripennis* and *A. chinensis* in Europe, especially if fruit trees are suitable hosts for ALB and coniferous trees for CLB)** was fulfilled during this project.

Concerning the achievement of deliverable **D4.2 (Review of the current knowledge on the dispersal behaviour of *Anoplophora* species; modelling the dispersal capacity of**

***Anoplophora* species in relation to biotic and abiotic conditions at an outbreak site)** it can be stated that it was partially fulfilled.

Preliminary results of an Italian study which was based on data of an infestation area in NE-Italy showed that it is unlikely that ALB will spread much farther than 2000 meters according with previously reported dispersal distance (Smith et al., 2001; 2004).

The Dutch team investigated the influence on dispersal of *Anoplophora* species by climatic conditions and estimated their lifecycles using an accumulated Degree Day Model. Thus, the development of *Anoplophora* beetles under Dutch conditions will take on average 3 years.

Main Conclusions

The obtained results and knowledge about new suitable host plants of *Anoplophora* species should be of high importance for monitoring and eradication measures in every infestation area in Europe and have to be considered when taking decisions on the surveys or eradication programs.

Papers, other publications and dissemination activities done

Oral presentation about preliminary results at the Austrian Plant Protection meeting (Österreichische Pflanzenschutztage 2011) in St. Pölten, 30.11.2011:

Lethmayer, C. & Hoyer-Tomiczek, U.: Asiatischer Laubholzbockkäfer (*Anoplophora glabripennis*) – auch eine Gefahr für den Apfelanbau in Österreich?

(= Asian long-horn beetle (*Anoplophora glabripennis*) – also a danger for the Austrian apple production?)

WP5: Investigating the potential efficacy of different management practices

There were 3 main topics for investigation:

1. Review the available information on management practices and attempt to fill relevant gaps
2. Evaluate the potential of a range of chemical treatment / treatment methods
3. Evaluate the potential for utilisation of biological control and/or enhancement of natural enemies

A desk study was undertaken to collate and interpret the current knowledge on the potential for controlling/managing infestations of *Anoplophora* spp (longhorned beetles). A review of the available information aimed to provide information on current management practices, identify relevant knowledge gaps, evaluate the potential of a range of chemical / treatment methods and evaluate the potential for utilisation of biological control.

There is a wealth of information on the variety of techniques that have been investigated to prevent, control and eradicate *Anoplophora* infestations, including cultural (e.g. siviculture, tree management, pest resistant clones), chemical (e.g. systemic, contact, fumigation), physical (e.g. heat, irradiation, exclusion) and biological (e.g. fungi, nematodes, bacteria, parasitoids) measures. Those that have received the greatest attention and which show the greatest potential for use in a practical situation, have been considered in this review.

Eradication is the goal for all infestations, however an integrated approach is essential for that goal to be achieved. The main strategies that have been vital to achieving eradication of *Anoplophora* infestations include tree removal and chipping, the use of protective insecticides, and public involvement.

Further research should investigate maximising the efficacy of existing control measures and investigating some of the more novel control measures, such as effects on the beetles' metabolism. The use of fungal bands may offer the most promising use of biological control agents, with efficacy enhanced by combining with an attractant or pheromone. The potential exists to augment eradication efforts by combining different methodologies. Other areas to consider may include pesticide rotation to reduce the risk of pesticide resistance and the use of alternatives methods for the treatment of wood packaging.

The longhorned beetle species *Anoplophora chinensis* and *Anoplophora glabripennis* have emerged in the last two decades as a risk to urban and woodland trees in Europe and there have been several outbreaks of these beetles in Europe. This review summarises the literature on biocontrol of *Anoplophora spp.* and discusses which are the strongest candidates for use in Europe. Some of the methods below could be useful for control, but are unlikely to be instrumental in achieving eradication. Below is a summary of the findings:

- **Entomopathogenic fungi:** Strong candidate as a biopesticide as fungal infection results in high mortality rates and has already been developed into a commercial product in Japan for *Anoplophora* control. *Beauvaria bassiana,* is already authorised for use as a biopesticide in the UK.
- **Parasitic nematodes:** Another strong candidate for use as a biopesticide due to high mortality rates and effective application methods have already been developed. Two nematode species, *S. feltiae* and *S. carpocapsae,* are also au for use in the UK and other parts of the EU.
- **Parasitoids:** Several parasitoid species (e.g. *Dastarcus helophoroides*) have been shown to be effective biological control agents in China but their specificity would need to be investigated further before use in Europe. Some native European parasitoid species (e.g. *Spathius erythrocephalus*) have potential to be used as a biocontrol.
- **Predators:** Two woodpecker species (*Dendrocopos major* and *Picus canus*) have been shown to be effective at controlling *A. glabripennis* numbers in Chinese forests and are also native to Europe.
- **Pathogenic bacteria:** Are currently not a strong prospect for use in biocontrol with no study yet advancing to field trials.

It was not possible within the scope of this project to undertake any significant R&D to attempt to fill any gaps in available information.

WP6: Investigating the biology of *Anoplophora* spp. in relevant EU climatic conditions

There were 5 main topics for investigation

1. Review current knowledge on the link between climate and the potential for *Anoplophora* population development.

2. Investigate the impact of EU climatic conditions on the life cycle of *Anoplophora* spp and make results available for incorporation into latest risk mapping/modelling effort.
3. Investigate if and how the layering and the vertical profile of meteorological variables could affect the short and long distance movement of *Anoplophora* (links to WP4)
4. Consider if factors like location, altitude, moisture of environment could affect *Anoplophora* dynamics
5. Update previous Climex models and/or produce other models with data from Objectives 1 - 4 to provide new models of the potential distribution of both species in the EU

Literature reviews were undertaken to understand the link between climate and the potential for *Anoplophora* population development and to feed the parameters into a revised Climex modelling approach. Temperature measurements were taken inside and outside trees over the course of the project to investigate if there is a large difference in temperature under different circumstances between where the larvae is feeding and the external temperature. It is of course the external temperature that is used to drive the modelling, so this could have a significant impact on the predicted life cycles.

The main findings were as follows:

- The rate of development of *Anoplophora* is restricted by temperature in the more northerly locations of its native and exotic range
- Models indicate that day degrees are likely to be the limiting factor for *Anoplophora* in northern Europe
- The minimum temperature for the development of the juvenile stages of both *Anoplophora chinensis* and *Anoplophora glabripennis* is around 10-13°C and the optimum temperatures for adults are around 23-24°C.
- By taking account of the natural variability in the rate of development in ALB larvae, on average it would take 3 years for complete development in the Netherlands, but 10% of a population could develop in 2 years, but it would take 6 years for 99% of a population to complete its development.
- Using simple application of day degrees, CLB and ALB are estimated to be able to complete their development in Denmark in 2, but with greater probability in 3 years.
- Larvae developing in tree branches or trunks exposed to sunshine could potentially develop around 1.9 times as fast as those in trunks or branches that are continuously shaded.

Contents

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Work Package 1: Project Management and Co-ordination

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Visit to Massachusetts to meet scientists and inspectors dealing with outbreaks and interceptions of Asian longhorn beetle

Dominic Eyre, 17 May 2011

22-25 March 2011

USDA-APHIS hosts
Alan Sawyer – Ecologist, Otis
Brendon J. Reardon – National program manager, Riverdale, Maryland
Christine Markham – Asian longhorn beetle program National Director, Raleigh, North Carolina
Clint D. McFarland – Project Director, Worcester
Dave Lance – entomologist / Assistant Director of Otis Laboratory, MA
Michael B. Stefan – National Science Program Leader, Raleigh, North Carolina
Patricia M. Douglass – State Plant Health Director, Wallingford, Connecticut
Phil Lewis – Entomologist working on ALB control methods
Scott Myers – Studies phytosanitary treatments, Otis
Scott Pfister – Director, Forest Pest Programs, Riverdale, Maryland
Rhonda Santos – ALB public Affairs, Worcester
Vic Mastro – entomologist / Director of Otis Laboratory, MA

EUPHRESCO visitors
Phil Northing – Co-ordinator of ANOPLORISK project, Fera
Derek McCann – Principal PHSI for Surveillance and Action, Fera
Dominic Eyre – Plant health entomologist, Fera
Hans Peter Ravn - Senior Scientist at Forest & Landscape, Faculty of Life Sciences, University of Copenhagen

Programme
Tuesday 22 March – At Otis Laboratory, Cape Cod, review of ALB programs, population ecology and discussions concerning climatic models and the ANOPLORISK project
Wednesday 23 March – Visited ALB outbreak site in Worcester, MA
Thursday 24 March – Tour of quarantine labs, discussion of hosts, phytosanitary treatments, chemical control
Friday 25 March – Met with Department of Homeland Security Officials and a tour of Boston Seaport

Adult Asian Longhorn beetle

Exit hole through metal tag made by ALB

Key points from visit

This visit gave us the opportunity to learn a lot about the biology and ecology of Asian longhorn beetle (ALB) and the strategies being used to eradicate it. We will disseminate this information to other EU partners in the ANOPLORISK project.

- The outbreak in Worcester, Massachusetts was first detected in 2008 and since then over 19,000 infested trees have been removed which is more than all the other outbreaks in the USA put together.

- Worcester is particularly susceptible to ALB due to the predominance of maple trees, favoured hosts of the beetle. Following tree losses due to a tornado in 1953, 70% of the street trees planted around the city were maples. The city is surrounded by forest that is dominated by Acers. The visit confirmed that ALB has infested woodland trees as well as trees within urban areas.

- Treating non-infested trees with imidacloprid by trunk or soil injection is a key part of the eradication strategy. This method has been shown to be 100% effective at preventing infestation and does not deter adults from landing on infested trees.

- Approximately 250 employed and contracted staff are involved in the ALB eradication campaign in Worcester. These staff are principally involved in surveying trees, removing infested trees and treating un-infested trees. Most of the funding for the ALB programme comes from Federal resources.

- The following collaborative initiatives have resulted from the trip:

1. Putting Anoplorisk colleagues in touch with Christine to discuss the training of dogs
2. Putting work package leader on ALB/CLB spread and modelling in contact with Al Sawyer to consider collaboration (e.g. sharing of model and Italian data)
3. Sending some acoustic sensors to get recordings of infested trees
4. Inviting APHIS representatives to the project meeting at the CLB outbreak site in Italy in Sept
5. Contacting Evan to discuss providing samples from the European outbreaks of ALB and CLB

ALB programmes in the USA

ALB eradication campaign have been funded in the USA, because 1) the science has supported the need to eradicate the pest and 2) experience and science have shown that it is possible to eradicate ALB. The dispersal rate is relatively low and it has a lifecycle of at least 12 months, both factors favour successful eradication.

APHIS is the lead regulatory agency for the ALB programme. In the early stages of outbreaks in New York and Chicago, different eradication strategies were used at each site. In order to try and bring best practice and a joined up approach to all of the eradication programmes a National Director was appointed in 2000 and there has been a National Strategy since 2002.

Management of the outbreak zone

The outbreak in Worcester was first detected in 2008. It was detected by a grandmother who had recently moved into the area and who was concerned that ALB adults may bite her grandchildren. She managed to establish that the beetles were ALB by using the internet and then contacted the USDA. The outbreak is thought to have started in the mid to late 1990s and is suspected to be related to wood packaging material delivered to local industry from Asia.

Christine Markham and Clint McFarland in front of the Worcester outbreak map

Section of the outbreak map for Worcester

The regulated area is now 104 miles^2 (266km^2) and enforced by local publicity, formal compliance agreements and random checks of vehicles leaving the area during the evenings and weekends. The outbreak covers five towns / suburbs – Worcester, Boylston, West Boylston, Shrewsbury and Holden. The City has been divided up into zones to help plan the management of the outbreak.

There are approximately 250 Federal, state and contracted staff working on the eradication campaign. Staff are given uniforms to give a unified image to the public. A tornado struck Worcester in 1953 and following this felled trees in public areas were mainly replaced with Norway maple, *Acer platanoides*. Norway maples now constitute 70% of all trees in public areas. Most of the budget (85%) for the eradication of ALB comes from federal resources. The ALB programme in Worcester has been well resourced due to the support of local politicians and industry.

There is one hardy-ornamental nursery within the infested area. The Worcester outbreak area was the first in the USA that included a nursery. A large number of the trees belonging to the nursery were destroyed. The state have bought non-host trees from the nursery for re-planting and have a project to develop systems to make the nursery stock safe. Garden centres are permitted to bring host material into the regulated area during the non-emergence period and this can be moved out of the regulated area before the next emergence period.

Survey Procedures

Each tree or group of trees is recorded with a reference number, although the trees are not individually labelled. Initially, un-infested trees were labelled, however, this is not done any more, because it gave the impression that trees were safe from infestation in the future.

The aim is to survey all trees within 1.5 miles (2.4km) of infested trees. The probability of ground crews detecting a low level of infestation is around 20-40%. This rises to 60-75% for tree climbers. Four negative surveys are required to confirm eradication.

Trees are rated for the level of infestation as follows:
Level A = oviposition sites only
Level B = oviposition sites plus 1-10 exit holes
Level C = oviposition sites plus 10-100 exit holes
Level D = oviposition sites plus 100+ exit holes

Within half a mile (approximately 800m) all host trees are surveyed visually. This is generally done by ground crews, but it is followed up with surveys by tree climbers and surveyors in bucket trucks in heavily infested areas.

Within 0.5 to 1 mile (800 to 1600m) of infested trees, the aim is to survey trees on an annual basis, however it is anticipated that the Worcester outbreak will not be delimited until 2012.

Sites which have a link to the infested area, e.g. tree service companies, landscapers are surveyed on an annual basis. Between 50-100 trees are surveyed on each visit. In order to ensure the quality of the survey work, surveyors are routinely challenged with simulated ALB damage, such as false egg laying sites or pits chipped into the bark. In addition, approximately 10% of properties are re-surveyed to check the accuracy of the survey work. It is important to note the location of this simulated damage to ensure that it is not interpreted as real damage at a later date.

In 2011 a lot of effort is being put into delimiting the outbreak. Surveyors are working from the infested areas outwards. The survey work is carried out year round. A total of 110 inches (2.8m) of snow fell over the 2010/2011 winter. Staff carried out the ground surveys using snow shoes.

Surveys in residential areas and woodland range from $322-778/acre ($800-1900/ha).

Ground surveyors
Ground surveyors work in teams. The team we met up with consisted of five people. They all had binoculars and each tree was inspected by more than one person. They use a palm top computer to record all trees present. The dbh (diameter at breast height) of each tree is measured and recorded. Experienced surveyors have the authority to label a tree for destruction, those with less experience can label a tree as showing suspicious symptoms and then others can confirm. GPS references are taken of infested trees, however, it is difficult to get an accurate reading from underneath the trees. The team that we met up with estimated that it would take five of them three hours to survey the 90 trees on the property = 10 person minutes per tree. Any piles of wood on the property are surveyed.

Tree climber explaining how to survey

Ground surveyors inspecting trees

Tree surveying

Tree climbers

We saw a team of four tree climbers at work. They use a rope placed around the join of a branch and trunk for support. Some of the tree climbers have got their jobs on the basis of their rock climbing skills, others are arborists who have trained to do the job. The cost of surveying a tree (12inch / 30cm dbh) with tree climbers is around $35-$50 in New Jersey and $70 in New York.

Tree Removal

More infested trees have been removed in Worcester than in all the other US outbreaks put together.

Table showing the number of trees removed due to ALB infestation or high risk of ALB infestation in the USA

Outbreak Area	Infested trees	High risk trees	Total
New York	6276	12192	18468
New Jersey	1500	221	1771
Illinois	729	21250	21980
Massachusetts	19265	10250	29515
Total	27821	43913	71734

Trees are removed with the assistance of cranes. These can lift trees from back gardens, over houses and on to the road. The chains /straps from the crane are attached to the tree, a tree surgeon then cuts off the base / large trunk of the tree with a chainsaw and it is lifted up an over houses. Branches are then lopped off the tree when it reaches the street. Most of the host removal work has been carried out by contractors. The cost of removing a tree with a diameter at breast height (dbh) of 33 inches (84cm) in Worcester is around $3000. The cost of removing trees in the next size category up (36inches+ / 91cm+ dbh) is $4800. The crane crew would be expected to remove three large trees in around 3 hours. Heavily infested branches are put aside for inspection by ALB programme staff.

Stumps are ground down to 8 to 9 inches (20 to 23 cm) below ground level. If this is not possible, a herbicide is applied.

Once trees in residential areas are identified as infested, a gap of a few weeks is left before the trees are removed to allow people to come to terms with the removal. Some people, who want to get rid of their own trees, have put a red mark on them in the hope that they will be removed.

We visited Dodge Park in Worcester where 65% of the trees are going to be removed. Trees can be removed at any time of the year. One exception occurred on Prall's Island, a bird sanctuary near New York where all removals had to be completed before the bird nesting season.

Infested tree being lifted over a house onto the street in suburban Worcester

Trunk of infested tree being cut through

Track laid down to allow forestry work

Trees being removed from woodland

Tree shredder

Tree removal in woodland

All host trees are being removed from 140 acres (57ha) of woodland on the edge of Worcester. The woodland included a mix of maples, hickory (*Carya*), oak and pine. A large part of the work is to lay down a wooden track for the heavy machinery to drive over. This reduces compaction of the soil. The trees are removed by a 'feller-buncher' which is used to grab trees and cut them off at the base with a 6 foot diameter (2m) spinning disk. The trees are then dragged out of the woodland, chipped and loaded onto waiting lorries for transport to a power plant. The stump of infested trees is removed. The stump of other host trees is treated with herbicide. A 'mower' then comes around to remove all small shrubs. Tree removal in woodland costs around $10,000 / acre or $25,000 /ha.

Holding and disposal of infested / suspect trees

The disposal facility used to be run by contractors, but now the work is being carried out by staff employed by the programme. There is a three stage process

1) Initially the trees are shaken to remove all soil etc.
2) Larger trunks (greater than 20 inch / 51 cm) are spilt
3) Trunks are then put through a wood chipper twice so that the chips are less than 1 inch (2.5cm) in two dimensions.

Wood chips are then considered safe. They are stored and are sold on to mulching companies or to 'co-generation' energy plants. During the period when adult beetles are not emerging, there is no set schedule for chipping. However, during the flight period, infested logs must be chipped within 48 hours. The machine used for chipping cost around $750,000. It uses between $30-50 gallons of diesel (110-190 litres) an hour. The site functions year round.

Tree trunk splitter

Tree trunk chipper

Chemical treatments

ALB staff must have the approval of property owners before they treat trees with insecticides, however approval is not required for removing trees. In New York, residents were informed that their trees were going to be treated and asked to contact the authorities if they had objections. Many of the trees being treated in Worcester are sugar maples, *Acer saccharum* which can be tapped for syrup. When these trees are treated with imidacloprid a tag is attached to warn residents not to eat syrup from the trees. As yet, the gap between treatment and the time at which the syrup can be safely consumed has not been established, therefore treated maple trees have been indefinitely deemed as unsafe. Water courses in areas where treatments are being carried out are being monitored for increased levels of imidacloprid.

All potential host trees within 0.25 mile (400m) of infested trees are treated with imidacloprid. APHIS used to use 4mL Imicide capsules with the Mauget trunk micro-injection system. However, using this system, it was necessary to wait beside trees until the material had emptied out of the micro-injection unit into the tree. The USDA had to employ 'tree-sitters' to watch treated trees until the insecticide had been absorbed, this was expensive and led to negative reports in the media. The USDA have also trialled drenching tree bases with imidacloprid. However, where there is compacted soil, which is often the case in urban areas, the solution tended to form pools on the surface of the ground which would be a hazard to humans, pets and wildlife.

Other compounds have also been tested for their toxicity to ALB. Although acetamiprid, thiacloprid and clothianidin have also been found to be effective, imidacloprid has been found to be the most consistent compound.

Equipment for injecting imidaclopird into trees

Warning on maple tree

Two methods of applying imidacloprid are used now:

1) **Soil injection** – this is the most effective method. A solution of insecticide is injected into the soil around the base of tree trunks. There are a number of restrictions on using this method including i) treatments should not be made where there is a danger of runoff , ii) treatments should not be made in close proximity to vegetable gardens and fruit bearing trees iii) trunk injection is used in woodland, because there is a limit to the amount of imidacloprid that can be applied to the soil per unit area.

There has been some local opposition to using soil injections in some areas and in such areas trunk injection has been used as the alternative.

The target level for imidacloprid to be effective against ALB is 7 parts per million in foliage. APHIS have tested soil applications to small and larger trees at three rates. The results are shown in the table below.

Amount of imidacloprid (parts per million) in the foliage of trees treated by soil injection

Application Rate	Small DBH trees (ppm)	Large DBH trees (ppm)
0.25 full rate	12	<7
0.5 full rate	15	8/9
Full rate	40	22

2) **Trunk injection** – In this system a (7/32 inch diameter hole = 5.5mm) is drilled at a slightly downward angle 3/4 inch into the wood (1.9 cm), approximately 6 inches (15cm) above the soil line. The number of holes required is determined in proportion to the diameter of the tree. The imidacloprid solution is then injected under pressure into the tree. An average time to inject a tree (10inch / 25cm dbh) is less than five minutes. The equipment costs around $3000.

Trees are treated between April and early June. Trials have shown that applications up to the time just before adult emergence can be effective.

Soil injections and trunk injections are effective against young larvae and adults feeding on the foliage, but are not effective against older larvae that have bored deeper into the trunk. Chemical treatments have been 99.9% effective at preventing infestation and the failures are thought to relate to improper applications or applications to trees in poor health. Trials have been carried out to determine whether adult ALB are deterred from feeding / laying eggs on trees with have been treated with imidacloprid and they have found that there is **no deterrent effect**.

No phytotoxicity has been observed when treating trees as recommended by APHIS. Normally, trees would be treated once a year for three years. However, some trees have been treated six times and no damage has been noted. However, iIf trunk injections are made with excessively high pressure, this can cause the trunk to crack open.

The cost of injecting trees varies from $4.00 to $8.50 /per inch (2.5cm) dbh depending on location. City centre locations are the most expensive to treat. Soil injections are cheaper and cost around $2.50 / inch dbh.

Over one million treatments have now been applied to about 350,000 trees in the USA, approximately 400,000 by soil injection, 180,000 by liquid trunk injections and 440,000 injecting trunks with Mauget capsules.

Foliar sprays / bark sprays are not currently used in ALB eradication programmes in the USA due to potential public opposition to their use.

Ann Hajek at Cornell University has tested the use of bands wrapped around trees and coated with an entomopathogenic fungi. Entomopathogenic fungi are grown within non-woven fibre bands and placed around tree trunks and branches where ALB adults become inoculated when walking across bands. *Beauveria brongniartii*, *Beauveria bassiana* and *Metarhizium anisopliae* have all been tested. This method is thought to have more promise for citrus longhorn beetle than ALB, because CLB tend to be active at the base of trunks whereas ALB can be active over the trunk and all the branches.

Re-planting

The loss of trees has had a major visual impact on some neighbourhoods and the loss of trees leads to an increase in the cost of keeping houses cool in summer and warm in the winter. Thus replanting has been identified as imperative.

The USDA's Forest Service has invested $4.5million in a replanting programme. The trees used in this programme are typically 14 feet (4m) tall with a stem diameter of 1-2 inches (2.5-5cm). Once in place, the care of trees is the responsibility of the property owner. Anyone within the regulated area can apply to have a tree. The replanting periods are Apr 11th – end June/early July and the end of August until mid November. Approximately 45 local people who have been out of work for a while have been employed on seasonal contracts to do the re-planting work. The location of re-planted trees has been tagged with a GPS to form part of a database.

In addition, the Worcester tree initiative was launched in January 2009 with the aim of planting 30,000 trees within five years. This is a charitable scheme which has partly been funded by donations from industry. Smaller trees are being planted by members of the public and voluntary groups such as the scouts.

Table of species used for re-planting in the Worcester ALB quarantine area

Shade trees		**Flowering trees**	
Honeylocust	*Gleditsia triacanthos*	Serviceberry	*Amelanchier* sp.
Sweetgum	*Liquidambar styraciflua*	Dogwood	*Cornus* spp.
Hophornbeam	*Ostrya* spp.	Cherry	*Prunus* spp.
Blackgum	*Nyssa sylvatica*	Crabapple	*Malus sylvestris*
Yellowwood	*Cladrastis kentukea*	Fringetree	*Chionanthus*
Hornbeam	*Carpinus* spp.	**Evergreens**	
Tulip tree	*Liriodendron tulipifera*	White fir	*Abies concolor*
Pin oak	*Quercus palustris*	Juniper	*Juniperus* spp.
Red oak	*Quercus rubra*	White pine	*Pinus strobus*
Basswood	*Tilia americana*	Colorado spruce	*Picea pungens*
Beech	*Fagus* spp.		
Dawn redwood	*Metasequoia glyptostroboides*		
Larch	*Larix* spp.		

In addition, the following species are listed as recommended replacement trees in the USDA's 'Landscapers guide to ALB and its host trees' Revised 2005: Japanese lilac (*Syringa reticulata*), Kentucky coffee tree (*Gymnocladus dioicus*), dawn redwood, southern catalpa (*Catalpa bignonioides*), English oak (*Quercus robur)*, swamp white oak (*Quercus bicolour*), white oak (*Quercus alba*), bur oak (*Quercus macrocarpa*), basswood, tulip tree, American hophornbeam (*Ostrya virginiana*), serviceberry, gingko (*Gingko biloba*), bald cypress (*Taxodium distichum*), honeylocust, Turkish filbert *(Corylus colurna*) and Littleleaf linden (*Tilia cordata*).

Publicity

APHIS has a Legislative and Public Affairs Officer, Rhonda Santos, who is based in Worcester, but also responsible for NY and NJ. She deals with all press enquiries regarding the outbreak and is also involved in interactions with local, state and Federal politicians. Local people and politicians have asked the likely timescale of the Worcester outbreak. However it has not been possible to answer this question definitively yet, because the delimitation of the outbreak has not been completed.

There is an annual public outreach campaign which is timed to start at about the time when adult beetles are likely to be around. This includes adverts on buses, bus stops, billboards, in newspapers, public service announcements on local radio and on the web.

Meetings have been held with legislators before public meetings so that legislators are well informed.

If ALB programme staff are approached by journalists on the street, they are advised to explain what they are doing, but contact the public affairs officer if they would like an interview.

Public meetings
Regular public meetings have been held in Worcester to inform residents about the ALB infestations and the eradication methods. Initially some of these meetings were attended by 500 people, but as the programme has gone on attendance has dropped and 20 attendants is typical.

Telephone enquiries
APHIS have found that three to five individuals on staggered shifts can handle calls from a community of 30,000 people.

Publicity materials
A wide range of publicity materials have been produced including:
i) A 26 minute DVD describing the ALB outbreak in Worcester
ii) An online game – freeze and collect. Players have to distinguish ALB from other beetles and collect them.
http://www.beetlebusters.info/beABeetleBuster.php
iii) Colour guides for Landscapers to help them to detect symptoms of ALB
iv) Lesson plans – so that teachers can teach school children about ALB from 3rd grade (aged 8)
v) Temporary ALB tattoos
vi) Mugs to celebrate the eradication of ALB in Chicago with disappearing ALB

Measures of the success of the publicity campaign

- There have been few negative articles in the local media
- Elected officials have been supportive
- There has been good feedback at public meetings
- Many people have made use of the publicity materials
- The programme is still high priority for Federal funding

Host list for ALB

Alan Sawyer provided us with his latest annotated host list for ALB.

Preferred hosts in US: *Acer, Aesculus, Betula, Salix* and *Ulmus*
Occasional to rare hosts in US: *Albizia, Cercidiphyllum, Fraxinus* [all the *Fraxinus* records were from one site and there is some doubt as to whether complete ALB development would be possible], *Platanus, Populus* and *Sorbus*
Questionable US records: *Celtis, Hibiscus, Malus, Morus, Prunus, Pyrus, Quercus, Robinia* and *Tilia*
No US record: *Alnus, Eleagnus, Koelreuteria* and *Melia.*

Tree Genera infested by ALB at different outbreaks sites

Host	New York	Illinois	New Jersey	Total
Acer	4072	1041	600	5713
Ulmus	78	223	14	315
Salix	94	17	12	123
Aesculus	65	6	0	71
Betula	49	9	1	59
Platanus	6	0	0	6
Fraxinus	0	64	0	64

Infestation rate adjusted for host abundance in Illinois

Host	No. Infested	Non-infested	Total	Proportion infested	Index (takes into account nearest hosts)
Acer	1046	5535	6581	0.159	0.309
Ulmus	218	2324	2542	0.086	0.167
Salix	9	94	103	0.087	0.169
Aesculus	16	103	119	0.134	0.260
Betula	8	92	300	0.027	0.052
Fraxinus	64	2802	2866	0.022	0.043

Studies on ALB movement and behaviour

History of outbreaks in the USA and the number of introductions

New York it is believed there were two separate introductions into
New York City (1996-2010) – only one infested tree was found in 2010.
It is believed to have been spread by a tree care company from New York to Long Island (1996-2007)
Jersey City (New Jersey, but believed to be related to NY City infestation) (2002-2003)

Illinois – there were two maybe three separate introductions. Beetles were found 1998-2003, and a single adult beetle was found at a new location in 2008.

New Jersey – all outbreaks are believed to stem from one introduction
Middlesex County (2004-2005)
Union County (2005-2006)
Richmond County, NY (2007, 2009)

Massachusetts
Worcester (2008-2011)
Boston (2010) – Six infested trees have been found

Life history studies
Ratio of number of emerging adults to initial number of egg pits is approximately 0.25

Finite rate of population increase (R_0) when resources are not limiting is approximately 3.0
Parameter estimates used in population model are as follows:

There is a 50:50 sex ratio for ALB.
95% of females mate
The survival to maturation is 95%
Each female creates 35 egg pits
There will be 0.75 eggs / pit
Egg survival is 85%
First instars survival is 80%
Middle instars survival is 85%
Biggest instars larval survival is 60%

Population spread

The infestation in Cataret, New Jersey was thought to have been started by ALB emerging from wooden spools used to transport paper.

Location and level of infestation of host trees in Cataret, New Jersey

No. Of trees	Exit hole count	Estimated year of first infestation	Mean distance from foci (m)
79	0	2004	632
28	1-10	2003	476
16	11-30	2002	125
2	31-70	2001	116
4	17-150	2000	52
2	151-310	1999	106
1	311-630	1998	85
1	631-1270	1997	0

APHIS found a new infestation of ALB in Boston in 2010. Two exit holes and two adult ALB were found. Life history data was used to predict how many emigrating females there might be under the assumption that there were two founders of the outbreak in 2008. The following estimates were made:
In 2009 there would be 1 emigrating female
In 2010 there would be 3 emigrating females (with a maximum of 10)
In 2011 there would be 9 emigrating females (with a maximum of 35)

In the initial stages of an ALB outbreak the population is likely to remain highly focused and spread will be slow. Outbreaks typically remain undetected for 5-7 years, although it may be possible to detect an outbreak within 2-3 years. After 5-7 years, beetles may spread over 100's metres or even a few km.

Heat treatments and fumigation of wood

APHIS trials have shown that:

- In a study involving 302 ALB larvae treated for 30 minutes at 56°C, all larvae died.
- a small proportion of emerald ash borer (EAB) larvae can survive a heat treatment of 55°C for one hour

- The new APHIS standard for EAB is 60°C for 60 minutes
- APHIS now have a standard for treating firewood
- To get the core of wood up to 56°C can take around 3-4 hours depending upon the original temperature of the wood and the size of the wood
- APHIS tested whether the submersion of black ash in water for 8 weeks, as practiced by Native Americans for transporting wood, had any impact on EAB. However, it does not have an impact on emergence.

Location and symptoms of infestation

Young larvae damage the phloem and can girdle trees. Older larvae can damage the xylem leading to structural damage. ALB won't attack dead trees. In a study in Illinois, there was no evidence that ALB has a tendency to attack stressed trees. They don't seem to attack the outer portions of the tree canopy.

Oviposition scar

Internal damage by numerous ALB larvae

ALB exit holes

Healed over exit hole

Field studies into ALB dispersal

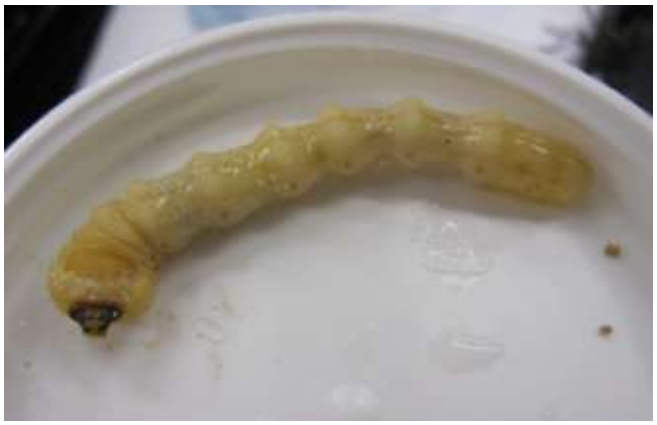
- Mark release studies in China have demonstrated that ALB tends to fly towards and land upon tree shaped objects. They will then spend longer feeding on favoured hosts such as Acers (*A. saccharanum* and *A. platanoides*) in preference to poplars
- No long range pheromones have been detected for ALB despite a large research effort over the last 14 years.
- Traps containing a male produced pheromone have been tested in Worcester. In 2009, 89 traps were used and 9 beetles were caught.

Studies on acoustic detection

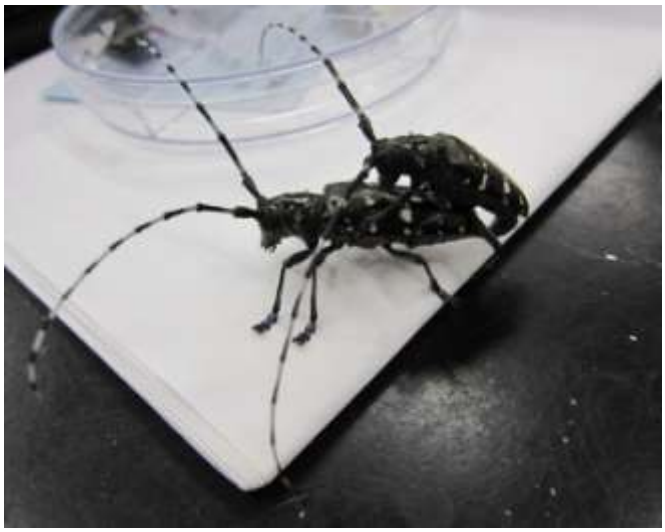
The US studies on acoustic detection have not yet led to a system that can be use in the field.

Otis lab quarantine facility

We were shown the quarantine facilities at Otis. ALB are kept in plastic jars similar to those used for storing sweets. Muslin cloth is placed inside for the females to lay their eggs on. In laboratory conditions, female ALB can be very long lived, for example there was one female in late March that had emerged the previous July. Females lay about 8 eggs per week and take 9 days to hatch.



ALB larva



ALB mating

ALB pupa

Other species held in the lab are *Lobesia botrana* (grapevine moth), *Sirex noctilio,* European gypsy moth, emerald ash borer and some parasitoids of this pest including *Spathius agrili* from China, a *Spathius* spp. from Russia and *Atanycolus picipes.* USDA and university scientists have found than emamectin benzoate is highly effective against emerald ash borer, but effectiveness of imidacloprid against that insect is variable. The Otis Lab has a facility for mass-rearing gypsy moth for the production of virus for control. At peak, 2-3 million moths were produced per year. This peak production occurred when the use of the Sterile Insect Technique was being developed for Gypsy moth. However, this wasn't considered cost effective once effective formulations of *Bacillus thuringiensis kurstaki* came available.

ALB legislation in the USA

- There has been a change in the plants for planting regulations. Many taxa will be put on a list of banned imports until a PRA has been produced
- A Federal Order from 2009 states that plants with a diameter of <1cm are not regulated for ALB, but artificially dwarfed plants are exempt from the 1cm threshold. There are no records of ALB developing within plants with a diameter of <1cm.
- Acers from Japan and Europe are banned.
- Artificially dwarfed plants are subject to post-entry quarantine. They must be held in greenhouses with screened vents and closed doors for 2 years.
- In 2009, the National Clean Plant Network was set up to protect U.S. specialty crops such as grapes, nuts, fruit trees, citrus and berries from the spread of economically harmful plant pests and diseases.

Meeting Department of Homeland Security and tour of Boston Seaport

- Customs and immigration became part of Homeland Security in a review following 9/11. Staff have the combined role of looking for pest and disease problems, material infringing intellectual property rights, CITES material, illegal drugs and contraband.
- Ships manifests must be sent to the US, 24 hours before the ship leaves the originating port. In some cases, holds are put on material before it has left port.
- If wood-borers are found on wood packing material (WPM) there are two options: 1) returning the whole shipment to the country of origin 2) it can go to a 'strip out area' where the product will be separated from the WPM. The WPM is sent back to the country of origin.
- Any shipment of heavy machinery etc. is likely to have WPM with it
- Methyl bromide fumigation is an option at Boston seaport and some containers are fumigated as a matter of course
- High powered X-rays are used to view the contents of shipping containers for items of security concern, drugs, plant health risks etc.

Boston seaport

Stone with partially marked wood packaging from Greece

Acknowledgements

We are very grateful to all the staff from APHIS for creating and producing an interesting and valuable programme, for their comments on a draft of this report and for their hospitality. This trip was funded by the Anoplorisk funders – FERA plant health policy and the Danish government.

Notes from visit to *Anoplophora* outbreaks in Italy on 12 and 13 Oct 2011

Dominic Eyre, Fera

Participants:

Hans Peter Ravn, Ruud van Donk, Dominic Eyre, Fera, UK, Giuseppino Sabbatini

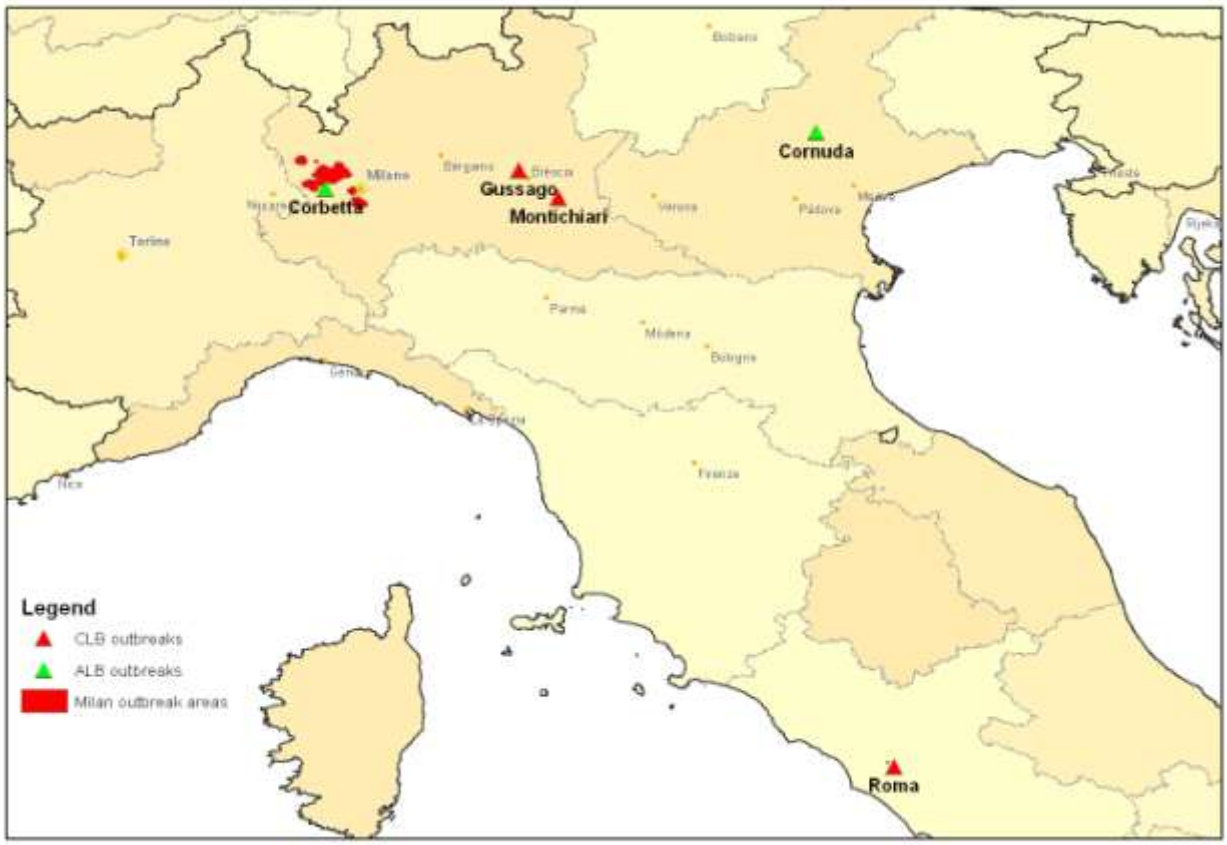
Itinerary

- Oct 11 Travel to Milan
- Oct 12 am: visit outbreak site and discuss outbreak; pm travel to Verona
- Oct 13 travel to Cornuda, study infested tree, presentation in municipal buildings in Cornuda, visit quarantine facility, travel back to Verona
- Oct 14 travel back to UK

Key points from visit

- Public support for the eradication campaigns of CLB in Lombardy and ALB in Veneto was low to start with, but after PR campaigns there is general public support.
- Public awareness of CLB in Lombardy is now very high. A new outbreak location was reported by 11 members of the public.
- Over 3 million trees have been surveyed in Lombardy
- Demarcated area in Lombardy now covers 400 km^2, there is a policy of eradication for the new outbreaks and containment in the core areas.
- The outbreak of ALB around Cornuda covers 70 km^2. With the detection rate of around 80% and the removal of infested trees it will be difficult to eradicate the pest with current resources
- ALB has not been found during surveys of the forested areas around Cornuda

Map of *Anoplophora* outbreaks in Italy



The data for demarcated area around Milan has been copied from:

http://www.regione.lombardia.it/cs/Satellite?c=Redazionale_P&childpagename=DG_Agricoltura%2FDetail&cid=1213305544054&pagename=DG_AGRWrapper

Detailed maps of the CLB outbreak in Rome are available at:

http://www.lazioanoplophora.it/index.php/carto.html?task=viewcategory&catid=7

Anoplophora chinensis outbreak in Lombardy

Viewing Infested trees and tree removal operations – Tuesday 11th October 2011

We met up with Beniamino Cavagna (PPS Lombardy), Mariangela Ciampitti (ERSAF Lombardy), Costanza Jucker (Researcher at University of Milan) and Matteo Maspero (Researcher at Fondazione Minoprio) in the garden of old hospital in Parabiago. This area is thought to be one of the first that was infested with CLB. We were shown the symptoms of infested trees, in addition to tree felling and root removal operations. During surveys, infested trees are mapped with GIS and marked with a band and a paint mark. There are twenty teams of two agriculture graduates who carry out the survey work. They are all trained for a week before starting surveys. Most (80-90%) exit holes are believed to be under ground level. In 2011, 200,000 trees were surveyed in Lombardy demarcated

area of which 1,000 were found to be infested. A total of 3 mill trees have been surveyed over the last four years 2008-2011.

In preparation for laying eggs, female *A. chinensis* create one longitudinal slit with their mandibles and then the perpendicular part of the 'T-shape' is created when she pushes her abdomen underneath the bark in preparation for laying an egg (Fig 1). Larvae tend to push frass out of the tunnels of trees where there are natural breaks in the bark thickness. In the Netherlands, young Acers e.g. 1-2cm diameter have been found at import to have corkscrew like damage that has been caused by CLB (similar to Fig 3, but twisting around the stem).

Fig 1: T-shaped slit cut into bark for oviposition by a female CLB

Fig 2: Tunnels formed by CLB larvae in a tree root

Fig 3: Tunnels formed by CLB larvae in a tree root

Fig 4: CLB tunnels in a cut trunk

Fig. 5: CLB larva removed from the base of a tree in the field

Fig. 6: CLB adult emergence hole

Fig 7: Frass close to the base of a tree infested with CLB

Fig 8: Matteo Maspero locating CLB larvae within a tree stump

We met the Austrian dog training team, Ute Hoyer-Tomiczek and Gabriele Sauseng. They were in Italy to train their dogs. One of the dogs is beagle, one is a mongrel and two are Austrian hunting dogs. It takes around a year to train dogs for Anoplophora detection.

Figs 9 to 11 Ute and Gaby from Austria telling Ruud van Donk and Hans Peter Ravn about training dogs to detect CLB

Tree removal

We were given a demonstration of how trees are removed in accessible areas. The trees that we saw being felled were all relatively young sycamores. They were cut close to the base with chainsaws (Fig 12) and then the stumps were ground down (Fig 13 and 14). At the start of the eradication campaign, tree roots were removed by diggers (Caterpillars / JCBs), but this was discontinued when it was found to be too expensive and damaged underground services (pipes and wiring). Infested trees are felled in winter. Approximately 18,000 trees have been removed over the course of the eradication campaign and 17,000 of these have been replaced with non host trees. The trees were loaded on to a lorry and taken to a central collection point.

Gas and electricity pipes, plus buildings, especially historical buildings can make treatment of roots difficult, impossible or expensive. Most (70%) of the infested tress have been found on private land which has made access difficult on occasions and also increased the cost of eradication measures. A team of 50 people are employed to remove trees in Lombardy and they can remove around 200 trees per day. Tree removal takes place between 1st October and 31 March. When necessary, police are used to allow the enforcement of eradication procedures.

Destruction of infested trees

In the middle of the season of tree removal, wood chipping is carried out over 24 hours and the wood chips are piled approximately 10m high. Trees are passed through a wood chipper and then used in power production.

Fig 12: Sycamore trees being cut down with chainsaws

Fig 13: Stumps of infested tree being ground down

Fig 14: Partly ground stump of infest tree, the material left after grinding can be seen on the left

Fig 15: Infested trees being loaded for transport to a secure area

Chemical and biological control

During the flight season, trees are treated three times with pyrethroids which are applied as anti-mosquito treatments. The parasitoid wasp *Aprostocetus anoplophorae* (Hymenoptera: Eulophidae) has been shown to be capable of providing 80% control of CLB in the laboratory. It has a patchy

distribution in the outbreak area, but more wasps are being bred in a laboratory and the authorities hope to have distributed the pest across the outbreak area by 2013. Costanza Jucker from University of Milano is studying the life cycle of CLB and parasitoids.

Lifecycle of CLB in Italy

In Lombardy, most CLB are thought to take 2 years to develop. In Rome CLB take one year to two years for development. The variation in development time is thought to be partly determined by the time at which of year in which the eggs are laid. If eggs are deposited in June-July => one year, if eggs are deposited in September => two years. But other reasons, currently unknown, can be involved in this aspect.

The dispersal rate for CLB is usually very short. They stay in the same tree, or in trees close together, until the density of the females get too high. It is thought likely that females will generally fly up to max 400m. There has been one documented example of dispersal of 600 m.

Publicity

Over 1 million letters have been sent to householders in Lombardy to inform them about the CLB outbreak. Initially, there was a lot of public opposition to tree removal, but now there is generally good co-operation. An example of the current levels of awareness and co-operation is that a new outbreak in Gussago was reported by 11 different local people. The large majority (>99%) of pest reports are found to be false alarms. *Monochamus* sp. are commonly mistaken for *Anoplophora*. Four people are employed to take calls from the general public.

The scale of the eradication campaign

In 2001 and 2002, householders were responsible for removing infested trees from their own gardens. However there were difficulties with inspection / quality control, for examples trees were being cut at the wrong height etc. Since 2004, ERSAF (Ente Regionale Per Servizi All'Agricultura e alle Foreste) have been responsible for all tree removal.

2008-2010 there was a budget of €10 million for tree removal, surveys etc.

2011 Jan-April – No tree removal took place due to a lack of funds

2011 Sep – 2013 A budget of €6 million has been agreed. The current focus of activity is on the fringes of outbreak zones rather than in the central areas which are more heavily infested.

The outbreak in the Milano area was discovered in 2006 and new attack in Brescia in 2008. The demarcated area covers approximately 400km^2 at present.

By 2013, approximately €20 mill will have been spent on the eradication campaign since 2005, including €1.5 mill for CLB research. The resources for the eradication campaign have all come from the Lombardy government.

Signs of successful effect of control: Area of infested trees is going down. 80% of infested trees were in 10 municipalities. In many municipalities, no CLB have been detected in since 2010.

Nurseries in the outbreak zone and movement of firewood

There are 10 nurseries in the demarcated area. All have a buffer zone around them or physical protection, including a nursery that receives bonsais from Japan. The movement of firewood over long distances is not considered to be a problem in Lombardy, because burning firewood outdoors is prohibited.

Outbreak of *Anoplophora glabripennis* in Cornuda, Veneto NE Italy

History of outbreak

The ALB outbreak in Cornuda was discovered in June 2009. By studying the age of exit holes, researchers have been able to conclude that the outbreak is most likely to have begun in 2004. Cornuda is at the boundary between the start of the foothills of the Alps and the north-eastern edge of the plain that stretches across northern Italy. The beetles are genetically similar to the ALB at outbreaks in Austria and New York state and different to the ALB that have been found in Lombardy (which have been linked to ALB populations in Korea). In 2010, ALB was discovered in the nearby town of Maser. There is a waste collection point for gardeners close to where this outbreak was first detected. In 2011, 1000 trees were inspected outside of the established demarcated zones around Cornuda and Maser. The trees were chosen on the basis of the distribution of host species and their proximity to roads. One infested tree was detected that was 3km from the nearest infested tree. Therefore, there is a possibility that there is a low level population spread over a wider area. After this find the demarcated area was extended to 7000 hectares.

Eradication procedures

There are two teams of 3 people who conduct the survey work from the ground. Between March and November there are 18 tree-climbers from the Veneto Forestry Service who are responsible for tree removal and tree climbing inspections. Six of these work year round. There are two students working year round on the ALB outbreak and one researcher who spends 3-4 months a year working on the eradication campaign. The owners are offered replacement trees when trees have been removed and approximately 50% have been replaced. The Genera considered to be hosts are *Acer, Ulmus, Betula, Salix, Aesculus, Prunus, Cerdidiphyllum, Populus,* (other Genera that were surveyed during the first year are *Carpinus, Fagus and Platanus* but no infested trees were detected and so these genera are not surveyed any more).

Trees are all surveyed from the ground and any trees that are suspected to be infested are then surveyed by climbers (1200 have been surveyed by climbers). The tree surveys are about 80% effective. Containment is considered a realistic aim, but resources are not thought to be adequate for

complete eradication. There are about 18,000 suitable host trees in the outbreak area which are checked annually, and only the infested ones are cut and chipped.. The outbreak area covers a combination of the town, agricultural areas and forested areas. No infested trees have been detected neither in random surveys of the forest nor along the forest edges.

Fig 16: Exit holes in infested sycamore tree in Cornuda

Fig 17: Frass pushed to the surface of tree by ALB larvae and liquid oozing from scarred parts of the tree (photo Ruud van Donk)

Fig 18: Large pile of frass that had fallen on to a stone below the ALB infested tree

Fig 19: Andrea Battisti (Padua Universtity) discussing ALB damage

Fig 20: Dying twigs visible at the extremities of the tree

Fig 21: Frass being collected for laboratory analysis by arborist from the forestry service

Number of trees surveyed and infested around Cornuda (updated at May 2011)

Species	No. Surveyed	No. infested	Proportion infested
Acer	2499	195	7.8
Ulmus	1351	250	18.5
Betula	1247	127	10.2
Salix	1550	59	3.8
Aesculus	74	14	18.9
Prunus	3006	9	0.3
Cercidyphyllum	6	2	33.3
Populus	812	3	0.4
Carpinus	1085	0	0
Fagus	165	0	0
Platanus	668	0	0
TOTAL	**12463**	**659**	

Publicity / public relations

Initially there was a lot of public opposition to the eradication work, but now the public are generally supportive. Working in schools has been found be very effective, because children are generally very inquisitive and they will report what they have learnt at home. Talking to gardening clubs is another effective means of communication. There have been hundreds of calls from the public with ALB records, but 99% of the reports have been false.

Observations and conclusions from research

There is a quarantine unit within two lockup garages in the quarantine area and a culture of ALB is being kept there.

- High humidity is very important for larvae up until their 3rd stage, but after this it is not so crucial
- In 50 oviposition pits made in maples only five have been found to contain eggs, whereas in 10 oviposition pits in Ulmus, nine have been found to contain eggs
- There are two types of scars at the oviposition sites 1) a narrow slit (Fig 22) – which are normal in trees with thin bark 2) a rounded scar (Fig 23) – which are normal in trees with thicker bark
- Adults will feed on the bark of larger trunks if they don't have the option of feeding on smaller twigs
- ALB population in Veneto is mainly monovoltine.

Fig 22: Narrow oviposition scar

Fig 23: More rounded oviposition scar

Outbreak of Anoplophora chinensis in Rome

The outbreak of CLB in Rome was discovered in July - August 2008 when some adults were found in the San Sebastiano Park and shortly after 12 trees (*Acer negundo* and *Aesculus hippocastanum*) were found with dozens of exit holes. A survey of trees within 1km was completed over a month and the only infested tress found were in San Sebastiano Park and some private gardens close to the park. The main hosts have been *Acer negundo*, *Aesculus hippocastaneum, but Corylus avellana*, *Platanus* sp. and *Ulmus* sp. were also attacked. Infested trees and all others susceptible ones within 20m have been removed. The exit holes would generally not be higher than 1m from the ground, but some holes have been found 6m above ground. Generally the exit holes are at soil level and so, during monitoring, it is necessary to pull up the grass around the base of trees. The origin of the outbreak is thought to be a bonsai grower in 2004/2005. More details on the history of the outbreak site in Rome are to find in the paper of van der Gaag et al., 2010 - Evaluation of eradication measures against *Anoplophora chinensis* in early stage infestations in Europe (EPPO Bulletin 40, 176–187) and on the web site www.lazioanoplophora.it with the complete mapping of the infested area.

There is a gap of 350m between the main hot spot (with infested trees showing frass and exit holes) and the furthest infested trees (at time of detection showing only frass) with no infested trees in between. In 2010, one infested tree was found 700m away from the original infestation. Some areas where it was not possible to destory the root system have been covered with a wire net to prevent CLB adults escape from the emerging site.

An experimental cage has been built at the cost of approximately €30,000 to study CLB. The steel mesh is 1mm in diameter and the mesh size is 4/5mm square. It has a double door with the wire net stretching 50cm below the soil.

In Rome, CLB has been shown to infest roots with a diameter of <2cm.

Outbreak of ALB in Austria (information from Ute Hoyer-Tomiczek)

In 2008, full development of ALB was confirmed in *Fraxinus* in Austria. No *Ulmus* have been infested in Austria, which contrasts with Cornuda where many have been infested. There have been mixed infestations of *Aromia*, *A. glabripennis* and *Cossus cossus* in the same trees. ALB has been detected in the roots of ALB in Brannau.

Acknowledgements

We are grateful for the EUPHRESCO funding for this visit and to all our hosts in Lombardy and Veneto for taking the time to show us how they are tackling *Anoplophora*.

Work Package 2: Development and testing of non-destructive detection methods

Participants contributing to WP 2: P1, P2, P3, P5, P6, P9

Work of the participants was focused on the following issues.

P1: Acoustic Detection of feeding larvae in host plants
P2: Assessment of X-Rays for their ability to detect *Anoplophora* infestation in young and mature host plants and in cut wood
P3: Biological Sensors: Education and assessment of Detection Dogs
P5: Contribution to sound library of P1
P6: Assessment of image guided methods for their ability to detect *Anoplophora* infestation in young and mature host plants and in cut wood
P9: Contribution to sound library of P1

Milestone or Deliverable		**Target date**	**Achieved**	
Number	**Title/Description**		**In Full**	**On-Time**
D2.1	Literature review on image guided detection methods	May 2011	Yes	Yes
D2.2	Produce summary listing of promising imaging based methods to be investigated	May 2011	Yes	Yes
D2.3	Development of a model system for the combination “model plant”, “variable inside holes” representing “different sizes of larval galleries”, different live stages (sizes) of insects.	Jul 2011	Yes	Yes
D2.4	Assessment of chosen methods according to D2.2 using the model system developed in D2.3.	Dec 2011	partly	partly
D2.8	Development of an improved algorithm for X-ray detection	Mar 2011	Yes	Yes
D2.9	Validation of the algorithm, based on available images in database	Apr 2011	Yes	Yes
D2.10	Training of inspectors / stakeholders	May 2011	Yes	Yes
D2.11	Evaluation of the tested methods concerning efficacy, applicability and costs	July 2011	Yes	Yes
D2.12	Proof of principle of CT as a detection tool for 3D detection	Dec 2011	Yes	Yes
D2.14	A robust, tested x-ray detection technique, and a SOP for using it (standard operating procedure)	Dec 2011	partly	partly
D2.15	A robust, tested acoustic detection technique, standard equipment and standard operating procedure	Dec 2012	partly	partly
D2.16	Detailed validation and clarity on the scenarios where this technique is appropriate	Dec 2012	Yes	Yes

Milestone or Deliverable		**Target date**	**Achieved**	
Number	**Title/Description**		**In Full**	**On-Time**
D2.18	• Evaluation of the potential for the use of ALB/CLB detection dogs in o areas of ALB or CLB infestations inspection of wood packaging material inspection of imported plants in addition to the visual inspection by inspectors and tree climbers	Dec 2012	Yes	Yes
M2.1	Final decision of imaging based methods to be tested.	May 2011	Yes	Yes
M2.2	Model system in place	Jul 2011	Yes	Yes
M2.3	Completion of laboratory and field tests on imaging based methods	Aug 2012	Yes	Yes
M2.4	Completion of a robust, tested x-ray detection technique, and a SOP for using it	Dec 2011	partly	partly
M2.5	Expanded library of feeding sounds collected and assessment of potential for speciation concluded	Jan 2012	Yes	Yes
M2.6	Conclude experiments on the validation of the detection methods and investigating limits of detection	Oct 2012	Yes	Yes
M2.7	Amended, practical, robust acoustic detection system available for utilisation by inspection services	Jun 2012	Yes	Yes
M2.8	Conclude the review on the future potential of acoustic systems in monitoring for A*noplophora*	Mar 2012	Yes	Yes
M2.9	Improvement of the assurance of the dogs of search and of indication	Dec 2011	Yes	Yes
M2.10	Elaboration of a training program for interested dog handler	Mar 2012	Yes	Yes
M2.11	Elaboration of guidelines for the practical work with detection dogs	Oct 2012	Yes	Yes
M2.12	Evaluation of the capacity, efficacy and costs of dog detections teams	Dec 2012	Yes	Yes

Acoustic Detection

Executive Summary

- The acoustic system has been tested in various situations from laboratory (York and FERA) through tests on native trees to use on imported Bonsai and recordings taken in Italy.
- Various sensors have been designed and tested with different resonant frequencies, housing design (including custom housings) and amplification factors.
- Sensitivity tests have indicated that a sensor with gain x20 has a detection limit at around 6m for a fractal dimension detection limit of 2dB signal to noise ratio.
- 8-channel and 16-channel multiplexed systems were developed for use by PSHI on imported Bonsai. The systems have been found to be successful and have additionally been employed in the laboratory at FERA for long term recording.
- A protocol for the use of the 8 and 16 channel sensors has been developed for the deployment of this equipment at Bonsai Nurseries
- Testing of the bite detection software has highlighted that there is still more work required to make this an effective, efficient system.
- A single sensor system for deployment in the field has been developed as well as USB-based sensors for use in the research laboratories.
- A sound library for feeding sounds has been created for 11 species of wood boring beetle including *Anoplophora glabripennis*, *A. chinensis, Agrilus planipennis*, several bark beetles and one Lepidopteran larva (*C. cossus*). The recordings include those taken during previous research projects. It is intended that this library will be continually updated after the end of the project to develop a comprehensive and useful facility.
- Discrimination between species has been shown to be feasible during a previous research project, but the software to enable this is no longer available. Discovery of the presence of *Otiorhynchus sulcatu*s larvae in the plants at FERA initially suggested that their feeding sounds are very similar to ALB; subsequent analysis has shown that this is sometimes the case highlighting the need for discrimination software if all the benefits of an acoustic detection approach are to be maximised.
- Two types of artificial larva have been designed and tested for use with the sensing systems.
- It is possible for a two sensor system to locate the approximate position of a larva to within 8-9cm if between the sensors, or whether it is on the distal side of either sensor. Higher resolution can only be attained if a much higher sampling rate than 44.1kHz is used.
- A forward-looking analysis of new systems and applications has been carried out indicating that future systems can be in two forms – stand-alone and wireless networks, Each system has application in different but overlapping scenarios.
- The work package has produced a total of two 16-channel systems, one 8-channel system, five single sensor systems, five USB systems, ten artificial larvae type 2, three type 1 and sufficient amplifier boards and components to make a further 100 sensors.

Overview

The primary goals were (i) to test the acoustic system developed previously under a range of circumstances and collect as many recordings as possible from a range of relevant species; this involved partners P1, P8 & P9; (ii) to take the current system and implement practical improvements to increase its ease of use (P1); (iii) validation of the detection methods and investigating limits of detection (P1) and (iv) perform a forward-looking review to provide a

specification for miniature and self-contained detection systems for deployment on individual trees with radio networking capability.

The deliverables associated with this Objective were:

D2.16 Detailed validation and clarity on the scenarios where this technique is appropriate
D2.17 A review of the technologies and applications for the expansion of the acoustic detection technique

Specific milestones were:
M2.4: Expanded library of feeding sounds collected and assessment of potential for speciation concluded.
M2.5: Conclude experiments on the validation of the detection methods and investigating limits of detection.
M2.6: Amended, practical, robust acoustic detection system available for utilisation by inspection services.
M2.7: Conclude the review on the future potential of acoustic systems in monitoring for *Anoplophora*.

Both deliverables and all milestones have been achieved. Outstanding issues are discussed in the conclusions to this Objective.

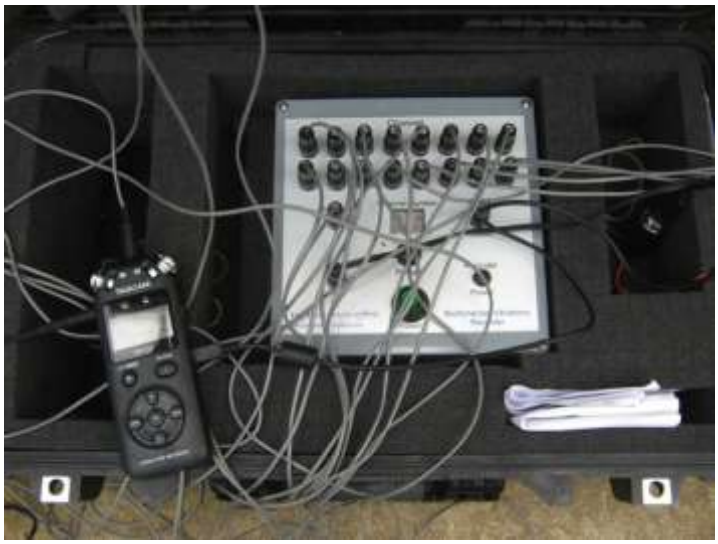
Improvements to System

Multiplexed System for Multiple Trees

The system developed previously provided a two channel detection system with software running under Windows utilising a novel detection mechanism. Whilst the two channel system is suitable for individual trees it is not suitable for large consignments. PHSI had a requirement in 2011 for urgently monitoring a large number of Bonsai trees that had been imported and were being held under quarantine. This required the development of a new approach which was a departure from the original objective. This new system was designed and implemented at short notice and initially comprised an 8 channel multiplexer. The concept was to acoustically sample each tree (8 in this case) for one minute sequentially and record on a stereo portable recorder, the second channel recording ambient sounds. The sensors would then be moved to another group of trees and recorded for 1-2 days. The 8-channel system was a prototype for a 16-channel system. A total of two 16-channel systems and one 8-channel system have been supplied and tested at 3 Bonsai Nurseries in 2011 and at 2 Bonsai Nurseries in 2012.

Both systems have also been used extensively to record from ALB larvae in the laboratory at FERA. The system is supplied in a robust wheeled case complete with sensors, recorder (Tascam DR-05), memory cards and cables. Two separate 12V sealed lead acid batteries are required, one to power the multiplexer and the other to power the recorder (only if extended recording periods are required).

The 16-channel unit has a LED display showing the current channel and internal switches to set the time interval to 1 or 5 minutes. Figure 1 shows the 16 channel system and its deployment at a quarantine site.



(a) 16 Channel System

(b) Deployed at Bonsai Nursery

(c) Sensors Attached to Bonsai

Figure 1 16-channel System Deployed at Bonsai Nursery

A number of improvements to the multiplexer system have subsequently been made as a result of feedback. These include: increasing the sample interval to 5 minutes and the addition of manual channel advance buttons to facilitate sensor testing in conjunction with artificial larva AL1 (see section on Artificial Larvae6).

Single Channel System for Research and Individual Trees

A number of simple single channel systems have also been implemented, consisting of amplified sensors and Tascam DR-05 portable recorder. This system has been used in the field in Yorkshire to obtain recordings of native wood boring beetle larvae and sent to Italy. Figure 2 shows the system in its case. It has also been deployed in Italy to record ALB larvae.

USB-based Sensor

In addition to simple amplified sensors used in the previously described systems, a USB-based sensor has been developed which acts as a self-contained sound card. It can be connected to any computer (PC, Mac, laptop or iPad) with a USB port and can be used with any recording software. There are three versions – self contained sensor, one and two channels. Figure 3 shows the self-contained and single channel versions.

Figure 2 Portable Single Sensor System

Shows two sensors at left (x20 and custom housing), sensor battery unit (centre) and Tascam DR-05 recorder at right.

Figure 3 Self-contained and Single Channel USB Sensor

Validation of Detection Methods and Limits of Detection

Sensor Design

In previous work the sensors were based on 2kHz resonant piezoelectric transducers (Chesmore and Schofield, 2010). Additional work has been carried out on the following sensors:

- piezoelectric resonant sensors of differing frequency and size;
- wideband piezoelectric sensors;
- MEMS (microelectromechanical system) accelerometer.

Figure 4 shows the format and sizes of the sensors in relation to a 1p coin. A miniature microphone is also shown but previous work has indicated that microphones are unsuitable.

The resonant piezoelectric sensors have continued to be the most sensitive, reliable and cost effective. The wideband sensors, whilst being capable of operating over the whole audio frequency range, were too insensitive. The MEMS accelerometer (type Analog Devices ADLX103 single axis) was found to be unsuitable for two reasons: (i) the frequency response is very poor, much lower than required for detection of ALB feeding sounds and (ii) very insensitive despite being the most sensitive device available (+/- 1.7g full scale) and with an amplification factor of 100 (see Figure 8 for an example of sensitivity).

In summary, any of the piezoelectric resonant sensors are suitable and have been incorporated in suitable plastic housings with built-in amplifiers (gains x20, x50, x90). The housings are made waterproof by sealing the removable bottom and the cable entry. The terminating end of the cable is via a sealed 6-way connector.

Each sensor is attached to 1, 2 or 3m of cable as required; it is possible to have longer cables if required. The amplifier in each sensor requires a power supply (9V battery box) in order for it to work. However, if the battery is not connected, the sensor has a gain of x1 and so can be used with or without amplification. The major drawback of an unpowered amplifier is that the sensor is not buffered and can easily pick up mains interference.

Several sensors (large size) have 6V lithium batteries built in which are powered only when the connector is attached. However, the weight of the sensor is increased and the sensitivity is reduced somewhat.

Examples of amplified sensors in different housings are shown in Figure 5, including a 3D printed custom designed housing made at York in Figure 6 also (illustrated at the right in Figure 5).

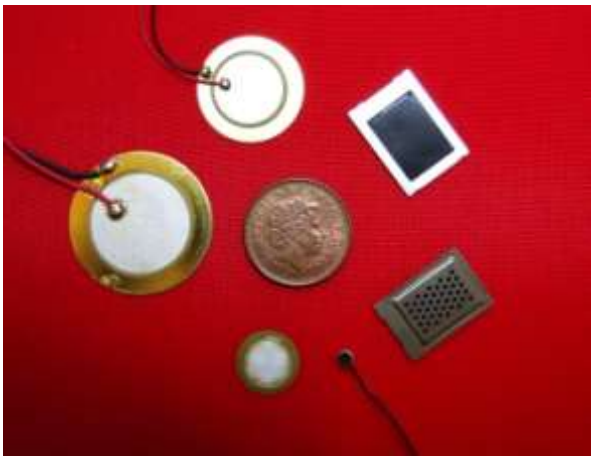


Figure 4 Range of Sensors Tested

From top clockwise: 6kHz piezo, wideband piezo type 1, wideband piezo type 2, microphone, 12kHz piezo and 2kHz piezo.

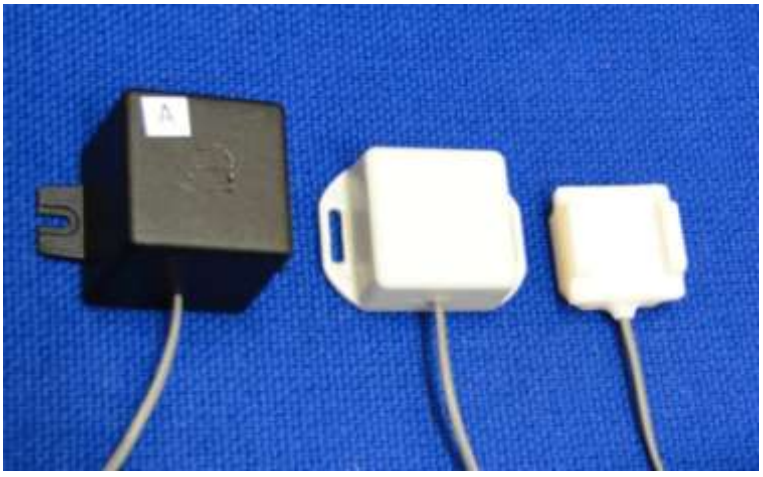


Figure 5 Sensor Housings

Sizes. left: 4x4x4cm; centre: 3.5x3.5x2cm; right: 3.2x3.2x1cm

Figure 6 Two Types of Custom Printed Housing. Circular housing has 3.5cm diameter and 1cm height.

Sensor Testing of Sensitivity and Limits of Detection

The sensors have been tested in a number of scenarios:

- In the laboratory using logs and timber to test sensitivity.
- On native trees at the University of York and surrounding region.
- In the laboratory at FERA.
- In Italy.
- On Bonsai in UK nurseries.

These are detailed in the following sections.

In the Laboratory using Logs and Timber

Figure 7 shows a typical set up on a Birch log (diameter 0.34-0.36m, length 1.4m) using a custom sensor and artificial larva AL2 which produces simulated feeding sounds. 10 seconds of sounds were recorded using an Edirol portable recorder at 44.1kHz sample rate. All sensor types have different responses as indicated in Figure 8. Here, two sensor types were tested – "old" are the 4x4x4cm (black) and "new" 3.5x3.5x2cm (cream) with amplifiers turned on (x20) or off (x1). Included in the figure is the response from the accelerometer which is much lower than the piezoelectric sensors.

The responses differ between sensors which can be attributed to the internal structure of the log. However, it is important to note that ALL responses are much higher than the limit of detectability (see Section cc) of 2dB indicating that any of the sensors can be successfully employed.

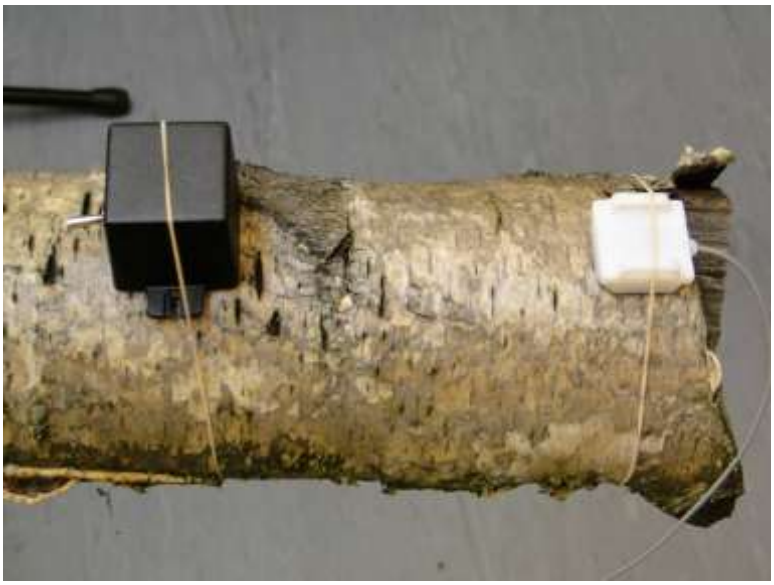


Figure 7 Sensitivity Test on Birch Log using Artificial Larva AL2

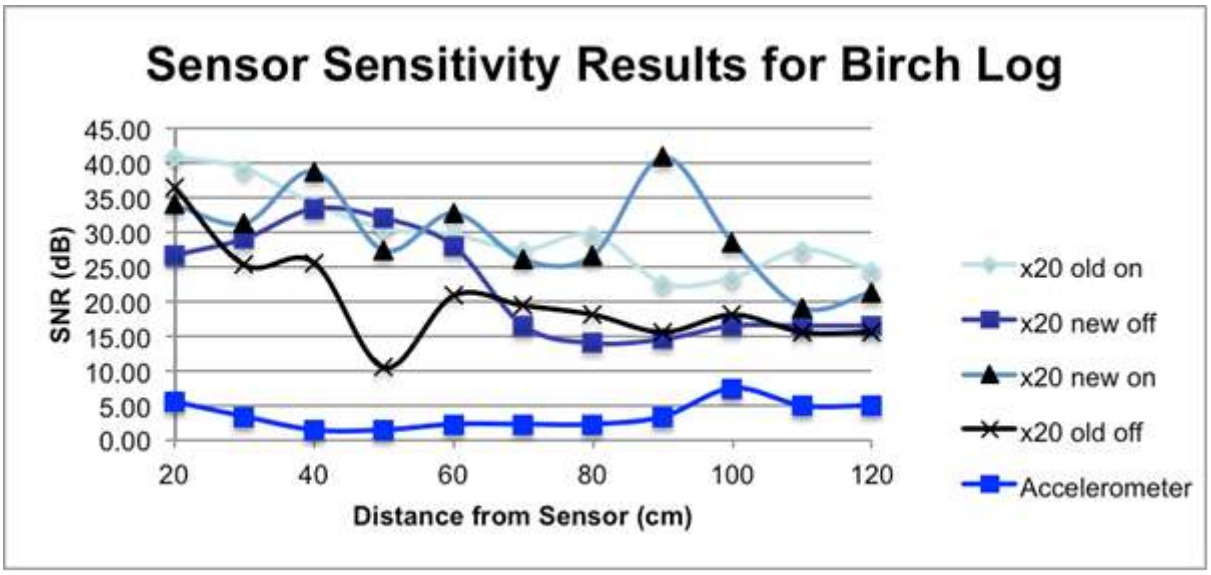


Figure 8 Sensitivity Tests for Sensors

Tests on Live Trees

Initial tests were carried out using x20 sensors attached to a live tree (*Tilia cordata*) at the University of York. The tree had a diameter at 50cm above ground (sensor location) of 18cm and 6cm at 2m. The artificial larva AL1 (buzzer) was used to test sensitivity at various heights and along the lower level branches to a distance of 3-4m. Figure 9 gives the results for the tree indicating that the signal to noise ratio at 4m is above 10dB. It is important to note that this location is along a major branch and not on the main trunk of the tree. Extrapolation of the graph to a detection limit of 2dB gives a maximum detection distance of 6m.

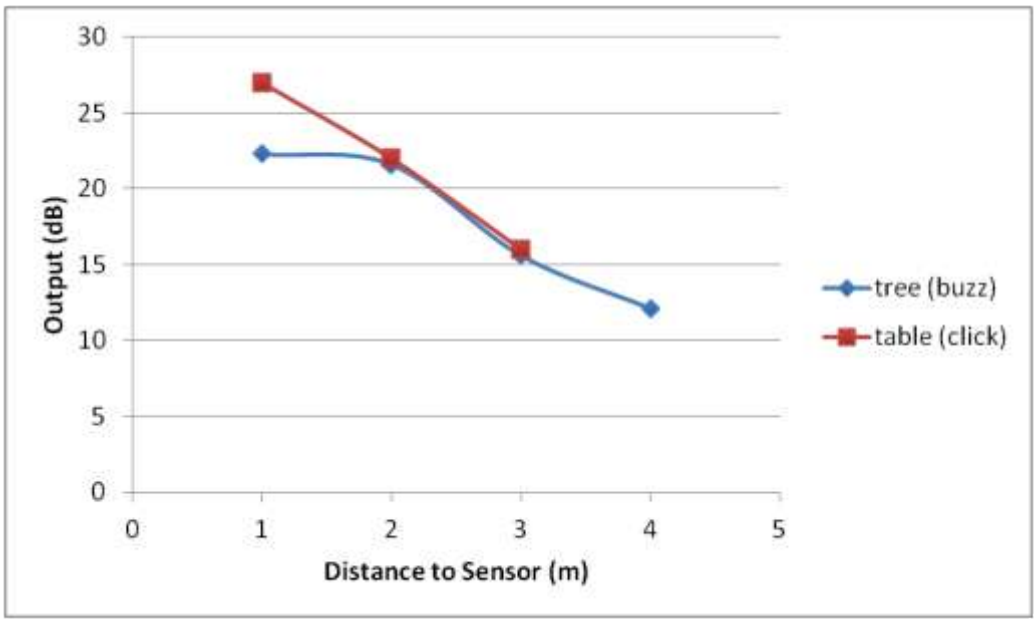


Figure 9 Sensitivity Tests on Live Tree

(buzz = artificial larva AL1; click = AL2)

A similar result is shown in Figure 9 for a test using AL2 on a 3m long wooden work surface using AL2 larva. Extrapolation gives a detection limit of 5.5m on a complex substrate including direct connection to the ground. The results given in this section are representative of all the tests undertaken.

Different sensor testing

The 8-channel and 16-channel systems have been used extensively for recording ALB in the laboratory at FERA. In addition to long term monitoring, sensors with different frequency responses are tested as indicated in Figure 10. Here, four sensors (x20, 2kHz; x20 12kHz; x50, 2kHz and x50, 12kHz) were attached to a stem containing one or more larvae together with standard x20 2kHz sensors. Results from the tests indicated that all sensors respond well and are suitable.

Figure 10 Sensors in FERA Laboratory

Black sensors are standard; grey are tests for different gain and resonant frequency

Culturing ALB at Fera

ALB were obtained from Franck Hérard & Nathalie Ramualde (EBCL, USDA, ARS, Montpellier, France). A total of eighty larvae were delivered to Fera in June 2011, of those that survived the journey were ten stage 2-3, seven stage 3-4, and thirty five pre pupal stage. Only the prepupal stages had been diapaused. Direct comparisons cannot be made, as larvae were being used for recordings, but it was observed that a diapause period was not essential. A higher proportion of the diapaused larvae successfully emerged as ad ults

(69%), but 47% of the non-diapaused larvae also emerged. Twelve larvae were also kindly provided by Massimo Facioli in December 2012, eight of which survived the journey.

The larvae were kept in glass or plastic jars, containing an artificial media (recipe supplied by Franck Hérard as before). The lids had a number of holes drilled into them for ventilation. The culture was kept in a CE room at 21ºc ± 2ºc, RH 65% and 16 :8 hr L :D. Larvae were regularly examined, and when the ratio of media to frass was found to have dropped to about 1 :4 the larvae was transferred to a fresh jar. This was approximately every 4 – 6 weeks, but varied according to the size and activity of the larvae. As the final stage larvae approached pupation, feeding activity stopped, and the larvae usually pupated on the surface of the media.

Occasionally pupation occurred within the media, which made successful adult emergence less likely. As the pupa matured, it darkened in colour from cream to brown, before assuming the adult black shortly before it emerged.

Whenever an adult was about to emerge, the lid was removed from the jar and the jar placed within a larger ventilated plastic tub that contained a fresh twig of *Acer negundo* (Boxelder maple), and a water source. When the adult had hardened up, the jar of media was removed and more *A. negundo* twigs and leaves added to the tub. The adult was kept isolated for between 7-10 days in order to allow it to feed and mature, before being released into a breeding cage.

The breeding cage consisted of a purpose built Perspex cage, with a fan and extra ventilation holes. These were all screened with a wire mesh to prevent the adults from escaping. Fresh twigs of *A. negundo* and *Salix* sp were placed into a tub of water within the breeding cage, with a rooted cutting of *Salix* sp for oviposition, and a water source. A limit of ten adults per cage was chosen, as the maximum that could safely be monitored during culturing.

Fresh twigs were added as required, depending on how quickly the bark was stripped from them, or if they dried out. The rooted cutting was usually left in place for about one week, until several egg pits had been made, before being replaced. The original cutting was then maintained in a separate cage, before being used for acoustic recordings.

30 – 50cm rooted cuttings of both *Salix* sp and *Populus* sp were produced, of between 30-50cm diameter, but *Salix* was the most successful. Rooted cuttings stayed fresh for longer,

giving the beetle larvae a longer feeding period. Young larvae tended to feed just under the bark in the cambium layer, before burrowing deeper into the trunk as they matured.

Freshly cut bolts of other species of tree were also introduced to the breeding cages at times, to see if the adults would lay into them. These were sealed at both ends with Parafilm to reduce moisture loss.

As well as eggs laid directly into rooted cuttings or bolts of wood, over 130 eggs were collected from elsewhere in the breeding cages. These were either lying on the cage floor, on the side of a food twig, or most often in the damp cotton wool used to plug the food twig into its water source. Larvae were successfully hatched from many of these eggs, using a variety of media such as damp blue roll, artificial diet, and inserting them under the bark of different thicknesses of twigs. The success seemed linked to the freshness of the egg when found, rather than the method; if the egg had been laid over a weekend and begun to dry out, it rarely hatched.

Adult survival times also varied, with males generally outliving females. Adults could live for over two months, but females in particular seemed to suffer injuries, with antennae, wing cases and legs being broken at times. Damage to ovipositors was also observed occasionally.

Freshly cut batons of a number of different tree species were offered within the breeding cages to see if adults would use them for oviposition. Some also had eggs placed under the bark, to see if larvae would develop on them.

Tree species	**Common name**	**result**
Acer negundo	Boxelder maple	Suitable host
Salix spp.	Common willow	Suitable host
Salix matsudana 'Tortuosa'	Contorted willow	Suitable host
Acer platanoides	Norway maple	Suitable host
Aesculus hippocastanum	Horse chestnut	Suitable host
Fraxinus excelsior	Ash	Not suitable
Populus nigra	Poplar	Suitable host
Prunus spp.	Ornamental flowering cherry	Not suitable

Laboratory acoustic monitoring.

In order to study the long term acoustic characteristics of ALB larvae in wood a modified version of the method employed for the bonsai monitoring was developed. Samples of wood under investigation were moved from the breeding cage (see culturing section) to a Perspex cages located in a quiet area of the quarantine facility as shown in figure 1.

Figure 1. Recording cage

This apparatus was used to evaluate the various acoustic sensors under development and to perform long term acoustic behavioural studies

To allow long term continuous recordings to be made, a compressed file format was selected for the recorder (MP3 44.1 kHz, 256 kbps). This format allowed 10 days of continuous recording on a 32GB memory card (as opposed to 50 hours for the uncompressed wave format). The process of waveform compression during the storage of audio results in a loss of some information. However, bite sounds could still clearly be identified in the decompressed MP3 waveforms when inspected with audio editing software. Where a higher quality recording was required an uncompressed 16 bit 44.1 kHz recording format was utilised. As with the bonsai monitoring trials, multiplexor units were utilised to allow the study of multiple samples with a single recorder. To reduce the risk from power cuts and mains electricity interference all the equipment was run from lead acid batteries.

Long term monitoring

Rooted cuttings and wood bolts from the culturing cage which showed signs of oviposition on the bark or which had artificially introduced ALB eggs were placed in the recording cage. Acoustic sensors were attached in close proximity to the oviposition sites as shown in figure 2. The sensors were connected to a digital recorder via a multiplexor unit.

Figure 2. Sensors attached to rooted cuttings

Continuous recordings from the samples were made using the MP3 format and a 1GB file size. The recorder was left to run for up to 10 days (maximum duration for memory card) after which the batteries and memory card were replaced and the recording recommenced.

Recordings were transferred from the memory cards to a PC for inspection and analysis using audio editing software (Audacity). Samples which showed no sign of insect activity after approximately 6 weeks of monitoring were removed from the recording cage and replaced with new samples from the breeding cages.

After some time (6 weeks to 3 months depending on size/sample) the wood bolts and rooted cuttings began to dry out. As this drying progressed activity from the larvae diminished and eventually stopped. At this stage the samples were removed from the recording cage and carefully dissected and inspected. From this it was often possible to assess the number of larvae that had been active in the sample and how active they had been.

A collection of recordings have been built up which are being used to investigate aspects of the ALB larvae activity in wood.

First detectable bite sound

It was seen as important objective to ascertain how early in the ALB development biting activity could be detected by the recording equipment and subsequent analysis method.

Of the recordings available only those that originated from samples that had been in the breeding cage for a comparatively short period (approx. 24 hours) or, had eggs manually introduced were looked at. In so doing some certainty could be made of the time between the introduction of eggs to the wood and the first sound. Of these, only samples that had shown clear evidence of insect activity (both acoustically and through the production of frass) for over a month were considered. Using these criteria three sample's recordings (C1, C3 & C6) were selected for study.

Code	Sample	Description
C1	Norway Maple Bolt	Oviposition by ALB female
C3	Boxelder Maple Bolt	Introduced egg
C6	Norway Maple Bolt	Oviposition by ALB female

A careful examination of the recordings from these samples was made tracing back to locate the first evidence of a distinctive bite sound. The early bite sounds are very difficult to locate visually as can be seen in figure 3 which shows (in the highlighted peak) the first detected bite for sample C1. The sound which corresponds to this peak when heard however was very distinguishable. In practise the only way to locate these early peaks was to listen, on headphones, to many hours of the recording.

Figure 3. Sample C1 –Highlighted first detected bite sound (10 minutes of recording visible)

Below are shown (at maximum Y axis magnification) the waveforms for the first bites located in the recordings. In all 3 figures the Y axis scale of the graphs are of a similar scale and the highlighted areas, which contain the bite sounds, are of the same time size (0.014 seconds).

Figure 4 Sample C1: First bite sound detected in 5th day after oviposition. Bite sound in highlighted area.

Figure 5 Sample C3: First bite sound detected in 4th day after oviposition. Bite sound in highlighted area.

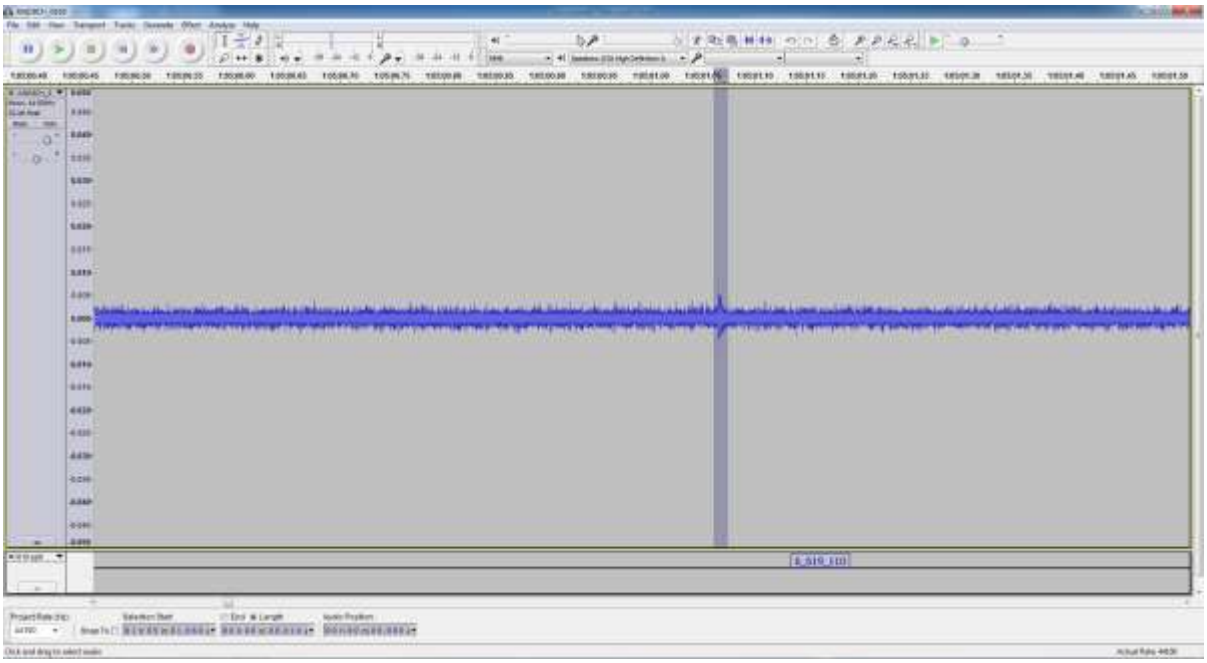


Figure 6 Sample C6: First bite sound detected in 4th day after oviposition. Bite sound in highlighted area.

The early bite sounds were very infrequent with up to several hours between events. As the larvae developed both the amplitude and the frequency of the bite sounds increased. Figure 7 shows activity from sample C1 14 days after the first bite was detected. The level of activity was still not consistent at this stage and recordings still showed long periods of little activity. Note the Y axis in figure 7 is of the same scale as for the initial bite (figure 4).

Figure 7. Highlighted 1 minute area – multiple bite peaks from sample C1 - 14 days after 1stsound detection.

Twenty eight days after the first bite was detected the amplitude of bite sounds from sample C1 had increased considerably as shown in figure 8. Note the scale for the Y axis is 10 times greater than for the earlier figures.

Figure 8. Highlighted 1 minute area - C1 28 days after 1st sound detection.

A single bite from figure 8 is shown magnified in figure 9. The highlighted peak time is 0.014seconds which is the same as for the first bites seen in the earlier figures. Therefore, in these examples, as the larvae grew the amplitude of the bite increased yet the duration remained broadly the same.

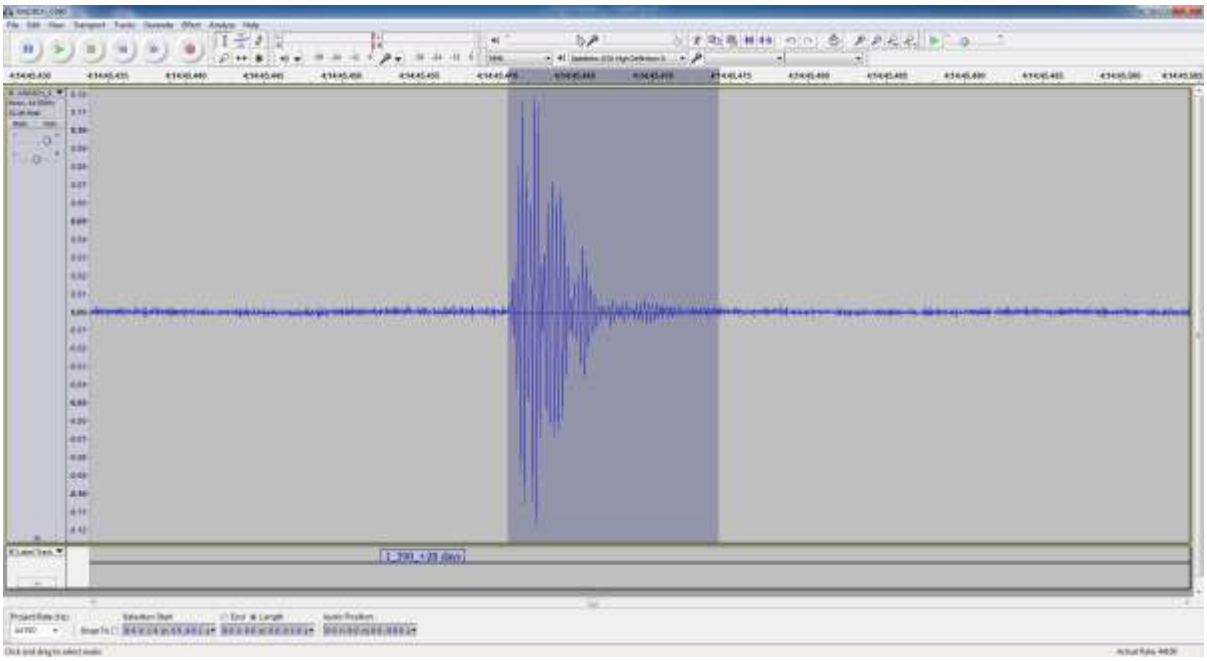


Figure 9: C1 - Single bite sound 28 days after 1st detection

Use of acoustics to screen imported bonsai trees for the presence of ALB

Principle

Amplified piezo sensors held in firm contact with the trunk of the bonsai sample under test were used to detect emanating sounds. The sensor's output was fed to the right channel of a stereo digital sound recorder. To collect reference ambient sounds an identical sensor, physically isolated from the test sample was connected to the recorders left channel. Recordings made on the reference channel helped to differentiate between captured sounds uniquely emanating from the sample and those common to the wider test area.

To allow multiple samples to be sequentially examined automatically, a multi-channel multiplexor unit was used as shown in figure 1. This activated, one at a time, the sensors attached to the sample bonsais routing their sound output to the recorder's right channel. The multiplexor was programmed to switch between samples at intervals (1 minute in the 1st year, 5 minutes in the 2nd). As all the samples were in close proximity (approximately 2m radius) only a single centrally located reference sensor was used.

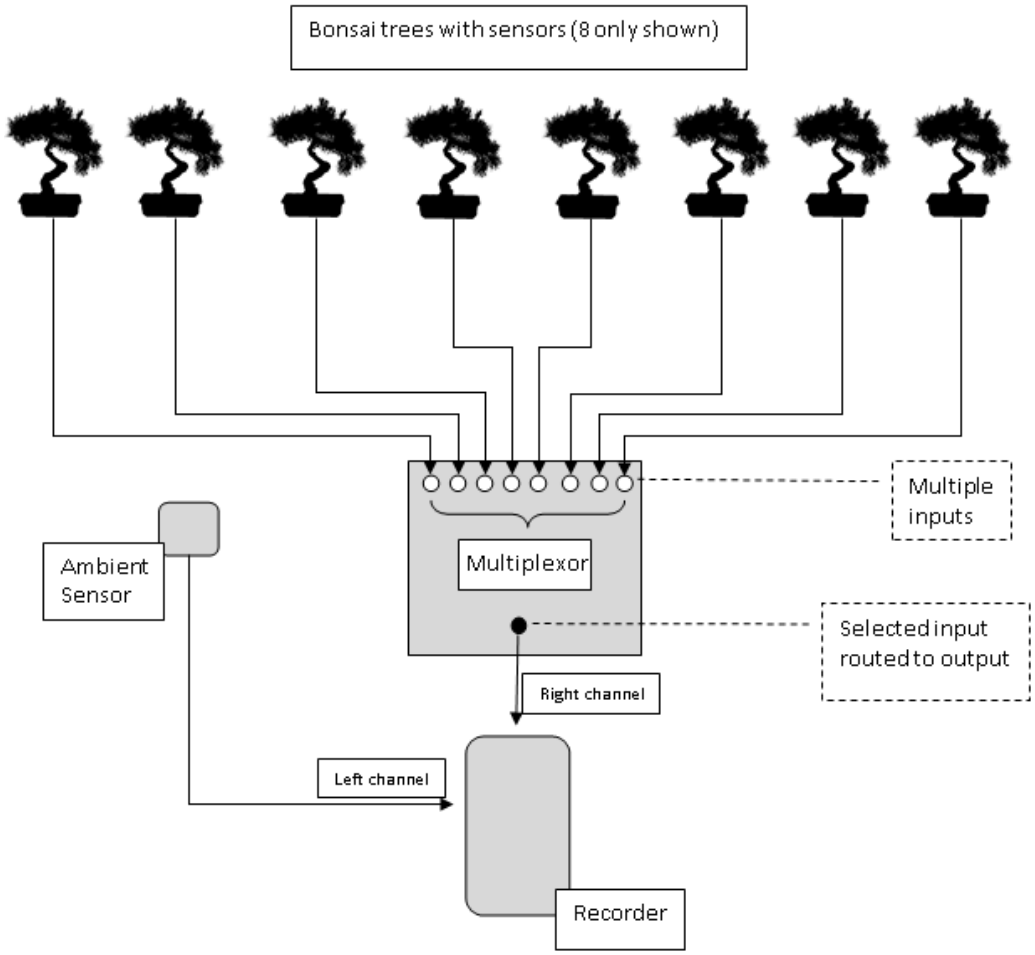


Figure 1. Schematic representation of bonsai audio recording equipment.

Sets of equipment were sent out to field inspectors who deployed them at the secure bonsai holding facilities around the country. The set up procedures sent out with the equipment are attached to this report as appendix??

Manual assessment of recordings.

Audio recordings in the form of multiple uncompressed 2GB stereo wave files were saved to 32GB memory cards by the digital recorder. At the sampling rate used (16 bit 44.1 kHz) this allowed each card to hold approximately 50 hours of recordings. When full the memory cards were removed from the recorders in the field and sent back to the laboratory.

The audio files were transferred from the memory cards to a PC for inspection and analysis using audio editing software (Audacity). Using a combination of visual and audio assessment suspicious sounds that resembled insect bite sounds recorded in the lab were identified.

The software graphically displays the recorded waveforms with amplitude on the y axis and time on the x axis. A typical example of a single 2GB file is shown in figure 2. The ambient channel recordings are displayed in the upper graph with and the sample channel on the lower graph. The example shows a 2GB file (maximum single file size) which corresponds to 3 hours 22 minutes of recording. The regular (5 minute) full scale deflections of the waveform seen in the right channel are deliberately introduced by the multiplexor unit each time the channel is switched and act as navigation points.

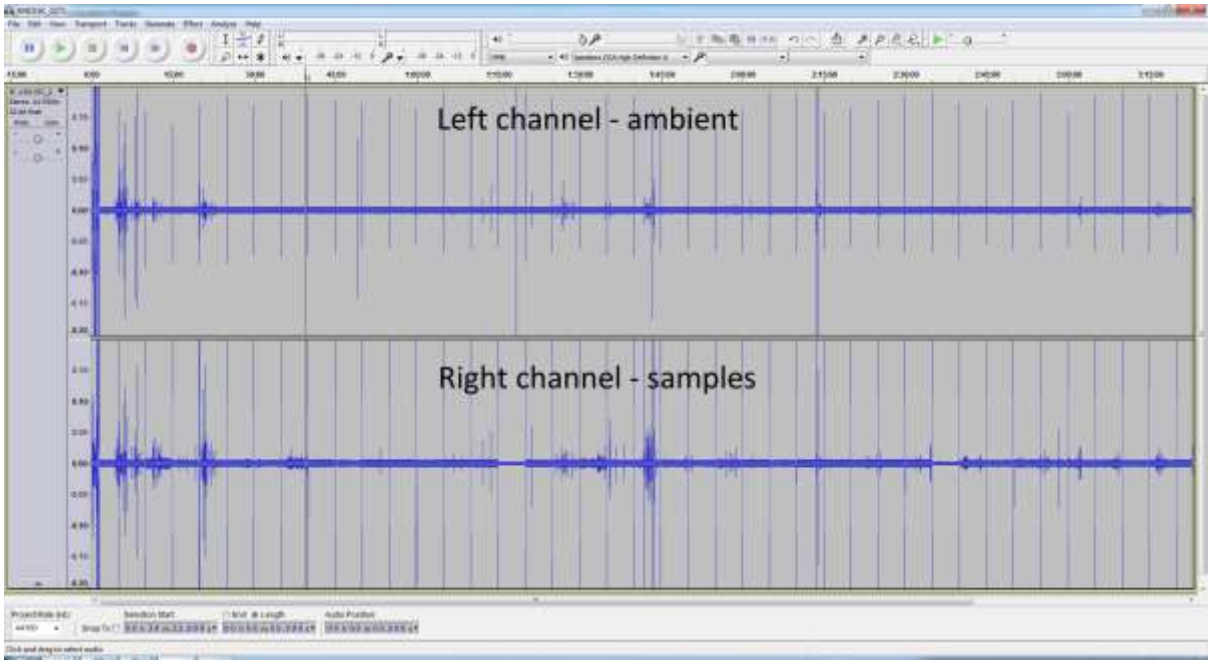


Figure 2. Typical waveform

To inspect the recording for potential bite sounds the view of the waveform is zoomed into as shown in figure 3. The software allows the wave files to be played back in real time while a

cursor indicates on the graph the play back point. The analyst can therefore check sounds that correspond to amplitude variations (peaks) in the waveform and compare them with bite sounds recorded from samples known to contain ALB larvae made in the laboratory quarantine facility.

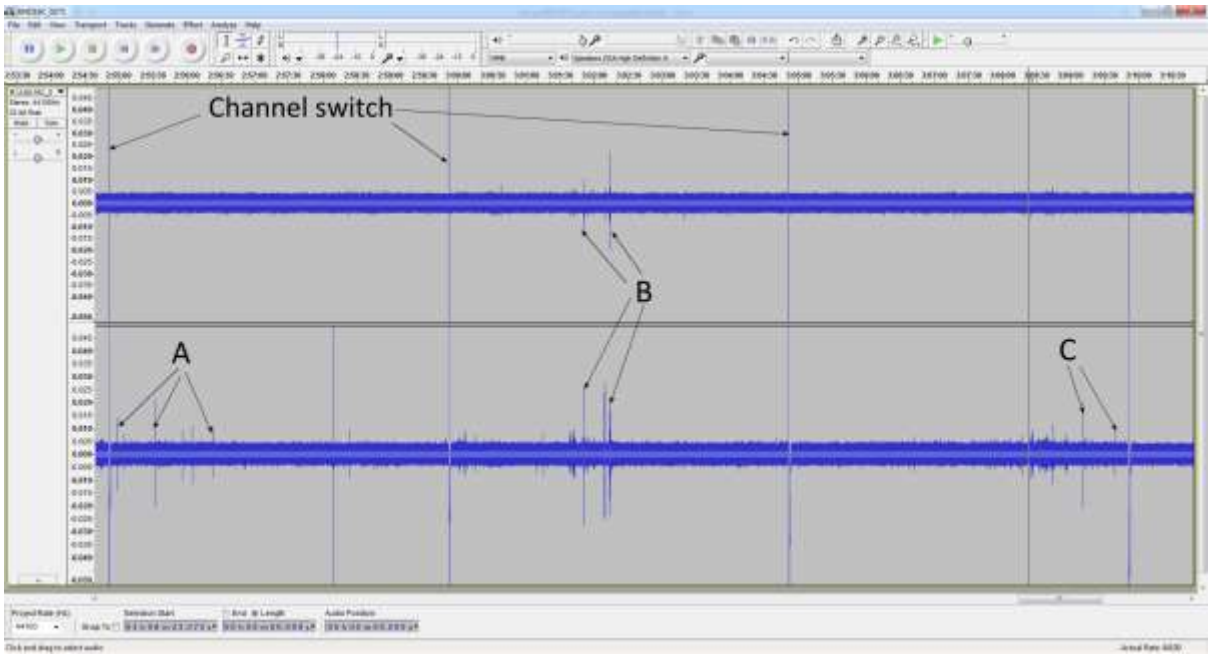


Figure 3. Peak examples

In the example shown, peaks labelled A from the sample channel were identified as suspicious and marked for subsequent closer inspection. Peaks marked B appeared in both the sample and ambient channels and were therefore discounted as sounds from the wider environment. Peaks marked C though only showing on the sample channel and looking (at this level of magnification) similar to bite sounds could be identified, through listening, as being sounds associated with a door being closed.

Depending on the number of peaks in the wave file the process of sifting out suspicious sounds can be very time consuming. Typically nocturnal recordings made in the bonsai holding facilities contained far less background noise and could therefore be processed quicker. Figure 4 shows a one hour section of a recording which contained very few peaks only one of which was suspicious.

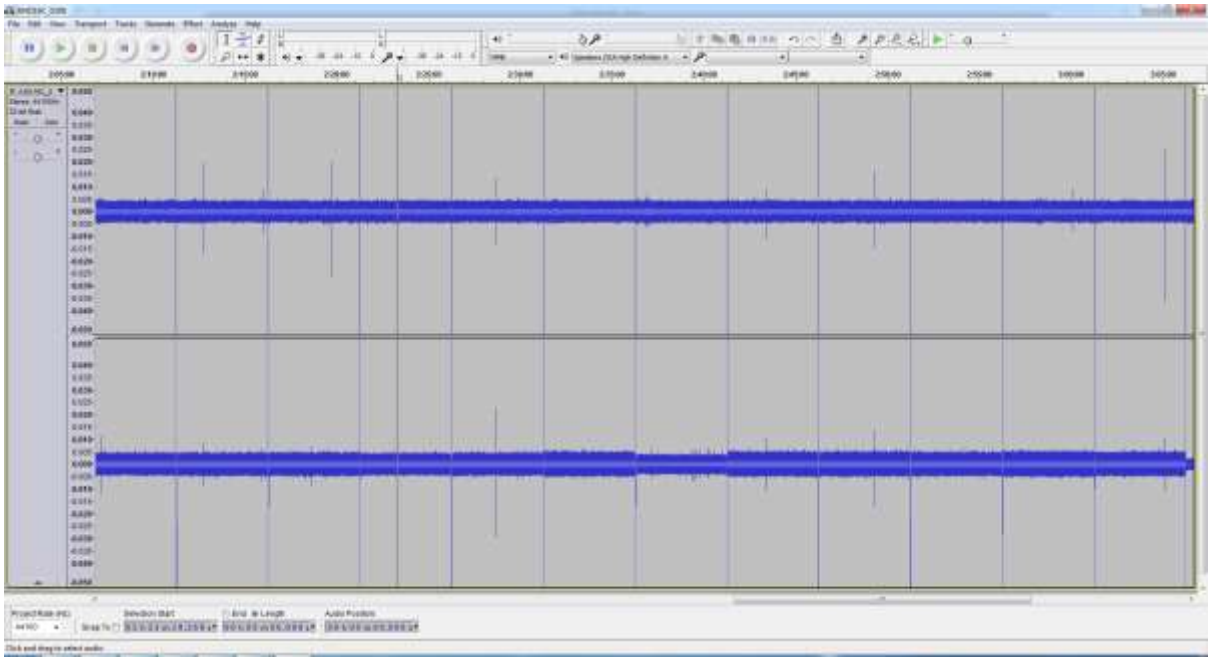


Figure 4. Few peaks

In contrast Figure 5 shows a 1 hour section of a recording which contains a great number of peaks. This recording was taken during the day when maintenance work was in progress (watering etc.). However, examination, of the waveform showed only 9 peaks to be suspicious.

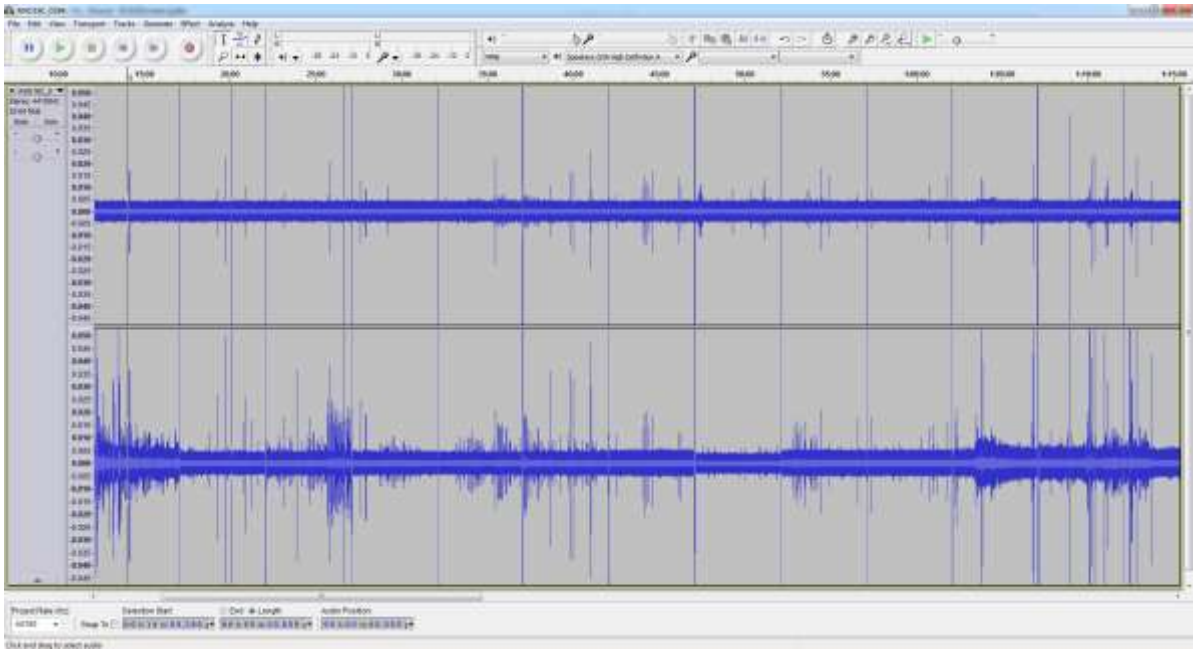


Figure 4 multiple peaks.

As a result of the assessment a compendium audio file was assembled of all the suspicious sounds for each sample. Depending on the number and frequency of these sounds a management decision was made on whether to recommend release of the samples from the secure holding facility.

Automatic Detection Software

During the development of this process for Bonsai growers, the software that was developed by the University of York to automatically detect suspicious 'bite' sounds was redeveloped to deal with the changing inputs and then rigourously tested. The testing was undertaken on data from the Bonsai growers and on laboratory recordings of ALB infested wood (and Vine Weevil infested pots – see Vine Weevil section) providing a range of recordings that have either

(i) No wood boring larvae but plenty of background noise
(ii) Wood boring larvae and not much background noise
(iii) Larvae feeding on the roots of the trees and not much background noise

It was clear from this testing that the software was not quite fit for purpose and it is probable that the change in complexity of the programme caused problems. The key issues were as follows:

(i) The software struggled with the large amounts of data from the Bonsai grower recordings – this has mostly been resolved but the latest version still has some stability issues when there is a lot of background noise
(ii) False positives – the noisy environment at the Bonsai growers nurseries led to a very high amount of false positives. These are where small sections of a variety of sounds are flagged as suspicious. A small amount of these are to be expected however the volume of them made assessing the data impractical.
(iii) False Negatives - many of the bites in the recordings with ALB larval feeding nactivity were not flagged by the software as suspicious. It is important that false negatives are absolutely minimised before this software s fit for purpose.

The latest update of the software has addressed a number of these issues, but there is still some work to do before we would be comfortable recommending the use of the system with just the detection software. Currently it must be coupled with visual and audio review of the files in Audacity to consider suspicious sounds. This makes it economically unviable and the Bonsai growers are more likely to choose 10% destruction than have the combined costs of delay in sales (as they must keep the plants in a quarantine environment for several months) and the full economic cost of multiple inspector visits and the time required to analyse the approx 48 hours worth of recordings from their premises.

Location of Larva using Two Sensors

Figure 15 shows a set up for the location of larvae inside the substrate using time of flight of feeding sounds. It shows a rectangular piece of wood representing a tree, log, etc. The sensors are labelled L and R corresponding to the left and right channel of the recording and placed at distance D_{LR} apart.. Possible locations for the larva are at X, Y and Z with respective distances to left and right sensors indicated on the figure.

Determination of the time of flight of a pulse from X, Y or Z to L and R depends on the speed of sound in wood, typically 3900ms^{-1} for hardwood but can be as low as 3300ms^{-1} for softwood.

The time of flight of the pulse is determined by:

$T = \frac{D}{C_W}$ where C_W = speed of sound in wood

In the following it is assumed that the time difference between left and right channels is measured with respect to the left channel (T=0). There are 4 cases that can be identified to locate the larva; these are:

Case 1 Larva to Left of L Sensor (position X)

$T = T_L – T_R = +T_{LR}$ i.e. T is equal to the time between L and R. D_{XL} is unknown.

Case 2 Larva to Right of R Sensor (position Z)

$T = T_L – T_R = -T_{LR}$ i.e. T is equal to the time between L and R but pulse arrives at R first.

D_{BZ} is unknown.

Case 3 Larva between L and R Sensors, closer to L (position Y)

$T = T_L - T_R £ \frac{T_{LR}}{2}$ and $D_{YL} = C_W(T_L - T_R)$

Case 4 Larva between L and R Sensors, closer to R (position Y)

$T = T_L - T_R £ -\frac{T_{LR}}{2}$ and $D_{YL} = -C_W(T_L - T_R)$

In cases 3 and 4 it is possible to estimate the location to within a resolution determined by the sample rate. For a sampling rate of f_S samples per second, this equates to $D_S = \frac{C_W}{f_S}$; the distances for 44.1kHz and 48kHz are 8.8cm and 7.5cm respectively for C_W = 3900 ms^{-1}.

A test was carried out using artificial larva 2 (pulsed) and sensors placed at 0.8m apart on a 1.6m length of timber with cross-section 0.03x003m (sample rate = 44.1kHz). Figure 16 shows waveforms for the three scenarios (X, Y and Z). It is evident that the time differences are small and it is possible to identify cases X and Z (Figure 16a) and c)) with relative ease. Individual samples are visible on the traces. However, it is very difficult to locate the start of the pulse for case Y since the number of samples corresponding to D_{YL} and D_{YR} is very small

(maximum of 9). It is easier to state that the larva is left of sensor L, right of sensor R or between L and R.

There are two options to improve determination of the location: (i) increase the spacing between the sensors to 2m and/or (ii) increase the sampling rate to 48kHz or higher; 96kHz will give 4cm.

In summary, location of larvae in a tree is possible and has application for locating the approximate position of a larva in, for example, a branch, thus enabling the branch to be removed without removal of the complete tree.

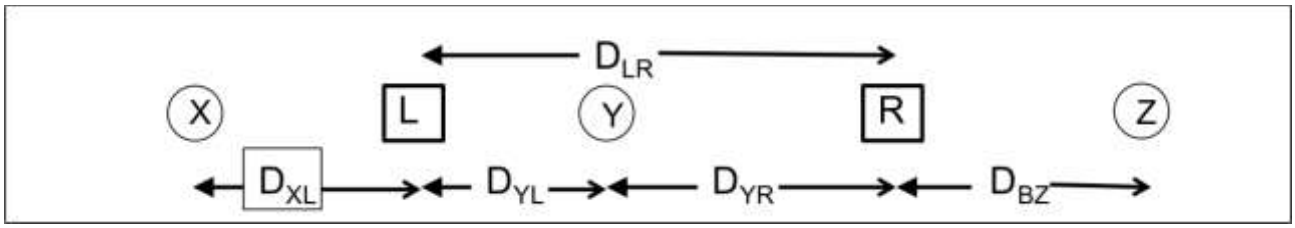
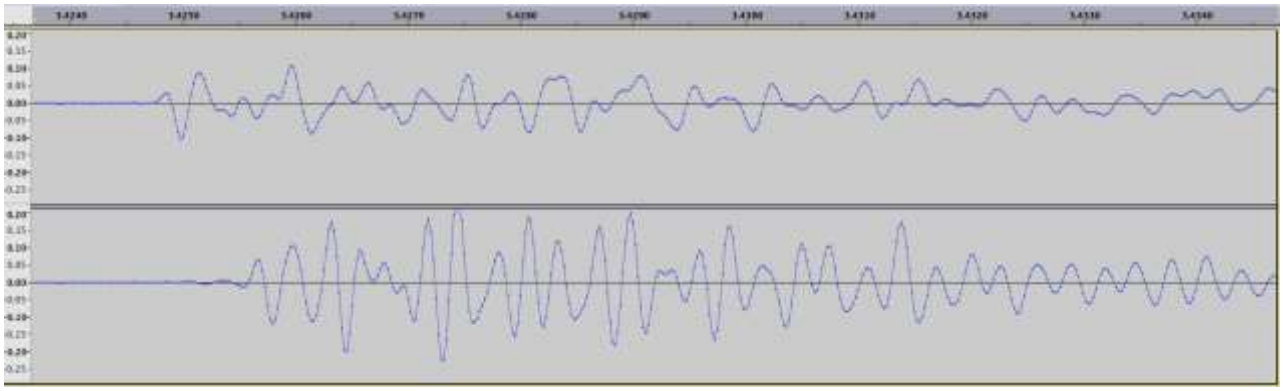
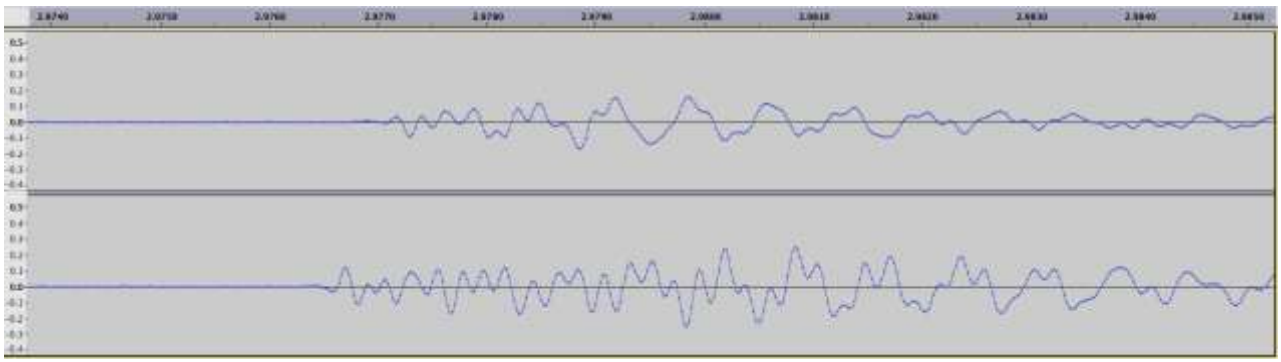


Figure 15 Schematic Diagram of Location of Larva in Wood

a) Left of L Sensor



b) Right of R Sensor



c) Between Sensors

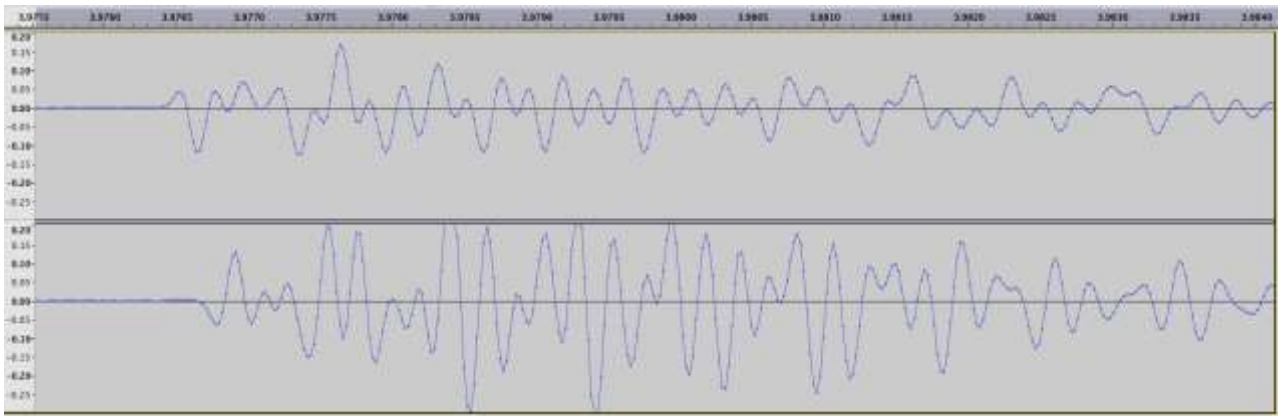


Figure 16 Tests of Location of Larva

Discrimination between Species

The research project PH0419 included preliminary research into the automated discrimination between different species based on feeding sounds using a combination of relational tree descriptors and artificial neural networks (Schofield, 2011). The tables below are confusion matrices taken from Schofield 2011 which indicate that it is possible to discriminate between two and three species.

Table 1 Discrimination between Two Species

Classified as	***A. glabripennis***	***H. bajulus***
A. glabripennis	93	7
H. bajulus	5	95
Confidence	94.9%	93.1%

Table 2 Discrimination between Three Species

Classified as	***A. glabripennis***	***A. planipennis***	***H. bajulus***
A. glabripennis	92	4	4
A. planipennis	7	37	6
H. bajulus	2	5	93
Confidence	93%	80%	90%

To date, very few examples of CLB feeding sounds have been recorded so it is not possible to make any assertion that ALB and CLB can be identified.

Vine weevil (*Otiorhynchus sulcatus)*

Currently there is no software available to discriminate between species and this causes an issue as there are some species whose feeding noises appear to be superficially similar.

Long term recordings were made from a rooted willow cutting taken from the culturing cage after potential signs of ALB oviposition on the bark were observed. This recording contained peaks which, on first inspection, looked and sounded like ALB larval bite activity. After recording was finished the sample was dissected, however no evidence of ALB larvae was found in the wood. An examination of the soil in which the sample was rooted revealed twenty 1cm long larvae (figure 2) which were subsequently identified as vine weevil (VW).

Figure 2 Vine weevil larvae

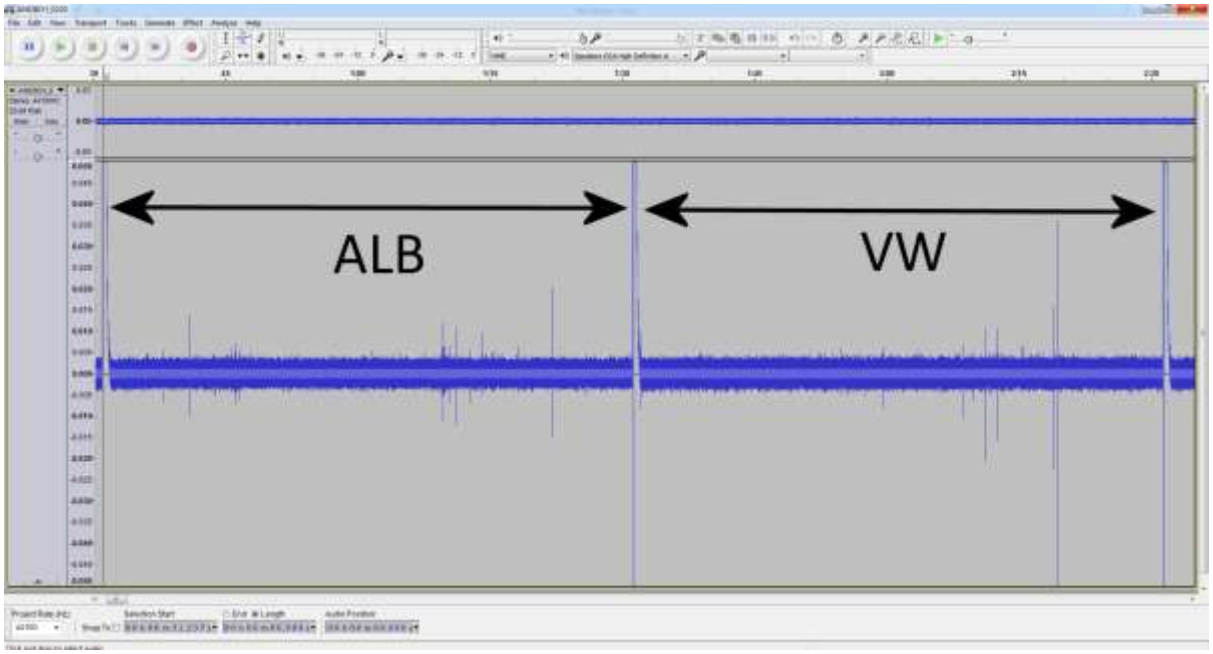


Figure 3 ALB and VW recording

An examination of the bite sounds found in the recordings from this sample was made and compared with those taken from ALB larvae.

Figure 3 shows a section from the multiplexed (1 minute interval) recording (uncompressed wav format, 16 bit 44.1 kHz, 1 GB file size) which contained the VW sample. For clarity the ambient recording channel is shown at reduced size as the top channel in the diagram. In the recording sequence the VW sample was preceded by another rooted willow sample which contained an ALB larva (confirmed by subsequent sample dissection as shown in figure 4) and no VW larvae.

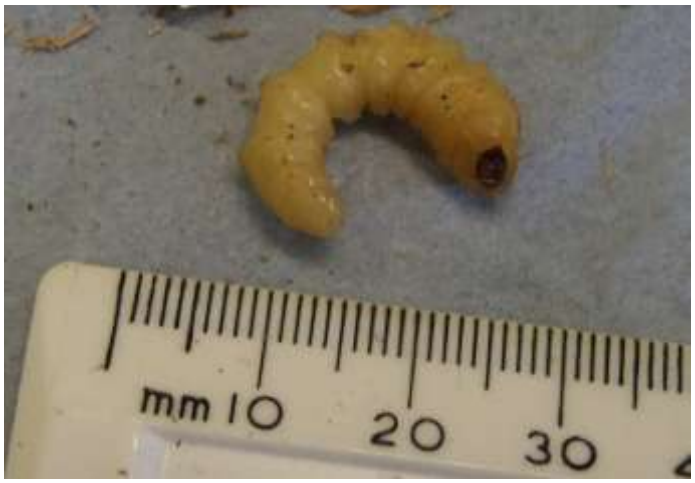


Figure 4 ALB larvae

Figure 5 shows five magnified example bite sounds taken from each of the ALB and VW sections of the recording. Each peak has been lifted in a 0.010 second time slice to aid comparison.

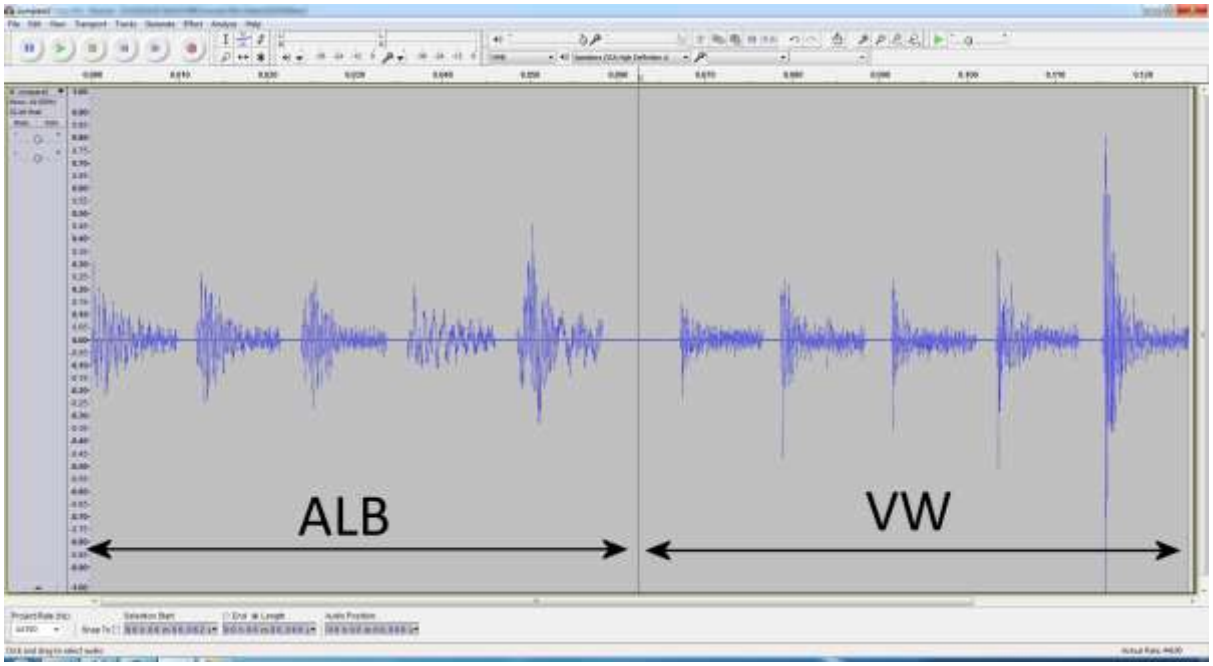


Figure 5 Five example bite sounds from ALB and VW

Zooming in even further, figure 6 shows a direct comparison of 2 of the individual bites from figure 5 (Top = 2nd ALB peak, bottom = 3rd VW peak).

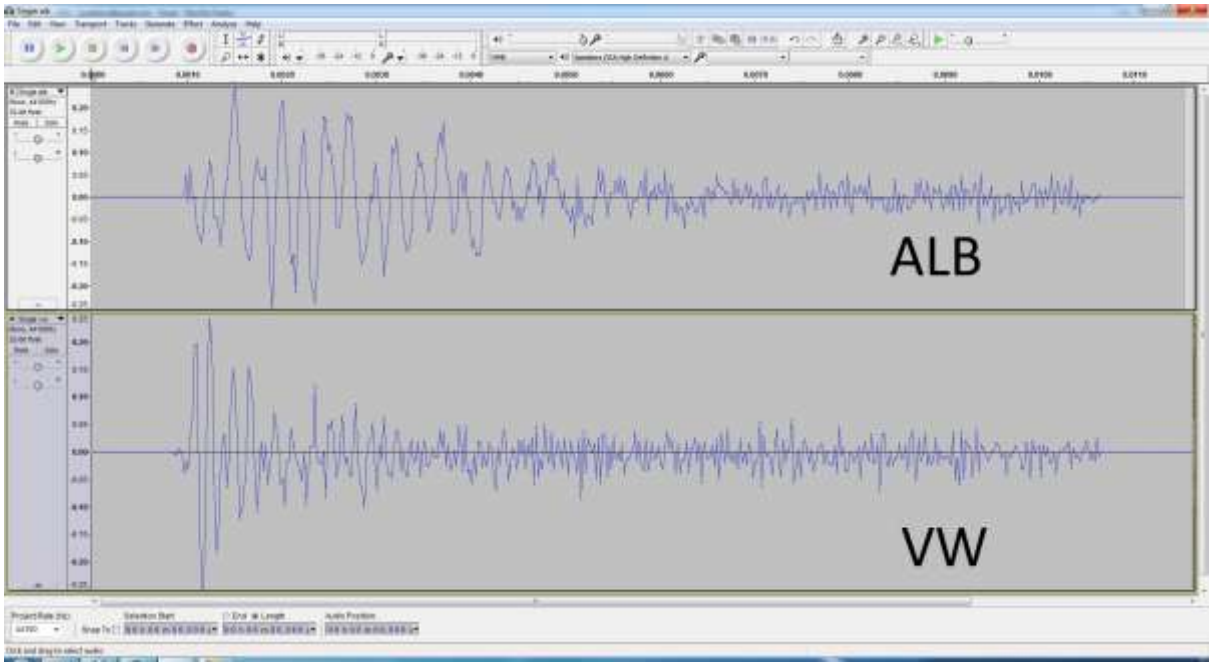


Figure 6 ALB and VW single bite comparison.

Visually it would appear that there are some differences between the peaks in the waveforms created by the 2 types of larvae. Figure 5 and 6 suggests that the peaks from ALB take longer to build up and subsequently decay from maximum amplitude. Conversely the figures also show a sharper maximum amplitude peak and smaller subsequent peaks in the decay of the VW bite sound. It is not known if any of the differences could be attributed to the different physical properties of the parts of the wood being bitten (roots versus under bark).

These observations are made with the benefit of certainty about origin of each peak. Looking at an unknown sample (e.g. bonsai tree) with no references, positively attributing a suspicious sound to that of ALB bite purely by ear is very problematic.

Initial inspection of the recording used in this study did not identify the VW bite sounds as not originating from ALB. By chance therefore, this contamination event has shown the fallibility of even an experienced analyst when presented with a species that makes sounds similar to ALB. Under these circumstances it is likely that a wrong diagnosis could be made.

Software

To further investigate the perceived differences between the ALB and VW sounds the 2 minutes of recording used in the previous section (figure 3) was run through the latest version of the detection software (Console_Bite_Detect.exe : University of York 15/08/11). The software only identified 1 bite candidate which on inspection was found to correspond to the first channel switch (i.e. not from the ALB or VW larvae).

As a bigger test for the software, composite files were made from all the ALB and VW one minute sections contained in the parent file from which the earlier peaks were drawn. From

the ~96 minutes contained in the 1 GB parent file 12 minute files for both species were assembled (figures 7 and 8). To eliminate any false positives associated with multiplexing, the peaks associated with channel switching were not included in the composite files.

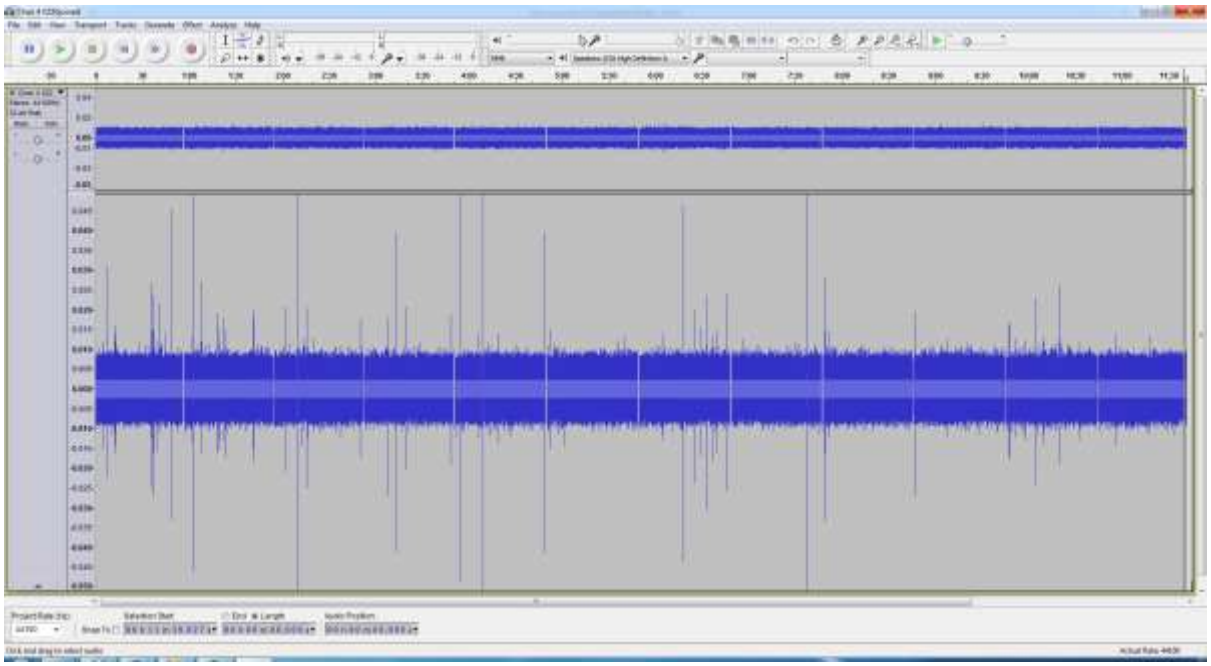


Figure 7 Composite file made from twelve 1 minute recording segments - ALB

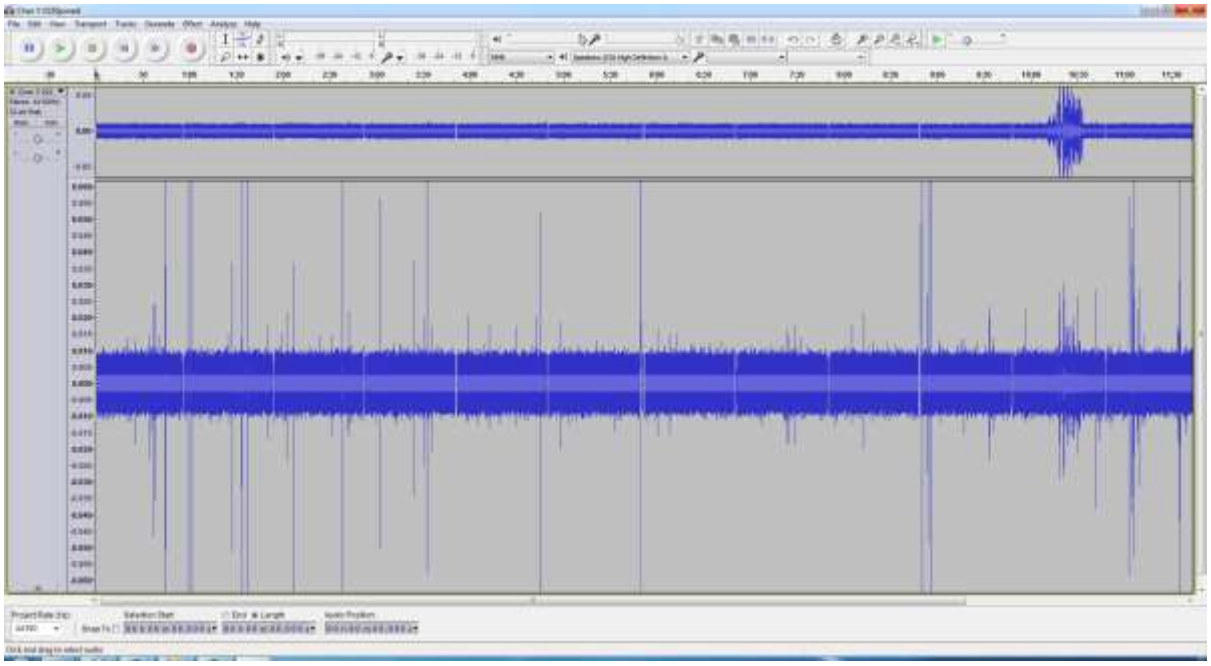


Figure 8 Composite file made from twelve 1 minute recording segments - VW

Both files were run through the latest version of the detection software. Three candidate bite sounds were located in the ALB file as shown in figure 9.

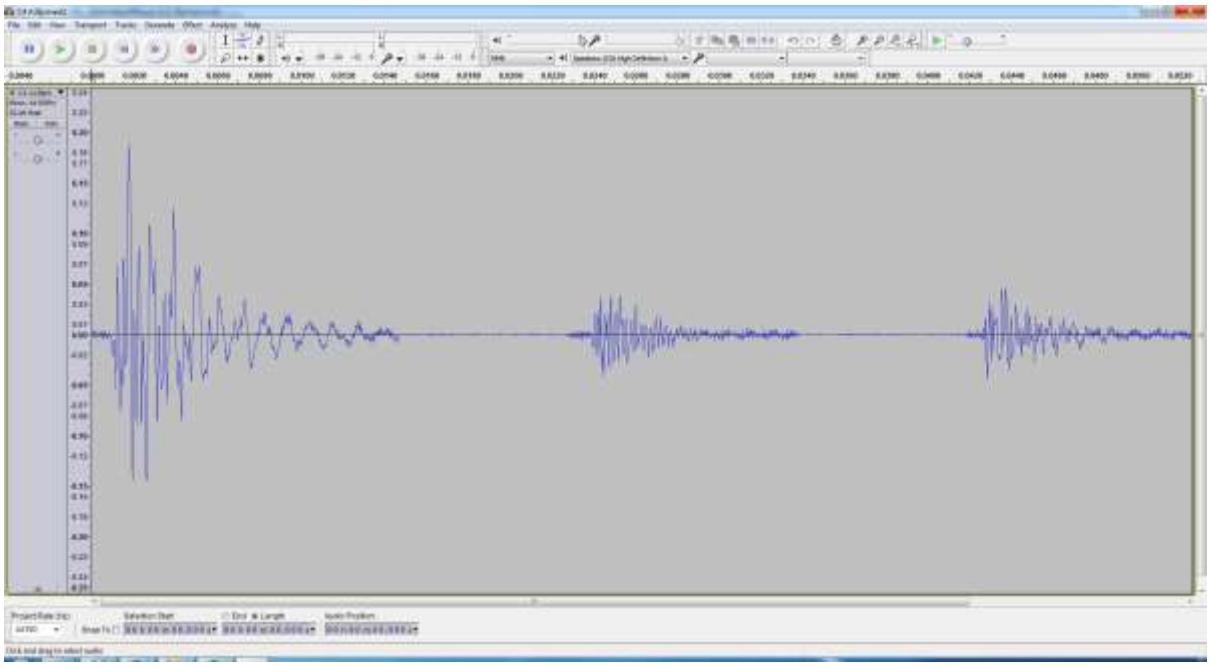


Figure 9 Candidate bite sounds located by software in ALB file

Four candidate bite sounds (false positives) were located in the VW file as shown in figure 10.

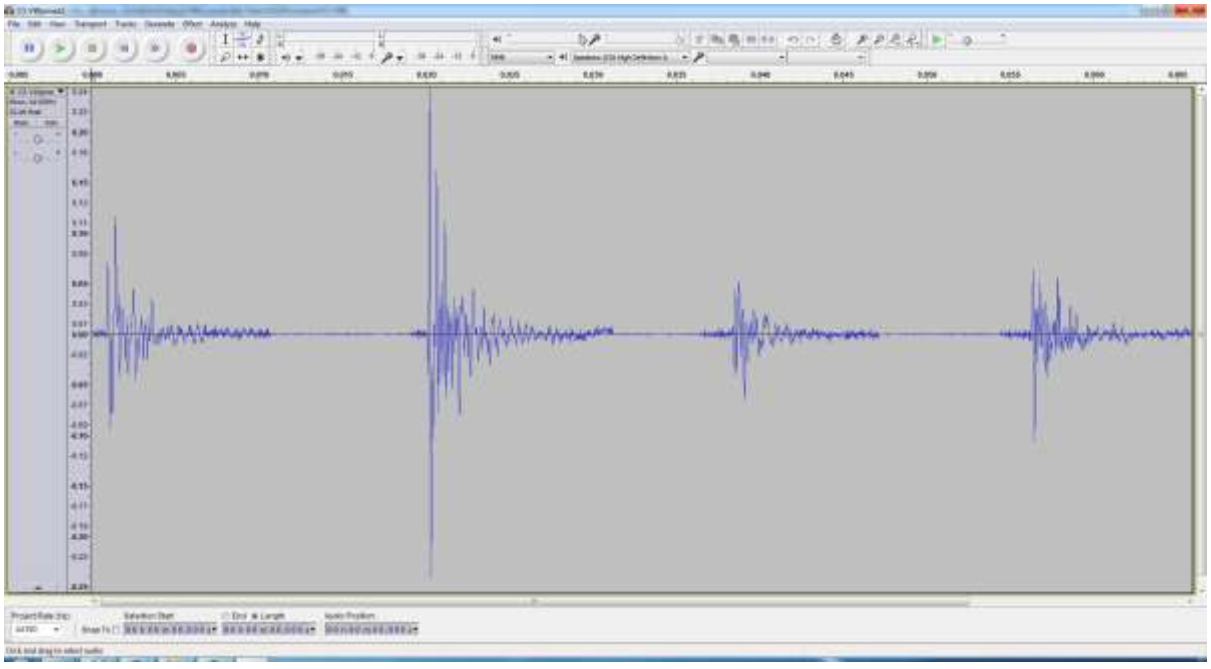


Figure 10 Candidate bite sounds located by software in VW file

A subjective assessment of the 12 minute ALB audio file was made by ear using headphones from which 65 distinct bite sounds were identified. This would indicate that the software was less than 5% efficient at locating bite sounds. The identification of candidate

bite sounds in the VW audio file would also suggest that false positives could be problem when processing recorded data with this software.

From this assessment of the detection software it would seem clear that it is in need of further development before it can be utilised in non-destructive analysis.

Sound Library

A sound library has been created that contains representative feeding and movement sounds of 11 species of Coleoptera and one of Lepidoptera. The library has been compiled from recordings taken in two previous research projects, Italy, Canada (from previous project), York University, FERA and in surrounding local nature reserves. Forestry Commission was tasked to provide material and sites for additional native species but there was little useful input.

L. cervus and *D. parallelepipedus* have been included as they provide examples of deliberate sound production (Harvey et al., 2011).

Table 3 gives a list of the species. The library is provided on CD but it is suggested that a web site is created for easy access for ANOPLORISK partners and other interested parties.

It is intended that this library is continually expanded beyond the end of the project to aim for a comprehensive library.

All sounds are recorded using either Edirol R4, R09 or Tascam Dr-05 recorders at 44.1kHz, 16 bits per sample and stored in .wav format.

It is imperative that no new files are added to the library in any compressed format such as MP3 as this severely affects the waveform.

Table 3 Species Included in the Sound Library

Species	Family	Order	Feeding Category	Notes
Anoplophora glabripennis	Cerambycidae	Coleoptera	Woody tissues	
Anoplophora chinensis	Cerambycidae	Coleoptera	Woody tissues	
Trichoferus griseus	Cerambycidae	Coleoptera	dead wood	
Rhagium bifasciatum	Cerambycidae	Coleoptera	dead wood	
Prionius corarius	Cerambycidae	Coleoptera	dead wood	
Agrilus planipennis	Buprestidae	Coleoptera	Bark	from Canada
Lucanus cervus	Lucanidae	Coleoptera	dead wood	stridulation sounds
Dorcus parallelipipedus	Lucanidae	Coleoptera	dead wood	stridulation sounds
Hylobius abetis	Curculionidae	Coleoptera	Bark	

Pityogenes chalcographus	Curculionidae	Coleoptera		
Otiorhynchus sulcatus	Curculionidae	Coleoptera	Bark	from Germany
Cossus cossus	Cossidae	Lepidoptera	Woody tissues	from Germany

Artificial Larvae

There was a requirement to create a means of testing sensors in the absence of the pest. Two such devices have been developed, one to give a good audio indication via a sensor and the second to provide a simulated feeding sound.

Buzzer-based Artificial Larva (AL1)

The first artificial larva was designed not to imitate the feeding sounds but to provide a good indication of the operation of a sensor attached to a tree or wooden material. The buzzer is based on a mobile phone vibrator which is a small motor with off centre weight. Applying 3V DC to the motor causes it to vibrate. It is housed in a small sensor box which also contains a 3V lithium cell. The device has an on off switch as indicated in Figure 18.

Piezoelectric Impulse-based Artificial Larva (AL2)

A second device aimed at testing sensors with more realistic signals used a piezoelectric speaker driven by an impulse generator producing impulses at approximately one per second. The device is powered by an internal 3V lithium cell and housed in a standard black sensor case as illustrated in Figure 18. The impulses generated are very similar to those of a Cerambycid larva but are longer by a factor of 2 (Figure 19); this is caused by the speaker vibrating the whole of the case. It is possible to reduce the pulse width but deemed unnecessary.

Figure 18 Artificial Larvae.

Left is AL1, right is AL2

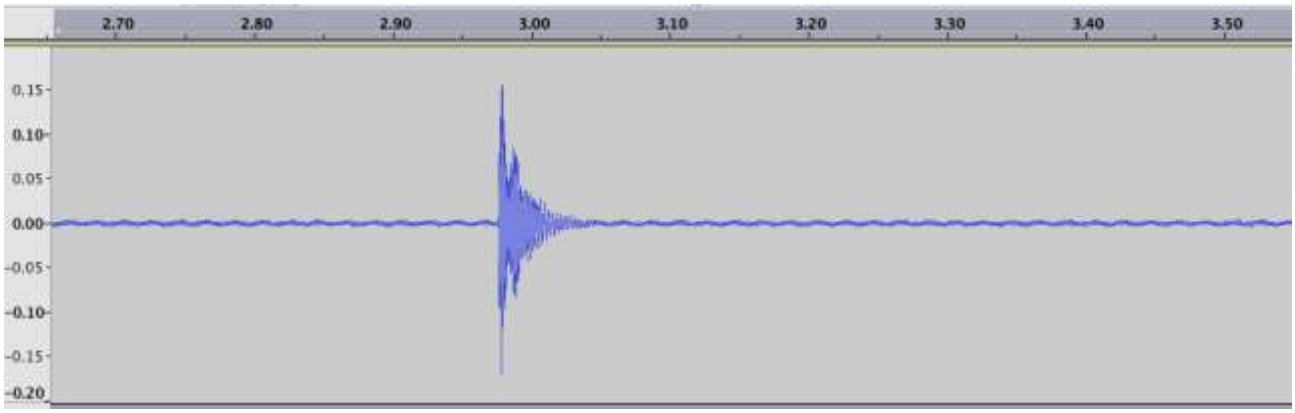


Figure 19 Single Pulse from Artificial Larva 2.

Horizontal axis is time in seconds. Vertical axis is amplitude (arbitrary unit)

Recommended Protocols

The following protocol has been set up and deployed for the UK PHSI to use with Bonsai importers who want to avoid the destructive sampling process (10% of all imported trees).

Deployment of ALB acoustic testing equipment in Bonsai Nurseries

Principle

A sensor held in firm contact with the test sample picks up sounds from the sample and passes them into one channel of a stereo digital sound recorder. The recorders second channel is connected to another sensor which is isolated from the sample and collects ambient sounds. By comparing the two channel's recordings it is possible to identify those sounds emanating only from within the test sample which may be the result of insect activity. To allow multiple samples to be examined a multi channel multiplexor unit is used. This activates, one at a time, 16 different sample sensors and sends the active sensors sound output to the recorder. The multiplexor makes these switches at 5 minute intervals incrementally from channel 1 to 16. After channel 16 the multiplexor returns to channel 1 and commences the cycle again.

Setup

An overview of the basic setup of the equipment with a single test sensor connected is shown in fig 1

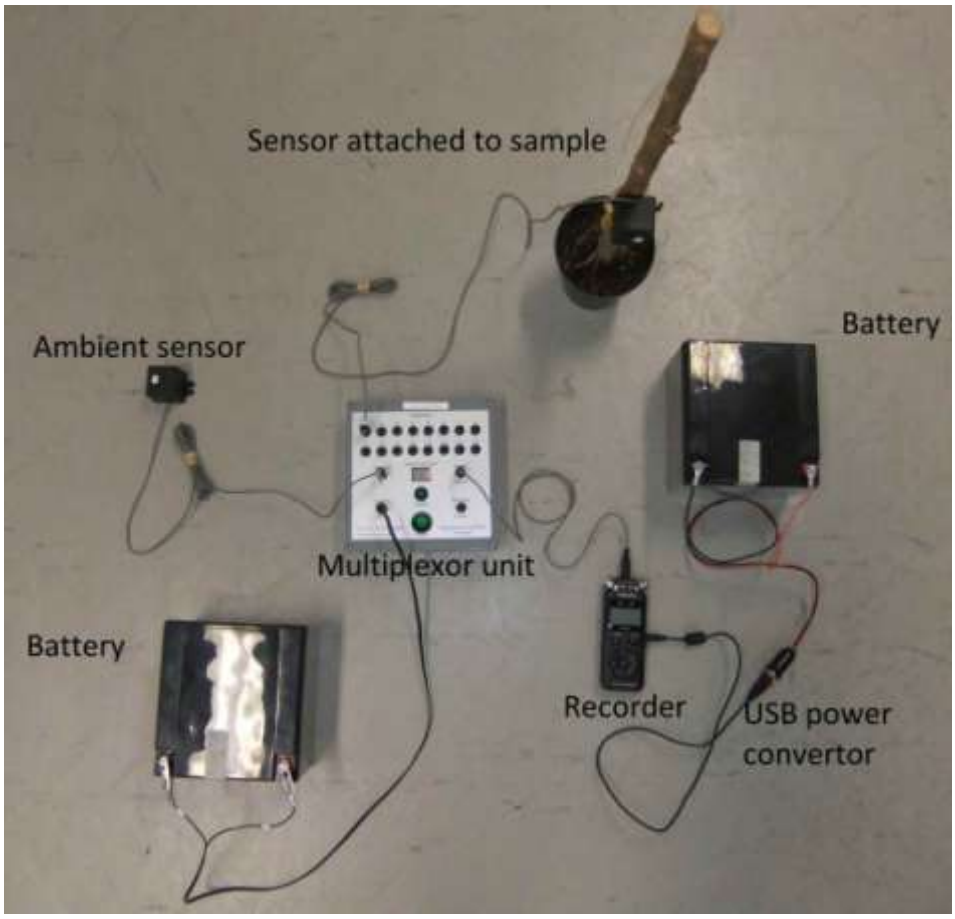


Fig 1

Procedure

1) Locate the multiplexor unit roughly central to the samples selected for monitoring. **Bear in mind that the multiplexor and recorder are not waterproof and will need to be protected from watering systems.**

2) Attach the required number of sensors (max 8) to the samples using rubber bands as shown in fig 2. Choose a spot on the main trunk that will allow good contact with the sensor but will not cause damage. It is not critical that the whole back of the sensor is in contact with the sample. The band should be tight enough to give a good contact between the samples bark and the back of the sensor but does not need to be excessively tight.
Make a record of the sample id and sensor number. Ideally take photos of the setup.

3) Connect the sensor wire plugs to the corresponding channel numbers on the multiplexor socket bank. The connector plugs should lock into position. Note: To remove a connector lift the plug collar and gently pull upwards.

4) Locate the ambient sensor (marked A) in a free standing position roughly central to the samples. Connect the sensor's plug to the corresponding socket on the multiplexor.

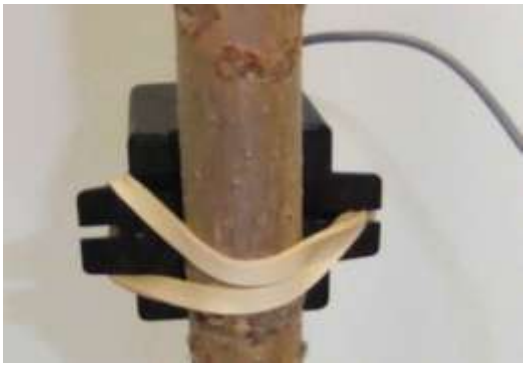


Fig 2 Sensor attachment

5) Connect the multiplexor Power Input cable to the multiplexor.

6) Taking care with polarity, connect the multiplexor to the battery using the crocodile clips. If the multiplexor unit display comes on switch the unit off using the power rocker switch.

7) Connect the Audio out cable between the multiplexor and the recorder (Jack socket on top of recorder between L and R microphones).

8) **Taking care with polarity**, connect the USB power convertor cables to the battery using the butterfly nuts. The red light on the convertor should illuminate.

9) Connect the recorder to the USB power convertor using the USB to micro USB cable.

<u>Recording</u>

1) Ensure the recorder is fitted with a micro SD card. Cards are fitted in the slot on the right hand side of the recorder as shown in fig 3. When fitting a card gently push the card in until it clicks into place. Pushing a fitted card gently in and then releasing it will eject the card.

Fig 3

2) Switch the recorder on by pressing and holding the on/off button see fig 4. An initial message will ask what mode to start in. Select “Bus power” by pressing the play/enter button when that selection is highlighted (as shown in fig 4).

Fig 4

3) Switch the multiplexor on using the rocker switch.

4) Put the recorder in record mode by pressing the record button **once**. The REC indicator will flash red.

5) Connect the headphones to the recorder (jack socket on left hand side of recorder). Sound being picked up the ambient sensor should be audible on the left channel while sound from the numbered sensor (as shown on the multiplexor display) should be heard in the right. Gently touching the active sensors should be very clearly heard. The display on the recorder will also give a visual representation of the sounds. If no sound is being picked up check the wiring is correct and all the connectors are pushed fully home.

6) To allow the operator to quickly check that all the numbered sensors are working correctly using the headphones, a manual channel advance mode has been added to the multiplexor unit. In this mode, the user can manually increment the channel using the push button (channel increment) on the bottom right-hand corner of the unit. This removes the need to wait for 16 x 5 minutes to check that all the channels are working correctly.

    a. To enter manual mode, press and **hold** the channel increment button then press the restart button once. Continue to hold the increment button as the display briefly shows 16 then switches to 0.

    b. Release the increment button. The active channel will now increase every time the increment button is pressed and the sound from that sensor will be heard on the right of the headphones.

    c. To exit from the manual mode, press the restart button without pressing the channel increment button. The unit will then display 8 briefly then start operating.

NB If in doubt which mode the unit is in, switch the multiplexor off, pause for a second and then switch it back on again. This will ensure the unit is out of manual channel advance mode.

7) When satisfied that the sensors are working correctly exit the manual channel advance mode as detailed in 6) c.

8) To start recording press the record button for a **second** time. The REC indicator will illuminate red continually. At the same time press the "Restart" button on the multiplexor this will force the unit to switch to channel 1 and start it's cycle.

9) To stop recording press the on/off button once. To turn the recorder off all together press and hold the on/off button.

Notes:

- The 32gb memory cards have capacity for approximately 50 hours of recording after which a "card full press play" message will be displayed. Approximately one cards worth of recording (i.e. ~2 days) is thought to be a suitable monitoring period for a set of samples.
  PLEASE TURN THE RECORDER OFF BEFORE REMOVING AND REPLACING THE MEMORY CARD. Attempted "hot swapping" of cards has previously resulted in file corruption.

- It would be most helpful for the subsequent analysis of the recordings if any pertinent observations made during the monitoring process are sent along with the memory cards. Such observations (especially supported by photographs) can help explain spurious sounds which might, for example, may be caused by dripping water, spider/slug and snail/ rodent activity, breezes causing foliage movement etc etc.

- It is not necessary to use all the sensors if there are fewer than 16 samples to be tested. Leave unused sensors unconnected and make a record in the notes.

Future Systems

Based on the recommended protocols discussed in Section 7, there are two potential future systems, one for sentinel trees and the other for outbreaks. The following sections describe the two systems but it should be noted that there is considerable overlap in design parameters and considerations such as power consumption.

Limitations of Current Approach

The multiplexed system in particular has several drawbacks:

- wired connections (trip hazard, potential for electromagnetic interference);

- storage of all recorded signals resulting in gigabytes of storage;
- limited to 8 or 16 channels;
- little flexibility in setup (e.g. maximum 3m distance from unit to sensors).

Wireless Approaches

In order to remove the problem with wires, it is possible to utilise wireless communications. There are a number of solutions ranging from very low data rate point to point VHF and UHF transceivers to 2.4GHz wireless network nodes capable of building large scale networks.

Considerations for selection of wireless modules include:

- Power consumption; generally higher for high data rates and/or longer communication distance.
- Communication distance is determined by the maximum available power output (legal requirement), required distance and the environment in which the system is to operate. For example, a highly cluttered environment will reduce distance significantly for higher frequencies such as 2.4GHz.
- Required data throughput. If full audio is to be transmitted then a minimum of 705,600 bits per second is required (one channel, 44.1kHz and 16-bit resolution). Large numbers of nodes in a network will reduce the data rate considerably. There are ways of overcoming this by restricting the amount of recording time in a manner similar to the multiplexed system.
- Whether the module is capable of supporting a network. If it does then an ad hoc wireless network has many advantages (see below) over point to point communications.

The most appropriate approach for this application is to use 2.4GHz wireless nodes such as Zigbee® or XBee (lower complexity version of Zigbee). For example, the XBee Pro (Digi International) has Zigbee connectivity and can operate from 250kbps to over 1Mbps over distances of 40m indoors and 3200m outdoors. It does, however, consume 200mA while transmitting which would be a significant drain on any battery. Power down mode reduces consumption to 3.5μA.

A wireless network has the capability for “multihop” communication whereby data from one node is transferred across the network which is too large for direct communication, i.e. communication between two nodes which are out of communication can be achieved via intermediate nodes. Such a system would be suitable for deployment of sensors both in quarantine environments (e.g. a replacement for the multiplexed system) and in outbreak sites where large areas can be covered.

Another wireless system worth consideration is Bluetooth® which allows connection to a smartphone for data downloading. The main limitation is that the maximum number of nodes is 7. Devices exist for long distance up to 100m.

Wireless System

The preferred solution for wireless sensors is XBee or similar to create a "sensor network" capable of monitoring a number of trees. It is envisaged that the system would have a base station connected to a computer (or stand-alone) for storing data and controlling the network. A number of limitations would have to be placed on communication speed including only recording for short intervals and analysing the data in each sensor. In this way only "potential events" will be recorded and transmitted for further analysis. Based on recordings at FERA (section 2.2.2c) a recording of 5 minutes will result in 30-50 bites if a larva is present. If each sensor is switched on for 5 minutes in 60 then the overall storage requirements and data throughput will be minimised, as will be power consumption. It is possible for such a sensor to be battery powered an operate for several days.

Long term monitoring (e.g. sentinel trees) will require energy harvesting to replenish the battery. Solar power or wind power would be suitable.

Stand-alone System

This is a single sensor system which records for 5 minutes in 60 minutes and stores candidate "events" on SD card for future retrieval. Calculations of power consumption using an ARM-type processor will give 55 days of operation on one 4Ah sealed lead acid battery. Assuming there are 20 candidate events per 5 minutes, and 2 seconds of each event is stored on the SD card then a 4GB card will be sufficient for the lifetime of the battery.

Such stand-alone systems will have application on sentinel trees, in research laboratories where larval activity is to be measured and on specific trees at outbreak sites.

Development cost of the stand-alone system is much lower than the wireless system and can be used in more scenarios.

Further enhancements to both systems can be achieved by "intelligent" monitoring. For example, if a suspicious event is detected then the system can be programmed to monitor for a longer period. Also, it may be possible to integrate species identification (at least powerful discrimination against noise) onto either system.

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X-ray Imaging for the detection of *Anoplophora* infestations in wood

Report P2 - AJM Loomans, National Plant Protection Organization, Netherlands Food and Consumer Safety Authority, Wageningen in collaboration with Roel Jansen (Wageningen University RC, the Netherlands) and Chris Mol & Ronald Wolf (Philips Research Eindhoven, the Netherlands).

Introduction

Inspection of import trees from Asia to detect the presence of long-horned beetles (*Anoplophora*) is necessary to avoid damaging infestations of such beetles in Europe. This inspection is currently carried out visually, by trained inspectors, on a relatively small sample of relevant tree consignments. This procedure has not fully prevented the outbreak of infestations in a number of European countries over the last years. In search of improvements, the Dutch Ministry has commissioned Wageningen University Research Centre (Greenhouse Horticulture) and Philips Research to explore the application of non-destructive 2D and 3D-imaging techniques. Both projects have been integrated within an international EUPHRESCO context, called ANOPLORISK. The work reported on here has been performed during the first year of this project (2011) and has been finished within the assigned time. Here we summarize both projects, based on the reports made by the subcontracting partners.

In previous research commissioned by the PPS (nVWA) the application of 2-dimensional (2D) X-ray imaging to the detection of beetle-generated boreholes, galleries and exit-holes was explored in wood pieces (de Kogel et al., 2010) and in intact trees (Jansen & Hemming, 2010). That research was performed with a commercially available luggage scanner [(Hi-Scan 6040i; Smiths - Heimann, Germany], as routinely used for inspection of luggage during air traffic and border control. The feasibility to detect boreholes with this method by human vision and machine vision (automated image analysis) was clearly demonstrated. However, human vision needed training and using machine vision a large number of unaffected trees was incorrectly classified as borehole affected. The complexity of the structure of the trees (roots, branches), prohibited clear identification of boreholes in stacked sets of trees and generated a significant number of false-positive findings in single tree images. This was our motivator 1) to further improve and validate the 2D x-ray detection method and 2) to explore alternative 3D - imaging approaches.

Figure 2-1. Pilot experiment - development and testing of a non-destructive 2D scanning method: 2D X-ray as a detection tool on individual model plants (pre-drilled wooden axles an *Acer* stems) and intact *Acer* trees.

Figure 2-2. X-ray image of an *Acer* tree infested by *Anoplophora chinensis* (left) and automated detection of gallery in the stem of the intact tree, marked in blue (right).

1) Optimization and validation of 2D X-ray for borehole detection in intact trees

The first objective of this 2D research performed by **Jansen & Hemming (2011)** from Wageningen University, was to improve the machine vision borehole detection method and to validate the performance of tree classification using the improved method. The research question associated with this objective was: what is the effect of improving the borehole detection method on the performance of tree classification?

Methodology

HALCON v. 10.0 software for machine vision was used for optimizing version 1 of the automated borehole detection method described in Jansen & Hemming (2010). The dataset for optimization consisted of 929 X-ray images generated during the previous research (Jansen & Hemming, 2010). The optimized borehole detection method was validated using a dataset consisting of 1204 X-ray images, not used for optimisation. The low performance of the previous borehole detection method of a whole tree was mainly the result of gaps in-between roots and branches which were incorrectly identified as “boreholes” (false positives). Therefore, during optimisation morphological operators were applied to delete these type of small structures before further processing and optimization was achieved by focusing the region of interest (ROI) to the main trunk and the root part only and by deleting the small structures before further processing. During validation, classification of the used (1204) dataset by the optimised borehole detection method resulted in a number of true positives (TP) and true negatives (TN), and false positives (FP) and false negatives (FN) . We used ‘accuracy’ [(TP+TN)/total] to test the performance of tree classification and ‘sensitivity’ [TP/(TP+ FN)] to test the ability to identify positive results.

Summary of results

After validation of automated detection of all 1204 images, the number of true positives remained the same, the number true negatives increased and false positives and true negatives decreased sharply. Optimization of the borehole detection method resulted in an increase in the accuracy of the method from 3% to 67% while the sensitivity remained at 83%. The validation resulted in an accuracy of 72% and a sensitivity of 56%. In spite of this improvement, this result clearly needs improvement before it can be implemented.

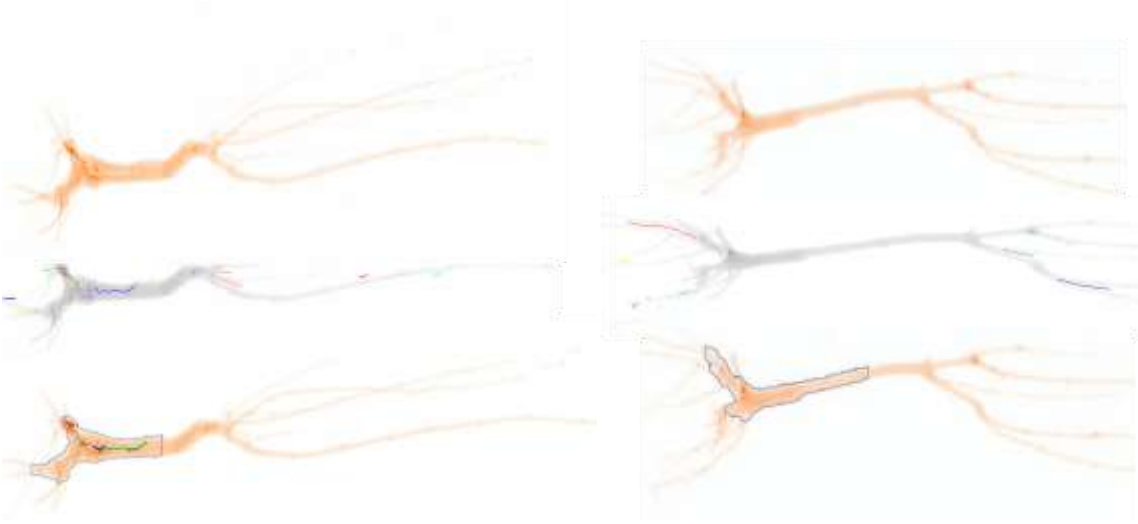

Figure 2-3. Illustrations of an image scan of a true positive n infested *Acer* tree (left) and true negative (right): raw image (top), automated image (middle) and optimised image analysis (bottom)

2) Improving borehole detection by combining machine vision and human input

The second objective of the 2D research was to test whether the borehole detection could be further improved by combining machine vision and human input. It was expected that the performance of tree classification would further improve by combining machine vision and human input. Therefore, the associated research question was: what is the effect of combining machine vision and human input on the performance of tree classification?

Methodology

To test the effect of combining machine vision and human input, a user friendly application was developed. This application consisted of the optimized borehole detection method integrated into a graphical user interface (GUI). Using this GUI, 100 X-ray images were shown to two inspectors from The Dutch General Inspection Service). These operators were trained for 30 minutes prior to the test and during the test images were shown one after the other for 10 sec. each.

Summary of results

When combining machine vision and human input, the total number of true positives and false negatives remained equal (9 each), but the number of false positives decreased and true negatives increased. Overall: combining machine vision and human input resulted in a further increase in accuracy up to 83%, sensitivity was 50%. Nevertheless, X-ray images of suspicious trees (true positives) were classified dissimilar. For instance, two X-ray images of suspicious trees were classified as negative by machine vision but classified as positive by both observers. The reason for negative classification by machine vision is the constraint that the boreholes have o be orientated nearly parallel to the centre line of the main stem. Also the opposite occurred: two X-ray images of suspicious trees (true positives) were classified as positive by machine vision but classified as negative by both observers. The classification

of the observers was similar: 88% of the images were classified equal. The combination of machine vision and human input resulted in an improved performance of X-ray assisted borehole detection for intact trees. We expect that training of X-ray operators will further improve this performance. However, the effect of operator training on the performance is mostly unknown. Therefore, we suggest to study the effect of operator training in order to quantify this effect.

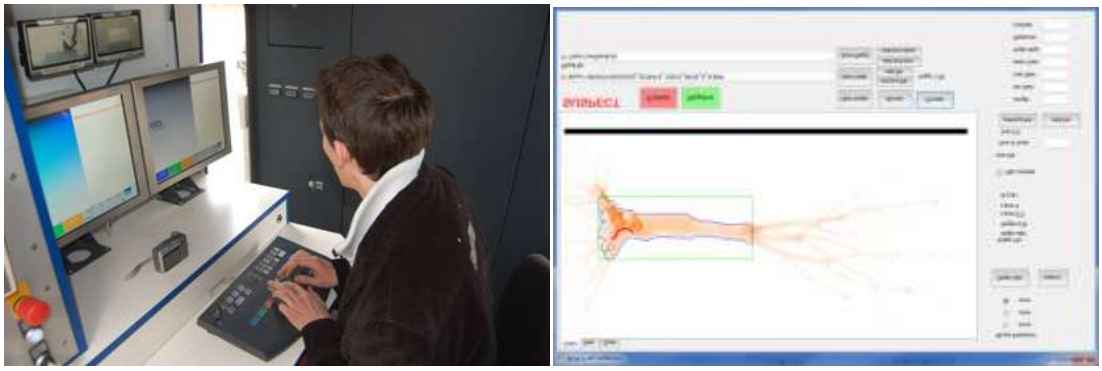

Figure 2- 4.Training of image recognition for inspectors (left) using optimized images from infested and non-infested *Acer* trees, including false end true positives and negatives (right).

3) 3D Imaging for the detection of *Anoplophora* in wood

Introduction

The investigation undertaken here was limited to establishing the proof-of-principle to detect and analyze the shapes and sizes of the various boreholes, with available 3D-imaging technologies. Main questions:

- Can 3D-imaging help to detect the presence of Anoplophora beetles non-destructively and economically in bundles of imported young/small trees at the time of import? (import inspection).
- Can 3D-imaging help to identify the presence of Anoplophora beetles from the shape and size of boreholes in mature trees? Can it help to establish the timing of infestation? (evaluation of field infestation sites).

This investigation was performed by **Chris Mol & Ronald Wolf (2011)** and was allocated at Philips Research (Eindhoven, The Netherlands) because of its expertise in 3D-imaging, related to Philips' commercial activities in Healthcare Imaging systems. In this project Philips Research has explored 3D imaging technique as currently employed in various healthcare applications. Here we summarise the main results, for a detailed report see Mol & Wolf, 2011).

Methodology

CT imaging was carried out with a (high-end) Philips Brilliance iCT scanner, which is able to image 256 slices in one circular rotation of the scanner. This scanner has a circular bore of 70 cm and a circular field-of-view size of 50 cm. The field-of-view is the circle in which the high resolution for medical applications is guaranteed. The tube voltage used was 100 kV. The slice-thickness was set at 5 mm. The machine resolution is in 0.3 – 0.5 mm in all

directions. The object was moved through the scanner with a speed of 20 cm/sec. No further optimization of the system to the object was pursued. The full scanning of this composite object lasted approximately 3 seconds and yielded 140 cross-sectional images.

In addition a Magnetic Resonance Imaging (MRI) was carried out with a (high-end) Philips MR Achieva 3Tesla system with a dual quasar gradient system. This MR system has a bore of 50 cm. Initially, the same phantom was used as with CT. Other imaging methods like ultrasound and nuclear imaging were reviewed, but these applications were not further explored for *Anoplophora* detection purposes.

Figure 2-5. Composite phantom for testing the capabilities of CT-imaging of wooden objects: wooden sticks with man-drilled boreholes of 2 and 3 mm , and 2 larger tree branches with a number of substantial boreholes from the long-horned beetle; oven-dried after harvest, and stacked dry at room temperature.

Summary of results

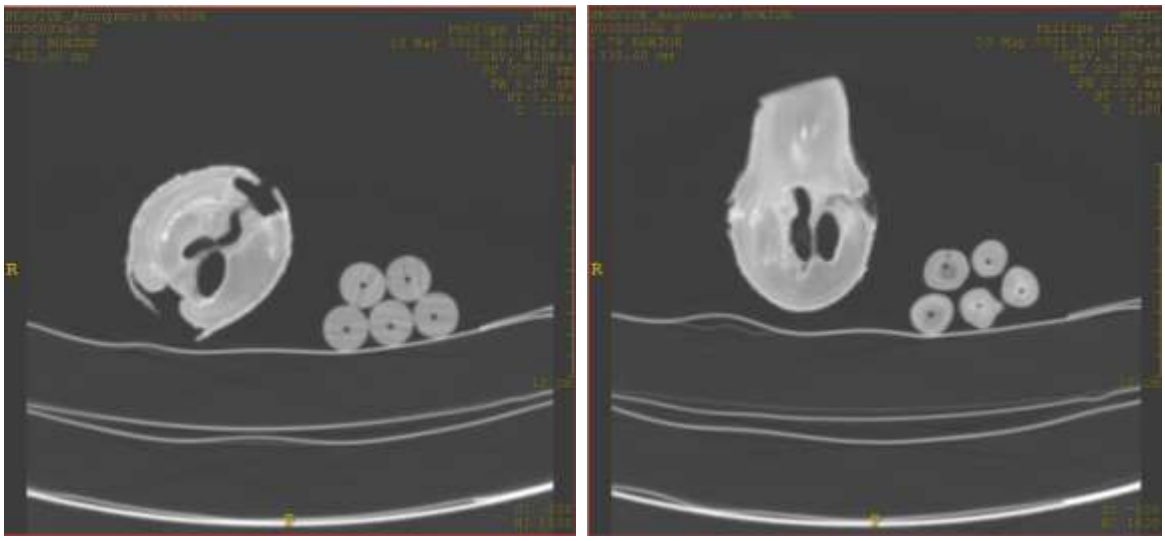

Figure 2-6. Cross-sectional CT images of a large tree trunk with beetle generated boreholes on the left and wooden phantoms on the right side. The structure on the lower half of the image is the folded (patient) mattress

CT scanning - Artificial boreholes in wooden objects can be identified easily, both in the sticks as well as in the small tree branches (figure 2-6). Also the boreholes in the larger tree

trunk are clearly delineated. Also various other structures inside the wooden tree trunk can be distinguished. Many year-rings can be identified and at some locations the wood is clearly more dense (X-ray absorbing) than at others, presumably related to the xylem content / wood density.

Imaging of boreholes and beetle larvae with CT - A further set of CT images was generated with the same CT scanner and scanning parameters. Subsequently, a 3D reconstruction was made. A range of differently oriented cross-sections and 3D surface images was generated from this clearly showing adult exit-holes, pupal; chambers and larval galleries. A CT scan was made of a tree trunk, which contained the (dry) remains of long-horned beetle larvae. The resolution along the scan axis was selected to be 1 mm, yielding 200 images over 20 cm. In below images, again cross-sections were selected to optimally visualize shape and size of the larvae in the boreholes. The small images on the right side of each figure show cross-sections in directions perpendicular to the direction of the main image (figure 2-7). Measurements show that the dimensions of the larvae can be measured well. The length is approximately 37 mm, the thickness here is between 5 and 8 mm.

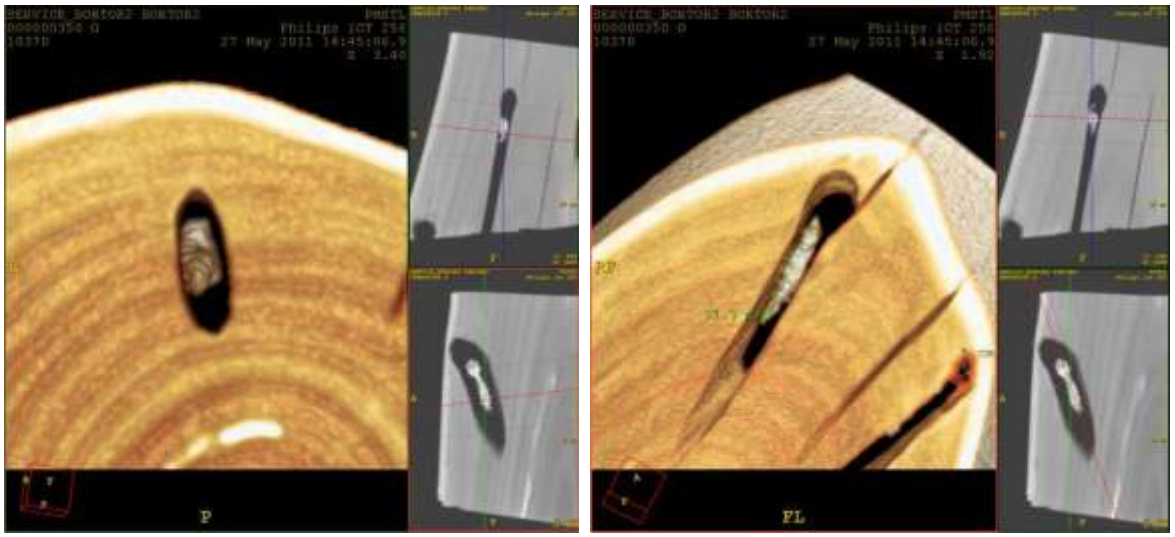

Figure 2-7. CT images of beetle larvae imaged inside a tree stump. The figures show a volume-rendered slab of the tree, oriented so as to optimize the viewing of the larvae. The small black white images on the right side of each image show the cross-sections of the tree in planes perpendicular to that of the main (coloured) image

Imaging of stacks of small trees with CT - In this experiment, approximately 40 small trees were packaged in - plastic bags in - a cardboard box, to approach the situation during import of such trees from Asia (figure 2-8 left). Some of the trees were specially prepared to mimic the presence of boreholes of long-horned beetles by cutting the trees, drilling holes between 2 and 4 mm, app. 3 cm deep, and glueing the tree parts back together. The whole image series consisted of 1350 images, each 0.36 cm apart, across the length of the 50 cm long box. Figure 2-8 (right) shows a single cross-section through this bundle of trees. This image was selected at a cross section approximately 1/3rd along the length of the box (at slice 403/1369), cutting through the main tree trunks of the tree bundle on the left side, while cutting through some of the branches of the right-hand side bundle, showing up as the smaller blobs. Structures were identified, where dark patches were found, not corresponding

to the artificial boreholes. Further exploration of the actual trees demonstrated physical deformities (discoloration of wood as a result of rot and die-back) in some of these trees, which could well correspond to (and explain) the CT findings. Although interesting from a more general quality inspection point of view, no detailed comparison was made between the CT images and the related tree deformities. We concluded that the 3D CT images contain enough information to identify boreholes of long horned beetles in bunches of small import trees. We expect not so much to miss relevant information (false negatives) but, if anything, to find too many 'abnormalities' (false positives). A quick estimate of through-put using a (Brilliance 16) CT scanner shows that at a rate of 250 boxes / 8 hrs (ca. 1 box / 2 min; 40 trees / box) and 200 days /yr around 2.000.000 trees could be inspected per year.

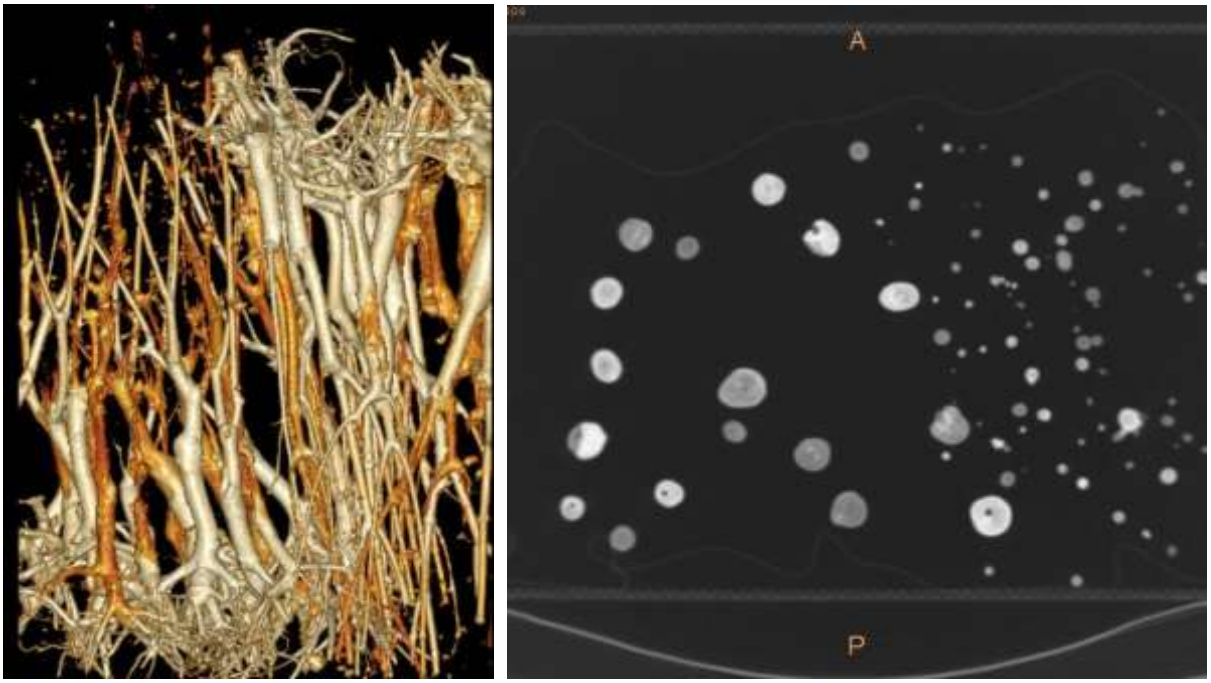

Figure 2-8. 3D-CT image of a stack of small (length 50 cm) *Acer* trees (left) and cross-section through the tree bundle showing demonstrating the clear delineation of artificial boreholes in individual trees, as well as further wood structure within the trees (right).

CT Image Analysis and 3D-processing – A normal output of CT imaging is a series of cross-sectional images (slices) through an object. This often does not give, to the untrained observer, a clear impression of what the imaged object looks like in 3 dimensions. Therefore, most high-end medical CT-scanners contain software to generate 3D images of entire volumes and of 'slabs' of imaged objects, in order to help medical interpreters. Automatic identification of boreholes from long-horned beetles in wood is not a standard, commercially available, product today. For this specific purpose a 'Philips Imalytics' working station (http://www.imalytics.philips.com/) was executed automatically by a software program to analyse the detection of boreholes in bundles of import trees. An Imalytics workstation contains many special tools to perform a wide range of image processing functions on CT (and other imaging modalities) image data. A first initial assessment showed it was feasible to identify in 3d the individual trees within a bunch (figure 2.9 left)) and boreholes within these trees (figure 2,9 right). This implies that in an actual implementation we could aim at a scenario in which no human interpretation of the CT-images by (trained) experts would be

necessary. A system that automatically alerts an inspector to actual beetle infestation would thus (ultimately) be feasible.

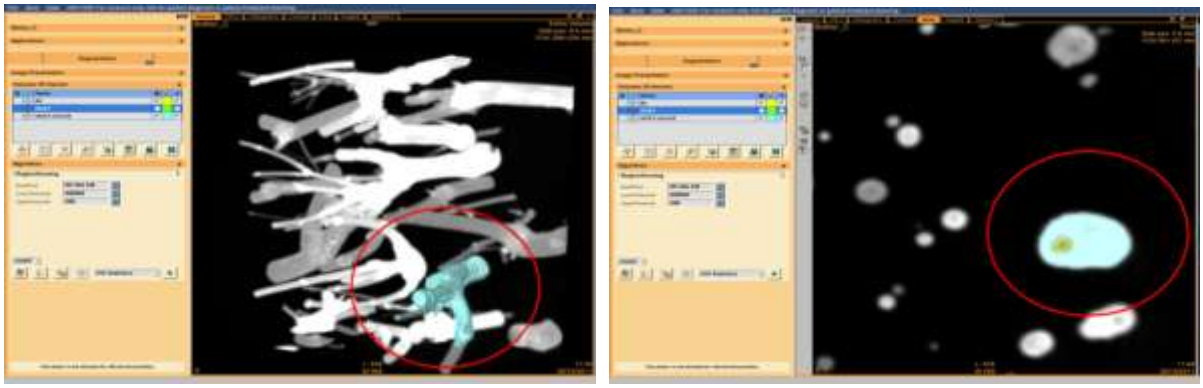

Figure 2-9 Screenshot of the Philips Imalytics workstation, while processing the images of a stack of trees, in the red circle identifying a single tree (left) and the automatic detection of an (artificial) borehole (right)

Summary of results

In the 3D project we have demonstrated (Mol & Wolf, 2011) that CT imaging is the method of choice for this application. MR images have insufficient resolution while other imaging methods are not suitable at all. After establishing this, we have applied CT imaging methods to various samples of trees, demonstrating that:

- air-filled bore holes down to 2 mm in wooden samples can easily be visualized by CT imaging (Figure 2-6);
- the shapes and sizes of bore holes in infested wood can be visualized in various 3D-display modes
- (dried) larvae of long horned beetles can be identified and visualized (Figure 2-7).
- pseudo *Anoplophora* bore holes in large (40 unit) bunches of small trees can be spotted visually in the CT cross-sectional images.We estimate that automatic detection of such bore holes requires substantial image analysis effort, but is quite feasible.

We conclude that the successful application of CT imaging for the inspection task at hand is technically quite feasible. Based on the observations of this study we also expect that making use of specific algorithms tuned to specific infestations, CT imaging could be used as a routine inspection tool also outside the infestation area covered in this report. When exploring logistic and financial scenario's for the application of CT imaging in plant inspection, a trade-off between cost and the probability of *Anoplophora*-detection is apparent. We expect that the current inspection procedure can be improved with CT imaging, but a follow-up project is necessary to identify the specific requirements to optimize the scanning and software technology for detection purposes.

4) General conclusions

The present research was based on X-ray images recorded on a system dedicated to luggage inspection (2D) and human health care (3D). Results show an increase in accuracy of inspection potential using X-ray instruments. Both type of X-ray instruments are not optimal for phytosanitary inspections, such as borehole detection in trees. Therefore, the design and build of an X-ray instrument dedicated to phytosanitary inspection needs consideration. Since the volume of suspect tree imports in the Netherlands is quite limited, 2D X-ray and also a CT scanner cannot be fully occupied by this application alone. To cover its fixed costs, it will be necessary to identify further (inspection) applications for CT imaging in this environment, whether other phytosanitary problems are detectable using X-ray. Attractive new applications may be found in the objective, quantitative measurements of plant characteristics, such as size, number of branches, root-structure, potentially leading to increased value in the plant business (e.g. via a quality label). The Dutch NPPO has started exploring further research and implementation of X-ray instruments in collaboration with Wageningen UR and Philips Research. When setting up a list of prerequisites for such an instrument, different stakeholders will participate, including personnel working at NAK Tuinbouw, NVWA, and the Dutch Customs Laboratory, as well as experts in phytosanitation. Furthermore, the effect of operator training needs exploration and quantification since we expect that training will improve the detectability of boreholes in intact trees.

In addition, also a system that automatically alerts an inspector to actual beetle infestation would (ultimately) be feasible, both for the 2D and 3D scanning technology. To execute such an image analysis task automatically (i.e. without input from the user) requires the development of dedicated application software that needs to be tuned to the precise application.

Project Reports

- Jansen, R.M.C. & Hemming, J. (2011). “Validation of X-ray for borehole detection in intact trees” Draft Wageningen UR Greenhouse Horticulture, Wageningen / Bleiswijk, December 2011, 26 pp.
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- Jansen, R.M.C. & Hemming, J. (2010) “X-ray inspection for boreholes in intact trees”. Rapport GTB-5006, 2010 Wageningen, Wageningen UR Greenhouse Horticulture (Wageningen UR Glastuinbouw).

Anoplophora detection dogs – practical use in different operation areas and development of an educational concept

Report P3 – U. Hoyer-Tomiczek, G. Sauseng, BFW, Vienna, Austria

The most important measure in any case of prevention of introduction and probably infestation of *Anoplophora glabripennis* (Asian longhorn beetle, ALB) and *Anoplophora chinensis* (Citrus longhorn beetle, CLB) is the detection and identification of infested plants, trees or wood packaging material. Because of the hidden life cycle of the beetles the visual detection is difficult and leads to false negative decisions by the inspector on several occasions. Destructive sampling can increase the finding ratio, but is not ideal as it is costly to the importer, time consuming for the inspector and not always possible.

For efficient, non-destructive inspection of plants at import or trees in infestation areas as well as of imported wood packaging material a new method by using dogs for detection of ALB was established at the Department for Forest Protection since February 2009. It is well known that dogs are used for detection of e.g. explosives, narcotics, fire accelerants, people or various biological materials (Browne, Felgentreu). Henceforward dogs can detect with their extremely high sensible nose scent traces of *Anoplophora glabripennis* (Asian longhorn beetle, ALB) and further indicate them. Due to the close relationship of *Anoplophora chinensis* (Citrus longhorn beetle, CLB) to ALB and overlapping scent patterns, the dogs could detect both species.

Dogs from breeds or breed mixtures with high working willingness, endurance, and high drive to find the scent source – like hunting dogs - are requested for this task.

A complex dog training program was installed, using a reward system response with playing or food. Dog and handler are working as a team. Within a playful training the team should work with happiness. *Anoplophora* detection dogs are trained for searching different developing stages of ALB and CLB in host plants and wood packing material. The imprinting process is done with scent material of all development stages. With an active indication like scratching or barking or passive indication like watching and sitting in front of the scent source the dog shows where the scent source is hidden. Training is performed under various conditions in different environments including real ALB/CLB infested areas. The dogs learn to know which materials should be investigated and to work systematically through an area.

The four presented Austrian detection dogs Jackson, Jolly, Andor and Aline have been worldwide the first trained *Anoplophora* detection dogs being able to detect ALB and CLB in many various situations. All four dogs are additionally trained also on other features. The description of the dogs in detail:

- Jackson:
  - born March 2007
  - trained as man trailing and cadaver dog
  - trained on ALB/CLB since February 2009
  - he is the “prototype” of an ALB/CLB detection dog
  - † 20.02.2013

- Andor:
  - born September 2007
  - trained as hunting dog
  - trained on ALB/CLB since May 2009

- Jolly:
  - born January 2009
  - trained on ALB/CLB since March 2009
  - trained as man trailing dog since May 2009
  - trained on drugs since August 2009

- Aline:
  - born June 2002, Andor‘s mother
  - trained as hunting dog
  - trained on ALB/CLB since March 2010

D2.18

Evaluation of the potential use of ALB/CLB detection dogs in different operation areas

D2.18.1

Use of ALB/CLB detection dogs for investigation of ALB/CLB in infestation areas

The detection dogs can be used for the following items:

- monitoring in the infested/demarcated area and buffer zone in public and private property to find infested trees,
- investigation of trees, stumps, roots due to suspicious symptoms,
- verifying of suspicious samples taken by tree climbers or inspectors,
- cooperation between detection dog teams and tree climbers,
- investigation of areas after preventive cuttings,
- investigation of (preventively) felled trees for identification of the dimension of infestation, in newly discovered infestation areas,
- monitoring of dense growing tree stands/forests,
- monitoring in agricultural and natural environment,
- investigation of the growing trees in nurseries within the demarcated area,
- investigation of public collection sites for green waste,
- investigation of fire wood.

The main testing, training and use of as well as monitoring with the four Austrian Anoplophora detection dogs were done in infestation areas of ALB and CLB in other European countries because in the Austrian infestation area no ALB infested trees and no beetles were found since June 2009. To ensure the results of the detection dogs, also experiences of their work in the year 2010 before start of the ANOPLORISK project are involved in this report.

The Anoplophora detection dogs worked in the following infestation areas:

1. ALB in Austria since 2009:
   - Braunau: currently no infestation known, use of detection dogs for investigation of areas after preventive cuttings, of suspicious trees and plants in nurseries
   - Oberaichet/St. Georgen: new ALB infestation area since July 2012: monitoring of the core area of infestation by investigation of (preventively) felled trees with dogs
2. ALB in Italy/Venetia, Cornuda + Maser:
   - (July 2010), May 2011
3. ALB in the Switzerland/Cantons Fribourg, Basel, Thurgau, Zürich:
   - Oct. 2011, May 2012
4. ALB in Germany:
   - Bavaria: Neukirchen: Nov. 2011
   - Baden-Württemberg: Weil/Rhein: May 2012
5. ALB in UK/Kent, Paddock Wood:
   - Aug. 2012
6. ALB in Switzerland/Canton Zürich, Winterthur
   - July – December 2012 by Swiss detection dogs, trained by BFW
7. CLB in Italy/Lombardy, Region Milan:
   - (July, Sept., Oct. 2010); May 2011, Oct. 2011; Sept. 2012
8. CLB in Croatia/Dalmatia, Turanj:
   - June, July 2011

D2.18.1.1.1. ALB infested area in Austria/Upper Austria, Braunau, 2009 – 2012

In the first ALB infestation area of Europe the detection dogs were used to investigate trees in private gardens and public areas, dense growing stands of trees and small forests, the stumps of trees of preventively cut areas, suspicious trees and samples taken by the ALB trained tree climbers. Additionally the public collection sites for cut plant material were target of the dog monitoring like also the nurseries within the demarcated area. Both locations are high risk sites because cut plant material can be infested by ALB and trees of a nursery inside a demarcated area can also be attacked by ALB and, if sold, can contribute to the distribution of the quarantine pest. In Braunau the dogs were able to detect several stumps with ALB infestation, a remaining stem part with a full developed ALB beetle inside, and confirmed a pupa as ALB found by tree climbers.


Fig. 1-2: Investigation by the dogs of suspicious trees and suspicious samples cut by tree climbers

Fig. 3-5: Monitoring of the stumps and shoots after preventive felling of trees along the main road in Braunau in the first spring (left and middle) and in the second spring after cutting (right).

In densely growing stands and forests the visual monitoring is very difficult and far from being satisfactory, especially if ground vegetation like nettles, blackberries and herbage exacerbate the through coming of the inspectors and if the growth of ivy makes the visual inspection impossible.

Fig. 6: Monitoring of a small, densely growing forest in Braunau with the detection dogs.

Fig. 7-8: Despite of the heavy smell of rotting the dogs are able to detect the ALB scent within the cut material on collecting sites.

Fig. 9-11: Monitoring of trees of a nursery within the demarcated area of Braunau with a detection dog. Due to stem protections visual inspection could not be carried out, but sniffing detection by the dogs is unproblematic makeable.

D2.18.1.1.2 ALB infested area in Austria/Upper Austria, St. Georgen, July-August 2012

At the end of July 2012 a new ALB infestation was detected in Upper Austria in St. Georgen at the ground of a stone importer who also imported stones from China. Immediately an intensive monitoring was started combined with preventive felling of all host trees, also fruit trees like *Malus* ssp., *Pyrus* ssp. and *Prunus* ssp. within a radius of 500 m around the core of the infestation. This was done to realize the size of the infested area and to eliminate the potential breeding material for ALB. The cut trees were either investigated visually by inspectors or by the detection dogs. The dogs checked the crowns of the laying trees branch by branch also inside the crown. If they showed any indication, that part of the crown was investigated carefully visually to find any symptom of ALB. Therefore two trees could be recognized by the dogs to be infested, one *Prunus* tree and one horse chestnut tree. The *Prunus* tree had only dead oviposition sites and starting larval galleries without living stages, but the horse chestnut tree had one exit hole and three additional larvae inside their pupa chambers.


Fig. 12-15: Investigation of the preventively felled trees with the detection dogs to realize the size of the infestation area and to find infested trees.



Fig. 16-18: Felling of the horse chestnut tree for preventive reasons and indication by the detection dog. Afterwards finding of the exit hole and the larvae inside the pupa chambers.

All together five infested trees were found, two of them by the detection dogs. During the other investigation time the dogs were not present. The investigation with detection dogs of preventively felled trees is much less time consuming than the visual investigation of inspectors and more reliable because the dogs can smell ALB scent even there where symptoms are not visible due to leaves, ivy or other hiding or covering things.

D2.18.1.2. ALB infested area in Italy/Venetia, Cornuda and Maser, July 2010 and May 2011

The dog monitoring in Venetia was thankfully supported by the Plant Protection Service of Venetia, especially by Dr. Marco Vettorazzo, and by the Forest Service of Venetia (Servizio Forestale), especially by Tiberio Zanini.

The main task of the detection dogs in Cornuda and Maser was to investigate places where still trees were standing in the surrounding of already felt infested trees and places where during the last years repeatedly trees were felt due to ALB infestation. These places were public green or parks, private gardens as well as windshield stripes in agricultural environments.

In total 13 places of interest were investigated and at eight of these places the dogs indicate one to three trees each, all together at least 13 trees. In 90% of indication two dogs indicated accordingly, often a third dog confirmed the indication by the two other dogs.

During the visual monitoring in autumn 2011 trees indicated by the dogs were checked carefully. In all cases of indication by the dogs ALB infestation was confirmed by the inspectors.

Fig. 19: Indication of a tree in a public park

Fig. 20: Dog investigation of trees along a ditch in an agricultural area

Fig. 21-23: Monitoring in a private garden, indication of a tree by the dog. Visual verification was complicated by the growth of *Hedera helix*.

Fig. 24-26: Investigation of a windshield stripe in an agricultural environment, indication by a dog and marking of the trees indicated by the dogs.


Fig. 27-28: Investigation of trees at the ground of a sports centre

D2.18.1.3. ALB infested area in Switzerland/Canton Fribourg, Brünisried and monitoring in endangered areas in Canton Basel, Basel and Canton Thurgau, Salenstein, October 2011, May 2012

In August 2011 a new ALB infestation in the Canton Fribourg was detected in the small village Brünisried. Because only one *Acer* tree and a hedge of *Acer* plants with ALB oviposition sites were found and no evidence on other symptoms or any hint for longer presence of this infestation, the Anoplophora detection dogs were requested for investigation of the infested zone.

The stays in Switzerland were financed and supported by the Swiss Federal Office for Environment (BAFU), especially by Rolf Manser and Martin Büchel. In Brünisried the detection dog teams were mainly supported by Bruno Suter of the local authority, in Basel by Stephan Ramin of the local Plant Protection Service, and in Salenstein by Ernst Fürst of the BAFU and local authorities.



Fig. 29-31: *Acer* tree in Brünisried/CH indicated by the dogs. Visual investigation of several branches yielded in finding of dead oviposition sites, starting larval galleries under the bark, but no living stages.

In Brünisried the dogs monitored all deciduous trees in the village and could find additional trees which were suspicious to be infested by ALB. One tree was part of the hedge and could be confirmed immediately by preparing young ALB larvae. Another *Acer* tree indicated by one dog in October 2011 was investigated again in May 2012 and repeated indicated by two dogs. Branches with suspicious symptoms were cut down and analysed. Oviposition bits, starting galleries of young larvae and feeding symptoms below the bark could be detected, but no living stages were found anymore. Therefore it seems that the hedged young larvae died due to temperatures around -10° degrees in November 2011 because they could not succeed to go deeply enough into the wood to be protected to freezing temperatures. Also wood packaging material was checked by the dogs to probably identify the source of infestation but without finding ALB infested material.


Fig. 32-33: Checking of wood packaging material in Brünisried to probably identify the source of ALB infestation.

In Basel ALB monitoring with the detection dogs was conducted in October 2011 and also in May 2012 in the close surroundings of the different ports because in one port living ALB larvae were found by the dogs in allegedly treated wood packaging material from China associated with granite stones. During these monitoring no ALB infested trees were found, only infestations by *Zeuzera pyrina* and *Cossus cossus* which were not indicated by the dogs. In summer and autumn 2012 monitoring with tree climbers confirmed the results of the dogs.



Fig. 34-35: Monitoring with detection dogs of *Betula* trees along a port in Basel. Symptoms of *Zeuzera pyrina* infestation could be observed, but were not indicated by the dogs.

In Salenstein dead ALB beetles were found in-between granite stones on wooden pallets from China in October 2011. It could not be verified anymore if maybe also living beetles could escape and infest trees in the surrounding. Therefore a monitoring with the detection dogs was performed in May 2012 but no infestation by ALB could be detected.

D2.18.1.4.1. ALB infested area in Germany/Bavaria, Neukirchen, November 2011

The stay in Germany/Bavaria was supported and financed by the Bavarian State Institute of Forestry (LfL), especially by Carolin Bögel.

The task of the detection dogs in this infestation area was the investigation of trees in places where ALB infested trees were found and felt before or where susceptible trees were dense or high growing. The detection dogs indicated one residue of a large, already felt *Salix* tree where oviposition sites were visible. Due to molecular genetically analysis at the Department of Forest Protection of the BFW six prepared eggs of this belonged to *Anoplophora glabripennis*.



Fig. 36-38: Monitoring with the detection dogs on a cemetery, the original infestation core in Neukirchen (left). Outside of the cemetery the detection dog indicated the residue of a big *Salix* tree (middle) on which oviposition sites were visible and ALB eggs could be prepared (right).

In different private gardens also storages of fire wood were investigated with the dogs because this kind of wood could be cut from ALB infested trees in the past and serve as a source for further new infestations.

Fig. 39: Investigation of fire wood by a detection dog

D2.18.1.4.2. ALB infested area in Germany/Baden-Württemberg, Weil/Rhine, May 2012

In Weil at the river Rhine in the port some living ALB beetle were observed in late summer 2011 obviously emerged from wood packaging material which was imported with granite stones from China. In the spring time 2012 the German detection dog team DI Hermann Meier and his dog “Rika” from the Plant Protection Service of the regional authority in Stuttgart, Baden-Württemberg, which were trained by the BFW in 2011, checked wood packaging material in the port of Weil. Besides of ALB infested wooden pallets the dog Rika also indicated one *Platanus* tree standing very close to the packaging units and at one branch in five metres height one ALB larvae could be prepared.

During the following monitoring in May 2012 the Austrian detection dogs and also the German detection dog indicated a *Prunus* tree accordingly standing close to the *Platanus* tree, but the inexperienced inspectors could not find ALB specific symptoms during the investigation after felling few weeks later.

Fig. 40-41: The German detection dog Rika indicated the *Platanus* tree next to the packaging units. At one branch (circle on the left photo) a young ALB larvae could be detected under the bark (circle on the right photo).

Fig. 42-44: The Austrian detection dog Andor (left), the German detection dog Rika (middle) and the Swiss detection dog Lara (right; also trained by BFW) indicated the *Prunus* tree next to the infested *Platanus* tree accordingly.

Later in July 2012 the German detection dog indicated additionally an *Acer* tree in the near surrounding of these both trees mentioned before. Two very small marks could be observed at the bark, being not obviously symptoms of ALB. This tree was visually monitored several times before by the inspectors without recognizing any ALB symptoms. Opening the stem of that *Acer* yielded in finding of two middle-size ALB larvae, indicated doubtlessly by the detection dog.

Fig. 45-47: The *Acer* tree indicated by the detection dog with the two small marks at the bark (left, middle) and the two ALB larvae inside their short galleries in the opened stem (right).

Due to this finding and result of the *Acer* tree the inspectors admitted that they probably missed some ALB symptoms on the *Prunus* tree which was indicated by five detection dogs.

D2.18.1.5. ALB infested area in United Kingdom/Kent, Paddock Wood, August 2012

The ALB infestation in Paddock Wood was detected in March 2012. An intensive monitoring was started but without ALB experienced tree climbers and all potential host trees within a radius of 100 m around infested trees were preventively felled (in total 5400 trees) and visually investigated at the ground to define the real size of the infested area. In this way the inspectors could find additional infested trees, all together 68, also one large poplar tree of 20 m height having oviposition sites and young larvae in the top part of the crown. In August 2012 the Austrian detection dog teams were invited to investigate the edges of the summarized 100 m zone of eight hectares during six working days to find probably additionally less infested trees or such with only early development stages. The stay in UK/Paddock Wood was financed and supported by the Food and Environment Research Acency (FERA), especially by Graham Brookes, Peter Scotting and Dominic Eyre and also by Nike Fielding of the Forestry Commission. The dogs monitored single standing trees, hedges and windshield stripes along main roads and agricultural fields as well as trees in private gardens. All together they indicated six trees to be infested by ALB. Visual verification wasn't possible in every case because of lack of ALB experienced tree climbers.

Fig. 48-49: The willow at the corner of the private garden was indicated by two dogs (left). Branches with suspicious symptoms were cut down and laid on the ground in a line-up for the dogs (right).

Fig. 50-51: The dogs investigated the willow branches on the ground (right) and identified one with an old dead oviposition site of ALB.

Fig. 52-54: Monitoring of windshield stripes along main roads with the dogs was exacerbated by strong wind. Accordingly indication by the dogs led to the felling of to neighboured willows.

D2.18.1.6. ALB infested area in Switzerland/Canton Zürich, Winterthur, monitoring by the Swiss detection dogs, July – December 2012

In Winterthur in the Canton Zürich of Switzerland in the middle of July 2012 a big infestation of ALB was discovered. Fortunately five dogs with their four dog handlers from Switzerland had successfully passed the BFW training courses for Anoplophora detection dog teams and could start to work in the infestation area immediately. Despite of less experience in real insets for ALB/CLB searches the dogs and their dog handlers did a very good job within the monitoring. After the first two weeks two dog handlers and their detection dogs were regularly twice per week in Winterthur for ALB monitoring. These dogs detected additional nine infested trees and several others at the beginning of the monitoring during the first two weeks which were not counted. Further six trees were indicated by the dogs but so far not confirmed due to the lack of obvious symptoms of ALB. In Winterthur the detection dog teams and the ALB trained tree climbers work hand in hand which yielded in very good results. Trees which were indicated by the dogs were carefully investigated visually by the tree climbers for symptoms. The tree climbers could find in one case only one oviposition site on an *Acer* tree in four metres height after according indication of the two dogs. If the tree climbers found any suspicious symptoms they cut down the branches or twigs and the dogs checked for ALB presence. In another case one of the detection dogs indicated from the ground a very large poplar. For verifying a probably ALB infestation the dog was lifted with a lifting platform up into the crown and the dog identified by sniffing the infested branch on which small ALB larvae were found. In all cases of indication by the detection dogs the finding of ALB was confirmed by sequencing analysis at the WSL (Swiss Federal Institute for Forest, Snow and Landscape Research, Birmensdorf). The dogs were also used for investigation of potential ALB host plants in nurseries and garden centres.



Fig. 55-57: The Swiss detection dogs at monitoring in the infested zone of Winterthur (left), checking potential host plants in garden centres (middle) and verifying from the lifting platform.

D2.18.1.7. <u>CLB infested area in Italy/Lombardy region, Milan and surrounding areas, (July, Sept., Oct. 2010); May, Oct. 2011; Sept. 2012</u>

Two visits were financed by the Minoprio Foundation, the other by the project ANOPLORISK. All visits in Lombardy region were thankfully supported by the Plant Protection Service of Lombardy region, especially by Dr. Beniamino Cavagna, and by the Minoprio Foundation, especially by Matteo Maspero.

The detection dog teams visited places and parks in Milan and Legnano with known CLB infestations where during the winter periods infested trees had been felled. The task was to check if still standing trees were also infested by CLB or not. Some of the parks have very natural character and include lakes and small ditches. In every location the dogs were able to detect additional infested trees which have not been recognized by the monitoring people before.



Fig. 58-60: City park in Milan with known CLB infested trees (left up) and unknown infested trees (right up) which were found by the dogs (left).

Fig. 61: City park in Milan: cutting of CLB infested trees (orange dots), but additional infested ones were overseen and found by the dogs (red arrows).

Fig. 62-63: Natural park in Milan with CLB infested trees along the water line.

Fig. 64-67: Group of infested trees at the water line of a lake in a naturalpark in Milan (middle): the dogs indicated several trees and saw dust of CLB could be found at the stem bases (left and right).

For the monitoring along the lakes and ditches it is necessary that the dogs have no problems with the water and are fearless to work there.


Fig. 68-69: Monitoring of trees at or in the water line of lakes by the detection dogs.

Also in the agricultural areas around Milan like in Montechiari and Assago the dogs could find infested trees which were unknown to the monitoring people. In one agricultural area of culturing rice the work of the dogs was exacerbated by water conducting ditches. The trees accompanying the ditches were infested by CLB and the investigation work also by the dogs had to be carried out from the water side.


Fig. 70-71: Investigations of trees along the ditches around the rice culturing fields by the dogs.



Fig. 72-74: Investigation of trees at a hill in the natural environment of Brescia requires self-dependent work of the dogs resulting in finding of some infested trees.

In all cases of indication by the dogs the confirmation of real CLB infestation was easily done by Matteo Maspero because the typical infestation symptoms were in most cases at the base of the trees.

During one of the stays in Italy/Lombardy region in May 2011 the work of the detection dogs was presented to 30 participants of an EPPO Training meeting of international Plant Protection Service inspectors.

D2.18.1.8. CLB infested area in Croatia/Dalmatia, Turanj, June and July 2011

In 2007 a CLB infestation was detected in one nursery in Turanj, Dalmatia, on imported plants from China. Thanks to intensive monitoring (every year monthly from May until October) and radical destroying of whole consignments of infested plants conducted by the Croatian Institute for Plant Protection a distribution into the surrounding of the nursery could be inhibited so far. Nevertheless the intensive monitoring of plants within the area of the nursery is continued for several years.

Both visits in Croatia/Dalmatia were thankfully supported by the Croatian Institute for Plant Protection in Zagreb, especially by Mr. sc. Andrija Vukadin, and by the Forest Faculty of the University of Zagreb, especially by Prof. Dr. Boris Hrašovec. One trip to Croatia was financed by the Croatian Institute for Plant Protection and the responsible ministry.

The task of the detection dogs in Turanj was to investigate all suitable host plants in the green houses and in the field area of the nursery, especially in the near surrounding of that green house where the infested consignments were stored in the past.

Fig. 75-76: Outdoor areas of the nursery in the near surrounding of the former CLB infested green house

Inside the green houses the dogs checked several hundreds of roses because CLB distributed on roses during the first year of infestation in that nursery, and hundreds of cherry laurel plants (*Prunus laurocerasus*) as well as other horticultural plants. The investigation of the roses and other host plants with the dogs at 38ºC and high air humidity in the green house was very exhausting and it was necessary to save the physical resources of dogs and humans under these extreme conditions. The monitoring of the roses also included the risk of injury of the dog noses due to the thorns.

Outside in the field area of the nursery next to the green houses the dogs investigated all species of suitable host plants, hedges of cherry laurel plants and especially Lagerstroemia plants because the original infested consignment imported from China consisted of this species.

Fig. 77-79: Monitoring of roses, cherry laurel plants and other horticultural plants in the greenhouse of the nursery in Turanj.

One Lagerstroemia plant was proved positive by the dogs. The investigation of the whole plant resulted in one small larva symptom at the base of the crown. Carefully destruction of the crown revealed in the finding of one CLB larva. Due to the molecular genetically analysis of the larva carried out at the Department of Forest Protection of BFW, Austria, it clearly belonged to the CLB population introduced 2007 to Croatia.

Fig. 80-83: Indication of one Lagerstroemia plant by the detection dog (left), the small symptom at the base of the crown (middle) and the gallery (right up) of the CLB larva (right down) found by destructive sampling of the plant.

No further plants within the whole area of the nursery could be found to be infested by CLB during both stays for investigation. Also outside of the nursery in the close surrounding no evidence for the presence of CLB was found.

D2.18.1.1-8 Summary of the results of the detection dog monitoring in ALB/CLB infested areas

- AT/Braunau:
  - finding of some infested stumps which had been overseen by visual control
  - finding of one developed ALB-beetle inside a remaining part of an infested stem
  - confirmation of ALB pupa found by tree climbers, molecular genetically confirmed by BFW
- AT/Oberaichet, St. Georgen:
  - 2 of 5 infested trees were found by the dogs during investigation of preventively felled trees
  - 3 other trees were detected by visual checking after felling (dogs not present)
- IT/Cornuda and Maser:
  - at 8 of 13 infestation points the dogs indicated suspicious trees
  - confirmation at all points during visual monitoring in autumn 2011
- CH/Brünisried:
  - 2 additional infested trees were found by the dogs and confirmed
- CH/Winterthur (Swiss detection dogs trained by BFW):
  - 9 infested trees were found by the dogs and confirmed by WSL
  - 6 additional indicated trees, but no confirmation so far
  - several additional not counted trees in the first days of felling
- DE/Bavaria, Neukirchen:
  - 1 additional infested tree stump were found by the dogs, confirmed by BFW
  - maturation feeding symptoms were indicated by the dogs
- DE/Baden-Württemberg, Weil am Rhein:
  - the infestation was detected by the German detection dog (trained by BFW) by indicating the infested *Platanus* tree
  - 1 additional infested tree *Acer* was indicated by the German detection dog and confirmed by finding of 2 larvae
  - 1 additional most probably infested tree *Prunus* was indicated by the several dogs, but inexperienced inspectors oversaw early symptoms
- UK/Kent, Paddock Wood:
  - indication of 6 trees by the dogs at the edge of the 100m zone
  - because of lack of experienced tree climbers probably confirmation next spring
- IT/Lombardy region, Milan and surrounding:
  - at every location the dogs detected so far unknown CLB infested trees, independent if in urban parks, in natural parks or in agricultural or natural areas
- CRO/Dalmatia, Turanj:
  - detection of 1 Lagerstroemia plant in the outdoor area of the nursery

- 90% according indication of at least 2 dogs (Andor + Jackson; Maisha + Waiko (CH))
- in (almost) all cases the dogs correctly indicated infested trees

D2.18.2

Use of ALB/CLB detection dogs for investigation of wood packaging material (WPM)

- importers of goods, especially from China, especially (granite) stones
- ports
- airports
- customs points
- packing-centers

- points of use/end user

Wood packaging material (WPM) from Asian countries was and is mainly the source for ALB infestation in Europe. Therefore the International Standard of Plant health Measures (ISPM No. 15) was introduced worldwide according to which WPM has to be treated either by heat treatment or by fumigation with Methyl bromide to ensure that possibly living insects within the WPM are killed before transfer to other states. The treatment according to that standard has to be marked on the WPM by a specific stamp. Despite of the present of the ISPM 15 markings very often WPM was infested by different insect species like *Anoplophora* ssp., *Apriona* ssp., *Sinoxylon* ssp. or other genera when arriving in European countries in the last years. Visual investigation led very often to overseen infestations because the symptoms are not always obvious or the insects are in inactive stages. Therefore the investigation with the detection dogs could increase the detection of infested WPM.

The experiences of the past showed that WPM of less quality and higher infestation rates is mainly associated with cheaper consignments like (granite) stones, especially from China, but also from India.

The Austrian Anoplophora detection dogs worked in the ports of Basel, Switzerland, in the port of Weil at the river Rhine, in ports at the rivers Danube and Enns in Austria as well as directly at the storage places of stone importers mainly in Austria. The best and easiest way to investigate WPM used for the transport of stones with detection dogs is directly inside the container if there was no fumigation conducted in the container before.


Fig. 84-85: Checking of WPM inside the container with detection dogs.

But also at the storage places in ports or at stone importers the dogs can check faster and more accurately the huge amount of WPM units. The WPM units mostly stand in lines of ten to twenty units, in levels up to four or five and several lines on to the other so that visual inspection only can be done for the outside units and everything of the inside standing units will be missed. The dogs can get the smell of ALB also from hidden units and indicate as near as possible to the scent source. In this situation the WPM units have to be separated and checked by the dogs again to isolate the infested unit(s) supported by visual inspection by the dog handler. Independent work of the dogs can be necessary if the dog handler cannot follow the dogs between the lines and on the top level of crates.

Fig. 86-88: Investigation of WPM of stones from China at a port in Basel (left) and at a stone importer in Austria (middle). Nails, metal straps and instable storage can endanger the dog (right).

Fig. 89-91: Indication of the dogs at different WPM units at a port in Basel. Visual investigation verified the infestation with living insect stages.

Very often infested WPM could be found by the dogs in which the development of the insects was almost finished and the beetles would have emerged only few weeks (sometimes days) later. The infested WPM had to be fumigated immediately inside of containers with allowed and suitable insecticides.

Due to the experiences of investigations of WPM at Austrian stone importers over seven months in 2012 the saving of time of an inspection with detection dogs compared with the visual investigation by inspectors is about 33 % with simultaneous increasing of the finding ratio of about 50 %.

Use of ALB/CLB detection dogs for investigation of imported plant material (D2.18.3)

- plant importers, nurseries, garden centers
- do-it-yourself-markets, discount-markets/centers
- ports, airports

The main way of introduction of CLB to Europe is within imported plants from Asia, especially from China. In the spring of 2010 the Netherlands imported large consignments of *Acer* plants from China and found CLB infested seedlings. Within the Standing Committee for Plant Health of the European Commission the discussion about a two years import ban of *Acer* plants from China was close to the decision. Therefore the Plant Protection Service of the Netherlands invited the Austrian detection dog teams to investigate a consignment of 40.000 young *Acer* plants from China for CLB infestation. Within only three days the dogs checked 15.000 plants and found five plants with CLB infestation. This result was one of the deciding aspects for the two years import ban from May 2012 until end of April 2012.

Fig. 92-94: 40.000 *Acer* plants from China in 40 crates at an importer in NL (left). Line-up of the crates (middle) and first investigation of the opened crates by the detection dog (right).

Fig. 95-96: Inspection of around 1000 plants of an indicated crate (left) and one of the detected CLB infested plants (right).

Fig. 97-98: Imported plants stored separately so that the inspection by the detection dogs is easier.

Additionally, the detection dog teams inspected several nurseries and garden centres in Austria in view of imported plants from other countries like the Netherlands, Italy, Germany, and of course China, and also during their stay in the United Kingdom. Fortunately so far no infestation of plants with CLB could be detected by the dogs in Austrian nurseries.


Fig. 99-100: Imported plants are mixed with native plants exacerbating the work of the detection dogs.

In Italy in Venetia the Austrian detection dog teams were enabled to investigate imported bonsai plants at a bonsai nursery. Due to the high value of the single plant the dog handler has to pay high attention that the plants were not damaged. Therefore it should be the best way to check bonsai plants for CLB infestation with dogs at the time of delivery when the plants are packed in small amounts on transport trolleys to enable the dogs to sniff from every side.


Fig. 101-102: Investigation of bonsai plants from China at a nursery on the table (left) and at the trolley of transport (right) with detection dogs.

Criteria for the use of detection dogs in different operation areas

In all infestation areas, in the nurseries as well as at wood packaging material small positive scent samples were hidden after the real investigation of the susceptible plants or WPM units

to verify if the dogs were able to find them. In all cases the dogs detected the positive samples, independent on the person who has hidden the sample and also independent on weather conditions. They worked successfully at heat of 38°C and high or low air humidity, strong wind, rain and also at low temperatures between 0-5°C and with snow.

There are some facts of high importance which have to be considered during the work with the detection dogs:

- dog handler and dog work as a team
- dog can trust in his dog handler
- dog handler has to read his dog always under different conditions
- verification of the dog's indication is obligation of the dog handler and/or inspector
- influencing factors which have to be regarded by the dog handler:
  - wind direction and strength
  - temperature
  - humidity / drought
  - time of the day (air circulation)
  - surrounding conditions: open or closed, natural or artificial area
  - size and shape of the trees
  - condition of the dog and dog handler

➢ The dog handler has to trust in his dog because his dog trusts in him!

Conclusion

Anoplophora detection dogs are universally usable, for investigation of wood packaging material in e.g. ports, for the import checking of plants in nurseries or other points as well as in infestation areas. . Well experienced detection dogs are able to detect ALB scent also in a height of at least six metres (experimental proofed) and also up to twelve metres due to experiences during real insets. They can also detect CLB scent in roots and under the soil where visual detection is impossible.

The scent detection by the detection dogs is independent of the development stage of the pest. Therefore a reliable indication of all stages is possible.

Anoplophora detection dogs are able to recognize ALB/CLB scent even there, where a visual detection of symptoms is not possible.

The investigation by Anoplophora detection dogs is non-destructive.

Anoplophora detection dogs are significantly more favorable in „acquisition", education and „maintenance" than appliances assisted detection methods.

Anoplophora detection dogs stand out due to ideal cross-country mobility.

Elaboration of a training program for interested dog handlers (M2.9)

In 2011 the two Austrian dog handlers Gabriele Sauseng and Ute Hoyer-Tomiczek of the Austrian Anoplophora detection dogs started to develop a training program for interested people to train them and their dogs on detection of ALB/CLB scent. The basic education consists of two obligatory consecutive courses of each one week. Every course ends with a theoretical examination for the dog handler and a practical examination for the dog and the dog handler as a team. A special certificate for the dog handler as well as for the dog indicates the successful participation. In the theoretical units the following items are taught:

- theory about the quarantine pests Asian Longhorn Beetle (ALB) *Anoplophora glabripennis* and Citrus Longhorn Beetle (CLB) *Anoplophora chinensis*:
  - biology, symptoms, location of infestation,
- theory about native insects:
  - biology, symptoms, location of infestation,
- theory about principles of detection dog training:
  - training concept, conditioning, indication, confirmation/reward,
- theory about the scenting physiology,
- examples of practical application,
- working out of strategies for the practical use in operation areas.

In the practical training the dogs learn to know the scent of ALB/CLB, to show a specific indication at the scent source and to find it in different environments, becoming more and more identical with real situations of use. The dog handler has to learn how to read and understand the behavior of the dog not to miss an indication and also to help the dog in difficult situations. Scent material is allocated during the courses by the trainer of BFW. Setup training courses are offered on request like done in September 2012 in Italy/Lombardy region on CLB for Swiss detection dog teams.

The following training courses have been conducted so far:

- Spring 2011 Vienna:
  - 1 inspector and his wife with 3 dogs from PPS of Northrhine-Westfalia/DE: for the ALB infested area in Bornheim/DE

- Autumn 2011 Ossiach/Carinthia:
  - 1 dog handler Hermann Meier with his dog Rika from PPS of Baden-Württemberg/DE,
  - 2 dog handlers with each one dog from PPS Carinthia/AT

- Spring/summer 2012 Ossiach/Carinthia:
  - 4 dog handlers with 5 dogs (from the PPS) of Switzerland
  - 2 dog handlers with each one dog from PPS Styria/AT

That means that in total 17 Anoplophora detection dogs have been educated so far by the two Austrian dog handlers and trainers Gabriele Sauseng and Ute Hoyer-Tomiczek in Europe: for Austria eight dogs, for Germany four dogs, for Switzerland five dogs.

In 2013 two sets of each two basic courses are offered in spring and summer in German language again and additionally one set of the two basic courses are offered in autumn in English language for participants from Italy, Netherlands, United Kingdom and other countries. The four German courses are already full booked with participants from Austria, Germany and Switzerland.

Unfortunately the first Anoplophora detection dog Jackson of Gabriele Sauseng died much too early in the age of six years in February 2013. Jackson was the “prototype” of an Anoplophora detection dog. If he wouldn’t have accepted so enthusiastically and teachable this scent work and wouldn’t have implemented it under the competent leading of Gabriele Sauseng, the idea of this detection work never would have developed to a successful and

internationally approved detection method for ALB and CLB. Without Jackson there wouldn't exist the Anoplophora dog detection method. Without Jackson this work will not be the same.

Acknowledgments

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They want to thank all national and especially international colleagues who believed in this method and offered the possibilities for training and monitoring in infestation areas in their countries and also sometimes financial support at the beginning.

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Non-Destructive detection techniques

Report P6 – N. Hoffmann and T. Schröder, Julius Kühn Institut, Institute for national and international plant health. Braunschweig, Germany.

Introduction

Background

As a result of globalization and international trade of plants for planting, tree destructive pests are arriving European regions from countries around the world [142]. Since several years exotic wood-boring insects such as the Citrus Longhorned Beetle (CLB), *Anoplophora chinensis* FORSTER form *malasiaca* (Coleoptera: Cerambycidae) have been detected repeatedly in EU member states at imported young trees [43].

CLB is a devastating organism on *Citrus* and ornamental trees in its native range in Asia and is able to be spread undetected within Bonsai and plants for planting. Host plants cover a wide range of deciduous tree species [165]. CLB is classified as quarantine pest in the EU. Infestations and outbreaks have been detected in Italy (established [56]), France (eradicated [55]) and the Netherlands (eradicated [54]) as well as findings of single beetles in different regions of e.g. Germany and in Switzerland [86].

According to the import prescriptions of the Commission Implementing Decision 2012/138/EG import inspections have to include destructive sampling of a specified amount of plants [58]. The background is that infestations with CLB almost show no external symptoms for identification by visual inspection. Destructive sampling by hand using rose scissors is time consuming, risky for the inspector and destroys parts of the plant consignment. Therefore efforts are underway for assessment and development of new technologies for efficiently but non-destructive inspection of plants.

Objectives

In the current study the assessment of a range of potential non-destructive testing and image guided techniques for detecting CLB larvae and larval galleries hidden in young host trees had to be conducted. This included the investigation, summarizing and adaption of existing techniques, given in literature, as well as development of new methods. In a secondary step, applicable principles/techniques had to be applied at appropriate testing objects.

Due to its quarantine pest status of CLB we had to use an adequate wood-boring model organism harmless in the EU member states but similar in larval size and galleries in the tree. Furthermore, for comparison of accuracy and to simulate naturally grown plants, standardized wood samples (“model plants”) of typical host trees were evaluated and applied for chosen detection technique.

Literature review of non-destructive image guided techniques

In the current research project task-related methods for non-destructive evaluation and detection of hidden larvae and larval galleries in living young trees should be found and assessed by given literature. Due to CLBs behavior and viability within living hardwood trees as host plants, the review was not only limited to living trees but included also the

assessment of potential techniques used within the framework of the analysis of defects in wood.

Methods

The literature review for non-destructive techniques to detect insect stages and damages in young trees was primary executed by using scientific databases according to “Web of Knowledge Portal” containing “Web of Science”, “Food Science and Technology Abstracts”, “CAB Abstracts”, “Biological Abstracts” and “Journal Citation Reports (JCR) – Science Edition”. Furthermore, and for description of basic properties and functions of the examined method, specific book and library research has been done. Superior technique-related terms (for example “thermography”, “ultrasonic”, etc.) were used and also combined with subordinated (e.g. “infrared”, “non-contact”) as well as task-related (e.g. “wood boring”, “larvae”, “insect”, “CLB”, “gallery”, etc.) keywords. In Total more than 300 papers and books have been included in the current review.

Non-destructive evaluation

DEFINITION

“Nondestructive materials evaluation is the science of identifying the physical and mechanical properties of a piece of material without altering its end-use capabilities and using this information to make decisions regarding appropriate applications” [149].

HISTORY

The method of non-destructive evaluation of materials is a steady educing process [19], founded by simple mechanical handlings (e.g. taping of ceramic) in prehistoric times. However, the economic meaning by implementation of physical applications for material inspection is relative young and (is) engendered by magnetic methods in 1868. Followed by magnetic powder processes and magnetic tomographics, eddy current measurements, x-rays, Fourier analytics, photographic, ultrasonic, fluorescents, radiographics and others, a range of techniques for non-destructive testing and evaluation are still under development [127].

Non-destructive evaluation of wood

In the background of worldwide ascending “emphasis to addressing forest and ecosystem health issues” [19] and its management for economical utilization of woody biomass, currently global research and development are conducted to verify capabilities of non-destructive testing technologies for assessment of wood and wood-based materials [19].

The first and even today used non-destructive evaluation method of wood is the visual inspection. Joining the development of scientific non-destructive testing in the early 20th century and the aim for assessment of wood properties by theory of elasticity, a number of technologies using mechanical behavior and electromagnetic radiation established for characterization of wood have been developed [27], shown in Table 1.

Table 1: Development of non-destructive evaluation of wood in chronological order

	time	method
in chronological order	before 1930	visual inspection
	since 1930s	theory of elasticity (static and dynamic methods, e.g. using acoustic vibrations)
	since 1960s	x-ray techniques microdensitometrie vibrational methods ultrasonic techniques
	since 1970s	mechanical characterization dielectric properties acoustical properties

In the large amount of potential imaging methods, the cooperation of various disciplines of scientific and engineering is inevitable. To get an overview of the opportunities for characterization procedures of wood, tools for systematically valuation are needed. The **classification** of non-destruction techniques in wood-science can be described according to following terms [27]:

- Scale of observation (according to the hierarchical structure of wood: group of trees in megascopic scale; tree [m] in mesoscopic scale; annual ring [cm] in macroscopic scale; cells [mm], cell walls [µm], fibrils [nm] in microscopic scale; cellulosic crystal in submicroscopic scale) [26] in [27]
- Type of wood product and the particular application (for example the detection of defects in timber as knots, determination of density, moisture content, etc.) [9]
- Physical properties of interest (dielectric, thermal, elastic, optical, mechanical, acoustic and electrical properties, weight, ionizing rays absorbation, ionizing radiation) [172]
- Characteristic of electromagnetic wavelength (Ionizing radiation, thermal, microwaves, ultrasonic, nuclear magnetic resonance [NMR]) [28]

The fourth classification according to the specific electromagnetic wavelength is summarized and presented in Table 2.

Table 2: Comparison between different non-destructive techniques used for structure characterization and defect detection of wood (adapted by BUCUR (2003b) [28])

Method	Parameter measured	Theoretical wavelength [m]	Sample	Structural feature observed
Ionizing radiation (noncontact, invasive, in situ, in vivo for trees)	X- or γ-rays Attenuation	10^{-12}…10^{-9} (0,001 nm … 1 nm)	Tree, logs, poles, lumber, wood-based composites	Density variation. growth rate, detection of metallic inclusions, knots, decay, influence of pollution, macrovoids in particleboards
Thermal (noncontact, noninvasive, in situ, in vivo for trees)	Temperature, phase magnitude	10^{-5}…10^{-4} (10 μm … 100μm)	Tree, forest, lumber, standard small clear specimens, wood based composited	Decay, knots, fiber direction, moisture distribution, effect of acid rains, rupture phenomena, subsurface integrity of composites
Microwaves (noncontact, noninvasive, in situ)	Dielectric constants in three anisotropic directions, dielectric loss, amplitude, polarization	10^{-3}…10^{-2} (1 mm … 1 cm)	Lumber, standard small clear specimens, wood based composites, forests	Slope of grain, knots, decay, cracks, moisture distribution, mechanical grading of lumber, lumber drying, voids in wood-based composites
Ultrasonic contact, noninvasive, in situ, in vivo)	Velocity, time of flight, pulse length, amplitude, energy	10^{-2}…10^{3} (1 cm … 1 km)	Tree, lumber, structural elements, poles, wood based composites	Knots, decay, slope of grain, detection of fungal attack in wood, delaminations and voids in composites
N M R (noncontact, noninvasive, in situ, in vivo)	Relaxation times – (spin-lattice and spin-spin) resonance frequency, magnitude	10^{-2}…10^{5} (1 cm … 100 km)	Tree, lumber, woo based composites, adhesion kinetics	Tree vitality, moisture distribution, knots, annual rings pattern, influence of climate, fungal induced diseases, preservative distribution, wood drying

For the current study the major limitation factor of non-destructive techniques is the material behavior at the point of observation time. The trees to be investigated are living but in dormant stage, whereas objects described in the mentioned test methods were either dried, larger or standing with liquid transport processes. Furthermore the defect can differ concerning size and distance to objects surface and important physical characteristics.

Given the stated factors above, there are no non-destructive techniques described in the literature to identify insects within young deciduous trees. Little evidence was given for detecting insects respectively holes, cracks or knots inside of wooden material and standing trees. A few methods deal with locating and tracing of high amount of insects and other pests and diseases (incl. fungal degradation), inducing altering processes of wooden objects.

The following chapters describe the literature findings of various non-destructive image guided techniques in wood and forestry science with respect on living and young trees and entomological background. The selection of chosen methods based on the structure of BUCUR´s classification (2003b) in Table 2 [28].

No methods have been assessed on its applicability on young wooden plants with stem diameters at the root collar of less than five cm. However, with respect to small-scaled and cut wooden materials and entomological backgrounds, few non-destructive techniques have been observed more or less successfully at least: Active thermography and larval galleries of wood worm borers [67], micro-computed tomography and wood wasp larvae [95], computed tomography and house borer larvae [105], microwave radar and house borer larvae [155] and non-contact ultrasound and cotton wood borer [65].

Computed tomography (CT)

After theoretically foundation of utilization of x-rays for internal image reconstruction at the end of 19th century, the technical implementation of *computed tomography* (or *x-ray computed tomography* or *CT*) gets started in the 1970th in radiological diagnostic. In addition to diverse biomedical applications, CT has offered increasing development in industrial control, airport inspection as well as material and natural science (e.g. in archaeology and geology) by now [6, 27].

In opposition to discrete tomography with fixed x-ray or gamma-ray sources, CT scanners are working with rotating generators and detectors circling around the material to be tested. Reconstruction of diverse slices of irradiation provides three dimensional data about internal structure of objects for precisely and detailed mapping of inhomogeneities, dimensions and position of pattern [23, 27]. CT technique is based on the physical behavior of the attenuation coefficient at penetration through material and influenced by the quantum energy of radiation and the chemical composition (dimension and density of object) of the object [22-23, 27].

In dependence of its dose and its biological activity, ionization radiation could induce somatic or genetic injuries in plants. The lethal dosage (LD_{50}, that is the amount of radiation, where 50% of population of a species will die) of deciduous trees with exterior radiation is ranged at ~ 5 … 175 Gy[1] [63]. The emitted energy of commonly used computer tomographs is ranged at 1…10 mGy [181] and is therefore not expected to affect plant growth.

In wood and forestry science the large sized and fast operating industrial CT scanner is used for visualization of inner defects (branches, cracks, decay, resin pockets) [6, 12, 75, 104, 135-136, 138] and structures (heart/sapwood, tree ring width, transition of wood to bark,

[1] 1 Gy (Gray) = 1 J · kg^{-1} ≈ 1 Sv (Sievert)

density) [22, 64, 115-116, 168, 170] of wood, especially timber and logs [6, 23]. To be aimed at optimization of yield of wood in grading and sorting, CT has been proven successfully to be an accurate analysis tool, but there are few reports in combination with entomological backgrounds.

The usage of CT miniscanner and portable CT scanner for identification of ALB damage in living trees has been simulated by CRUVENIL *et al.* (2003). Drilled boreholes and presence of inserted model organism of *Dichotomius anaglipticus* (Coleoptera: Cerambycidae) were detectable and could be reconstructed when energy was at 59.6 keV. However, image contrast resolution at 662 keV did not reach for displaying [45].

Quite similar results could be aimed by detecting old house borer larvae, *Hylotrupes bajulus* (Coleoptera: Cerambycidae), in pine, spruce and fir wood containing different thickness. CT-images were analyzed as a function of the degree of destroying and larval size and resulted in comparable data to those attaining with radiograms. Little and young larvae could not be visualized at 15-mm thick wood samples regardless of whether status of consumption has been observed. However, older individuals could be recognized in any wood diameter class successfully [105].

EL-MALLAKH (1993) could verify internal tunnel systems of carpenter ants, *Camponotus sp.* (Hymenoptera: Formicidae) at damaged conifers timber beams of *Pseudotsuga menziesii*. An early generation of computer tomograph, designed for reconstruction of human brain, enabled clear differentiation of hidden areas and intact wood by contrast values of images [53].

A special field of application in the framework of CT is *micro-computed tomography* (or *high resolution computed tomography* or *µ-CT*), that is working in small pixel ranges (µm) and has been conceived for small-sized components in nondestructive material testing [100].

Recent examinations of JENNINGS *et al.* (2011) peruse the identifiability of wood boring insects. *In vivo* micro-CT scanners were appropriate to recognize tunnels of woodwasps larvae (Xyphydriidae) within small logs (200 - 300 mm in length; 40 – 50 mm in diameter) of deciduous tree *Anodopetalum biglandulosum* and dead larvae inside in both longitudinal and cross-section successfully. In addition the differentiation between tunnels filled with insect frass and solid wood could be determined. However, high-resolution imaging confines the dimension to be noticeable at 0.3 mm in diameter. Micro-CT scanners permit sizes of objects to be analyzed up to 68 mm in diameter with a scannable length of 200 mm in total [95].

TARVER *et al.* (2006) examined micro-CT scanning for obtaining of internal feeding patterns of cowpea seeds. Insect frass damages, induced by cowpea bruchid, *Callosobruchus maculates* (F.) (Coleoptera: Chrysomelidae) could be clearly visualized and distinguished according to susceptibility and resistance of seeds and virulence of weevils [178].

In the meantime several CT devices and providers in material testing with properties for analyzing internal wood structure could be found (for example “InVision CTX” scanner [6], “SkyScan” devices [171], Siemens “Somatom”, etc.). Concrete examinations at logs and round timber has been in progress using a computer tomograph (Microtec ® CT LOG) at the Forest Research Institute Baden-Wuerttemberg [22-23].

Thermography

Since applications of *infrared* (*IR*) *thermography* developed in the 1960th in civil sector, it was primary used to monitor thermal bridging in buildings and overheating processes, for

example of engines and devices in electronic and energy industry. Today several utilizations in metrology and especially quality management can be observed. Further applications are found in air-conditioning, medicine, remote sensing, environment analysis, process monitoring in the meantime [74, 92, 118].

IR-thermography is described as a non-destructive, non-contact (and non-intrusive when passive) detection technique for imaging of temperature-dependent (thermal) radiation. By implementation of surface temperature distribution it can be used for assessment of structural and behavioral patterns under object surface [118]. IR-thermography benefits that objects with temperatures above absolute zero (0 K or -273 °C) emit individual electromagnetic radiation (*thermal* or *Planck´s radiation*) caused by inner molecular motion. This radiation, also called *infrared*, is located above visible spectrum (0.35 – 0.74 µm wavelength) and cannot be sensed by human eyes at common ambient temperature. For visibility and measurements of thermal distribution on objects surface and also for discovery of blemishes infrared cameras (or thermal cameras) are used [3-4].

The thermal radiation has to pass more or less long distances from measuring object to instrument (IR-camera). This way through media could affect results whereas transmission strongly depends on wavelength. Caused by atmospheric (air) absorption (radiant uptake), especially by steam and carbon dioxide, measurements between 0.4 µm and 30 µm wavelength are inappropriate for long distance. To minimize falsifications in results, the usage of three spectral bands with higher transmission in an “atmospheric windows” at 0.4…0.8 µm (1: Visible [VIS]), 3…5 µm (2: Mid infrared [MIR] or Mid-wavelength infrared [MWIR]) and 8…12 (14) µm (3: Long-wavelength infrared [LWIR]) for thermographic observations is common [21, 92, 166].

ADERHOLD and MEINLSCHMIDT (2005 and 2011) distinguish between measurements of emissivity differences and temperature differences. The latter deal with heat flow properties at warming or cooling processes, called *heat flow thermography*, and is most common nowadays. In characterizations of MALDAGUE (2001) infrared thermography is basically classified in *passive* and *active* thermography, containing both attributes: emissivity and heat flow.

The term “thermography” is commonly used individualized when applying ***passive thermography***. Features of investigation are naturally higher or lower energized than environment and emit thermal radiation [118]. In simply cases the cool-down period of objects is process-related and only be observed with a thermographic camera. Surface areas with trapped vacancies decrease heat flow and refrigeration will occur faster, due to reduced heat flux from inside. Application examples are delaminations or air inclusions [5].

When heating or cooling processes are not integrated in manufacturing the measuring objects has to be stimulated by heating impulse immediately before or while observation (***active thermography***). Simple case is an electric heater passing conveyer. Heating front invades the object while surface is cooling down. When passing vacancies the heat flow is disabled hence surface above these blemishes will be longer warm [5]. The speed of invading heat is an important factor and depends on thermal properties like density, heat capacity, thermal conductivity and the bonding quality between top surface layer and the base material. Subsurface anomalies will decrease the surface temperature more slowly [125]. Common processes of active IR-thermography follow with optical impulse, maybe halogen lights, laser or radiant heater. Larger pulse duration is adapted for detecting large

depth or materials with lower heat conductance basically. However, when objects surface (reflection) is high reflected or when measurement is not possible due to geometric(s) reasons (inspection of cavities), hot or cold mediums (for example air) can be supplied for analyses [5].

The general setup of both thermographic methods used in the current investigation (active and passive) is shown in Fig. 1.

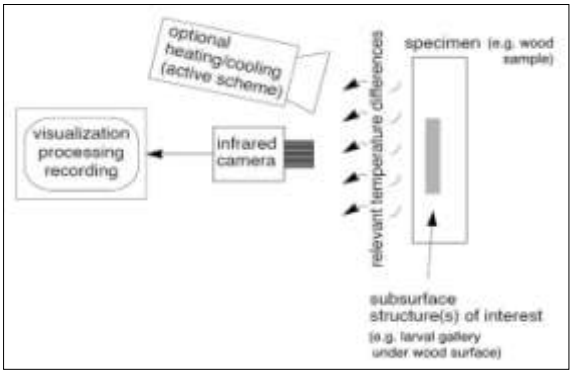


Fig. 1: Schematic setup of infrared thermography for nondestructive testing; (adapted from MALDAGUE (2001).

The described thermography techniques are primary used in nondestructive inspection and control for materials needed in building, engine and electronic sector. Studies in plant protection equally inspections in wooden materials gain in importance recently. The apportionment of latter fields seems to be reasonable with respect of water content, density and other properties which influence heat flow, thermal radiation and selected spectral bands for observation.

CATENA and CATENA (1990, 2001, 2002, 2003, 2008) investigated mature urban trees (different species under various phenological conditions [37]) for the assessment of stability and vitality by passive IR-thermography (*Treethermography*®). Thus allows an “early detection of various kinds of alteration, including decay and bark necrosis” [39], and provides quantification of vital and reactive tissue of trunk-, branch- and also root-areas. This characteristic bases on thermal conductivity of wooden tissue humidity and its decreasing by reduction of liquid content. In that case, zones with cavities (absent tissue) or decay (less heat-conveying liquids) offer lower surface temperature in comparison with healthy areas [38] and is patterned as a black-and-white (grey level) or pseudo-colour ‘thermal map’ [40] in [39].

Major limitations of *Treethermography*® are given when bark surface is wet or obscured by moss or other vegetation or when the tree-surface is heated by direct exposure to sunlight. These conditions induce misinterpretations. Also, the sensitivity of the IR-system is affected by the tree specified bark properties such as thickness and insulating characteristics [39].

Further restrictions are given in accuracy of measuring results. The thermal images do not distinguish between different kinds of alterations and do not determine precisely dimensions of features such as cavities [39].

To sum it up, Catena´s IR-system “only works on standing trees” due to thermal conductivity, “and not on timber or wood products, which requires invasive systems or other types of non destructive testing” [37].

Additional characteristics of vitality and soundness of forestlands and single individuals were observed by LEUZINGER *et al.* (2007, 2010) and WHITE *et al.* (2007). Dependent on the species diversity, location of the tree, its leaf size, stomatal conductance and canopy constitution, the surface temperature of a mature forest [111] or urban trees [112] is primary tree-specific and induce cooling effects during hot periods, evaluated by thermal aerial cameras in the spectral range of 7,5…14µm [112]. Spectral moisture indices in SWIR and NIR regions were useful for estimating mountain pine beetle red attack on mature pine forests [191].

Other observations in the context of plant protection deal with pathogens, pests and diseases (fungal infestation [39, 80, 83-85, 109] and (mass) attack of insects [60-61, 87, 119, 160, 163, 175, 191]) as well as abiotic factors [109] inducing alterations of plant tissue.

In the field of *remote sensing* the infrared spectrum is widely used for analyzing vitality of vegetation, canopies and other green compartments of environment. IR-images are taken with thermal cameras in ground level or higher flight altitude (airspace or outer space) in longer distances for inventorying, monitoring and mapping of earth resources (objects, areas or phenomena) [113].

Due to NILSSONs foundation of the dependence of leaf-temperature on degree of transpiration [134], HELLEBRAND *et al.* (2004, 2005, 2006) observed fungi infections on wheat plants by infrared cameras in the thermal range under laboratory conditions [85]. The infections resulted in an initial increase of transpiration rate caused by enhanced mycelium surface with small transpiration resistance. The energy consumption in the evaporation process of liquid water reduced the body temperature in comparison with ambient air. Thus the infected plants with higher transpiration had lower temperature than healthy plants [83-84].

Caused by natural temperature variation in the field occurring within the crop canopy (e.g. due to different plant height and plant density yielding shadowing effects) commercial thermal vision systems with comparatively low resolution cannot be used alone for recognizing infections in plants. Additionally, the measured temperature depends on the angle of the camera relative to the position of the irradiation of sun and heterogeneity of air motion within the canopy that may induce different cooling rates [84].

Additionally investigations of remote sensing data are reported in context to thermal detection of habitats, movement and behavior of insects, larvae and other invertebrates in dependence of temperature tolerances [82, 91, 94, 143, 148, 156, 163-164]

Thermographic measurements are already established in *timber industry* for automatic inspection of defects and machine based scanning procedures of surfaces [3]. Infrared cameras are able to detect heterogeneous on large-scaled product lines and invisible defects within lumber and wood-based materials [3, 125].

Applications ranged from not refrigerated hand-held cameras over cooled high-resolution systems to active heated lumber scanning techniques. A major object of study is to analyze parameters affecting wood quality and properties induced by defects (e.g. knots, holes,

dents) or divergences in density, soundness or moisture content [3-4, 98, 103, 117, 123, 125, 128, 140, 157, 182, 194].

There is a large quantity of provider dealing with thermographic tools, e.g. bigger companies like “InfraTec Corporation” offering a wide spectrum of thermal cameras. Furthermore consolidations working with thermographic equipment for purposes of scientific and research. Active thermography was particularly applied for detecting galleries of wood worms, *Anobium punctatum* L. (Coleoptera: Ptinidae), in a dried lath at the Fraunhofer Institute for Wood Research, Wilhelm-Klauditz-Institut in Braunschweig [67] (Fig. 2**Error! Reference source not found.**).

Fig. 2: Detected galleries of a wood worm, *Anobium punctatum* L. (Coleoptera: Ptinidae), in a dried lath using active thermography (WKI intern)

Radar

The discovery of electromagnetic signals to determine the presence of metal objects goes back to the beginning of the 20th century and offered an increasing development in locating of objects in military, marine and aviation as well as weather reconnaissance, astronomy, telecommunication and speed and motion monitoring [47]. In context to wood inspection, *microwave radar* is a relative young non-destructive and non-invasive evaluation tool and new studies in image interpretation started in the middle of the 1980th [27].

The term radar is an acronym for “Radio Detection and Ranging” and uses electromagnetic waves in radio spectrum (approximately 10^{-3}…10^{5} m or 300 GHz…3 kHz) for detecting presence and distance of objects. Signals are emitted by transmitting antenna and electromagnetic waves are partially reflected by objects and received by antenna. In dependence of current time and amplitude it is necessary to determinate the distance and angel (between the radar equipment and test object) as well as the approximate size of the test object [47]. In the case of sensory measurements, the radiated power of transmitted electromagnetic waves is relatively low and harmless and is ranged below the 1000th part of commonly used mobile phones [155]. Hence phytotoxic consequences on plants can be excluded and measurements with living trees are safely.

For imaging studies of solid wood using radar systems it is necessary to determinate the dielectric behavior of wooden object [28], and, for analyzing defects or invertebrates within the samples, any scattering objects affecting reflectance. When combining the relative permittivity of surrounding air ($\varepsilon_r = 1$) and material of objects (wood: $\varepsilon_r \approx 4…6$; insect: $\varepsilon_r \approx 30$; human: $\varepsilon_r \approx 50$; water: $\varepsilon_r \approx 80$), and its size in comparison to spatial curvature, dielectric contrasts are determinable/will be determinate and provide the basis for radar analysis [155].

The monitoring of movement of biotic (e.g. insects, particularly termites) and abiotic (e.g. raindrops and wind) components will be achieved by the difference between emitted and

reflected radar signals. SACHS *et al.* (2008) availed the principles of operation for detecting larvae of old house borer, *Hylotrupes bajulus* (Coleoptera: Cerambycidae) within timber rafter of spruce, using adapted ultra-wide-band radar (UWB-radar). The high-frequently electromagnetic waves (2…4 GHz) penetrated through the wood specimen and larval movement was recognized successfully. A major limitation based on anisotropy of wood. In that case measurements in axial direction will not be feasible. Furthermore conditions require minimum sizes of insects whereas sample thickness should not exceed deduced limits for detectability [155]. BUCUR (2003b) assessed the smallest detectable defect size by the ratio d / λ > 1, (d = length of the defect in the plane normal to the microwave vector; λ = wavelength) [27]. Appropriate radar systems are still in development and are currently available at the Institute for Information Technology, University of Technology Ilmenau, in laboratory scale (Fig.3) [155, 180]. Quite similar results are given in tracking analysis of MANKIN (2004) using microwave radar for sensing movement and vibration of adults of four different beetle species in various size, number and behavioral activity. Two different types of bioassays were appropriate for detecting insects under different conditions. First one enabled direct visual comparison with radar observation, when insects were visible in petri dishes or rearing jars up to 30 cm in distance. Second bioassays could reveal movement of preinfested stored products (boxes of corn meal mix and four mix) insects in a variety of circumstances (artificially infestation with 5-100 insects) [120].

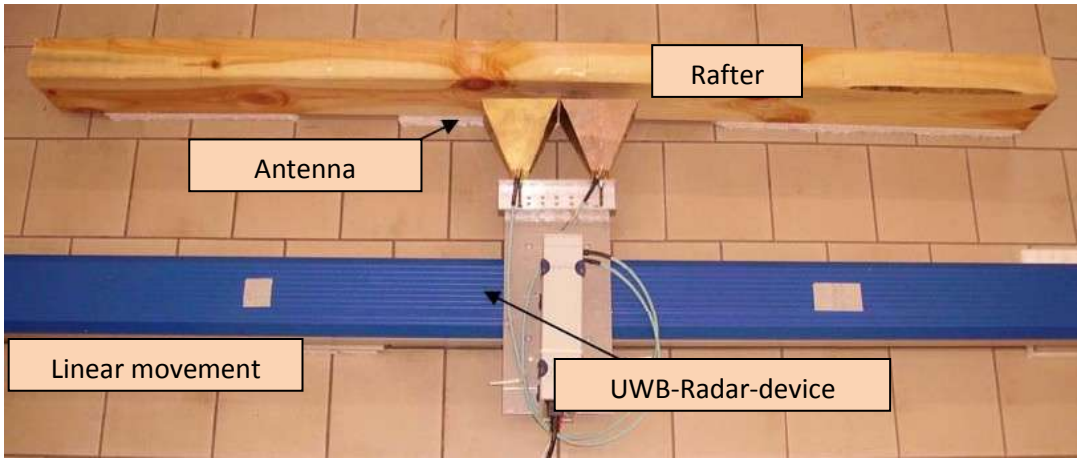


Fig. 3: UWB-radar system for detection of old house borer larvae, *Hylotrupes bajulus,* hidden in timber rafter of spruce (adapted by SACHS *et al.* (2008) [155])

Furthermore investigations concerning tracking and motion parameters (not hidden but visible) using microwave radar deal with various kinds of insects and other invertebrates for monitoring and behavioral evaluation [17, 93, 177, 184, 192, 201]. Main emphases approach precision targeting of termites respectively nests of termites [59, 195, 197-198]. A further observation deals with the detection of large-scaled damage on trees induced by insect feeding [2].

In the wide range of radar devices, Ground Penetration Radar (GPR) is an encouraging technique for characterization of wood. GPR is originally developed for high resolution reconstruction/imaging of earth´s subsurface in frequencies between 10 MHz and 1 GHz. Pulsed electromagnetic energy is propagated into ground or solid materials to measure travel times time and amplitudes of reflected signals (e.g. different layers of earth down to 100

meters for designing three-dimensional maps) [36, 47, 193]. An application in forestry and landscaping is the root-finding using GPR [199].

Task-related studies (in wood analyses) for detection of internal defects (e.g. knots, resin defect) and for characterization of wood (e.g. moisture content, density, anatomy) have been demonstrated by using GPR [36, 79, 147, 199] and further microwave radar technologies [7, 10, 46, 57, 66, 88, 137, 161].

Electrical resistivity tomography (ERT)

The *electrical resistivity tomography* (ERT), also electrical impedance tomography (EIT), belongs to one of the oldest surveying techniques in geophysics for exploration and analyzing sub-surface structures. The non-destructive, but intrusive testing method has gained in importance in tree diagnostics and control for an early identification of physiological alterations, quality assessment and stability of trees in forests and urban environment. Today modified ERT-systems can be used to get insights of standing trees and its tissue without injuring or destroying the plants. As a result two-dimensional images can be achieved in different cross sections to map defected and anomalous parts of stems (for example detection of early stages of decay, wet wood and different types of heartwood) [187]. Furthermore, biological parameter, for example estimation of the area of conductive sapwood respectively the differentiation between heartwood and sapwood, can be assessed by ERT [15]. The technique benefits that it can be used mobile and independent in various terrains by acceptable, little equipment and minimal safety-related risks [188].

The parameter *electrical resistivity* or its reciprocal, the electrical conductivity, is a physical property and defines the behavior of material to traverse electric current. Single-path (one-dimensional) evaluation of electrical resistivity does not allowed exactly determination of sizes of defects within the trees [24]. Furthermore, regions in cross section will be missed at single measurements and structures of interest (e.g. cavities) will not be detected with a high probability. Thus, the tomographic application (ERT) using more paths in radial and axial symmetry will provide more precise results. By connecting the surface of material by electrodes the spatial resistivity distribution within objects can be reconstructed and is the main task of ERT. Thus, inferences about inner structure of material can be made by non-destructive way [72].

In general four electrodes needed for tomographic imaging at least (Fig. 4). Electric current [ampere] is injected to testing object over two adjacent input electrodes (A and B). In dependence of resistivity distribution in the material electric fields are generated. Using other pairs of electrodes (M and N) voltage differences [volt] are measured at different reading points (principle of dipole – dipole configuration, **Error! Reference source not found.**). By sing the measured electric current, voltage differences and a configuration factor, the specific electrical resistance [ohm-meter] can be calculated for actual configuration of electrodes (two-dimensional). Eventually the tomographic principle requires a variety of configurations (measuring planes) along the stem for three-dimensional imaging. The number of electrode pairs at different reading points will affect the accuracy of measurement withal. In tree diagnostic an axial symmetric configuration around the stem is proved. The metallic configuration ring contains 24 needle electrodes and could be arranged in several cross sections in equidistance to the circumference of tree (Fig. 5**Error! Reference source not found.**) [188, 190].

The imaging of measured results (electrical resistivity) depends on specific inversion algorithms that have to be adjusted to the shape of measuring object. In tree assessment

modern reconstruction algorithms has been improved for inversion of cylindrical objects and is applicable for cross section geometry of standing trees [72, 77].

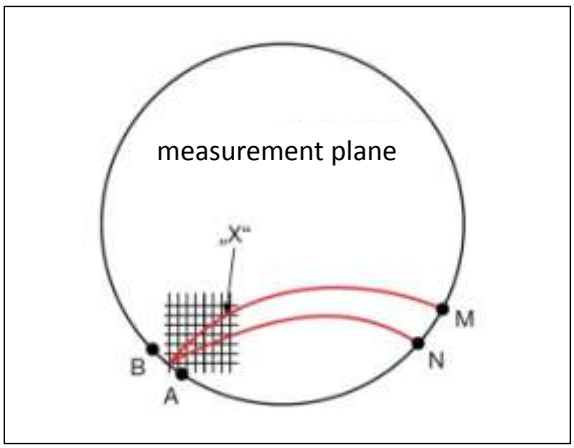


Fig. 4: Four-point configuration of electrodes; dipole – dipole (adapted by WEIHS *et al.* (2007) [188])

Fig. 5: Configuration-ring of electrodes at standing tree of the University of Applied Sciences and Arts (HAWK) Göttingen/Germany

The inner conductivity of electric current of material depends on the constitution (mobility and number) of charge carriers (ions) and is described by Ohm´s law and Maxwell's equations. It is primary regulated by the water saturation, the geometry of cellular lumina and the solutes (ionic concentration). With respect to wooden materials the cells are connected with each other and the ions will be transported by cellular fluid (electrolytic conductivity) and will affect conductivity values [121].

In general three crucial external factors will affect the properties of charge carriers, thus the electrical resistivity, of wood: water content, structural composition (anisotropy, density, components, species) and temperature [130].

Due to hydrophobic cellulose molecules, ***water*** content is the most influencing parameter for electrical resistivity. Highest values are given at kiln-dried status and wood (will) function as isolator. Increasing moisture content indicates a higher conductance because the electrical resistivity declines [130]. In the framework of tree diagnostics usually low-frequency alternating current will be conducted via apoplastic pathways (space outside the plasma membrane or collectivity of cell walls and stromas). An enhancement of water content and ionic concentration in apoplast (for example as a consequent of tree rot and injury) will reduce resistivity [188]. However, cavities of air and the absence of water induce ascending resistivity values [154].

With respect to the ***structural properties*** particularly the anisotropy defines the degree of resistivity. Due to its increased ion mobility in fibre (axial) direction electrical resistivity is reduced to a part of 2.5 to 8 in comparison to lateral (radial or tangential) direction [51] in [121]. In dependence of wood species and components it is minimally affected by wood density and will decrease with growing density [130].

The ***temperature*** at the point of measurement is a further parameter for electrical resistivity of wood. Increasing temperature induces a reduction of the Ohmic resistance. In addition the influence of temperature is coupled with wood moisture and takes highest values at dried wood [121].

To draw inferences from presence of larvae or boreholes inside of material about electrical resistivity, these external factors has to be kept constant or minimal. Thus, for comparability of results, homogenous and knotless wood samples of similar tree species are required at analyses. In addition wood moisture and temperature have to been hold constant and controlled. Due to higher measurement accuracy the implementation should solely performed in radial direction.

ERT finds application in tree diagnostics for imaging defects (epically fungal infections and decay, red rot, false heartwood, etc.) [13, 18, 24, 52, 96, 108, 126, 131, 154, 179, 188-190] and inhomogeneous moisture content distribution [14]. Further observations deal with sub-surface detection of location and distribution of roots [16, 150, 200].

One-dimensional (single-path) tools for measurement of electrical properties at standing trees: “Shigometer”, “Conditiometer AS1”, “Vitamat”. This devices require drilling of boreholes or installing of large electrodes within stems, that could induce injuries, water loss and fungal infection [154].

Two-dimensional resistivity equipments (e.g. “GeoTom” and “Solartron”) are currently in use for a variety of assessments in tree diagnostic and survey at the “arboriculture group” of University of Applied Sciences and Arts in Göttingen [107].

Ultrasonic

The basic principle to visualize structures and tissues using ultrasonic illustrates/depicts an early development and may find its first technical application in marine navigation (sonar and echo sounding) at the beginning of the 20th century. Technological advance offered the utilization of various non-destructive evaluation methods, for example in diagnostic sonography, material testing, sewage purification, production technique and a range of biomedical ultrasonic applications [11, 90].

Ultrasonic characterizes mechanical oscillations in frequencies above the audible range from human ear (approximately 16 kHz…40 GHz), that can propagate in solid, liquid and gasiform mediums [106]. The propagation in solid wood can be used in transversal (deflection of wave is vertical to propagation direction) or most common applied longitudinal (direction of vibration and propagation concur) waves and is relative to anatomical characteristics (orthotropy and inhomogeneity) [74]. Due to energy distribution and flow that are determined by different properties of wave parameters (time of flight, amplitude, frequency spectra of the waveform, the phase, etc.), image reconstruction depends on reflection, absorption and transmission of ultrasonic waves while/when passing medium [28]. Ultrasonic can be generated by using mechanical or electromechanical (piezoeltric, magnetostrictive, electrodynamic, electrostatic transducer) methods/transducer by contact (coupled) or non-contact (air-coupled) scanning [27, 110].

Non-contact ultrasonic was applied to detect larvae of the cottonwood borer (CWB), *Plectrodera scalator* (Coleoptera: Cerambycidae) as a model organism for ALB within wood packing materials. FLEMING *et al.* (2005) prepared 1-inch-thick wood samples of dried and green aspen and red pine by drilling boreholes and inserting insects to capture ultrasonic images of cavities, larvae and larval motion as a function of its position inside specimen, the

wood species and its moisture content. Artificially drilled holes could be verified successfully, as well as larvae and larval movement on top of the wood samples. However, neither the insect nor it´s movement could be identified when the larva was inside the samples (no differences between the hole and the larva in the hole). The transmission of ultrasound is dependent on moisture content of wood, dried samples showed higher signals. Additionally red pine reached higher transmission levels comparing aspen [65], maybe caused by higher density.

In context to non-contact ultrasonic similar results could be recognized by analyzing characteristics and defects (cracks, knots, delaminations) at wooden materials successfully [69, 158, 174].

As a particular technique, *ultrasonic tomography* works noninvasive and low energized by 3D-imaging in cross section from different directions [28]. Observations for analysis of quality are reviewed for decay detection on standing trees by MARTINIS *et al.* [122] and SOCCO *et al.* [173] and on oak blocks by NAJAFI *et al.* [129]. Ultrasonic propagation properties in dependence of anisotropy at wood pieces and logs (for simulation of sound propagation in a tree trunk) were investigated by SATO *et al.* [159].

A host of studies deal with *coupled ultrasonic* methods for wood analysis (characteristics, anatomy, moisture content, density, etc.) and protection (for example fungal decay, knots, cavities etc.) on solid wood as well as wood-based materials using coupling mediums [1, 8, 25, 30-35, 73, 81, 101, 124, 133, 151, 169, 176, 179, 185]. Comparable measurements are implemented concerning standing trees and tree diagnostics [29, 41, 44, 50, 99, 102, 196].

Ultrasonic devices are listed in Brashaw *et al.* (2009) corresponding to its important providers (e.g. CBS-CBT, CNS Farnell, GreCon) [19]. Additional tools in arboriculture are mentioned by Rust and Weihs (2007): Silvatest and Arbosonic Decay Detector [154].

Sound tomography

In opposition to ultrasonic tomography, devices of *sound tomography* (or *impulse* or *sonic tomography*) have been established in arboriculture and tree diagnostics for several years. Based on induced impulses and its relative sound velocity it is used for two- or three-dimensional imaging of internal structure of solid materials [70, 152, 154]. The tomograph is arranged in orbital order at stem surface by several sensors (pins) connected to bark. Stress wave propagation will be initiated by hit with a hammer at each sensor. Due to differences in speed of wave transmission, an inspection of defects, fungal decay, cracks or cavities will be realizable by sonic tomograms [42, 114] and results could be deployed for interpretations of more wood physical properties e.g. modulus of elasticity and weight capacity [154]. An increase of accuracy will be obtained at higher wood density [78], and wave transmission speed is generally contingent on anisotropy (faster in axial direction by contrast to cross-sectional direction) [132]. RUST (2000) defined limits of cavities sizes when holes corresponded 1/10 of tree diameter [152].

Most sound tomography observations deal with assessment of stability and rupture safety at standing trees for identification of internal decay [20, 48, 70, 97, 141, 146, 167, 186] or cavities, holes and cracks [42, 114, 162] respectively for general evaluation of technical basics and accuracies [71, 78, 144, 183].

No investigations could be found related to insects within small host trees. Sizes of pins and hammer beats will destroy plant tissue or restrict measurements at thin trees basically. Thus, sonic tomography is not an encouraging detection method for this study [153].

Following sound tomographic tools are available and applied: Arbotom [145], PiCUS Sonic Tomograph [139], Fakopp 3D Acoustic Tomograph [62] and Fakopp 2D.

Overview of task-related image guided techniques

According to the stated literature listing two matrices were designed and shall give an overview of arrangement with respect to the field of application respectively the non-destructive technique.

Matrix 1 - An overview for task-related classification

The first matrix illustrates the counts of literature findings at task-related consideration (Table 3). The given columns represent the different stages of a tree at the point of observation time. The specific characteristic or property that will be analyzed or described is shown in congruent lines. In total many investigations deal with assessment of structural properties, defects and decay at or within bigger sized wooden objects, concerning both cut wood and standing trees. However, no literature regarding younger trees or tree seedlings could be found. It is noticeable that few investigations expose single individuals of insects.

Table 3: Matrix 1: Overview of non-destructive image guided techniques for task-related classification (including methods: computed tomography, thermography, radar, ultrasonic, electrical resistivity, sound tomography)

	tree seedlings (young and living)	**trees** (living and standing)	**wooden materials** (cut or treated)
insects / larvae (single individual)	-	+	+
holes, knots and cracks	-	+	++
pests and diseases (incl. fungal degradation/decay, mass insect infestation)	-	++	++
basic properties (e.g. moisture, density, structure)	-	++	++

Body of literature (findings):

In total: more than 300 paper and books

Task-related: ca. 165 paper and books (counts: - nothing, + few, ++ many)

Matrix 2 – An overview for technique-related classification

In the second matrix the most promising examined image guided techniques and their subordinated classification are shown (Table 4). According to literature findings the theoretically applicability has been assessed. Additionally the testing and assessment activities in ANOPLORISK at the status December 2012 are represented.

Table 4: Matrix 2 – An overview of nondestructive image guided techniques for technique-related classification: (including the methods: x-ray, thermography, radar, electrical resistivity, ultrasonic, sound tomography)

Technique		Theoretical applicability according to literature	Testing / Assessment in ANOPLORISK
General principle	**Subordinated tool**		
X-ray **(**γ-ray)	2D scanning	Applicable (larval galleries)	done (P2)
	Computed tomography (CT)	Applicable (larvae and larval galleries)	done (P2 + P6)
	High resolution computed tomography	Applicable (larvae and larval galleries)	equipment not available
Thermography	Passive thermography	Applicable (larvae and larval galleries)	done (P6)
	Active thermography	Applicable (larvae and larval galleries)	done (P6)
Radar	Microwave UWB-radar little array (few antennas)	Applicable (larval motion)	done (P6)
	Microwave UWB-radar large array (many antennas)	Applicable (larvae and larval galleries)	equipment not available
Electrical resistivity	Electrical resistivity	Applicable (larvae and larval galleries)	-
	Electrical resistivity tomography	Applicable (larvae and larval galleries)	done (P6)
Ultrasonic	Coupled ultrasonic	Applicable (larvae and larval galleries)	done (P6)
	Non-contact ultrasonic	Applicable (larvae and larval galleries)	done (P6)
Sound tomography	Sound tomography	Not applicable (size of sensors, size of wavelength)	-

Derived hypothesis and objectives

Computed tomography (CT)

Computed tomography was assessed using a computer tomography (Microtec ®, CT LOG, Brixen/Italy) of the Forest Research Institute Baden-Württemberg where the technology is used to analyze inner features of logs. Wooden model plants (*Acer pseudoplatanus* L.) as well as young plants of the same species were assessed. Following a preliminary study to investigate the principle applicability, an observer blinded study was carried out with the following tasks: identify artificial larval galleries of different sizes; identify different locations of artificial larval galleries; identify living larvae in artificial larval galleries; identify artificially packed frass in artificial larval galleries.

Thermography

Infrared thermography was investigated using wooden samples of three European tree species (*Acer platanoides* L., *Acer pseudoplatanus* L., *Salix alba* L.) to capture thermal images of inserted wood-boring larvae (*Cossus cossus* L.) and larval galleries at long-term measurements at the Fraunhofer Wilhelm-Klauditz Institute Braunschweig (WKI). Various kinds of sensitive cameras were tested and compared concerning accuracy and responsivity. Due to different cooling-down behavior of treated (borehole and inserted larvae) and untreated zones of model plants, active excitation with various heating sources should be sufficient to draw inferences about presence of vacancies. Furthermore, the attempt was to visualize larval motions and activities based on differences in their temperature in comparison with ambient test objects.

Radar

Microwave movement radar was used for detection of larval activity induced by inserted *Cossus cossus* larvae in prepared wooden samples (*Acer pseudoplatanus* L.) at the Institute for Information Technology Ilmenau. Furthermore specific larval galleries at typical frass zones of CLB at young trees were occupied with *C. cossus* and analyzed using radar waves. Given high-frequently UWB-radar equipment of the Institute for Information Technology Ilmenau recorded a movement or vibration of larvae as a result of time-dependent differences in the receiver signal. Secondary object was to evaluate influences or interferences on larval detectability induced by surrounding and defect-free wood. In addition the spatial locating of hidden larvae using more than one antenna was exposed.

Electrical resistivity tomography (ERT)

In this study the use of electrical resistivity tomography was tested by a small-scale adapted ERT-system at wooden samples of *Acer pseudoplatanus* to identify boreholes and larvae (*Cossus cossus* L.) inside of wood. The system was oriented to the principle of electrode configuration ring of the “arboriculture group” at the University of Applied Sciences and Arts in Göttingen. Based on the property of increasing resistivity values by reduced water content it was assumable that entrapped air in wooden stem (boreholes) will increase resistivity intensively. The steep reduction in moisture from green wood to wood-free cavities was expected to help identifying hidden zones. However, the relatively higher water content of larvae compared to wooden material ought to reveal areas with declines in resistivity when inserted larvae are at measuring plane.

Ultrasonic

Transmission ultrasonic was tested for detecting predrilled boreholes and inserted larvae (*Cossus cossus* L.) inside of wood samples of *Acer pseudoplatanus*. In a first measurement

series, differences of ultrasonic travel times and velocity of propagation at boreholes and untreated areas were measured by coupled transducers in different measurement layers at the Federal Institute for Materials Research and Testing (BAM) in Berlin. The illustration in B-Scans helped to differentiate treated zones.

In a second test series, non-contact (air-coupled) transmission ultrasonic was applied for identifying boreholes and larvae at the Engineering Office Dr. Hillger in Braunschweig. Due to differences of ultrasonic wave amplitudes between wooden zones and blemishes, an automated flatscan will produce C-Scans to image the total array of wood samples and reveal treated areas.

Material and methods

Model organism Cossus cossus L.

The goat moth, *Cossus cossus* L. (Lepidoptera: Cossidae), a species native to Germany, was used as a surrogate for the studies (model organism). The goat moth larvae show similar symptoms concerning larval and borehole size and host plant spectrum in comparison to CLB, though the species is a moth and not a longhorned beetle (Fig. 6). *C. cossus* passes 8 larval stages up to 10 cm length in adult stage. It drills oval boreholes up to 2 cm in diameter in its major host plant genera similar to those used by CLB such as *Acer* L., *Salix* L., *Populus* L., *Fagus* L. and *Betula* Mill.

The goat moth larvae (at least 4 different larval sizes) were collected in June 2011 (Thermography), April/May 2012 (CT, ERT) and July 2012 (Radar, ERT, Ultrasonic) from in total three infested goat willow trees (*Salix caprea* L.) near Braunschweig in Germany (Fig 6 and Fig 7). The larvae were reared under laboratory conditions at mean temperature of 18 °C and 60-70 % rel. air humidity in separated glass jars (Fig. 8). According to Friedrich (1983) the goat moths were fed by dried granary bread and quartered apple pieces on saw dust used for animal bedding which functioned as soil substrate and wintering ground [68].

Fig. 7: Cut branch of infested *Salix caprea* with larval galleries of *Cossus cossus*.

Fig. 6: Four larval stages of *Cossus cossus*

Fig. 8: Glass jars for rearing of *Cossus cossus* larvae

A second insect species, the native meal worm, *Tenebrio molitor L. (Coleoptera:* Tenebrionidae), was used for preliminary tests for thermographic measurements though they are not related to wood. Larvae had a length of up to 30 mm in total.

Model plants

To be able to work with more or less homogenous, defect free, standardized test plants it was decided to use two kinds of "model plants": wood samples and young trees (lower part of the trunk including root which is the most affected part of CLB).

Wood samples

The wood samples *Acer pseudoplatanus* L., *Acer platanoides* L. and *Salix alba* L. were obtained by harvesting three to eight years old trees of the test/field area of Julius-Kühn Institute in Braunschweig and of a forest near Northeim in Germany. Shoot axes were prepared after cutting into 250 mm long samples with three diameter classes. For the experiments, three different holes sizes with a length of 100 to 150 mm were drilled into the samples in axial direction. Furthermore, for identification of the factor "hole position", two different borehole positions, center and edge area has been prepared (Table 5, figure Fig. **9** a-d and Fig. **10**). To mimic larval galleries entering the wood from the cambium layer, skewed running boreholes were drilled (only for CT-investigations). For control, the end of the borehole inside the sample was marked by an outer line (Fig. **9**e).

Table 5: Parameter of wood samples: Stem diameter, borehole diameter and borehole position.

Stem diameter		Borehole diameter		Borehole position
class	diameter [mm]	class	diameter [mm]	class
A	10 – 20	a	2 (3)	center
B	21 – 30	b	5	edge
C	31 – 40	c	10	

To simulate living plants the usage of green wood was required. For this purpose and to prevent losses of wood moisture the end faces of the samples were sealed with paraffin wax (Sigma-Aldrich Germany, Paraffin wax congealing range 45-50 °C; Fig. **9**e) and subsequently stored in air-sealed plastic bags at 6 °C. All samples were adapted to room temperature at least 24 hours before carrying out the investigations.

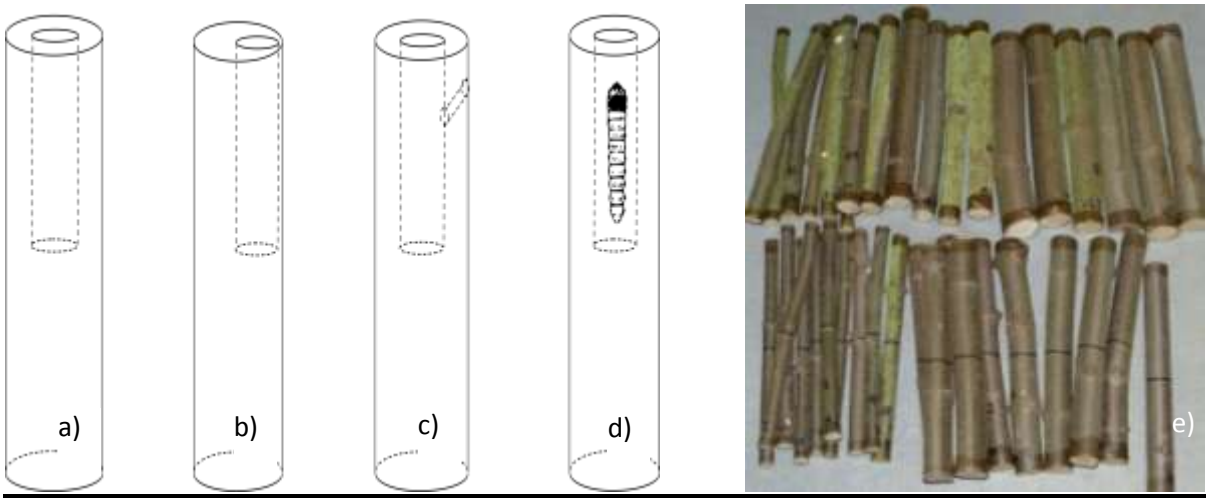


Fig. 9: model plants with artificially drilled boreholes: a) drilled in centre, b) drilled at edge, c) with skewed borehole, d) borehole with larva, e) . model plants with paraffin way sealed cut ends and mark of drilling depth

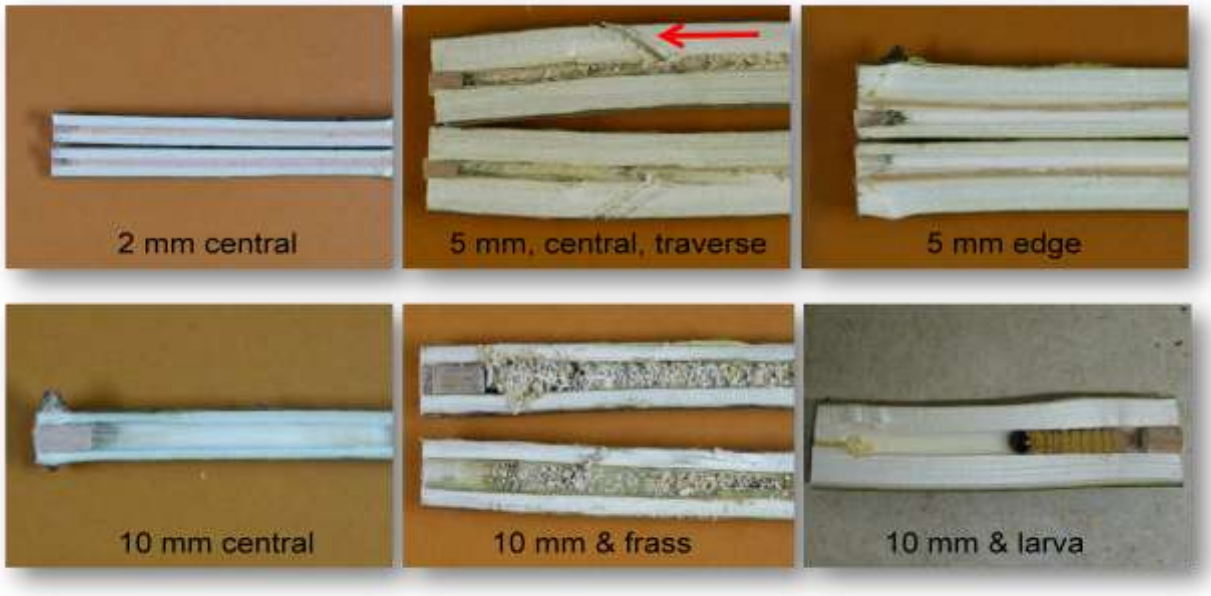


Fig. 10: Examples of boreholes of different sizes and locations with traverse larval gallery, artificial frass and *Cossus cossus* larva.

Young trees

Living trees of *Acer pseudoplatanus* L. and *Acer platanoides* L. with 2.5 – 3.0 m in length and 35 – 40 mm in diameter in the middle part were ordered by the nursery "Wilhelm Ley GmbH Baumschulen" in Meckenheim, Germany. For obtaining young tree samples, the upper parts of the tree including crown were removed by cutting and the lower stem and root zone area (80 – 100 cm in total length) was used for further studies. Entire samples were cleaned with water and bulky roots were cut and removed. Main boreholes with 5 and 10 mm in diameter and 100 to 150 mm in length were drilled in axial direction at upper front plane and stronger roots and in radial-axial direction at lateral stem surface. In addition only for CT-scanning, traverse holes were drilled to mimic larval galleries from larval feeding area in the cambium layer to the inner part of the tree. The ends of inner borehole zones were marked by outer lines (Fig. 11).

Fig. 11: Young tree samples of *Acer pseudoplatanus*. Left: Control; middle: 5 mm boreholes; right: 10 mm boreholes.

For detection of larvae or larval motion inside the wood samples, suitable goat moth larvae were artificially inserted in predrilled boreholes (Fig. 12). Subsequently plugging with a dowel of the opening of the hole prevented larval escape.

Fig. 12: Inserted *Cossus cossus* larva in a model plant (wood sample of *Acer pseudoplatanus*) with predrilled borehole. Current picture shows a cut wood sample sealed with a glass slide to observe larval behavior.

Non-destructive image guided techniques

Computed tomography

Model plants and organism

The sample material was prepared and stored according to the method described in chapter 0. In total 118 wood samples and 10 small trees of *Acer pseudoplatanus* were used. Wood samples contained three diameter classes: a = 10 – 20 mm; b = 21 – 30 mm and c = 31- 40 mm. Table 6 gives an overview on the range of the sizes of the used model plants after cross-measuring in the middle of each sample. Within each diameter class six control samples (without holes and larvae) and 30 samples with three different borehole sizes were used: 2 = 2 mm borehole diameter; 5 = 5 mm borehole diameter and 10 = 10 mm borehole diameter. Each five of the boreholes were located in the middle of the sample (following the pith) or located at the edge. For the samples containing two and five mm boreholes, two skewed boreholes from the outer side of the bark to the middle of the longitudinal borehole were drilled in the same diameter to mimic the larval gallery produced by the larvae when they move from the cambium layer to the wood. Based on the available size of the model organism larvae in each two of the samples with boreholes of five and 10 mm diameter, one larva each was inserted in the boreholes. In addition 10 samples of the diameter class “b” (21-30 mm), containing five boreholes of five mm and 10 mm, were artificially packed with borings to mimic larval frass.

Besides the model plants, 10 young plants as described in chapter 3.2 were assessed as well. Three plants did not contain any borehole (=control). Seven plants contained one 10 mm borehole from the centre of the root in the middle of the stem, one five mm borehole in one of the bigger roots and one five mm borehole from the cut end in the stem down to the root system. In addition five skewed boreholes of five mm diameter were drilled. Table 7 gives an overview on all analyzed variants.

Table 6: mean diameter of model plants used for CT-investigation.

	Diameter class of model plants			
	a (10 - 20 mm)	b ≤ 30 mm	c ≤ 40 mm	b_{frass} ≤ 30 mm
number	36,0	36,0	36,0	10,0
mean	17,2	26,1	35,0	27,2
minimum	13,5	21,5	30,5	23,5
maximum	20,0	30,0	40,0	30,0
standard deviation	1,8	2,4	3,0	2,6

Table 7: Variants of young trees for CT-scanning analysis. “X” indicate the relevant borehole.

No.	Test variants			
	control without any hole	borehole 10 mm centre of root/stem	borehole 5 mm in root	borehole 5 mm in stem
I	X			
II		X	X	X
III		X	X + 10 mm diagonally to root collar	
IV		X + skewed borehole	X	X
V	X			
VI		X	X	X + skewed borehole
VII		X	X	X + skewed borehole
VIII		X	X	X
IX		X	X	X + skewed borehole
X	X			

Table 8 gives an overview of all tested combinations of the CT observer blinded study.

Table 8: Test variants of CT scanning, observer blinded study.

No.	Diameter-class	borehole-diameter	location of borehole	replication	with larvae	with skew boreholes	with frass
	a, b, c	K, 2, 5, 10	Z, R		I	II	III
	a = 10-20 mm; b = to 30 mm; c = to 40 mm	K = control without borehole; 2 = 2 mm; 5 = 5 mm; 10 = 10 mm	Z = borehole in center; R = borehole at edge	1 to 5	I = larve according to borehole size (only 5 and 10 mm in the center)	II = shew drilled borehole (only 2 and 5 mm)	III = additional samples filled with frass
1	a	K		1			
2				2			
3		2	Z	1			
4				2			
5				3			
6				4			
7				5			
8			R	1			
9				2			
10				3			
11				4			
12				5			
13		K		3			
14				4			
15		5	Z	1	I		
16				2	I		
17				3	I		
18				4		II	
19				5		II	
20			R	1			
21				2			
22				3			
23				4			
24				5			
25		K		5			
26				6			
27		10	Z	1	I		
28				2	I		
29				3	I		
30				4		II	
31				5		II	
32			R	1			
33				2			
34				3			
35				4			
36				5			
37	b	K		7			
38				8			
39		2	Z	1			
40				2			
41				3			
42				4			
43				5			
44			R	1			
45				2			
46				3			
47				4			
48				5			
49		K		9			
50				10			
51		5	Z	1	I		
52				2	I		
53				3	I		
54				4		II	
55				5		II	
56			R	1			
57				2			
58				3			
59				4			
60				5			
61		K		11			
62				12			
63		10	Z	1	I		
64				2	I		
65				3	I		
66				4		II	
67				5		II	
68			R	1			
69				2			
70				3			
71				4			
72				5			
73	c	K		13			
74				14			
75		2	Z	1			
76				2			
77				3			
78				4			
79				5			
80			R	1			
81				2			
82				3			
83				4			
84				5			
85		K		15			
86				16			
87		5	Z	1	I		
88				2	I		
89				3	I		
90				4		II	
91				5		II	
92			R	1			
93				2			
94				3			
95				4			
96				5			
97		K		17			
98				18			
99		10	Z	1	I		
100				2	I		
101				3	I		
102				4		II	
103				5		II	
104			R	1			
105				2			
106				3			
107				4			
108				5			
109	b	5	Z	1			III
110				2			III
111				3			III
112				4			III
113				5			III
114		10		1			III
115				2			III
116				3			III
117				4			III
118				5			III

CT scanning

Model plants were placed randomly on a tray fixed in the CT-scanner (Fig. 13a). In each case the borehole was located to the starting point of the scanner. Three scans were necessary to investigate all wood samples. In comparison to that, only two plants each could be scanned. The plants were placed on the same tray in a way that the scan ran from the stem down to the root system (Fig. 13b).

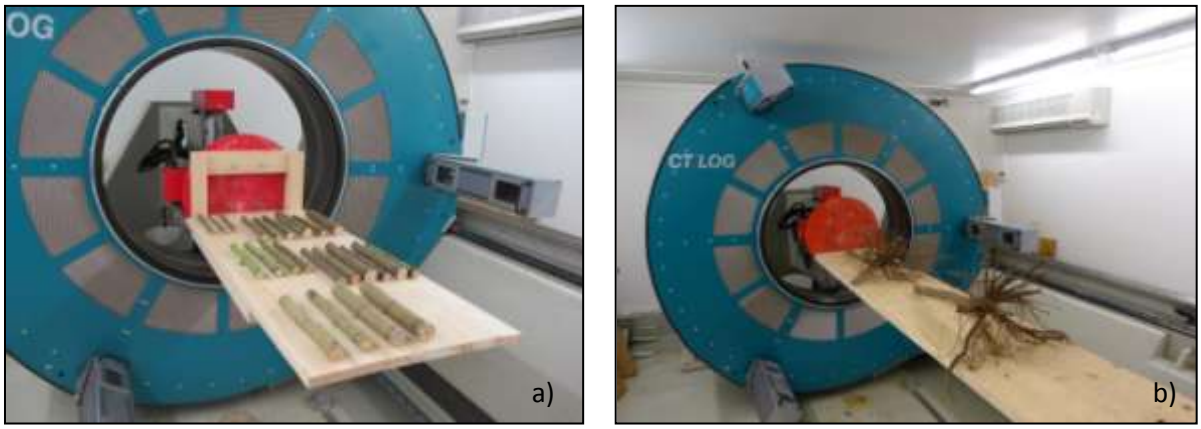


Fig. 13: Computer tomograph (Microtec ® CT LOG) at the Forest Research Institute Baden-Wuerttemberg. a) model plants on a tray; b) young plants on a tray.

CT measurements were carried out applying an accelerating voltage of 180 keV and a beam current of 11 mA. Model plants as well as plants were scanned with an axial feed of five mm per scan. The reconstruction of each cross section consisted of 900 planes. The determination of the characters to be assessed: artificial larval galleries of different sizes; different locations of artificial larval galleries; living larvae in artificial larval galleries; artificially packed frass in artificial larval galleries was carried out as an observer blinded study on the basis of the 2D pictures. To assess an improvement of the visual inspection the single slides were combined to a film using Microsoft Movie Maker 2012.

To evaluate the potential of a CT-Scanner, 3D pictures and films were created by the Forest Research Institute Baden-Württemberg.

Thermography

Thermographic equipment

The investigation was carried out in a stepwise approach. In a **first preliminary test** two infrared imaging cameras a VarioCAM® hr research 600 (Jenoptik, Germany) and a ThermaCAM™ B20 (FLIR Systems GmbH, Germany) were used to verify if meal worms can be identified in adequate contrast to background. Furthermore thermal images of wooden samples with boreholes and inserted larvae were taken and analyzed. A **second preliminary** investigation was conducted for observation of time-depending cooling processes of samples (*A. pseudoplatanus*) and boreholes induced by different heating procedures by active thermography. For this purposes three heating sources were applied: blow-dryer, infrared carbon emitter, and black body emitter.

For assessment of larvaes motion activities and quantifiable inspection in wooden samples, two long-term measurements about 1) 20h and 2) 24h were run with a high-performance thermographic dual-band system Geminis 327 k ML (IRCAM GmbH, Germany, 3.7 - 5.0 µm and 8.0 - 9.4 µm), used in metrology, research and industry, at the Fraunhofer Institute for Wood Research in Brunswick. Pre-investigations approved a higher contrast and granularity in LWIR range compared with MWIR range. The technical data of all applied cameras are described in Table 9.

Table 9: Main feature of IR imaging cameras

	VarioCAM® hr research 600	**ThermaCAM™ B20**	**Geminis 327k ML (dual – band)**
Spectral range	7.5 - 14 µm (LWIR)	7.5 - 13 µm (LWIR)	3.7 - 5.0 µm (MWIR) 8.0 - 9.4 µm (LWIR)
Resolution	30 mK at 30°C	100 mK at 30°C	15 mK (MWIR) 25 mK (LWIR)
Measuring range	-40 - 1200 °C optional 2000 °C	-40 – 500 °C	n.a.
Absolute measurement accuracy	± 2 °C or ± 2 %	± 2 °C	n.a.
Image size	640 x 480 pixel	320 x 240 pixel	640 x 512 pixel

Thermographic long-term measurements

The goal of **long-term measurement 1** was to determinate activities of four larval stages of *Cossus cossus* accompanied by differences in temperature. Furthermore the assessment of maximum divergences of thermal properties between background objects (wood chips, apple and solid wood) and larvae has been studied during 20 hours.

Four goat moth larvae with various body sizes were placed in a plastic box covered with wood chips, solid wood pieces (*Salix caprea*) and quartered apple (Table 10). The thermography set-up was positioned directly above and focused on the test objects. After

recording the thermal imaging video, 10 representative pictures of the 20 h lasting film were chosen for detailed thermal assessment. The acquisition of data and evaluation of temperature was done by thermal determination lines that were defined for each picture and each test object.

Table 10: Test objects of long-term measurement 1

test object	characteristic	code
background 1	wood chips, apple	b1
background 2	solid wood (*Salix caprea*)	b2
larvae 1	100 mm (length)	l1
larvae 2	70 mm (length)	l2
larvae 3	50 mm (length)	l3
larvae 4	30 mm (length)	l4

The second observation (**long-term measurement 2**) aimed for precisely identifying and determination of larvaes activities inside natural host material. Additional goal was to detect boreholes without larvae. Pre-drilled (10 mm borehole diameter) wooden samples (*A. platanoides*, *S. alba*) were inserted with *C. cossus* larvae of higher development stages between 60 and 100 mm body length. For obtaining different activation status, and for assessing effects of bore dust or strands, larvae were instated at different times: 1) 20 days before (boreholes filled with bore dust at the time of measurement) and 2) immediately before starting thermal measurements (boreholes without bore dust at the time of measurement). For control of larval behavior and to get insight at any time, two samples were divided in center in longitudinal direction and then bonded with tape before imaging.

Eight samples (stem diameter 20 – 42 mm) fitted with one goat moth larvae each were placed in a 40 x 25 x 25 cm glass container, which, to minimize means of escape, sited in a major plastic box (Fig. 14). At this, six wooden samples were chosen and two furthermore larvae functioned as control for larval temperature outside of samples. In total, eight representative pictures served as the basis for determining of temperature profiles of eight test objects (Table 11).

Fig. 14: Thermographic setup for long-term measurement 2

Table 11: Test objects of long-term measurement 2 (wood samples [*Salix alba*, *Acer plata-noides*] with inserted *Cossus cossus* larvae; larva 1 and 2 outside of specimen for control).

		wood		larva		
test object	**tree species**	**diameter [mm]**	**borehole characteristic**	**larval size [mm]**	**larval time in wood**	**code**
sample 1	*Salix alba*	22	bore dust	60	20 days	s1
sample 2	*Salix alba*	42	bore dust	90	20 days	s2
sample 3	*Salix alba*	20	no bore dust	80	0 days	s3
sample 4	*Acer plat.*	25	no bore dust	80	0 days	s4
sample 5	*Salix alba*	36	bore dust	100	20 days	s5
sample 6	*Acer plat.*	33	no bore dust	90	0 days	s6
larva 1	-	-	-	80	-	l1
larva 2	-	-	-	60	-	l2

The thermal determination lines were located in center axis in axial direction along the total sample over each frame. Larvae 1 and 2 were scaled over its longest straight-line when larvae was visible (Fig. 15).

Fig. 15: Thermal test setup of long-term measurement 2. Solid wood samples (*Acer platanoides*, *Salix caprea*) with axial boreholes in center and inserted *Cossus cossus* larvae in each sample. Left: Color image with marked positions at the end of boreholes. Right: Thermal image with measurement setup of thermal determination lines (s1 to s6). Test object l1 shows one of two “free” larvae.

The thermal determination lines illustrated the output values and represented the absolute temperature at measuring point. One pixel confirmed one measuring point of determination line and resulted in 0.035 cm per pixel (one value of temperature per pixel).

Radar

Model plants and organism

The given sample material was prepared and stored according to chapter 0 and 0. At the time of radar measurements smallest larvae were grown up and borehole had to be adjusted to 3 mm in diameter. In total 84 wood samples and 18 young tree samples of *Acer pseudoplatanus* L. including controls and 39 larvae (*Cossus cossus* L.) were used for analyzes. For each category, three of six samples were separately filled with one larva, appropriate to borehole diameter, at wood samples. Furthermore, to simulate larval frass, additional 12 specimens were plugged with boredust (Table 12). Two larvae for each category were fitted at three young tree samples, first one at the upper stem region, and second one in the root zone (Table 13).

Table 12: Parameter and number of wood samples (*Acer pseudoplatanus* L.) and larvae (*Cossus cossus* L.; appropriate in size to borehole diameter) for larvae, borehole and frass detection using radar. One larva separately fitted at three samples of each category. C = Control.

stem diameter class [mm]	**borehole diameter** [mm]	**number of samples** (larvae and borehole detection)	**number of larvae**	**number of samples** (frass detection)
	C	6	-	-
A	3	3 / 3	3 / -	-
(10 – 20 mm)	5	3 / 3	3 / -	-
	10	3 / 3	3 / -	-
	C	6	-	-
B	3	3 / 3	3 / -	-
(21 – 30 mm)	5	3 / 3	3 / -	6
	10	3 / 3	3 / -	6
	C	6	-	-
C	3	3 / 3	3 / -	-
(31 – 40 mm)	5	3 / 3	3 / -	-
	10	3 / 3	3 / -	-
Total		**72**	**27**	**12**

Table 13: Parameter and number of young tree samples (*Acer pseudoplatanus* L.) and larvae (*Cossus cossus* L.; appropriate in size to borehole diameter) for larvae and borehole detection using radar. Two (one of two) larvae separately fitted at stem and root zone at three samples of each category. C = Control.

borehole diameter [mm]	**number of young trees** (larvae and borehole detection)	**number of larvae**
C	6	-
5	3 / 3	6 / -
10	3 / 3	6 / -
Total	**18**	**12**

Radar equipment

An ultra wideband (UWB) radar system (4.5 GHz bandwidth, Meodat GmbH, Germany), transmitting much wider frequencies compared to conventional systems, was used for analyses. Three antennas (DRH20, RFSpin, Czech Republic), operating in a spectral range

of 1.5 GHz and transmission power of 1mW, were configured adjacently in horizontal axis enable to spatial location of larvae inside the samples (Fig. 16). For obtaining reliable sensitivity to insects, receiver were stabilized/fixed against test objects on a fixed construction and measuring range of antennas was shielded to external moving objects. Thus, electromagnetic absorbing materials in background environment were used for investigation (Fig. 16).

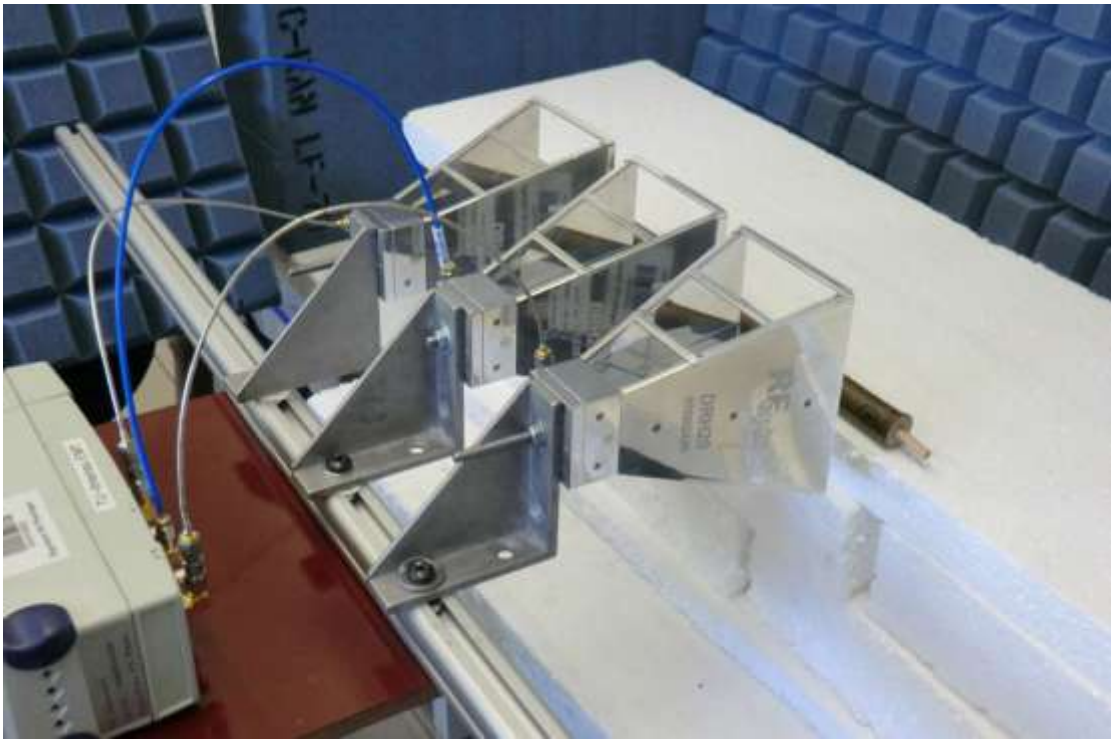


Fig. 16: Measurement setup of radar: Radar device, three antennas, wood sample and electromagnetic absorber material

Radar measurements

To draw inferences about precision and applicability of radar system, measurements were conducted as a blind study. Hence, subjective failures induced by personal operators could be hold down. The samples were positioned in front of antennas homogenous in measuring distance (approx. 10 - 15 cm) to minimize external effects or keep them comparable. In total 188 measurements were executed, in particular four measurement series could be distinguished: Single measurements and repetitions (wood sample), long-term measurements (wood sample), simultaneous measurements of several samples (wood sample) and single measurements as a function of position of tree (young tree), confer Table **14**.

Table 14: Number and time of radar-measurements as a function of testing object, type of measurement and repetition

Testing object	**Type of measurement**	**Repetition**	**Number of measurements**	**Measurement time** [s]
wood samples	single measurements	day 1	84 (3 replicates)	150
		day 2	27 (3 replicates)	150
		day 3	27 (3 replicates)	150
	long-term measurements	-	2 (2 replicates)	600
	simultaneous measurements of several samples	-	12 (3 replicates)	150
young trees	single measurement	-	36 (3 replicates)	150

Radar measurements using wood samples

Single measurements and repetitions of separate sample had been carried out in a three-repetition-step over three days with a measuring time of 150 seconds per sample. The first measurements contained each category of parameter for larvae, borehole and frass detection (Table **12**). It became apparent early that radar assays were solely responsive to larval motion and vibration and no detection could be found without any insect. Thus, samples free of larva had been excluded from following analyses and samples with inserted larva, to address larvae activity and concomitant false negatives at absent motion, were analyzed separately at second and third repetition.

Based on single measurements of second day, two samples with undetected larva (false negatives) were analyzed in two ***long-term measurements*** over a time of 600 seconds to verify if larvae were merely idle and motionless at the time of single measurements.

To analyze influences and interferences of adjacent samples on larval detectability, ***simultaneous measurements of several samples*** were executed. One sample fitted with active larva was measured first, and afterwards, additional larvae-free samples were added to measuring field (fields of view) next to the first specimen. Following numbers of samples per measurement were observed at three replicates over 150 seconds: 1, 5, 10 and 15.

Radar measurement using young trees

Larval motions were studied at ***single measurements as a function of the position of the young tree***. So, in a first step, inserted larva was measured at the upper part of the trunk. A secondary larva inside of the root zone was assayed subsequently. For each measurement, corresponding measurement position had to be modified and the upper and lower part of the

tree had been analyzed separately (Fig. 17). In addition, boreholes without any larvae were investigated at young trees.

Fig. 17: measurement setup of radar: Three antennas and young tree (root zone)

Analyses of signals

All signals in front of antennas were recorded and analyzed by ultraANALYSER software (ILMENS, Germany) with 16.57 measurements per second (time vector ≈ 0.06 s). Based on differences between actual and preceding signal, moving objects could be illustrated in a radargram as a function of time. Eventually, to display in a radar image, the values of amplitude had been color- or grayscale coded.

The detected movements and vibrations (activity signals) were summarized in a defined detection range and all peak readings were graphed in a normalized scale. Thus, two activity curves of larvae, one for each radar channel, were displayed per measurement. The sensitivity of the receiver to larval motion was set at threshold (movement) level of 5.8×10^{-5} (transformed in limitations between 0 and 1 at 0.3625) to define (minimal) larval activity and a concomitant detection signal. Hence, the detection could be realized in automated mode when (absolute) value had been achieved or exceed. Interference signals (external noise e.g. handy superimposition) were filtered and removed of evaluation.

Electrical resistivity tomography (ERT)

Model plants and organism

The sample material was prepared and stored according to chapter 0. In total 85 green wood samples of *Acer pseudoplatanus* L. including five repetitions of each sample parameter (stem diameter, borehole diameter, borehole position), except A10e, were analyzed for borehole detection (Table **15**).

In addition 15 *Cossus cossus* L. larvae (chapter 0) were inserted in secondary samples of *Acer pseudoplatanus* L. (5 repetitions at stem diameter class A, B and C; borehole diameter 10; central borehole position) for larvae detection according to chapter 0.

Table 15: Parameter and number of samples (*Acer pseudoplatanus* L.) for borehole (100 to 150 mm in length) and larvae (*Cossus cossus* L.; appropriate in size to borehole diameter) detection using ERT

stem diameter class [mm]	**borehole diameter** [mm]	**borehole position** (centre; edge)	**number of samples** (borehole detection)	**number of samples with inserted larvae** (larvae detection)
A (10 – 20 mm)	**2**	c	5	-
		e	5	-
	5	c	5	-
		e	5	-
	10	c	5	5
		e	-	-
B (21 – 30 mm)	**2**	c	5	-
		e	5	-
	5	c	5	-
		e	5	-
	10	c	5	5
		e	5	-
C (31 – 40 mm)	**2**	c	5	-
		e	5	-
	5	c	5	-
		e	5	-
	10	c	5	5
		e	5	-
Total			**85**	**15**

ERT equipment

The measurements have been conducted using a mobile multiplexer (GeoTomMK8E1000 RES/IP/SP, Geolog Fuß/Hepp GdbR, Germany) with internal 12V power supply. Coupling to sample surface was performed by a small-scaled adapted configuration system following the principle of custom-made electrode ring of the HAWK Göttingen. Caused by harmful/high injury and overlarge of pins at the phloem (bark) of small trees, the setup was adjusted to small samples. Two acrylic tubes (40 mm and 55 mm inner diameter; 250 mm length) were perforated with 24 equidistant holes in three measurement planes. These perforations were used for attachment of cannulas (Sterican® Kanülen 0.8 x 40 mm, B. Braun Melsungen AG, Germany), functioned as electrodes, and has been forced through the sample´s bark. The

reverse side of cannulas was fixed to a 24-pole broadband cable to be connected to the GeoTom multiplexer (Fig 18). Low frequency current was used in a dipole – dipole configuration.

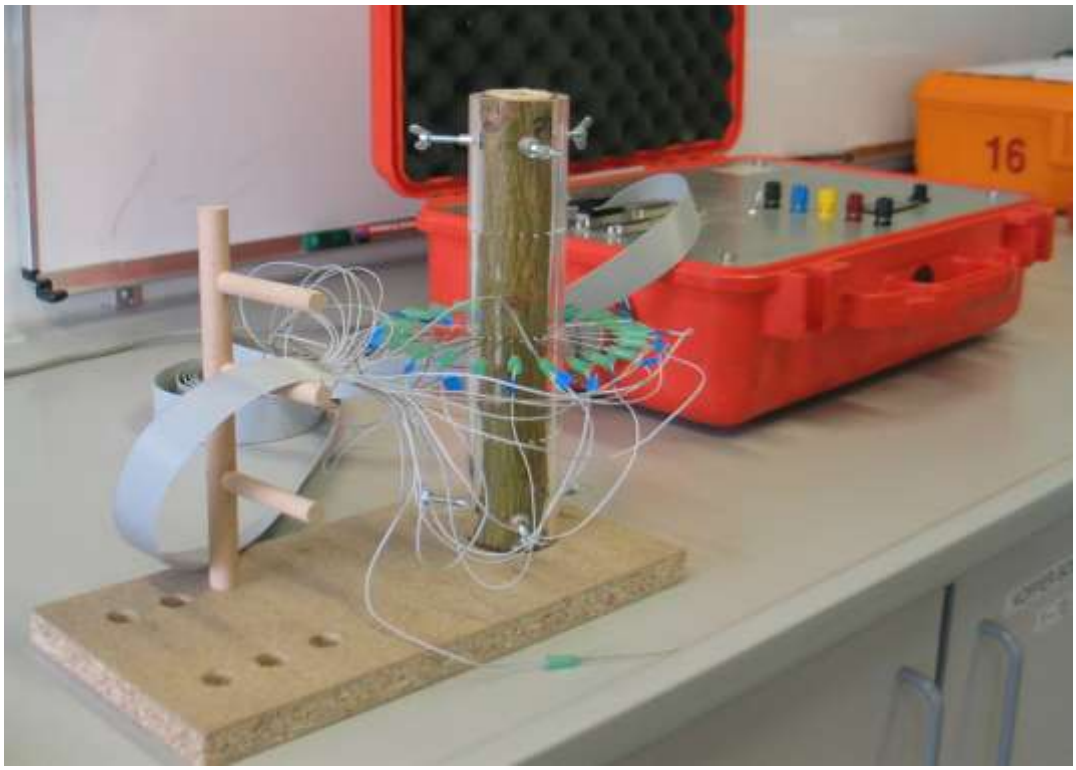


Fig. 18: ERT - Modified electrode configuration system, wood sample and mobile multiplexer GeoTom

ERT measurement

For comparison of untreated (defect-free) and treated (with borehole) wooden zones at the same sample, three measuring planes (MP) were performed at configuration system. The first plane (MP 1) was uniformly used for coupling of untreated areas and served as control, and the upper tier (MP 3) was applied for detecting predrilled zones. An additional measurement plane (MP 2) should offer transition areas between treated and untreated areas (Fig. 19).

The ERT measurements were executed for each sample at MP 1 and MP 3 using 24 electrodes in azimuthal direction around the stem in 15° angle to the next electrode. Cannulas were pricked through the sample´s bark carefully to get contact to the wood. Afterwards coupling of electrodes were tested and 264 resistance values in 11 depth levels were recorded via GeoTom software (GeoTom version 7.18, Geolog2000, Fuß/Hepp GdbR, Germany).
In addition secondary measurements using 12 electrodes (angle 30° to next electrode) have been conducted for one sample of each variant of sample parameter. The background of 12-point-measurement was the determination of the accuracy and resolution of ERT in dependence of the number of electrodes. The assessment of ERT using 8 electrodes (angle 45° to next electrode) has been tested in preliminary tests.

For larval detection separate samples with 10 mm boreholes (MP 3) in centre were inserted with larva compatible in size. In total three measurements were executed per sample using 24 electrodes: untreaed MP 1 (control), MP 3 with borehole and MP 3 with borehole and inserted larva.

The complete number of measurements for detecting boreholes and larvae is listed in Table 16. Two measurements of A5c failed at borehole detection as a consequence of the high number of knots on the sample surface.

Table 16: Number of ERT-measurements in dependence of type of detection, number of electrodes and measuring plane (MP)

Type of detection	number of electrodes	number of ERT-measurements		
		MP 1 (control)	MP 3 (borehole)	MP 3 (borehole and larva)
Borehole detection	24	84 (5 replicates)	84 (5 replicates)	-
	12	16 (1 replicate)	16 (1 replicate)	-
Larvae detection	24	15 (5 replicates)	15 (5 replicates)	15 (5 replicates)

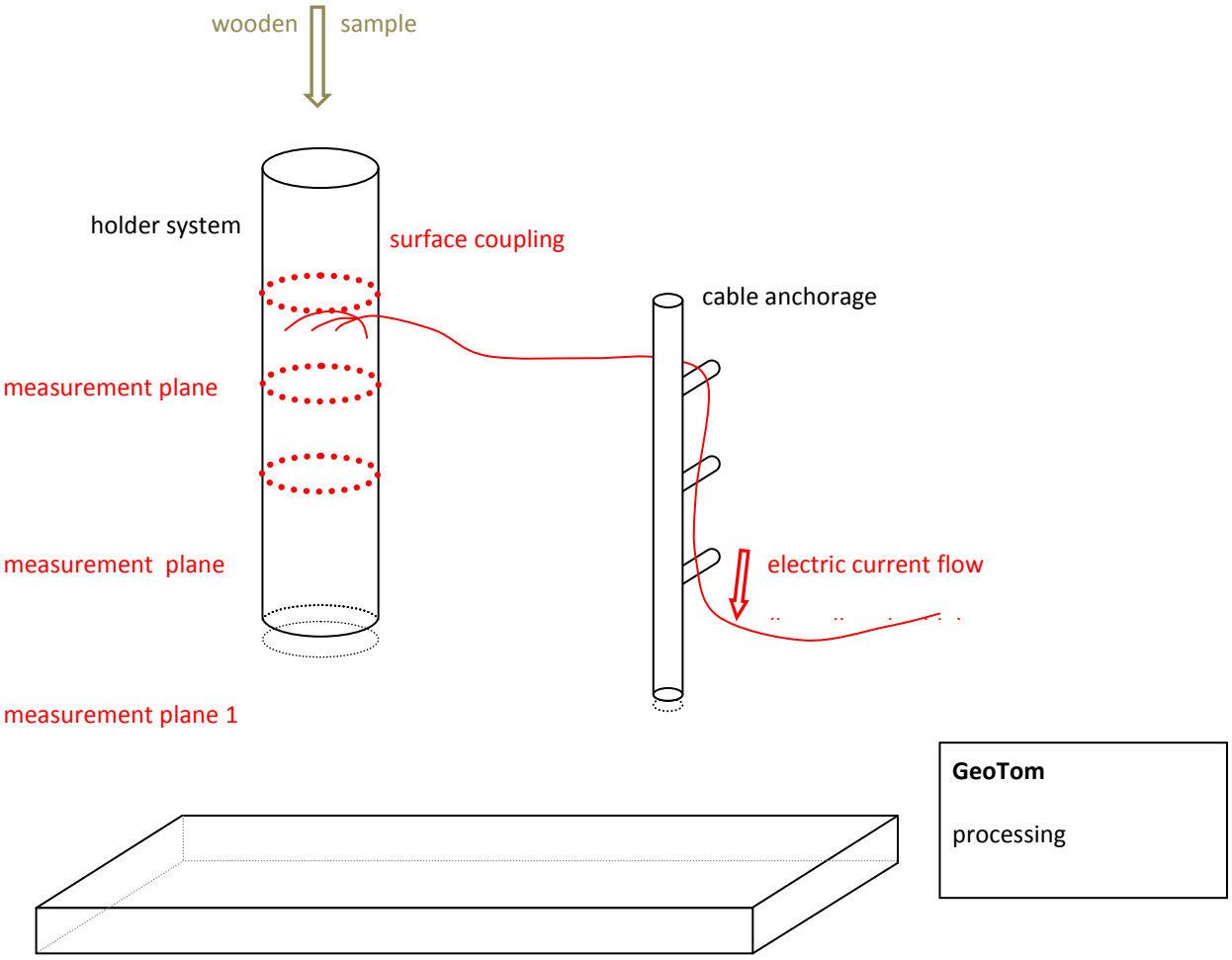


Fig. 19: ERT – Measurement system (schematic setup)

Inversion

The data inversion was achieved by the software "DC2dTree" (DC2dTree – Impendance Tomography on trees with tree shapes, version 0.9.2, C. Rücker & T. Günther, resisitivity.net productions, Germany), that adapted mathematical algorithms to tree shape [76]. A higher resolution was obtained via tuning of maximum mesh area. For comparison of treated and untreated areas the upper and lower thresholds (ohm) of MP 1 and MP 3 of one sample were adjusted for interpretation (adjustment of scale).

Image analysis

For assessment of accuracy of resistivity tomograms, samples had been cut at analyzed measuring planes and cross sections were scanned via color flat bed scanner (CanoScan LiDE 100, Canon Deutschland GmbH, Germany).

Ultrasonic

The investigation was arranged in two measurement series using coupled and non-contact transmission ultrasonic systems and will be considered separately. The techniques basically differed concerning coupling to wood sample and its applied wave length of transducer, but also in scanning procedure (and corresponding measurement time) as well as output parameters.

Model plants and organism

For borehole detection six wood samples (*Acer pseudoplatanus*) of several categories were prepared according to chapter 0 and analyzed with both ultrasonic techniques. Three secondary specimens were provided for detection of larvae and larval frass and measured by non-contact ultrasonic. Two boreholes (ca. 60 – 80 mm in length; 10 mm in diameter) were drilled at each front plane, and one inserted with *Cossus cossus* larva (appropriate in size) and the other fitted with bore frass. An overview of applied model plants and organism is shown in Table **17**.

Table 17: Wood samples, parameters and attributions for ultrasonic measurements

wood sample	**stem diameter class** [mm]	**borehole diameter**[mm] **and position** (center or edge)	**type of detection**	**ultrasonic method**
1 (38 II)	C 36.5	10c	Borehole detection	Coupled and non-contact ultrasonic
2 (59 III)	B 21	10c		
3 (60 II)	C 31	10c		
4 (60 VII)	A 18.5	5c		
5 (60 III)	B 26.5	5e		
6 (38 V)	A 16.5	2c		
7 (64 III)	C	10c (larva) / 10c (frass)	Larvae and frass detection	Non-contact ultrasonic
8 (64 II)	B	10c (larva) / 10c (frass)		
9 (64 I)	A	10c (larva) / 10c (frass)		

COUPLED ULTRASONIC

Coupled ultrasonic equipment

An in-house ultrasonic system of the Federal Institute for Materials Research and testing (BAM, Berlin, Germany) for acquisition of travel time (including frequency generator, oscillograph, amplifier, PC-processor etc.) and two transducers for longitudinal waves LD20 (ACSYS, Moskau, Russia), working without couplants, were used for experiments (Fig. 20 and Fig. 21). The measurements were executed with 100 and 120 kHz center frequencies with a sampling rate of 1 MHz and a measuring area of ± 200 mV.

Fig. 20: Ultrasonic system of the Federal Institute for Materials Research and testing.

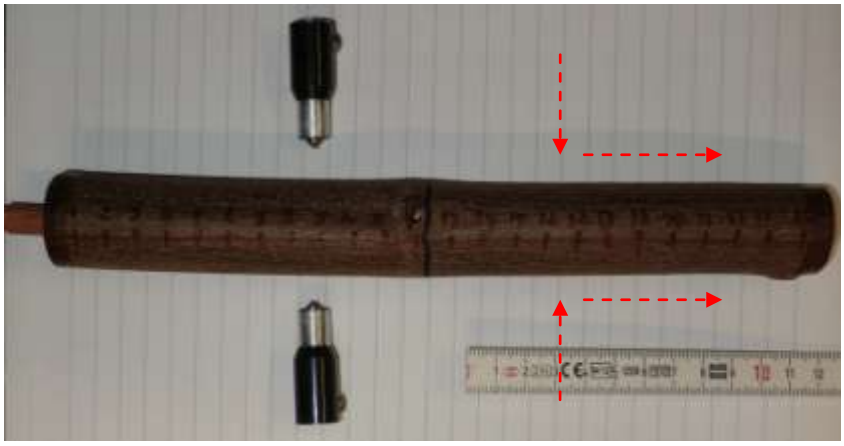


Fig. 21: Couple transducer LD20, wood sample and measurement setup (arrows)

Coupled ultrasonic measurements

Before starting analyzes, measurement points (connecting points of transducers) were marked in a distance of 10 mm in axial direction at two oppositely disposed indication lines at the surface of wood samples. It should be ensured that the opposing points were in axial-parallel order to stem axis. The transducers were connected by hand at both measuring points of corresponding measurement plane and initial impulses were generated. Henceforth, the analyses were implemented plane for plane in axial direction and started at borehole zone (Fig. 21). In total 24 time sequences of ultrasonic amplitudes were recorded per sample unit and graphed in (so called) A-Scans over 1 ns (1000 µs or 0.001 s). Per recording less than 10 seconds including positioning of transducers were needed.

For comparison of treated and untreated wooden zones, points were allocated in this way that the transmission direction crossed the borehole at borehole areas (Fig. 22, line 1). For control, a secondary transmission way next to the borehole was chosen at sample number

38 II (Fig. 22, line 2). The transmission with transversal waves at 55 kHz for control, resulted in lower differences between treated and untreated zones. Henceforth, analyses using longitudinal waves at 120 kHz were executed.

Fig. 22: Transmission directions at coupled ultrasonic measurement

Analyses of coupled transmissions

All recordings and analyses were executed by the in-house software “Ultraschall-Analyse” (BAM, Berlin, Germany), developed and applied for analyses of pulse-echo and tranmission. The reported/recorded A-Scans of separate measuring plane were graphed in a B-scan presentation. The B-scan displayed the time of flight (travel time t) of the sound energy in dependence of the position of transducer. By comparison of travel times and derived velocity of propagation of borehole zones and untreated planes, interferences of presences of boreholes could be drawn.

For determination of Δt emitted travel pulses have to be considered and subtracted from received travel time. Evaluation had been executed by comparing typical A-scans of treated and untreated zones.

NON-CONTACT ULTRASONIC

Non-contact ultrasonic equipment

The wood samples were tested with the imaging ultrasonic system USPC 4000 AirTech Industrie by air-coupling using transmission technique (Fig. 23). Ultrasonic signals were edited with the ultra-low preamplifier AirTech 4028-1. In dependence of stem diameter the transducer AirTech 300 and AirTech 200 were applied with initial impulses of 300 kHz respectively 200 kHz (Fig. 25). The samples were scanned automatically using the manipulator FlatScan 1000-03 AirTech (Fig. 24). All equipments and compounds were developed and produced by the Engineering office Dr. Hillger in Braunschweig in Germany.

Fig. 23: Ultrasonic system USPC 4000 AirTech (Source: Engineering office Dr. Hillger)

Fig. 24: FlatScan 1000-03 AirTech

According to Table **18** the transducer worked in dependence of its sound beam diameter in different distances to testing object (near field length):

Table 18: Sound beam diameter and near field length of air-coupled transducer

Transducer	**Sound beam diameter [mm]**	**Near field length [mm]**
AirTech 200	3	18
AirTech 300	2	12

Non-contact ultrasonic measurements

The samples were individually adjusted at scanning system is this way that the boreholes were optimal assignable in display. To reduce wave propagation on the surface of samples, foam material was pinched between anchorages and sample (Fig. 25). The automated scanning was executed with a measuring point distance (index step) of 0.5 mm.

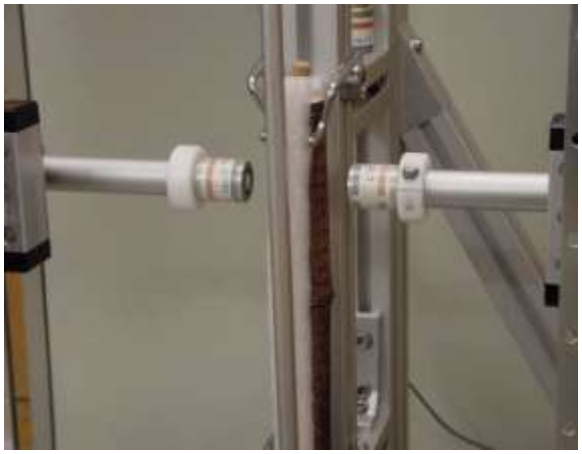


Fig. 25: Air-coupled transducer AirTech 200 and wood sample at scanning process. Foam material reduced wave propagation on the surface of samples.

Interpretations/Analyses of air-coupled transmissions

Data of transmission were analyzed with the software Hillgus for Windows (Engineering office Dr. Hillger, Braunschweig, Germany). In contrast to the line-based B-scans of coupled ultrasonic devices, entire areas could be scanned and reconstructed in C-scans using air-coupled transducers. The C-scan benefits that plan-type views of the position and size of sample features (vacancies, boreholes, boundary layers, etc.) could be provided. Similar to x-ray image, all acoustic structure scattering and reflecting the ultrasonic waves within and on the surface of the object, will be displayed in C-Scan.

With decreasing angel of sample surface to the transmission direction, ultrasonic waves would be scattered and propagated at surface and transmission was considerably weakened / disturbed in (Fig. 26, red arrows). Thus, only the inner zones of samples (in radial direction) could be analyzed with higher accuracy and edge regions were excluded from evaluation (Fig 26, green arrows).

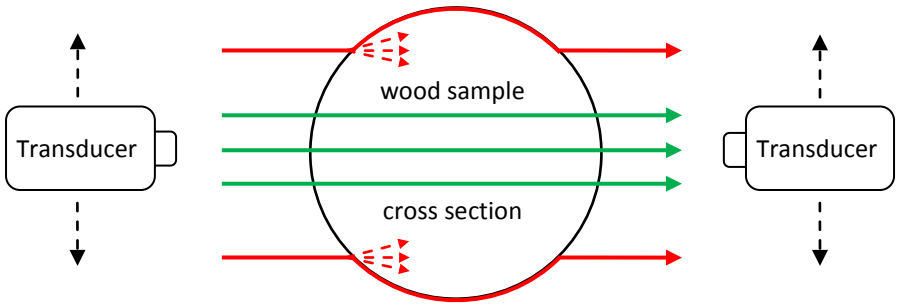


Fig. 26: Top view of transmission of ultrasonic waves through wood sample (cross section), schematic setup. With decreasing angel of sample surface to transmission direction, waves are scattered (dashed red arrows) and propagated at surface (red arrows) and transmission is weakened. Best transmission without distribution is given at nearly 90° angel (green arrows).

Measurement of wood moisture content

The moisture content of the wood was determinate according to DIN 52183 (1977) via gravimetric assessment after non-destructive measurements. The samples were placed in a forced-air oven (Memmert, Type UL50, Schabach, Germany) maintained at 103 °C until reaching a constant ovendry weight basis [49].

Statistical analysis

Statistical analysis was carried out using SigmaPlot 11.0 (Systat Software Inc., USA). At **long-term measurement 1**, mean comparison between each test object at observation time was realized by t-test, when both groups were normally distributed, or *Mann-Withney* u-test, when one group showed no normal distribution. The chosen p-value was defined at significance level of p = 0.01.

The **second long-term measurement** used fixed determination lines for tracing abnormalities or outliers in temperature that would be induced by larvae in center regions of wood specimen. Evaluation had been done by analyzing of temperature profiles along each test object per picture.

Results

Computed tomography

The aim of the study was to evaluate whether using CT-scanning it is possible to detect boreholes including their size and location, larvae and larval frass in model plants and young plants.

The 118 used model plants had a moisture content of 83,7 % in average (Table 19).

Table 19: Mean moisture content of 18 selected model plants.

	m.c.
number	18,0
mean	83,7
minimum	67,1
maximum	99,0
standard deviation (%)	10,2

Results of 2 D image analysis:

Using two dimensional (2D) slides, it was possible to identify larval galleries of two millimeter when located outside the pith and larval galleries of five and 10 mm in both cases in the centre (following the pith) and the edge. This result was independent of the diameter class of the model plants. As the pith occurs in the 2D pictures as dark/black dot, having more or less the same size as the two mm bore holes, it was difficult or sometimes not possible to detect the borehole in the sample. In no case a false positive bore hole (identifying a borehole where none was) was detected.

Skew drilled boreholes could be identified in all cases. Keeping in mind the starting of the hole in the picture (from inside to outside or from outside to inside) it was possible to describe the direction of the hole: from the stem down or upwards.

Larvae in the boreholes occurred as white fillings which became bigger from the first observation (head or tail end), followed by filling the hole and decreasing in diameter (again head or tail end). The contrast from black, unfilled holes to white larvae was very clear.

Artificially packed frass (borings) showed a grayish color and could be differentiated from empty bore holes as well as from larvae.

Figure 27 shows 2 D pictures of boreholes of different sizes, boreholes with as well as skewed boreholes.

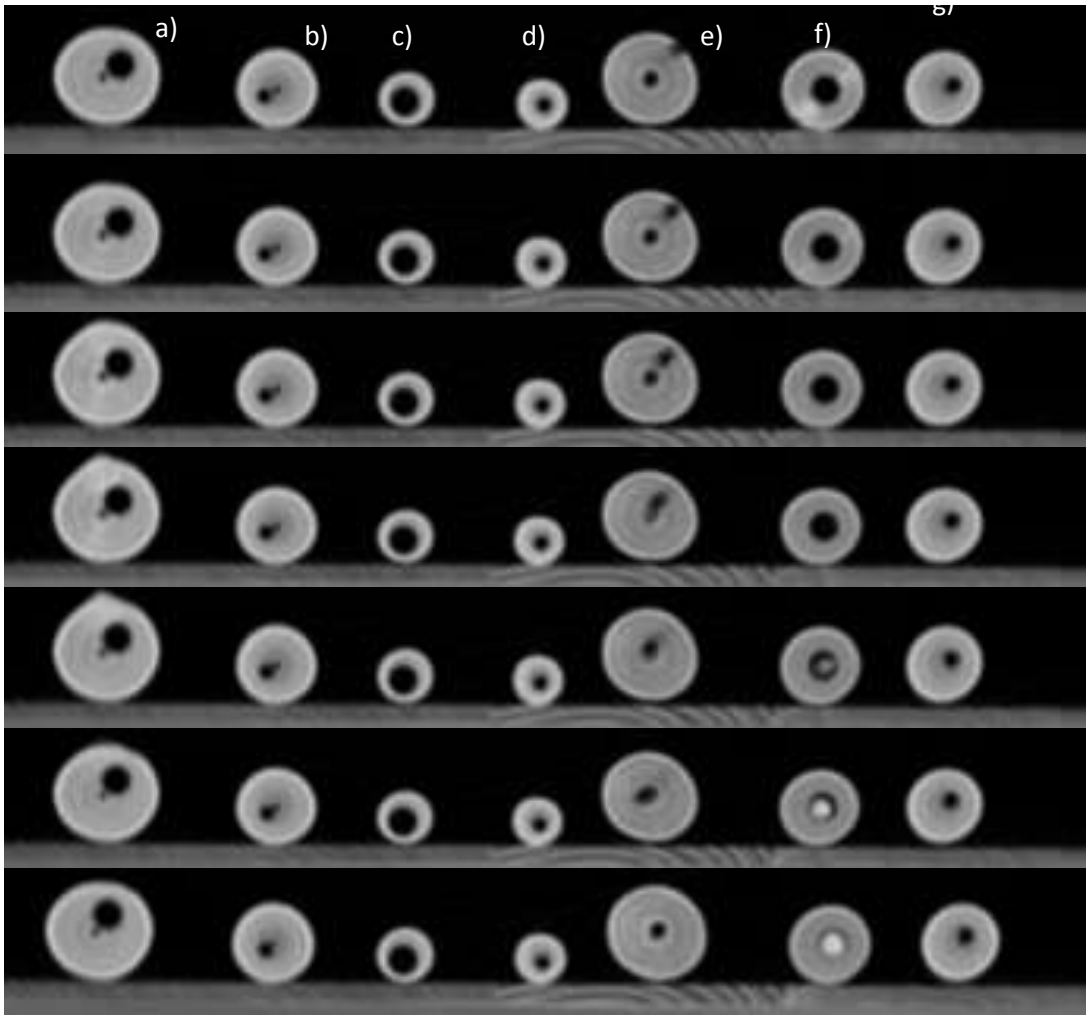


Fig 27: different sizes and locations of artificially drilled boreholes, with and without larvae, visualized using a CT-scanner. a) 10 mm diameter borehole, location at edge; b) 5 mm borehole, edge; c) 10 mm borehole, center; 5 mm borehole center; 5 mm borehole, center, skew borehole from line 1 to 5 from outside to center; 10 mm borehole, center, with larva from line 5; 5 mm borehole following pith (= center). Pictures from line to line represent a movement of the CT-scanner of 5 mm.

Results of the observer blinded study.

Model plants

118 model plants and 10 young plants were scanned without knowing with variant according to

Table 8 was placed at the tray in the SC-scanner. Evaluation was carried out on the basis of viewing the complete Scans once after the treatment. The following rating was given:

- "larval galleries" yes or no
- Diameter of the galleries (2, 5, 10 mm)
- Position of the galleries (center or edge)
- Larva inside yes or no
- Frass inside yes or no

Table **20** to

Table 24 show the results of the observer blinded study for the model plants.

Table 20: Observer blinded study: percentage of observed criterion "larval gallery" and "diameter of gallery". (Z = position in center of model plant following the pith; R = position in edge area). (*model plants without any borehole)

Criterion	Diameter gallery	n	correct observation (%)	False negative (no hole observed, but was there)	False positive (hole observed, but none was there)
Larval gallery (yes / no) & diameter	2 mm				
	Z	15	60	6	0
	R	15	100	0	0
	5 mm	35	100	0	0
	10 mm	35	100	0	0
	Control*	18	100	0	0

Table 21: Observer blinded study: percentage of observed criterion "larva". (*: Model plant without larvae, but with or without boreholes and frass)

Criterion	Diameter	n	correct	False negative	False positive

	gallery		observation (%)	(no larva observed, but was there)	(larva observed, but none was there)
Larva (yes / no)	5 mm	9	100	0	0
	10 mm	9	100	0	0
	Control*	100	100	0	0

Table 22: Observer blinded study: percentage of observed criterion “larval frass”. (*: Model plant without larval frass, but with or without boreholes and larvae).

Criterion	Diameter gallery	n	correct observation (%)	False negative (no frass observed, but was there)	False positive (frass observed, but none was there)
Larval frass (yes / no)	5 mm	5	100	0	0
	10 mm	5	100	0	0
	Control*	108	100	0	0

Table 23: Observer blinded study: percentage of observed criterion “larva”. (*: Model plant without diagonal galleries, but with or without boreholes, larvae or frass)

Criterion	Diameter gallery	n	correct observation (%)	False negative (no traverse observed, but was there)	False positive (traverse observed, but none was there)
Diagonal larval galleries (yes/no)	2 mm	6	100	0	0
	5 mm	6	100	0	0
	Control*	106	100	0	0

Table 24: Observer blinded study: percentage of observed criterion “position of gallery”. (*: Model plant without galleries; a = 10-20 mm log diameter; b ≤ 30 mm; c ≤ 40 mm).

Criterion	Diameter gallery	n	Correct observation (%)	Center, but observed edge			Edge, but observed center			No gallery observed
				a	b	c	a	b	c	
Position of gallery	2 mm	30	80	0	0	0	0	0	0	6
	5 mm	35	88	0	3	0	1	0	0	0
	10 mm	35	74	2	3	1	2	1	0	0
	Control*	18	100	0	0	0	0	0	0	0

Young plant

Seven plants with different borehole sizes and locations were analysed with a CT-scanner. Three trees without any hole served as control. The analysis was performed as an observer blinded study.

All boreholes were detected to 100% and the size could be described as well. Fig. 22 shows the true defect in the tree (stem, root part and roots) in the left raw and the related 2D CT-image in the right raw.

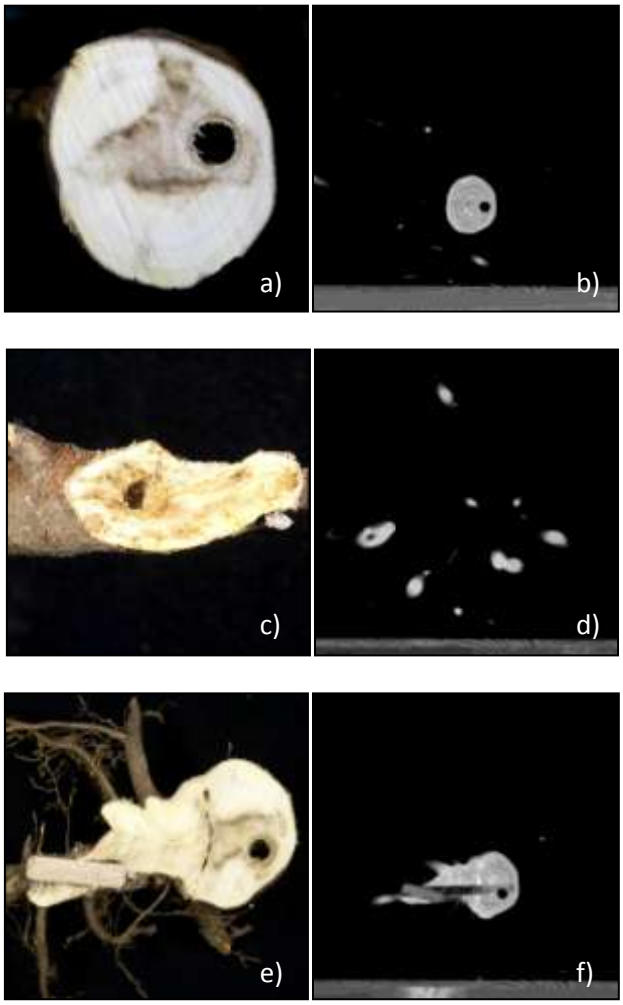


Fig. 22: boreholes in plants (left side) and visualization (2D-images) using a CT-scanner (right side); a + b: 10 mm borehole in stem; c + d 5 mm borehole in roots; e + f 10 mm borehole in root collar and 10 mm cross drilled hole with wooden peg.

Results of 3 D image analysis:

Because the 2D images already resulted in a 100% detection rate, 3D analysis was only carried out to show the potential of 3D imaging.

Based on the 2D pictures created by the CT-Scanner a software was used at the FVA Freiburg to combine them to 3D images. In addition it was possible to create films either on 2D or 3D basis to be able to analyze the trees and model plants several times.

Figures Fig. 23 to Fig. 26 show examples of 3D images. Fig. 23 shows the 3D images of the test arrangement which was shown in its natural picture in Fig. 13a. Fig. 24 shows model plants in scan direction with boreholes as well as a larva. Fig. 25 and Fig. 26 display slices of 3D images to visualize internal parts of test logs.

Fig. 23: 3D image of model plants on a tray after CT-scanning.

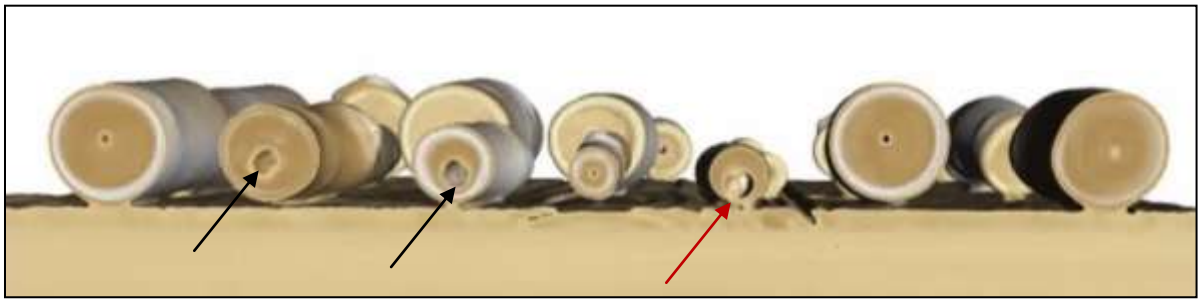
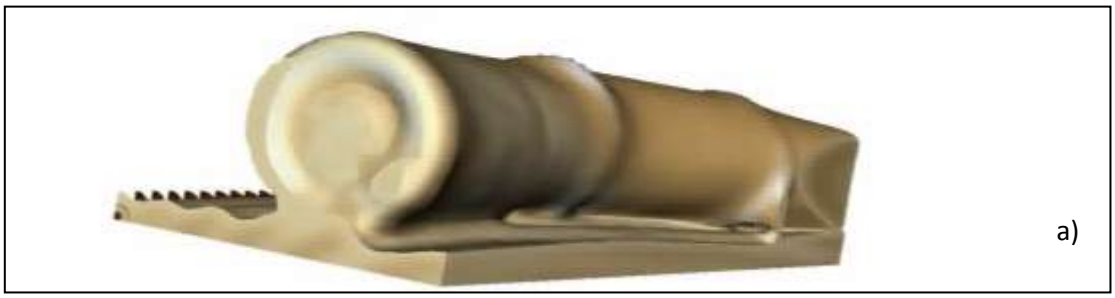


Fig. 24: 3D image of model plants on a tray after CT scanning: arrows indicate artificial boreholes: 3rd from left also contains a larva.



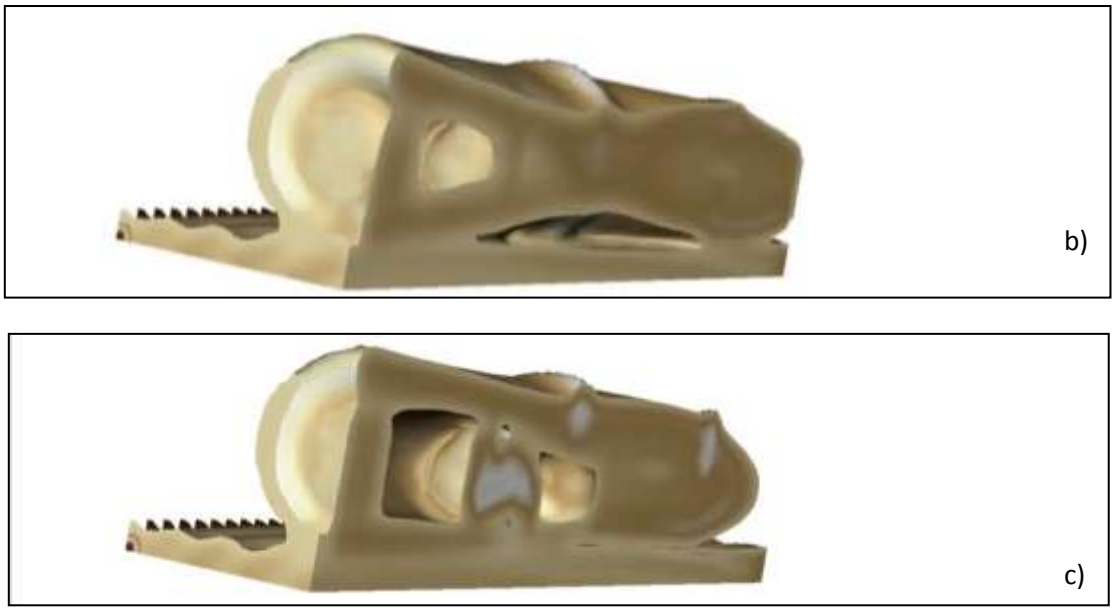


Fig. 25: 3D image of a single model plant with different slices: a) outside, b) wooden part, c) with bore hole.

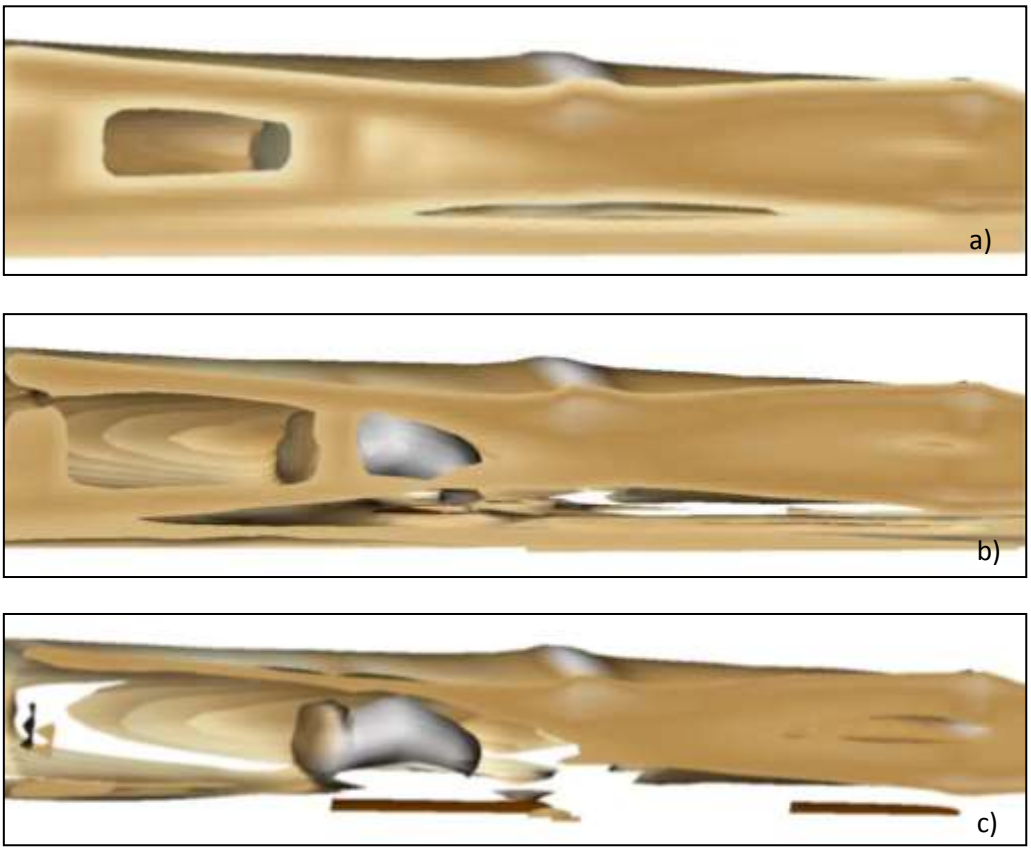


Fig. 26: 3D images of a model plant with different slices: a) wooden part with beginning borehole, b) + c) larva in borehole.

To sum it up it was possible to detect larval galleries of five to 10 mm diameter to 100%. Also 100% detection rate was reached for larvae and frass as well as for skewed running boreholes.

Only in cases where a two millimeter borehole was located in the pith, its detection failed in 40% of the tested samples. As under practical conditions it is not possible to have an internal larval gallery without any connection to the cambial zone and these simulated skewed running larval galleries were detected to 100%, it will be possible to detect that something entered the stem. In addition, as small larvae creating larval galleries with a size of two millimeter only occur in the cambium area and the outer sapwood, it is very unlikely to be faced to such small galleries following just the pith. Therefore under natural conditions it is expected to find bigger larval gallery sizes in the stem, which are detectable with CT.

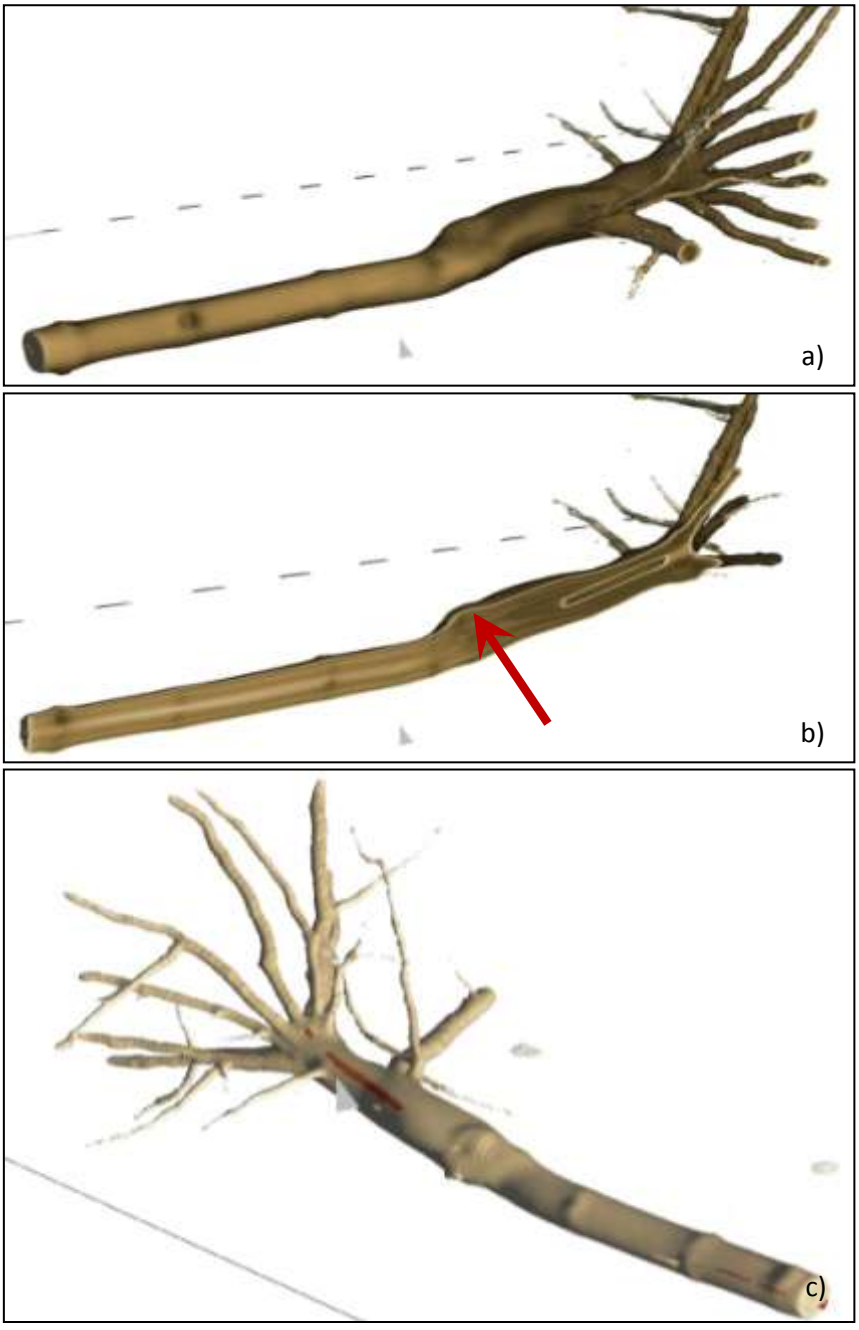


Fig. 27: 3D images of young plants after CT-scanning. a): control plant without any hole; b) plant built up in slices to visualize a 10 mm diameter borehole in the center of a root/stem (arrow); c) whole plant in 3D with outside visualization (red) of internal holes.

Thermography

Results of **preliminary test 1** revealed that meal worms could be recognized by thermal imaging under laboratory conditions. Both standardized passive systems VarioCAM® hr and ThermaCAM™ B20 identified organisms when larvae were on surface of background and no external objects blocked intermediately the thermal scans. However, in dependence of physical properties of the background the total measured temperature differences between larvae and background were very low and under 0.5 K. Highest contrast of approximately 1 K was captured between cold underground of plastic container and moving larva. In contrast, temperature changes between wood chips or solid wood and organism were vanishingly low. Furthermore neither boreholes (2, 5, and 10 mm in diameter) nor larvae inside holes could be visualized by given passive thermal systems. Zones with cavities and inserted organism had no measurable effect on surface temperature.

Similar results were given at **preliminary test 2** when active heating via blow-dryer, infrared carbon emitter and black body emitter and measuring following cooling behavior. Even by using the high sensible dual-band camera Geminis 327 k ML no alternation between treated and untreated wood sections could be visualized by heating impulses and active thermography.

Long-term measurement 1

Cossus cossus larvae could be displayed at 20 hours observation in thermograms when moving on the surface of background objects. As seen in Fig. 28, larvae (l1 – l3) appear brighter, hence warmer, compared to the colder wood chips and apple ground (b1). In contrast, temperature differences between larvae (l4) and solid wood (b2) were evanescent low, whereas wood samples even increased in level. At later points of view (especially at night), larvae showed reduced motion activity and decreased temperature changes. The detection even on background 1 was aggravated crucial.

This assessment is confirmed by analyzing mean temperature data of determination line (Fig 30). Viewing the complete test objects, in particular background 1 was exhibited in relatively low mean temperature. Consequently the highest difference of 0.164 K was given comparing background 1 (minimum temperature of 22.89 °C) and background 2 (maximum temperature of 23.055 °C). Divergences between background 1 and larvae were marginal ranged anymore, from 0.093 to 0.16 K. In addition mean variances among background 2 and larvae emerged in values smaller 0.071 K, miminal in measurability.

A confrontation of temperatures of larvae 1 – 4 resulted in very low divergences at thermal analyses and is ranged between 22.984 °C to 23.051 °C. In terms of larval size, the highest temperature could be recognized at the biggest larva 1; lowest value was given at the smallest larva 4. However, overall no distinct trend of differences in temperature was ascertainable, that could draw inferences from larval size to temperature changes.

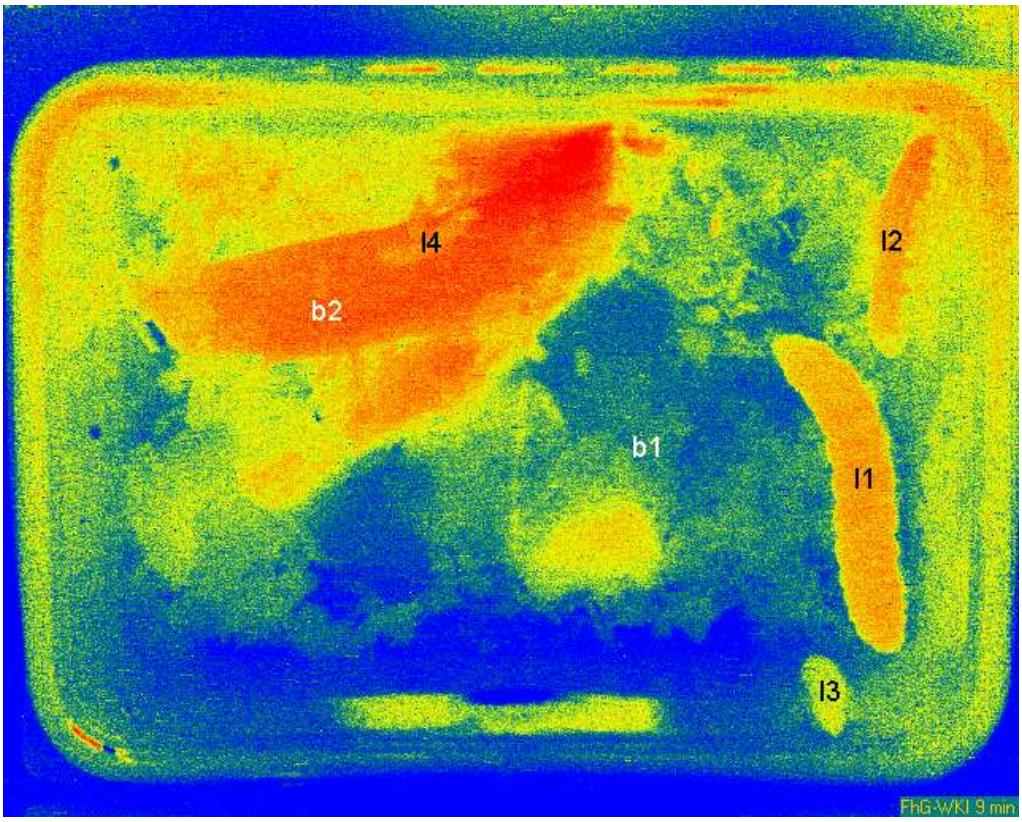


Fig. 28: Thermogram 1 at 9 min at high larval activity in shortly after beginning 20 hours long-term measurement 1. b1 = background 1 (apple, wood chips), b2 = background 2, l1 = Larva 1 [100 mm], l2 = Larva 2 [70 mm], l3 = Larva 3 [50 mm], l4 = Larva 4 [30 mm]. Adequate differences in temperature between larvae and b1. Inadequate differentiation between solid wood and l4.

Statistically mean comparison confirmed no statistical significances between all groups using significance level p = 0.01. Overall only small differences in temperature between each group of test object can be recognized. By comparison of background 1 and larva 1, a maximum mean difference of 0.16 K is been listed (p = 0.041). Residual groups resulted in lower differences ($p < 0.05$). The smallest divergences are given by relating solid wood and larva 1. As a consequence, larvae and background objects could not be imaged or differentiated adequate by thermal measurements.

By consideration of individual frame (point of view) it is recognizable that maximum differences in temperature between larvae and background 1 occurred in relatively high values (0.18 K) a few time after starting measurement. The divergences fell down at night to acquire a minimum level (< 0.05 K) when larvae reduced movement. The measurement was largely impossible, when larvae were hidden behind backgrounds or totally stopped motion. The highest variances were given during the next day (0.35 K) after 15 hours measurement.

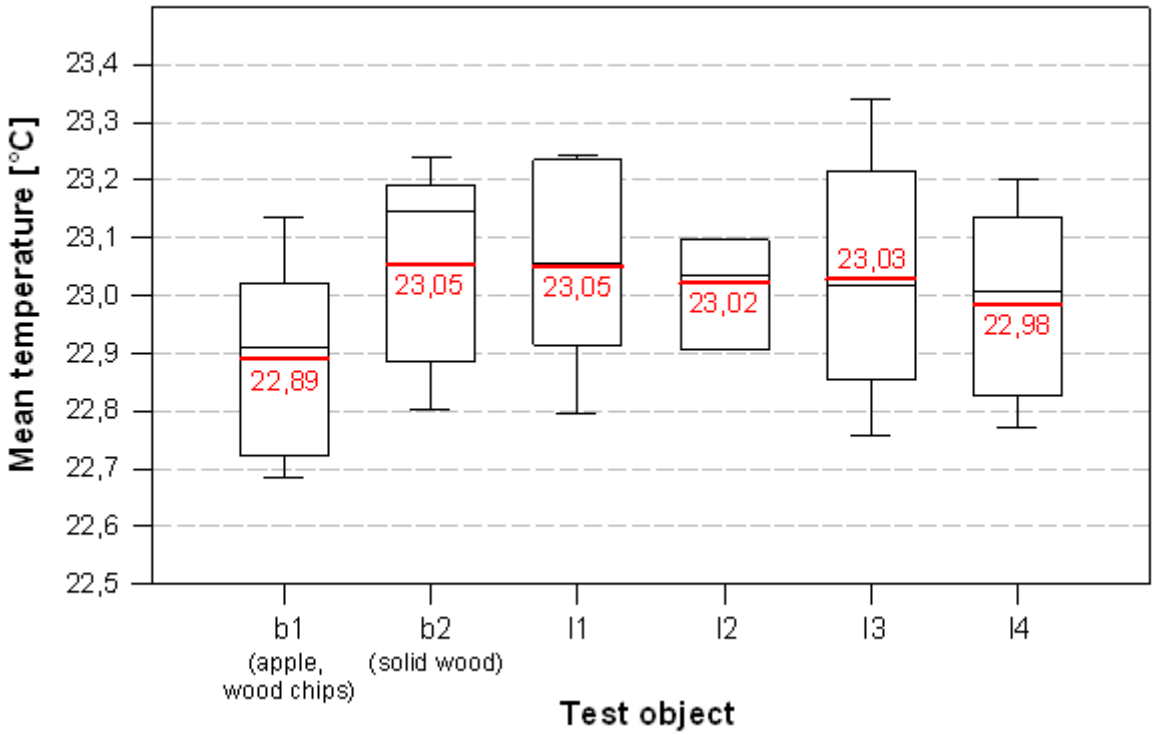


Fig. 29: Mean temperature of test objects (b1 = background 1, b2 = background 2, l1 = larva 1 [100 mm], l2 = larva 2 [70 mm], l3 = larva 3 [50 mm], l4 = larva 4 [30 mm]) at long-term measurement 1; averaged by determination lines.

Long-term measurement 2

Inserted goat moth larvae could not be visualized by thermal dual-band equipment inside of solid wood samples at 24 hours recording. Thermograms displayed no measurable temperature changes in marked areas, where larvae had been positioned. Over the whole samples, so including larval motion along total borehole regions, no increases in temperature could be recognized.

Additionally no differences in density, affected by abnormalities between solid wood and borehole or solid wood and bore dust, could be detected in thermograms.

As illustrated in Fig. **30**, a rapid reduction in temperature at edge regions, displayed in blue ranged areas in thermogram, could be identified. Caused by water less at front plane induced by evaporation, regions cooled down unleashing decreases of temperature (Fig. **30**, arrow 1). Due to adequate differences in temperature, anterior parts of moving larva with bore dust around were successfully identified outside of specimen by thermal imaging. However, back segments, hidden in wood specimen, were not recognizable (Fig. **30**, arrow 2).

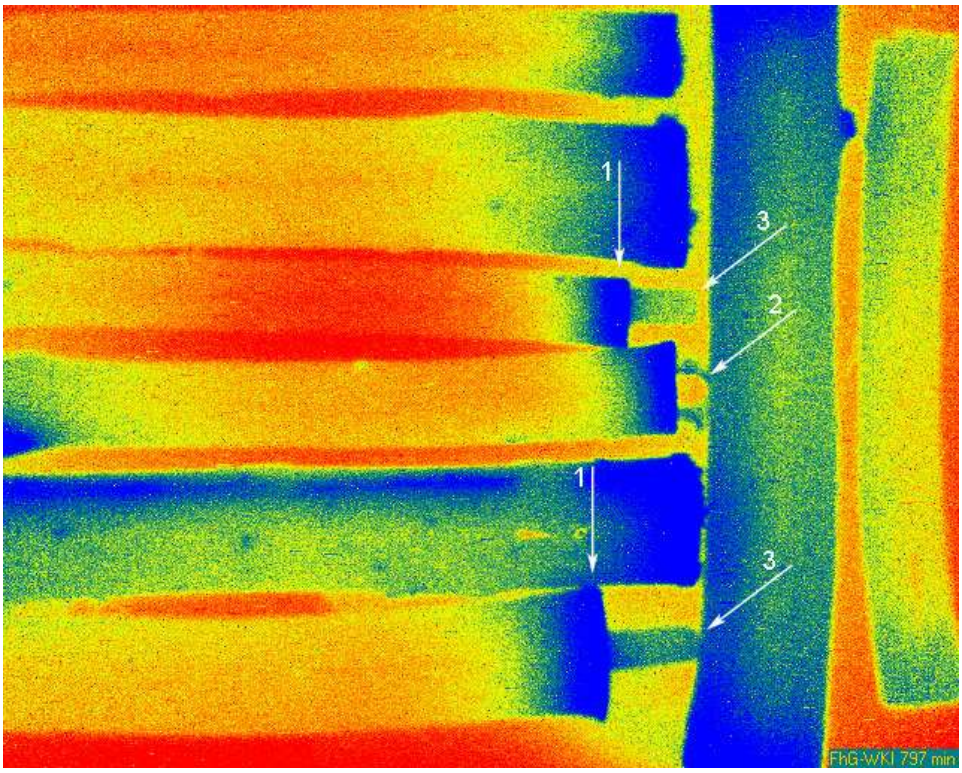


Fig. 30: Thermagram of evaluated frame of long-term measurement 2. Solid wood samples (*Acer platanoides*, *Salix caprea*) with axial boreholes in center and inserted goat moth larvae (*Cossus cossus*) in each specimen. 1: darker regions resulted from cooling processes induced by evaporation at front plane. 2: “head” of a moving larva with bore dust around. A back segment inside of wood was not recognizable. 3: Plugs for preventing larval escape (not to be confused with larvae).

Mean temperature profiles in center regions of specimen samples could not reveal temperature changes that would be initiated by body emissions of *Cossus cossus* larvae or given boreholes and -dust. Overall similar, homogenous trends of temperature and no abnormalities could be detected. Regardless of whether sample or frame (point in time) has been observed, the maximum differences in temperature remained always below 0.5 K (in most cases ranged between 0.2 – 0.3 K) at the particular specimen. The trend of temperature along the wooden axles is displayed by using two examples of frames in Fig. 31 . To minimize falsifications in data analyses caused by evaporative cooling, last pixels close by front planes had to be removed from investigation.

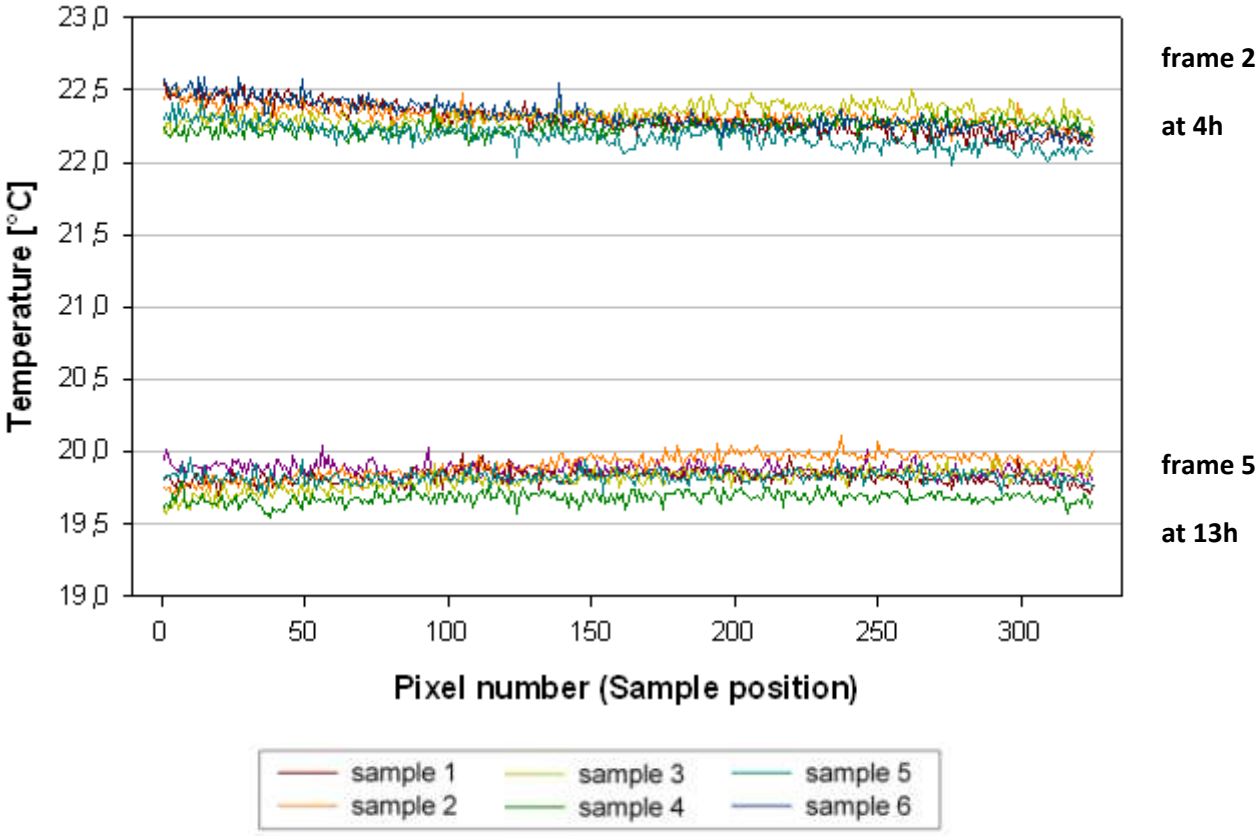


Fig. 31: Axial temperature profiles along the center of wood samples 1- 6 with boreholes and inserted *Cossus cossus* larvae at 24 hours long-term measurement 2. Two examples are represented: Frame number 2 (after 239 min; at daytime) and frame number 5 (after 797 min; at night). No measureable effect of temperature change induced by larvae, boreholes or bore dust was verified.

As illustrated in Table **25**, arithmetic averaging of data per sample resulted in means between 21.059 °C (sample 5) to 21.224 °C (sample 3). Standard deviations resided below 0.070 °C for each specimen.

Single frames also eventuated in relatively homogeneous values without remarkable variances between samples. It is notable that the measurement started with highest absolute temperatures to change into minimum at night (frame 5 and 6). Additionally even larvae outside of specimen (control) showed marginal variances compared to samples (Table **25**).

Table 25: Mean temperature of test objects per frame at long-term measurement 2. Sample 1 – 6: Solid wood samples (*Salix alba* and *Acer platanoides*) with borehole (10 mm in diameter) and *Cossus cossus* larva inside. Larva 1 (80 – 90 mm length) and larva 2 (60 - 70 mm length): Control - *Cossus cossus* larva outside of sample, when visible.

	test object							
	sample 1	**sample 2**	**sample 3**	**sample 4**	**sample 5**	**sample 6**	**larva 1**	**larva 2**
frame 1 (116 min)	22.946	23.046	23.027	22.954	22.913	23.058	22.909	n.a.
frame 2 (239 min)	22.297	22.317	22.331	22.241	22.180	22.322	22.256	n.a.
frame 3 (330 min)	21.581	21.646	21.705	21.596	21.520	21.665	21.723	n.a.
frame 4 (524 min)	20.147	20.193	20.307	20.197	20.097	20.205	n.a.	20.277
frame 5 (797 min)	19.876	19.824	19.907	19.805	19.682	19.826	n.a.	n.a.
frame 6 (822 min)	19.965	19.921	19.988	19.904	19.762	19.907	n.a.	n.a.
frame 7 (1232 min)	21.053	20.992	20.982	20.929	20.863	20.998	21.047	20.827
frame 8 (1349 min)	21.670	21.606	21.548	21.513	21.457	21.625	21.631	n.a.
Total (1440 min)	*21.192*	*21.193*	*21.224*	*21.143*	*21.059*	*21.201*	*21.901*	*20.392*

Radar

Results confirmed that inserted *Cossus cossus* larvae could be detected in wood samples and young trees in a high ratio using microwave radar. Larval motion induced steep increases of activity signals above threshold level and could be determined in an automated way / mode under laboratory conditions. Radargrams and –images as well as larval activity curves were appropriate to differentiate between presence and absence of larvae in wood, given the strict premise of minimal larval movement. Thus, with respect to larvae detection, reiterated measurements resulted in 19 false negative assessments, but 169 test objects were true analyzed. In addition the position of larvae inside the wooden axles could be located approximately by two-channel-locating. A higher number of interfering objects exacerbated larval detectability distinctly. Additional, radar measurements at root zones on young trees seemed to be more difficult.

However, radar principle was not appropriate for identifying boreholes and larval frass in wooden samples or young trees.

LARVAE DETECTION

In general, all diameter classes of *Cossus cossus* larvae could be detected using radar principle and sensitivity of assays was adequate to identify small larvae (3 mm in diameter) in a distance to antennas of 10 – 15 cm. No correlation between detection accuracy and larval size and, therefore, borehole diameter could be found and receivers revealed minimal movement. Nevertheless, the minimal larval motion was required for a true detection and for activating threshold level in radargrams and activity curves.

The total number of true and false assessments of all measurement series for larvae detection is illustrated in Table **26**.

Table 26: Number of true positives (TP, larva and signal), true negatives (TN, no larva and no signal), false positives (FP, no larva but signal) and false negatives (FN, larva but no signal) for larvae detection as a function of testing object, type of measurement and repetition

Testing object	Type of measurement	Repetition	TP	TN	FP	FN	Total
wood samples	single measurements	day 1	24	57	0	3	84
		day 2	21	0	0	6	27
		day 3	26	0	0	1	27
	long-term measurements	-	2	0	0	0	2
	simultaneous measurements of several samples	-	7	0	0	5	12
young trees	single measurements	-	8	24	0	4	36
Total			**88**	**81**	**0**	**19**	**188**

Wood samples

The wood samples given in this study were appropriate for radar analyses. One measurement per sample reached for analyzing total wooden zones and, additionally, an assessment of spatial position of larva inside specimen could be promoted/confirmed. No relationship between stem diameter class and tracing accuracy of detection/tracing was assignable. A mean wood moisture content of 81.9 % (center) and 74.1 % (edge) was determined.

Single measurements and repetitions

In total, 24 of 84 recordings revealed elevations of activity signals at first single measurements (day 1). Thus, three of 27 recently inserted larvae were not traced and

seemed to be in dormant state. Remaining 57 samples without any larva (boreholes, frass and controls) were not false assessed and approved to be true negative detected.

The second day provided/identified 21 larval activities and six larvae remained undetected at 150 s recordings. An example of movement amplitudes/signals respectively activity curves is shown in Fig. 32 at small *Cossus cossus* larva (3 mm in diameter) inserted in a wood sample of stem diameter class C. The discolorations in motion radar image (above) at channel 2 sign larval motion immediate after starting measurement and at 64, 91 and 148 seconds, but no/less deflections marked at channel 1. Thus, the larva was located on the right side of measurement field of antennas and no movement could be identified in the left area. Additional, according to running time (propagation time) of electromagnetic wave, the distance to moving object, starting from transmitting antenna, could be determined. The moving intensity could be illustrated/analyzed more accurate using separate peak reading of defined detection range (red-framed distance area to be analyzed), graphed in normalized scale (below). Hence, larval activity curves exceed threshold level of 0.3625 several times as a function of motion intensity.

On day 3 single measurements offered best results at 27 fitted samples concerning larval movement and 26 larvae were true positive detected in total (Table 26).

Long-term measurements

Two critical individuals offering no movement at single measurement on day 2 were controlled at repeated recordings extended to 600 seconds. The extensions and concomitant positive detections could confirm that radar sensitivity reached for analyzes and detectability depended on actual motion state of larvae (Table 26).

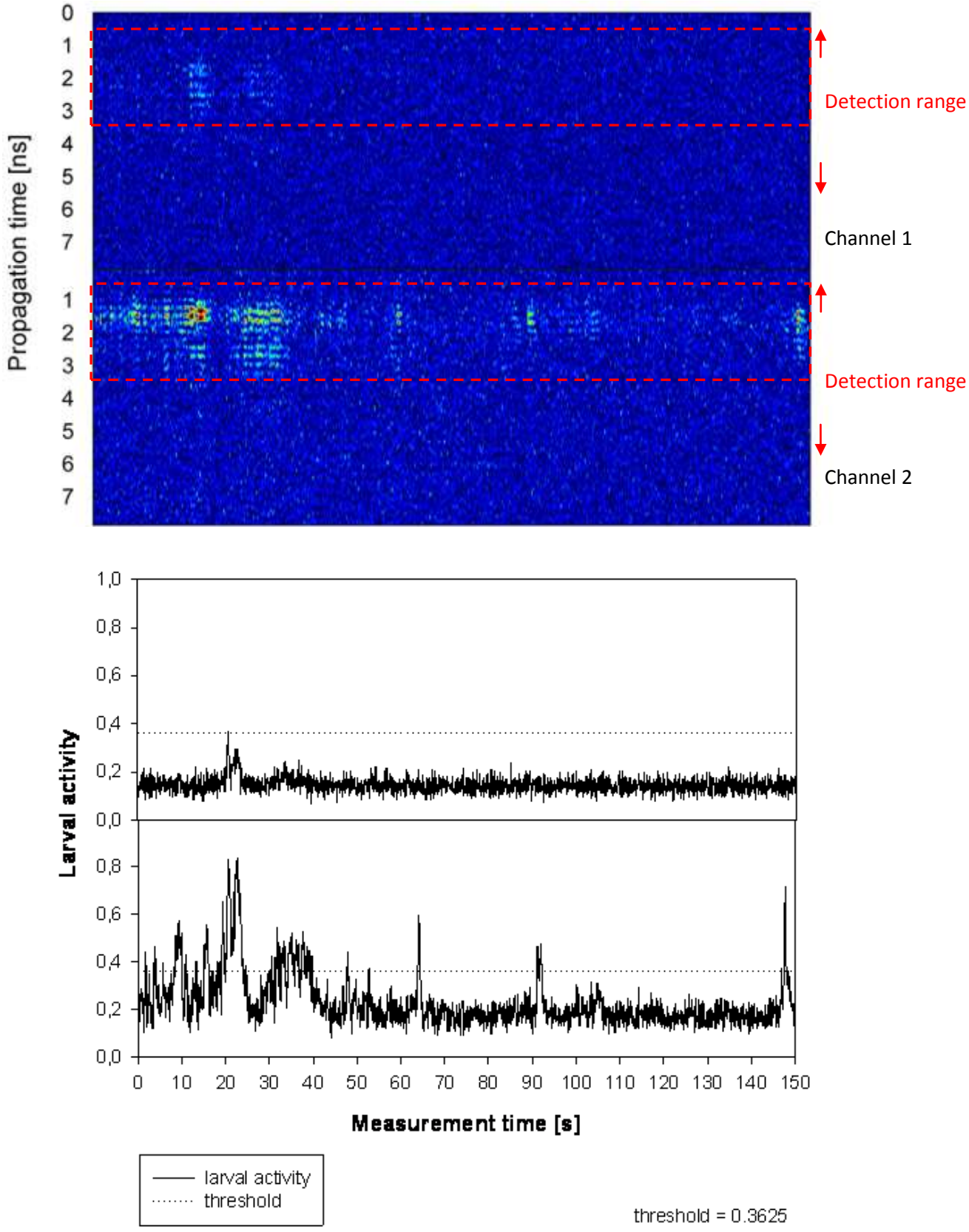


Fig. 32: Motion radar image (above) and larval activity (below) at single measurement of wood sample 57-C3L of *Acer pseudoplatanus* L. (stem diameter class: C; borehole and larval diameter: 3 mm). Channel 1: no larval motion and detection; channel 2: larval motion and detection at several observation points.

Channel 1

Simultaneous measurements of several samples

The addition of disturbing objects to specimen fitted with larva aggravated detectability as a function of number of samples. Due to adding of boundaries through mediums with different permittivity (air to wood resp. wood to air) with a higher number of object surfaces, values of refraction (changes in direction of an electromagnetic wave due to changes in its velocity of propagation) increased and signals had been scattered. Thus, larva could be identified by measuring one and five samples simultaneously at three repetitions. Higher amount of specimens (10 and 15), except one measurement, resulted in false negative assessments (Table **27**).

Table 27: Number of true positives (TP, larva detected) and false negatives (FN, larva not detected) for larvae detection as a function of additional samples at simultaneous measurement. First sample is fitted with one active larva (control).

number of samples per measurement	TP	FN
1 (Control)	3	0
5	3	0
10	0	3
15	1	2

Young trees

Multiple measurements are required for evaluation of young tree samples, according to higher length of trees. In total, 36 recordings (ever one measurement at upper and lower part of tree) at 18 young tree samples resulted in 32 true and four false evaluations. Similar to measurements of wood samples, boreholes and larval frass were all true negative assessed. However, according to larva detection, four of 12 larvae could not be identified in root zones (Table **28**). The measurements had to be arranged in a higher distance between object surface and receiver/antennas (approximately 20 to 25 cm) due to bulky offshoots of the roots. Furthermore, primary roots offered dimensions above 5 cm in diameter. As a consequence the sensitivity of detectabilty decreased and signal attenuations were induced.

Table 28: Number of true positives (TP, larva detected) and false negatives (FN, larva not detected) for larvae detection as a function of position of measurement and larva at young tree

position of larval measurement at young tree	TP	FN
upper stem zone	6	0
lower root zone	2	4

Electrical resistivity tomography (ERT)

The given ERT system was appropriate for detailed imaging of resistivity distribution of separate cross sections of wood samples. In total all recorded tomograms displayed reduced resistivity in outer zones close to the bark compared to inner regions. A detection of boreholes was possible as a distinct function of borehole size. However, the directly identification of *Cossus cossus* larvae seems to be more difficult and an adequate differentiation to borehole could not be achieved. In general, the evaluation was restricted to relatively comparison of (both) tomograms within separate samples due to differences of resistivity between untreated (MP 1) and treated (MP 3) wooden zones. Hence, thresholds in resistivity could not be given, but mean differences within separate samples offered distinct tendency and correlation between the detectability and borehole diameter size.

BOREHOLE DETECTION

24 electrode measurement

Boreholes could be detected frequently as circular zones with high resistivity in proximity to the stem centre at measuring plane 3, but often displayed deviant in pattern with respect to borehole size, shape or position. However, 47 samples (56%) could be indicated "true assessed" by directly comparison of treated and untreated areas of separate sample and adjustment of ohm scale. In contrast, left over 37 samples (44%) shows inadequate differences in borehole zones or untreated measuring planes increase resistivity.

The correctness of identification was not affected by stem diameter or borehole position, but detection accuracy increases with ascending borehole diameter. In total, 92% (95%)* of 10 mm, 69% of 5 mm and 14% of 2 mm boreholes were detected successfully (Table 29).

Table 29: Detection accuracy (number of true and false assessments) of ERT at *24 electrode* measurement for borehole detection as a function of borehole diameter. Comparison of untreated (MP 1) and treated (MP 3) areas of separate sample and adjustment of resistivity scale. n = 84 (99)*

	True Assessment (Borehole detected)		**False Assessment (Borehole undetected or confused with other tissue)**	
Borehole diameter class [mm]	**1** (differences in resistivity sufficient)[1]	**2** (differences in resistivity low but sufficient; dissenting in hole-size, -position and/or -pattern)[2]	**3** (differences in resistivity inadequate; no clear distinction between hole or other tissue)[3]	**4** (differences in resistivity inadequate)[4]
2	**-**	**4**	**12**	**14**
5	**12**	**8**	**5**	**4**
10	**21 (33)***	**2 (5)***	**-**	**2**

[1] Example: Fig. 33; [2] Example: Fig. 34; [3] Example: Fig. 35; [4] Example: Fig. 36

* inclusive 15 samples for larvae detection, comparison of borehole zone without larvae (MP 3) and untreated area (MP 1)

10 mm boreholes could be detected successfully using ERT and apart from two samples, all treated areas were displayed as regions with higher resistivity in tomogram (Fig. 33). Predominant similar pattern in size, shape and spatial position of boreholes could be offered and only two samples differed when comparison with cross section.

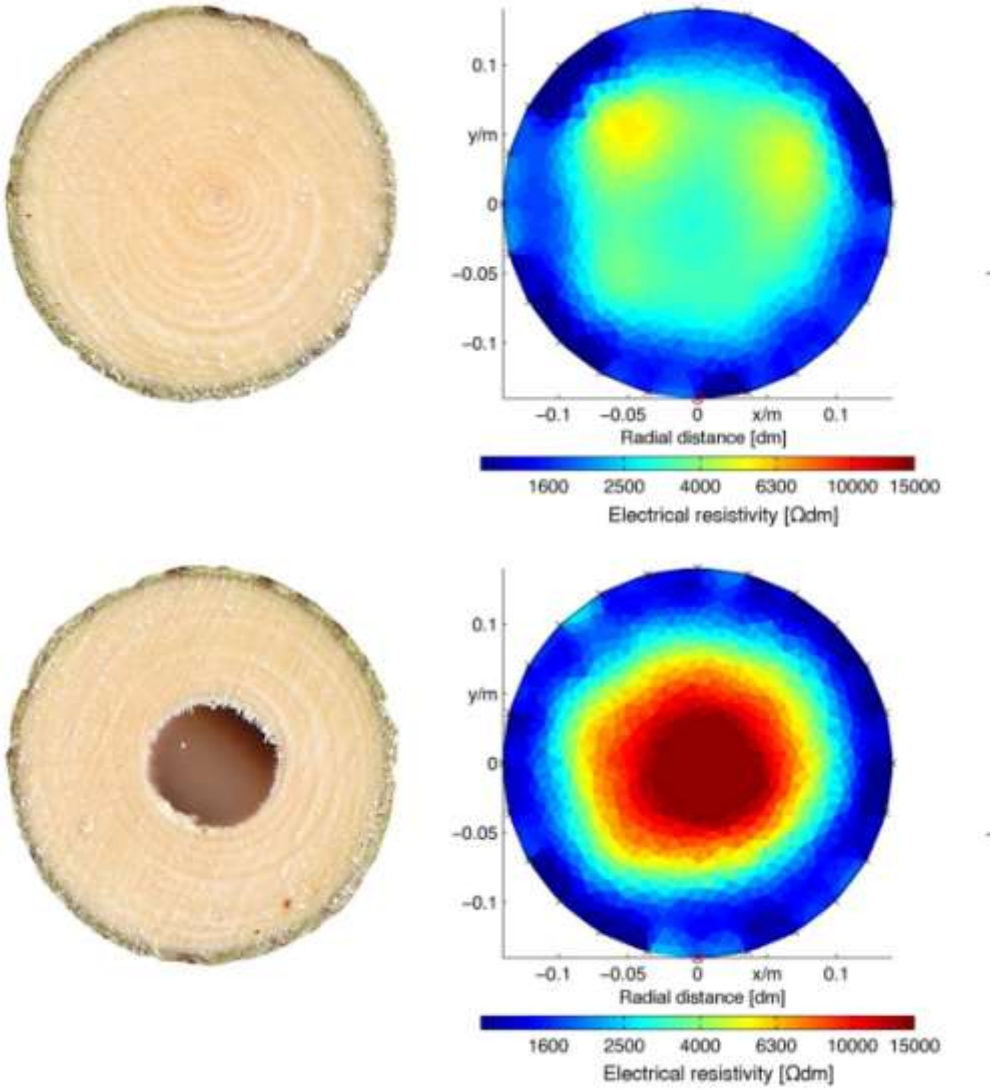


Fig. 33: Cross section and tomogram of sample 51-B10c of *Acer pseudoplatanus* (stem diameter: 29 mm, borehole diameter: 10 mm, borehole position: center). Above: Measurement plane 1 (MP 1) without borehole (control), below: Measurement plane 3 (MP 3) with borehole. True assessment 1 = borehole detected and accurate imaging.

Tomograms with **5 mm** boreholes in diameter showed several deviations in color, but 2/3 of boreholes could be identified at least. Although the drilled zones were often inhomogeneous and differed in size and circular shape to cross section, distinct increases and deviations of resistivity at borehole zones were displayed in tomogram at 20 samples (Fig. 34). However, no sufficient distinction between borehole and other wooden tissue could be determined at remaining 9 specimens.

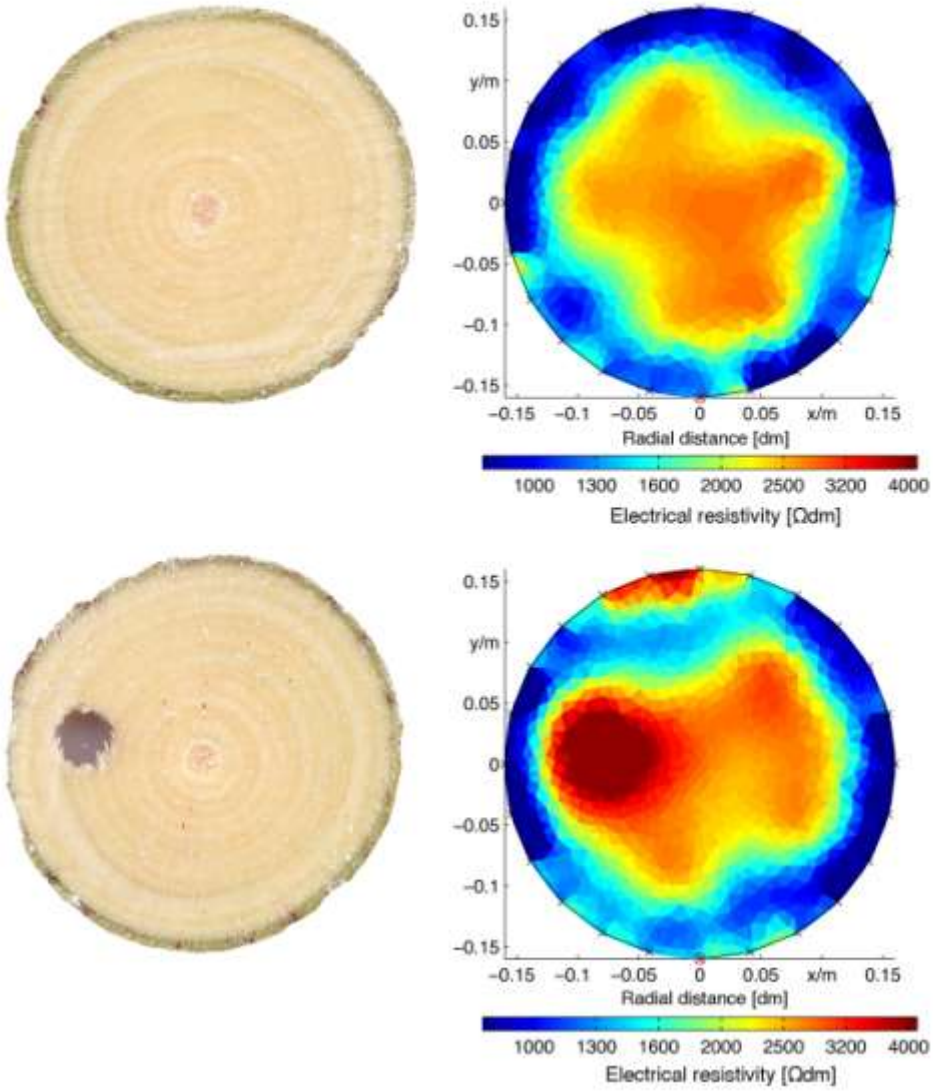


Fig. 34: Cross section and tomogram of sample 71-C5e of *Acer pseudoplatanus* (stem diameter: 32 mm, borehole diameter: 5 mm, borehole position: edge). Above: Measurement plane 1 (MP 1) without borehole (control), "false zones" with little increased resistivity; below: Measurement plane 3 with borehole, divergences in borehole shape and size. True assessment 2 = borehole detected. Differences in resistivity sufficient, but divergences in pattern (borehole shape and size).

Crucial results were obtained at **2 mm** boreholes and most analyzes do not offer drilled zones using ERT. In many cases tomograms displayed inhomogeneous and not assignable zones with higher resistivity at healthy cross sections and no clear differentiation between untreated (MP 1) and treated (MP 3) areas could be realized (Fig. 35). Further pattern indicated higher number of "false boreholes" at control plane and resistivity values actually exceeded those of borehole zones (Fig. 36). Merely 4 samples have been assessed successfully.

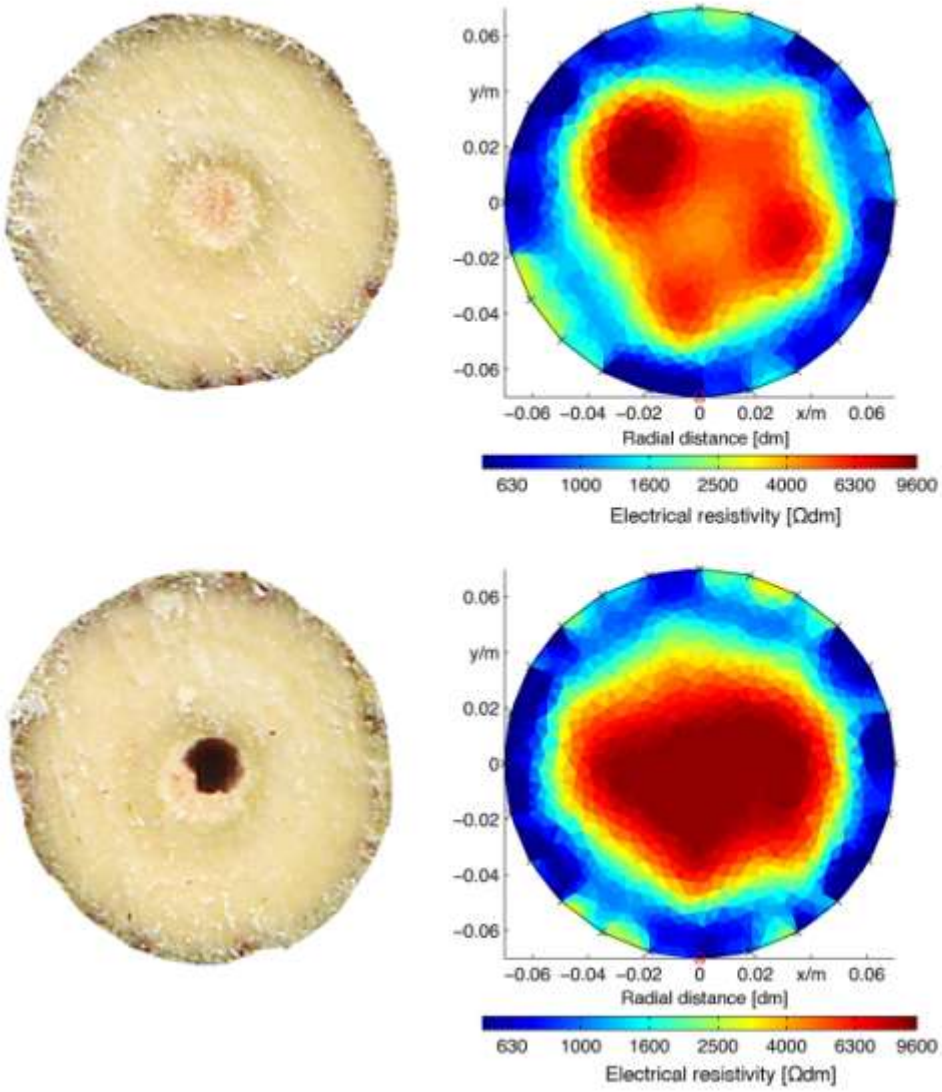


Fig. 35: Cross section and tomogram of sample 4-A2c of *Acer pseudoplatanus* (stem diameter: 14 mm, borehole diameter: 2 mm, borehole position: center). Above: Measurement plane 1 (MP 1) without borehole (control), "false borehole" with high resistivity; below: Measurement plane 3 (MP 3) with borehole, divergences in borehole shape and size. False assessment 3 = borehole not detected, differences in color inadequate.

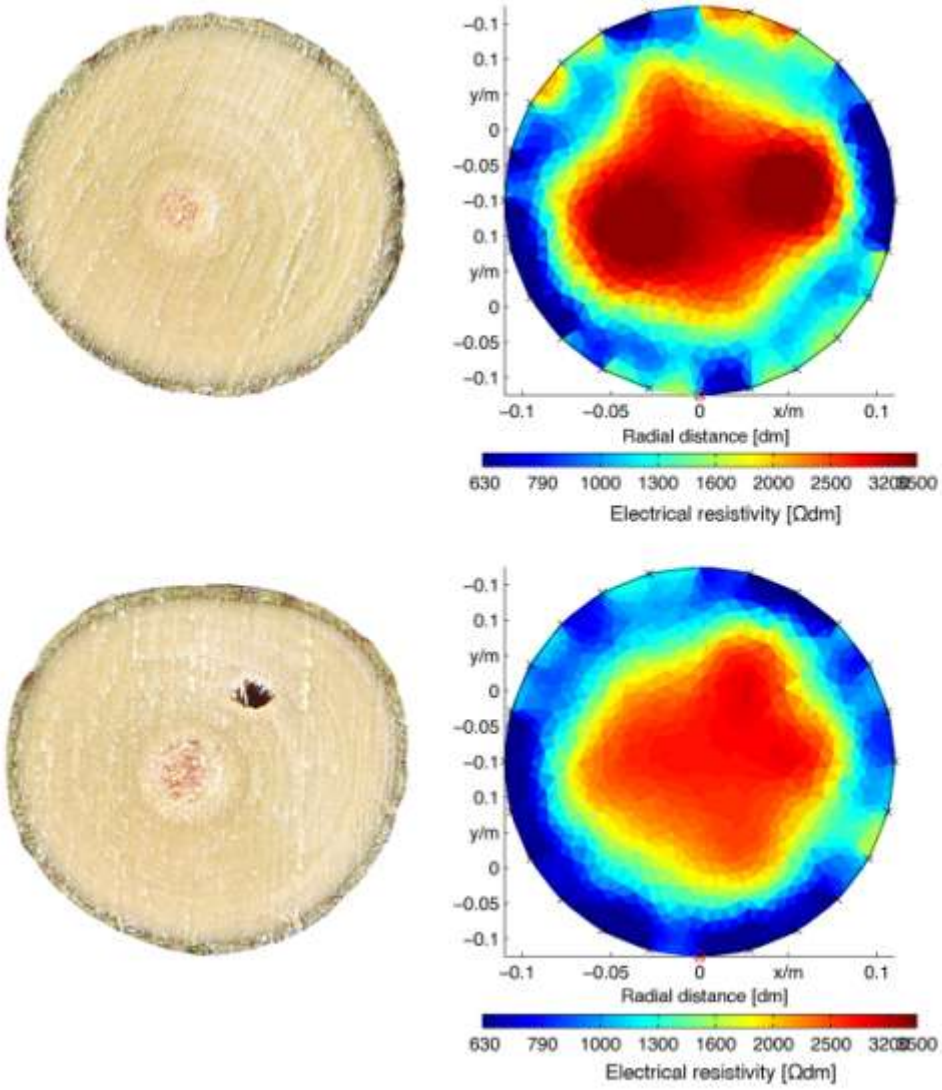


Fig. 36: Cross section and tomogram of sample 32-B2e of *Acer pseudoplatanus* (stem diameter: 22 mm, borehole diameter: 2 mm, borehole position: edge). Above: Measurement plane 1 (MP 1) without borehole (control), "false boreholes" with higher resistivity; below: Measurement plane 3 (MP 3) with borehole, divergences in borehole shape and size. False assessment 4 = borehole not detected and higher resistivity at untreated tissue.

12 electrode measurement

In summary, results of borehole detection using 12 electrodes were comparable to 24 electrode measurement. Strict correlation could be observed between borehole diameter class and detection accuracy. Thus, 10 mm boreholes could be identified in tomogram successfully and were represented in zones with higher resistivity values. However, 5 mm and particularly 2 mm boreholes tomograms revealed no clear differentiation between borehole (MP 3) and other regions (MP 1) regarding to resistivity values (Table 30). With respect to stem diameter and borehole position, no dependency concerning detection accuracy or borehole locating could be observed.

Table 30: Detection accuracy (number of true and false assessments) of ERT at *12 electrode* measurement for borehole detection as a function of borehole diameter. Comparison of untreated (MP 1) and treated (MP 3) areas of separate sample and adjustment of resistivity scale. n = 16

	True Assessment (Borehole detected)		**False Assessment (Borehole undetected or confused with other tissue)**	
Borehole diameter class [mm]	**1** (differences in resistivity sufficient)[1]	**2** (differences in resistivity low but sufficient; dissenting in hole-size, -position and/or -pattern)[2]	**3** (differences in resistivity inadequate; no clear distinction between hole or other tissue)[3]	**4** (differences in resistivity inadequate)[4]
2	**-**	**2**	**1**	**3**
5	**-**	**2**	**1**	**2**
10	**4**	**1**	**-**	**-**

Compared to 24 electrode measurements, ohm values decreased in total when using 12 electrodes. However, the number of electrodes would not indicate significantly changes in detectability, but detection accuracy for assessment of borehole pattern or size increased in some cases (**Fig. 37).** In summary boreholes were displayed in higher difference at four samples (3x 2 mm, 1x 10 mm) at 12-point-measurement, but four other specimen (1x 2 mm, 2x 5 mm, 1x 10 mm) differ in higher contrast using 24 electrodes as well (Fig. 38).

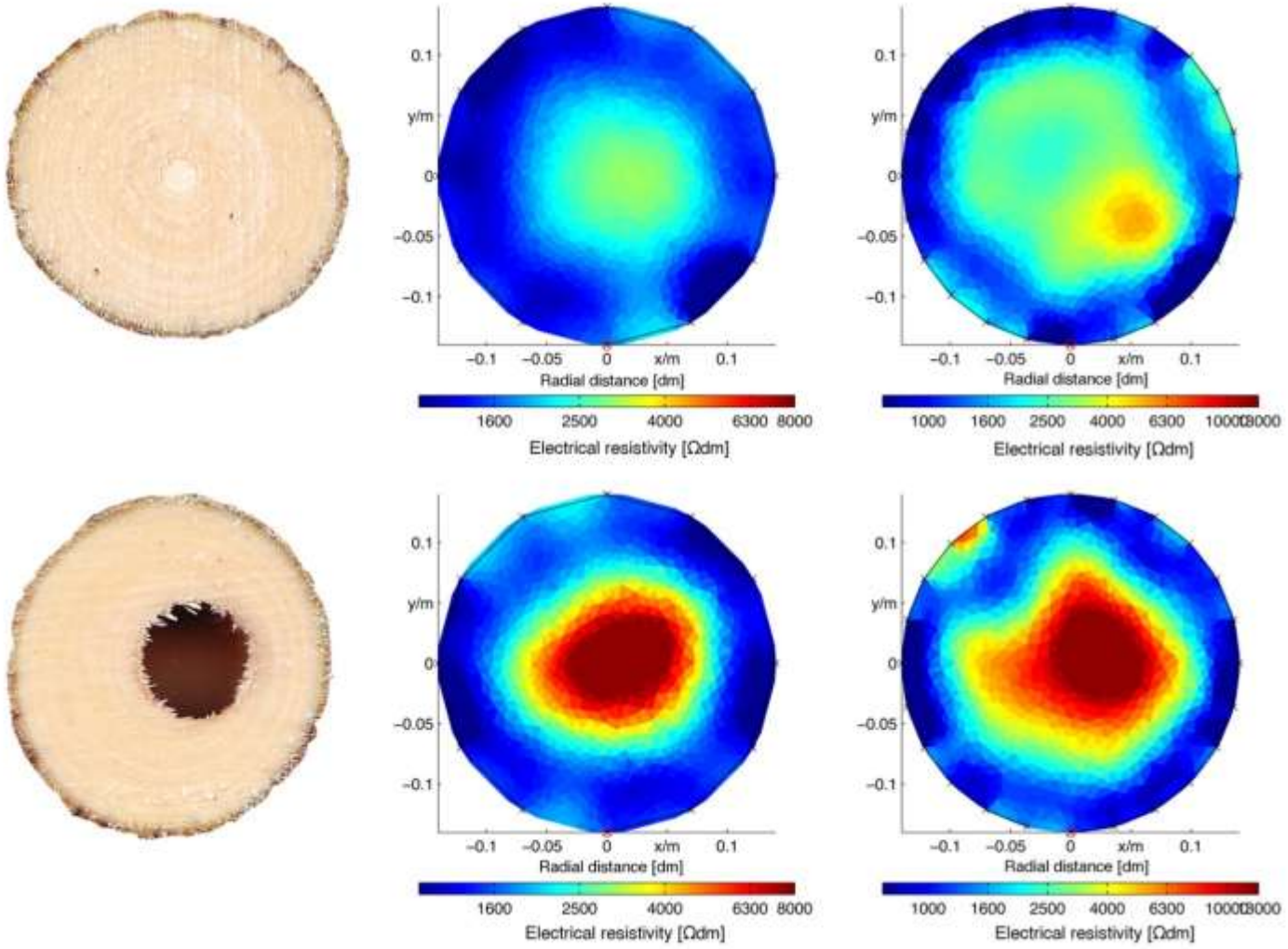


Fig. 37: Cross section (left) and tomograms using 12 electrodes (middle) and 24 electrodes (right) of sample 46-B10c of *Acer pseudoplatanus* (stem diameter: 28 mm, borehole diameter: 10 mm, borehole position: centre). Above: Measurement plane 1 (MP 1) without borehole (control); below: Measurement plane 3 (MP 3) with borehole. 1 = Correct assessment and borehole detected

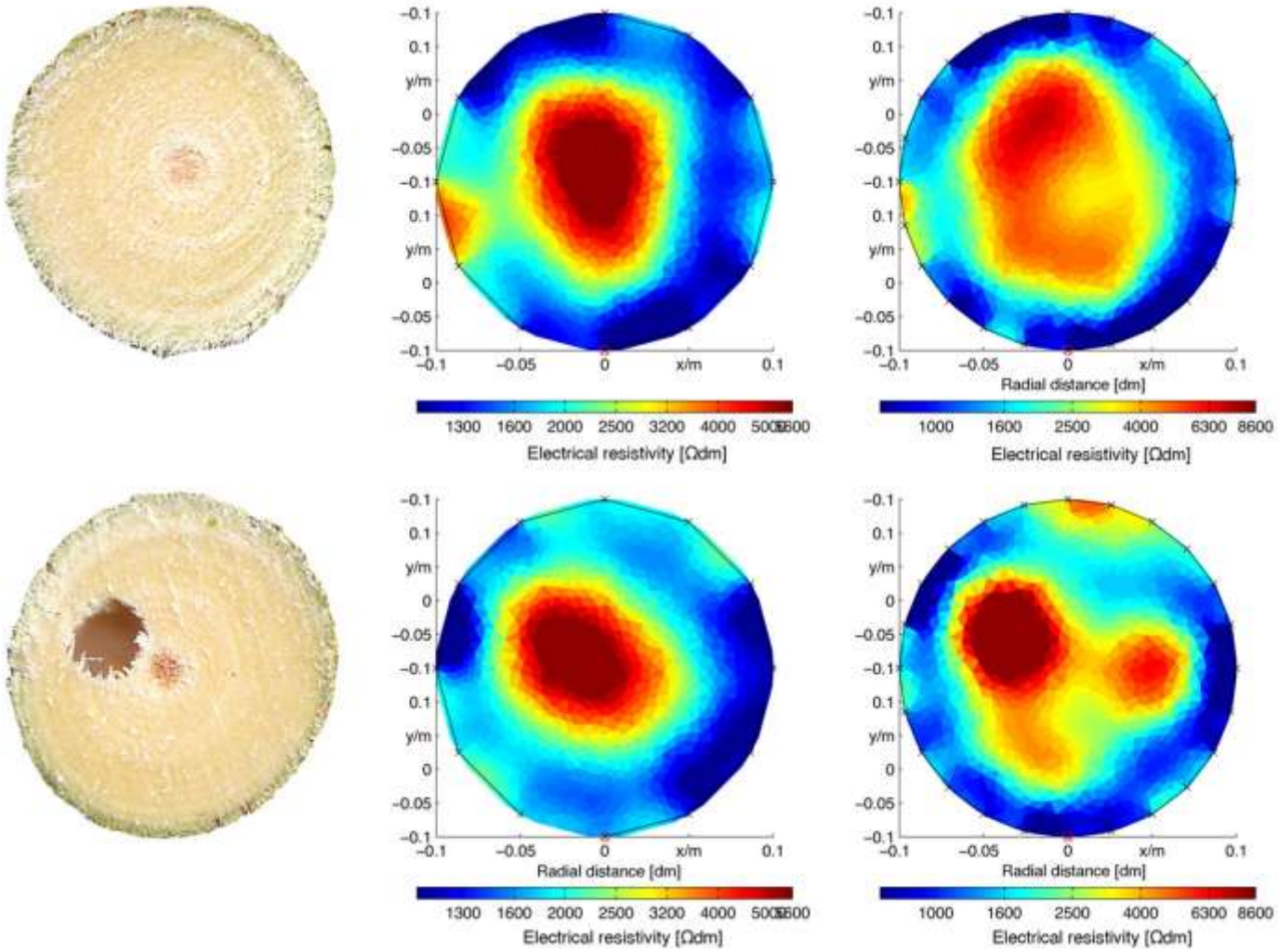


Fig. 38: Cross section (left) and tomograms using 12 electrodes (middle) and 24 electrodes (right) of sample 16-A5e of *Acer pseudoplatanus* (stem diameter: 20 mm, borehole diameter: 5 mm, borehole position: edge). Above: Measurement plane 1 (control, MP 1) without borehole (control); below: Measurement plane 3 (MP 3) with borehole.

LARVAE DETECTION

The presence of larvae could not be determined sufficiently in boreholes using 24 electrodes for ERT analyzes. Regardless of whether stem diameter class had been analyzed, differences in resistivity between borehole and occupied borehole were very low. Nevertheless, in all tomograms, presence of larvae induced little but homogeneous reduction in resistivity values (Fig 39).

However, by comparison of MP 1 and MP 3, boreholes, occupied as well as free of larvae, could be differentiated successfully according to intense increases of resistivity. In total the divergences of unloaded cavities exceeded those of larval inserted boreholes (Fig 39).

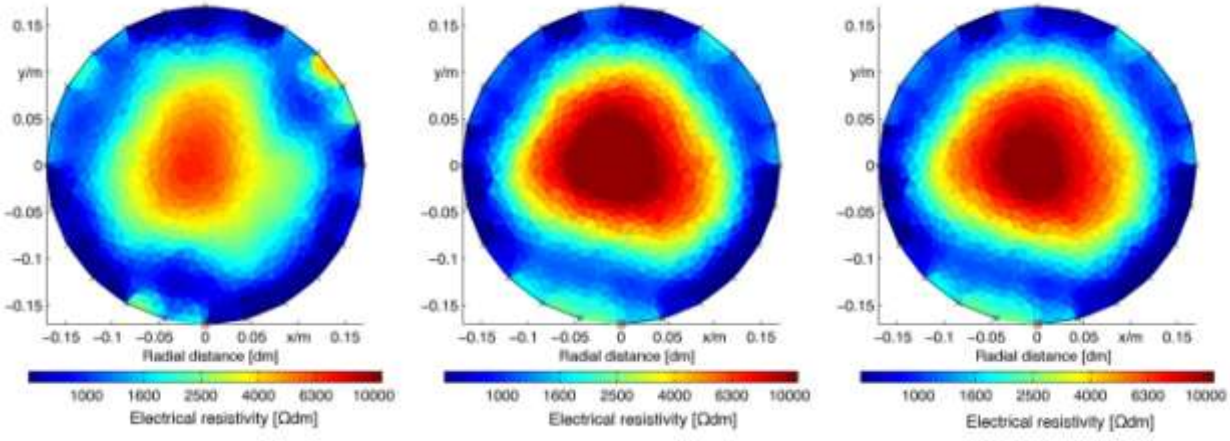


Fig. 39: Tomograms of sample 22II-C10c of Acer pseudoplatanus (stem diameter: 34 mm, borehole diameter: 10 mm, borehole position: centre). Left: Measuring plane 1 (MP 1) without borehole; middle: Measuring plane 3 (MP 3) with borehole; right: Measuring plane 3 (MP 3) with borehole and inserted larva.

Ultrasonic

Ultrasonic measurements could moderately identify/reveal prepared boreholes with a high dependence of the stem and borehole diameter under laboratory conditions. Hence, merely slight differences in ultrasonic travel time (and velocity of propagation) and wave amplitudes between treated and untreated wood areas could be (mainly) observed at large ratios of borehole / stem diameter of ca. < 0.2 (Table **31**). Thin stem Vacancies with 10 mm in diameter could be detected successfully at three specimens with outer stem diameter of < 20 mm. Wood samples containing 5 mm boreholes could be positive/true analyzed at 18.5 mm diameter specimen, but the larger stem size (26.5 mm in diameter) was more crucial to identify / difficult to assess. Little borehole diameter (2 mm) resulted in false assessments and vacancy could not be displayed in ultrasonic image (B- or C-scan). Due to ultrasonic wave length resp. frequency, thin sample diameter could not be transmitted sufficiently and ultrasonic waves were propagated on the surface of specimen. The identification of borehole position could not be considered.

In view of ultrasonic techniques (coupled and air-coupled) chosen in this study, results largely resembled each other concerning detectability and detection accuracy,

but vacancies of three samples could not be recognized clearly using air-coupled transducers (Table **31**). However, non-contact ultrasound benefited that larger areas could be analyzed in an automated and time-saving way. Furthermore, the assessment of approximate location of boreholes could be determined in C-scans.

Inserted *Cossus cossus* larvae caused no noticeable attenuation in air-coupled wave amplitudes and could not be distinguished from intact/untreated wooden zones. Compared to borehole zones merely little differences at one sample could be determined/observed. The plugged larval frass resulted in comparable C-scans of boreholes.

Table 31: Detectability of boreholes as a function of stem and borehole diameter, ratio of borehole/stem diameter and ultrasonic method

	parameter			ultrasonic method	
wood sample	**stem diameter [mm]**	**borehole diameter [mm]**	**ratio borehole/stem diameter**	**coupled ultrasonic**	**non-contact ultrasonic**
38 II	C (36.5)	10	0.27	detected	low detected
59 III	B (21)	10	0.48	detected	detected
60 II	C (31)	10	0.32	detected	detected
60 VII	A (18.5)	5	0.27	detected	detected
60 III	B (26.5)	5	0.19	low detected	not detected
38 V	A (16.5)	2	0.12	low detected	not detected

COUPLED ULTRASONIC

Borehole detection

Boreholes could be detected by optical comparison of travel-times of treated and untreated zones in edited B-scans using coupled transducers. Thus, borehole areas indicated increases of times-of-flight and could be differentiated from intact wood tissue. In Fig. 41 the ultrasonic travel-time (y - axis) is illustrated as a function of the axial position (x – axis) at sample number 59 III. The vertical dotted line at sample position 130 mm marks the border area of borehole on the left side and control zone on the right side. By comparison of borehole and control areas, increases of travel-times were ascribable for the six samples.

Another way of determination of differences between travel-times was the directly comparison of determined travel-times and velocities of propagation of separate A-scans at borehole and untreated zones. Fig. 40 shows the A-scans of emitted travel pulse (upper), that had to be subtracted from travel pulses at borehole (middle) and control A-scan (below). Furthermore, the velocity of propagation was determined using the known measuring

distance. All differences in travel-time and velocities of propagations are displayed in Table 32.. The measurement uncertainty for determined travel-times was calculated with ± 2 µs.

Table 32: Determined travel-times (t) and velocities of propagation (v) and differences at borehole and untreated zones as a function of stem and borehole diameter using coupled transducers

wood sample	**Stem diameter [mm]**	**borehole diameter [mm]**	**t_1 [µs] borehole**	**t_2 [µs] control**	***difference in t [µs]***	**v_1 [m/s] borehole**	**v_2 [m/s] control**	***difference in v [m/s]***
38 II	C (36.5)	10	110	102	*8*	1217	1659	*442*
38 II*	C (36.5)	-	101*	101	*0*	1738	1825	*87*
59 III	B (21)	10	101	96	*5*	955	1235	*280*
60 II	C (31)	10	105	100	*5*	1192	1476	*284*
60VII	A (18.5)	5	97	94	*3*	1028	1233	*205*
60 III	B (26.5)	5	102	98	*4*	1152	1395	*243*
38 V	A (16.5)	2	94	91	*3*	1100	1375	*275*

* secondary measurement with transmission direction next to the borehole).

Concerning Table 32 differences in travel-times and velocities between regions with and without borehole could be recognized. The control measurement at sample number 59 III with transmission next to the borehole could ascribe significantly lower differences. However, the differences within particular category (transmission with borehole or without borehole) are similar to difference within all samples in total. The significant attribution of travel-times or velocity of propagation to the condition with or without a borehole is aggravated conformably.

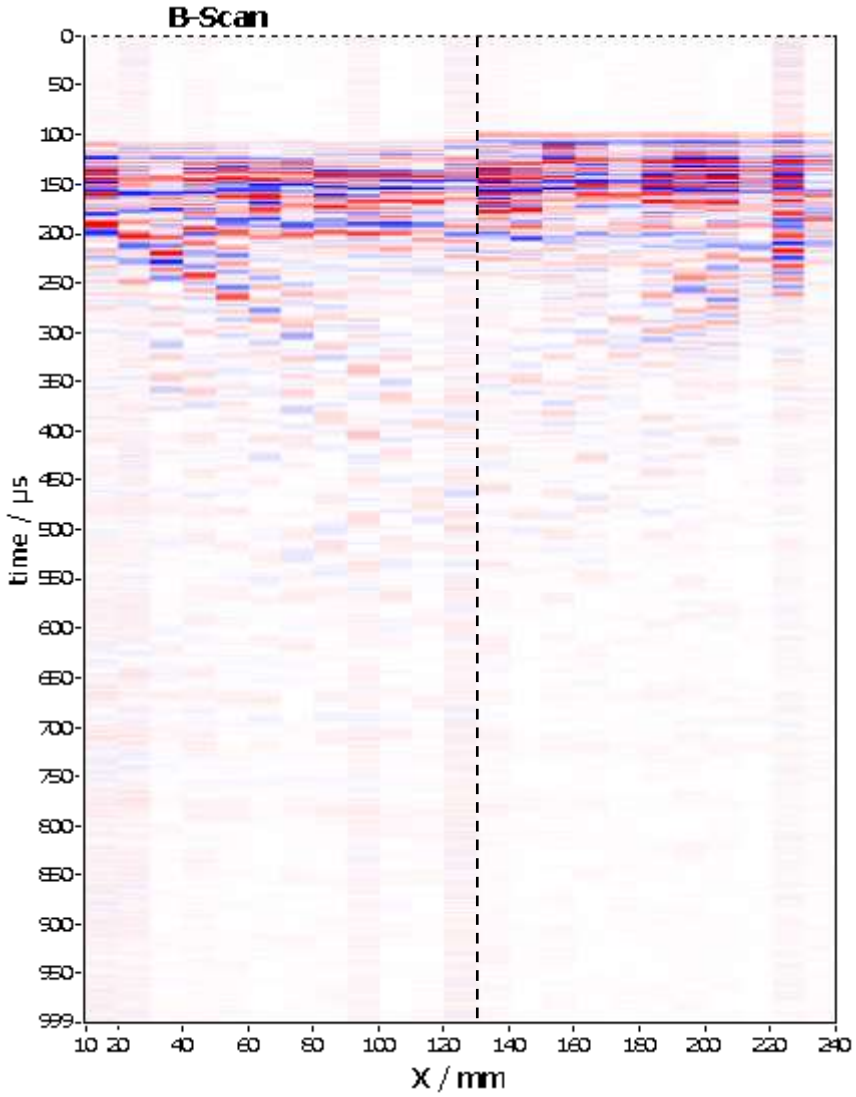


Fig. 41: B-Scan of sample 59 III (Stem diameter: 21 mm, borehole diameter: 10 mm, borehole position: center) using transmission ultrasonic; longitudinal waves at 120 kHz

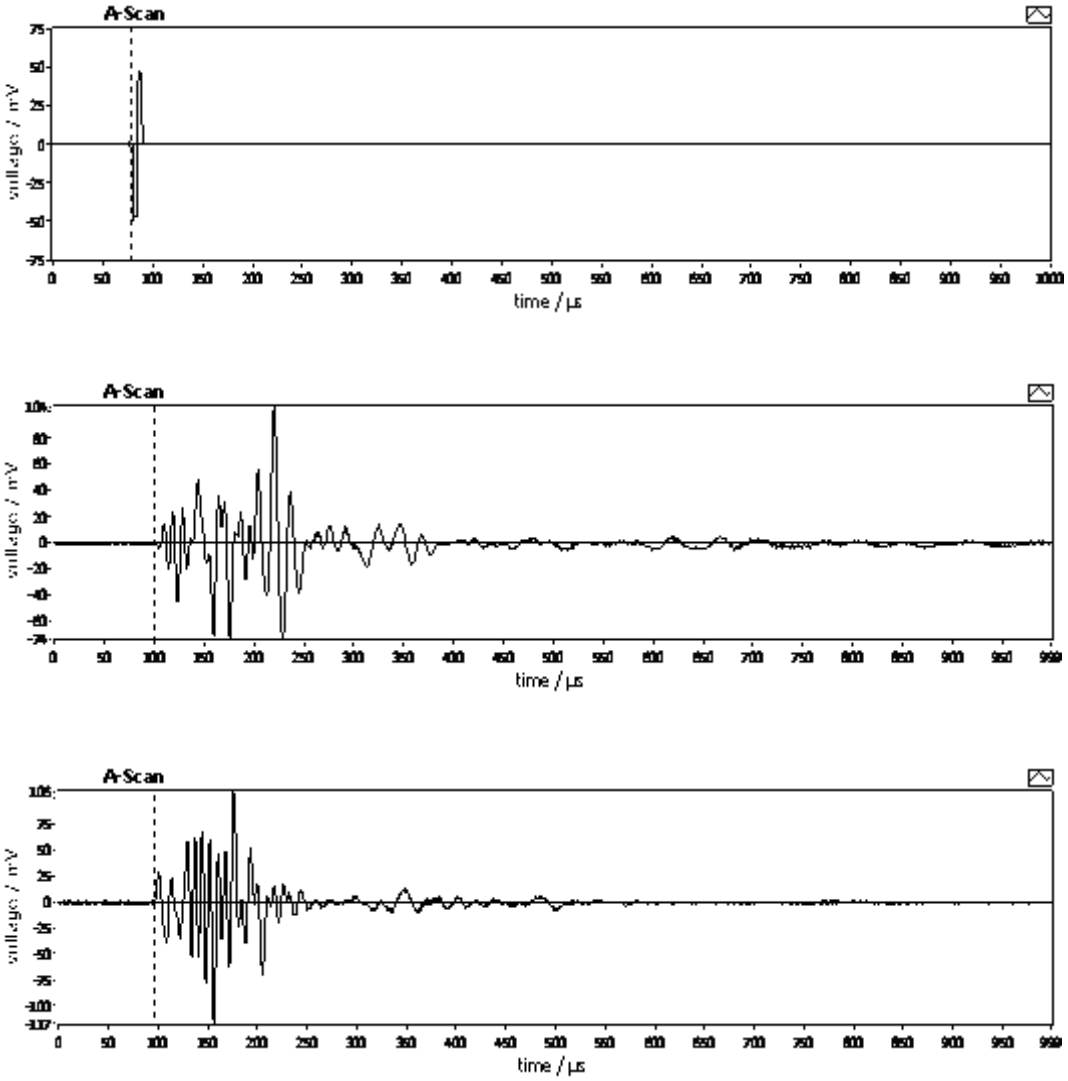


Fig. 40: Separate A-Scans of sample 59 III (Stem diameter: 21 mm, borehole diameter: 10 mm, borehole position: center) using transmission ultrasonic; longitudinal waves at 120 kHz. Upper: emitted travel pulse (= 79 µs); middle: travel pulse at borehole (= 101 µs); below: travel pulse at untreated zone (= 96 µs)

Borehole detection

In general boreholes could be represented in C-scans due to attenuation of ultrasonic wave amplitude. Hence, borehole zones were displayed in darker (percentage of maximum amplitude) or colored (absolute intensity of amplitude in dB) regions and could be distinguished from untreated wood areas (Fig. **42**). The dectability was strictly affected by the ratio of borehole / stem diameter and the applied frequencies of transducers. Thus, an adequate transmission could be realized at stem diameter ≤ 21 mm using 300 kHz and boreholes of 5 and 10 mm in diameter were detected. As a result of high damping of wood structures at large diameter, 200 kHz transducer had to be used at samples with diameter > 21 mm, but only 10 mm boreholes could be identified in C-scans. 2 mm boreholes were not detectable at all.

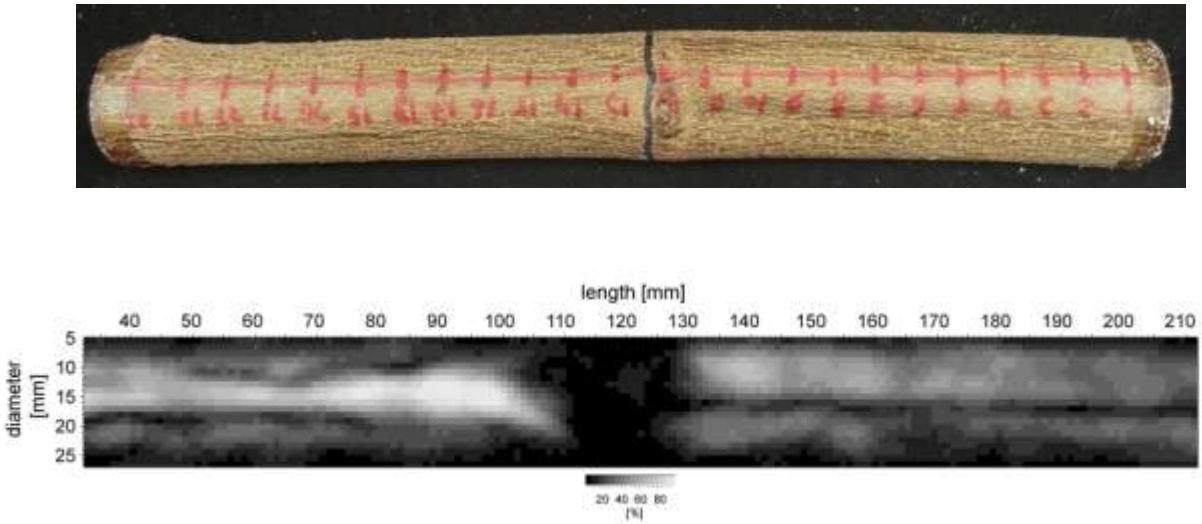


Fig. 42: Color image (above) and gray scaled C-scan (below) of sample number 60 II (stem diameter: 310 mm, borehole diameter: 10 mm, borehole position: center) with borehole on the right side using 200 kHz. Attenuation in wave amplitude [%] at borehole (ca. 130 – 210 mm) and knot (ca. 110 – 130 mm), displayed in darker regions.

All results of non-contact ultrasonic measurements using 200 and 300 kHz transducers are shown in Table **33**.

Table 33: Detectability of boreholes using C-scans as a function of stem and borehole diameter and ultrasonic wave length

wood sample	stem diameter [mm]	borehole diameter [mm]	200 kHz	300 kHz
38 II	C (36.5)	10	detected	no transmission
59 III	B (21)	10	detected	detected
60 II	C (31)	10	detected	no transmission
60 VII	A (18.5)	5	not detected	detected
60 III	B (26.5)	5	not detected	no transmission
38 V	A (16.5)	2	not detected	not detected

Larvae detection

The presence of larvae could not be detected sufficiently using air-coupled transducers. Neither 200 kHz nor 300 kHz frequencies were appropriate for differentiation between borehole and borehole with an inserted larva or with plugged larval frass. Thus, the attenuations in ultrasonic wave amplitudes at all treated zones (borehole, borehole with inserted larva, borehole with plugged bore frass) resembled each other and an imaging of larvae in C-scans could not be realized (Fig. **43**).

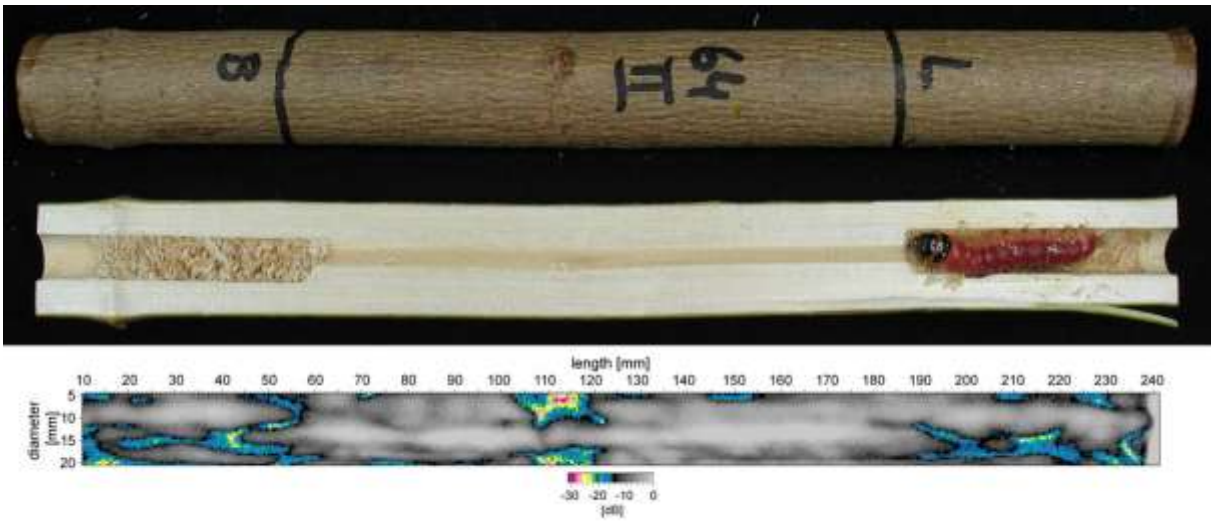


Fig. 43: Color image (above) and color scaled C-scan (below) of sample number 64 II (stem diameter: 250 mm, borehole diameter: 10 mm, borehole position: center) with plugged bore frass (left) and inserted larva (right) in boreholes. Attenuation in wave amplitude [dB] at borehole regions, but no differentiation to bore frass or larva, displayed in colored regions.

Discussion

Computed tomography (CT)

An observer blinded study using a CT scanner Microtec ® CT LOG resulted in a 100% detectability of larval galleries of different sizes, accurate decision on the occurrence of larval frass and larvae when 2D pictures/films were assessed by human assistance. Only in cases where a two millimeter borehole was located in the pith, its detection failed in 40% of the tested samples. As under practical conditions it is not possible to have an internal larval gallery without any connection to the cambial zone and these simulated skewed running larval galleries were detected to 100%, it will be possible to detect that at least something entered the stem. In addition, as small larvae creating larval galleries with a size of two millimeter only occur in the cambium area and the outer sapwood. It is very unlikely to be faced to such small galleries following just the pith in the case of beetles such as CLB as target organism. Therefore under natural conditions it is expected to find bigger larval gallery sizes in the stem, which are detectable with CT.

While most of the investigations using CT-scanner on wood analyzes relate to defects in wood such as knots, cracks, bark and resin pockets [23]. Only very few papers are available on insect damage and are aimed on wood quality as well rather than identifying living organism in imported living plants.

JENNINGS *et al.* (2011) used an *in vivo* micro-CT scanner to recognize tunnels of the woodwasp *Rhysacephala warraensis* within small branches (200 - 300 mm in length; 40 – 50 mm in diameter) of deciduous tree *Anodopetalum biglandulosum.* The branches were dried after storing them for insect emergence for 12 months in contrast to fresh ones (average m.c. 83,7 %). In addition the differentiation between tunnels filled with insect frass and solid wood could be determined as well as dead larvae. Though they proposed that this technology can be used for detection of insect damage in timber, the used scanner was limited to sizes of objects up to 68 mm in diameter with a scan able length of 200 mm in total [95]. With the scanner used in the current investigation, logs with a diameter up to 78 cm and a length of 500 cm can be analyzed. The length limitation is based on the size of the building sheltering the scanner.

The principle use of CT-scanners for the detection of cerambycid larval galleries and larvae has been shown by CRUVENIL *et al.* (2003). The authors could detect bore holes and larvae of *Dichotomius anaglipticus* (Coleoptera: Cerambycidae) using a CT miniscanner with an accelerating voltage of 59.6 keV. Using an accelerating valtage of 662 keV resulted in worse contrast resolution. The CT scanner used in the current investigation was run at 180 keV accelerating voltage which gave a good contrast resolution, to identify even small boreholes of two millimeter in diameter.

Quite similar results could be aimed by detecting old house borer larvae, *Hylotrupes bajulus* (Coleoptera: Cerambycidae), in pine, spruce and fir wood containing different thickness. CT-images were analyzed as a function of the degree of destroying and larval size and resulted in comparable data to those attaining with radiograms. Little and young larvae could not be visualized at 15-mm thick wood samples regardless of whether status of consumption has been observed. However, older individuals could be recognized in any wood diameter class successfully [105].

Though the current investigations were carried out with artificially drilled boreholes as well as inserted larval frass which may not represent natural conditions to 100 % it could be proven that CT-scanning has the potential to identify boreholes down to two millimeter, larval frass and larvae in living trees. Currently, human assisted analysis of the tomograms assembled to a film by a software, is needed to identify the mentioned defects. Additional software is necessary, but already

available, to create 3D images and films. The latter is complex but may be improved in short future. As CT-scanner can be found on airports regularly for inspecting luggage, the software adaption on the inspection of plants for planting during import inspection may result in additional product profitability.

Thermography

The evaluation of the experiments using goat moth larvae as model organism and typical hardwoods do not support hypothesis that infrared thermography can be used for detecting insect larvae, boreholes or bore dust within green solid wood.

Larval motion and presence do not affect temperature changes of specimen samples. Due to poikilothermic behavior of most insects, larval motion (motor) activity (muscle activity, regulation of nervous system) depends on ambient minimum temperature. Although a few species have specific regulation systems for independent heating body temperature and above ambient temperature (for example *Sphingidae*, *Bombus*), most insects are adjusted to environment [89]. In this case our *Cossus cossus* larvae largely adapted to temperature in solid wood and environment (ambient temperature and backgrounds) and no thermal energy modifies surface temperature.

According to preliminary tests and long-term measurement 1, larval temperature is minimally affected by activation energy. Larvae had been stressed induced to increasing motion, when starting or ending the experiments. Hence, the temperature changes occurred in relatively high values (but even below 0.5 K in average) and larvae could be identified when organisms were moving on top and no external objects blocked intermediately the thermal scans. Active larvae (for example when climbing along the box wall) showed higher temperatures compared to motionless individuals.

An additional factor for maximum variances could be found by cooling effect as a result of evaporation. HELLEBRAND et al. (2005) verified that fungal infected wheat plants with higher transpiration have reduced body temperature compared with healthy plants [84]. Thus, the high water content of apple and it´s passing to adjacent wood chips (background 1), inducing evaporation processes affected in a decrease of temperature. This phenomenon was also given at front planes of wood samples at long-term measurement 2 (Fig. **30**).

Over the course of observation, especially at night, animal functions turned down and body temperature adapted to ambient objects in a higher level, thus a differentiation was more difficult. Compared with visual assessment (for example by photo camera), that recognized moving larvae via basic color contrast, thermal imaging is no appropriate application for larvae detection on surface.

Furthermore it could be illustrated that larvae could not be identified inside of wooden model plants at long-term measurement 2. The same result was given at the first long-term measurement when larvae were hidden below solid wood or soil background. Thus, the effect of thermal adaptation on environment may superimpose the effect of motion activity and is insufficient for revealing.

As a second result no differences in surface temperature will be initiated by variances in density (holes, cavities, dust) inside of hardwood samples. Zones with cavities and bore dust have no measurable effect on surface temperature.

An explanation for this phenomenon could be given by internalization that specimen samples had indeed similar water content to living trees, but no (living) reactive tissue that could affect water

flux. Hence a measurable difference in thermal conductivity to hidden zones (decreasing by reduction of liquid content) is not given [38].

The effect of water is one of most limitation factor by using active IR-thermography in wooden materials via heating sources (blow drier, IR emitter, halogen spotlight). In conditioned, moist model samples of *Acer pseudoplatanus* primary bark tissue and components accumulate water, protect water loss and increase moisture. Water has very high thermal capacity and is able to storing heat well [113]. Hence the heated samples have long cooling duration and imitate boreholes do not affect the cooling process.

Radar

The radar study could basically confirm a realizable and positive detection of *Cossus cossus* larvae in different stages (3, 5 and 10 mm in diameter) inside wood samples (*Acer pseudoplatanus*, between 10 and 40 mm in diameter) according to lowest larval movement. Boreholes and simulated larval frass were not identifiable using small arrays in this study.

In general, the premise of larval motion was the superordinate restriction for positive detection and could not be controlled by visual inspection at hidden larvae in this study. Thus, the correct interpretation of signals was severely exacerbated. Future measurements using prepared wood samples with integrated viewing panels along boreholes and video recordings may help to monitor behavior of larvae and may approve/confirm/support correlations between real larval activity and signal intensity.

The larval dimensions affected detection accuracy in a higher degree. Based on the divergences between radar amplitudes (signals at presence and absence of larva) SACHS *et al.* (2008) deduced limits of detection as a function of volume of wood pest and distance to wood sample. Thus, a higher detection probability with increasing larval size can be expected. Regarding the fixed measuring distance of 10 cm to antennas, the minimal larval size of < 0.001 cm^3 will be identified accurately at minimal divergences of movement (< 0.001 dB). With respect to goat moth larvae, the estimated/extrapolated volumes of < 0.14 cm^3 at smallest larval size of 3 mm in diameter (larva with 5 mm in diameter ≈ 0.98 cm^3; larva with 10 mm in diameter ≈ 7.85 cm^3) were not exceeded [155]. Hence, actually all larval sizes given in this study could be identified and motion was recognized adequate.

With increasing distances between measuring object and receiver, sensitivity of detection will be reduced. According to this, measurements at more distant root zones at young trees (partially > 30 cm) could not reveal inserted larvae at (four/several) samples. Furthermore, high diameter above 5 cm induced attenuations of propagation signals resulting in false assessments.

An additional and more intensive distortion effect could be observed at simultaneous measurements with several disturbing samples. Thus, refractions of radar waves, initiated by passing through different boundaries of wood surfaces, resulted in decreases of detection accuracy. Furthermore heterogeneities in density und structure of wood (e.g. annual rings, cracks, knots) will induce reflections that have to be attended.

The detection probability is further (heavily) dependent of measurement time within larvae have to move. This unpredictable factor has to be observed in further studies to assess larval specific behavior and concomitant to optimize appropriate measurement time. However, recording can be

stopped and finished previously when the motion is still detected during this calculated observation time.

The location of larvae was limited to a nearly assessment of direction where insect was moving. The two-channel-way investigation offered classification of the left or right area of measurement field (in that case the whole wood sample or upper or lower part of the young tree) with an accuracy of a few centimeters at the chosen electromagnetic range [155]. Due to higher length of the young trees, multiple measurements increased temporal efforts per sample. However, sequently recordings will help to trace object of interest by allocation of measurement number.

Generally, the chosen broadband radar benefits that detection range can be restricted and defined specifically. Hence, secondary external interferences factors (radio, wlan, signals of mobil phones, television, etc.) can be excluded partially or filtered separately from recordings. For improvement of radar detectability a higher transmission power could increase reflections of the testing object. Furthermore, an enhancement of frequency would enable the identification of insects with less volume at the same sensibility [155]. Nevertheless, to determine lowest larval movements, sensory equipment as well as infestation material has to be fixed at adequate constructions and shielded to disturbing vibrations. As shown in this study simple absorption material and polystyrene could be sufficient to work under practical conditions.

Electrical resistivity tomography (ERT)

In this study, accuracy of ERT for borehole detection resulted in a distinct correlation to diameter of cavity. Tomograms of 47 samples displayed regions of high resistivity at borehole zones using 24 electrodes, 37 vacancies could not be identified. In total 92% of boreholes with 10 mm, 68% with 5 mm and only 14% with 2 mm in diameter were detected. Very similar results were given at 12-point-measurement and detectability ascended with borehole diameter size. However, the detection accuracy concerning borehole size, shape and position decreased, but 12-point-measurment benefits in time saving at electrode coupling and measurements.

Inserted *Cossus cossus* larvae could not be distinguished from boreholes adequate and resulted in merely marginal reduction of resistivity. The larvae detection was limited to comparison with untreated wood zones (control).

The ratio of electrode spacing (s = space between two electrodes) and size of object to be detected (d = size or diameter of detecting object), named s/d-ratio, can be used as an index for the detection potential or minimum detection size. The smaller the spacing of current electrodes (A and B), the finer the detection rating and more little vacancies can be identified. Consequently, a small s/d-ratio < 1 indicates accurate detection, a relation > 1 effects false measurements and objects will not be identified/be missed. Concerning the radial measurements in this study the object size corresponds to absolute borehole diameter and the s/d-ratio can be illustrated as a function of borehole diameter and number of electrodes (Table **34**).

Table 34: Mean s/d-ratio and attribution to detection accuracy as a function of number of electrodes and borehole diameter class

Number of electrodes	Borehole diameter class	Mean s/d-ratio	Detection accuracy			
			1	2	3	4
12	2	3,14	0	2	1	3
	5	1,45	0	2	1	2
	10	0,77	4	1	0	0
24	2	1,58	0	4	12	14
	5	0,68	12	8	5	4
	10	0,38	21	2	0	2

With respect to measuring results for borehole detection a high correlation between s/d-ratio and detection accuracy can be achieved. Thus, decreasing s/d-ratios correspond with ascending detection probability due to increasing diameter values of cavities. However, the well detected boreholes of 5 mm in diameter at 12-point-measurement cannot be explained by the high s/d-ratios > 1, and, in contrast, the low s/d-ratios < 1 could not indicate a correct detection of 5 mm boreholes at nine samples of 24-electrode measurement (Table 34Table **34**).

It is noticeable that no absolute thresholds of resistivity values [ohm meter] could be used for detectability in this investigation. The resistivity is strictly affected by heterogeneities (e.g. in moisture content, electrolyte concentration or density caused by knots, distribution of annual rings, ratios of early and late wood, ratios of juvenile and adult wood etc.) of wood that will differ between each sample and could superimpose the variances of treatment (borehole, larvae). Thus, the identification of blemish (borehole or larva) was limited to a comparison of treated and untreated zone of separate sample. For the displaying in tomogram, the resistivity scale had to be adjusted for both comparison images. A clear attribution of resistivity to the presence of borehole or larvae at unknown trees or samples could not be realized.

Ultrasonic

The evaluation of two ultrasonic devices (coupled and non-contact) could confirm the hypothesis, that prepared boreholes in wood samples could be generally detected using transmission technique. However, the detectability is limited to minimal stem diameter (in dependence of wave frequency) and large ratios of stem / borehole diameter about < 0.2. Thus, 10 and 5 mm boreholes could be recognized, but boreholes with 2 mm in diameter were not displayed in B- or C-scans. In dependence of the ultrasonic device merely moderate borehole detection accuracy could be achieved and variances in position, size and pattern had to be noticed.

The usage of determined travel-times and velocity of propagation with coupled transducers benefits that depths of reflectors (boreholes) could be investigated in B-scans. However, due to variances of travel-times between all samples, induced by damping and further effects of inhomogeneities in wood structure (e.g. porosity, annual rings, density), correct interpretation of results could only be executed by comparison of untreated and treated wood tissue within the same sample. Hence, borehole detection is limited to relative differences in travel-times and velocities within separate sample, and, additionally, real imaging could not be realized.

In contrast, automated and time-saving scanning technique using air-coupled transducers provided plan-type C-scans, comparable to radiographs/x-ray images, for an identification of approximately borehole size and position. However, the differentiation between boreholes and other reflecting, inhomogeneous wood structures (e.g. knots, annual rings, early and late wood) could not be realized in three cases. Thus, attenuations in wave amplitudes at borehole zones were not sufficient or couldn´t be assigned unambiguously to borehole and detection was aggravated at image analyses.

In general the increase of ultrasonic wave frequencies (or conversely the decrease of wave length) enables a higher resolution and finer detection accuracy, but image depth will decrease. Thus, 300 kHz transducers provided more precisely description of vacancies compared to 200 kHz, but transmission was limited to samples with stem diameter < 20 mm. In diagnostic sonography, hand-held sensors devices are applied with frequencies about 1 to 40 MHz (that is more than 100x of frequencies used in this study). The homogeneous structure and high water content of human tissue benefits with low power output needed for transmission. However, huge damping effects (due to large attenuation coefficient) of ultrasonic signals will superimpose a correct transmission at wooden materials and transmitting power cannot be achieved.

A further difficulty for achieving high transmission using air-coupled transducers is the circular shape of trees and tree/wood samples. Thus, deflections of ultrasonic waves on the surface of samples were initiated when transducers scanned at radial peripheral regions (Fig.). Results could be improved when entire sample surface is located in rectangular to transmission direction. For this, a scanning procedure with movement mechanism (for example rotating transducers around the stem) is necessary.

Summarized evaluation of image guided techniques

For an overall assessment of image guided methods applied in this study, two final matrices should give an overview of the potentials of techniques.

Matrix 3: Summarized evaluation for borehole and larvae detection

The first assessment was carried out using three basically categories (accurate, limited, not possible) in dependence of the detection (= the general detectability + the detection accuracy) of the three borehole diameter classes and the presence of larvae (Table 35).

In total, the CT scanning system provided best results and all borehole diameters as well as larvae could be identified with high detail and in a time-saving way successfully. However, passive as well as active thermography could not reveal neither boreholes nor the presence or motion of larvae. The UWB radar devices benefits with high sensitivity for identification of larval movements, but borehole detection could not be provided with little array. The usage of ERT was appropriate for imaging of 10 mm and higher proportion of 5 mm boreholes in tomograms, but inserted larvae could not be distinguished from boreholes. Detection could be only ensured by relative comparison of untreated and treated at separate sample. The identifiabilities of both ultrasonic devices (coupled and non-contact) were limited to higher ratios in borehole/stem diameters, but the larvae detection was not possible. Concerning to ERT measurements, merely differences between treated and control areas of separate sample could support the revealment of boreholes at coupled ultrasonic. Non-contact transducers benefits in automated scanning method and imaging in C-

scans, but the identification of boreholes is aggravated due to partly low attenuation in ultrasonic wave amplitude.

Table 35: Matrix 3: Summarized evaluation of image guided techniques for borehole and larvae detection

Technique	Borehole detection (borehole diameter in mm)			Larvae detection
	10	5	2	
CT[1]	accurate	accurate	accurate	accurate
Thermography	not possible	not possible	not possible	not possible
Radar	not possible	not possible	not possible	accurate
ERT[2]	accurate (limited)	limited	not possible	limited
Coupled Ultrasonic	limited	limited	not possible	-
Non-contact ultrasonic	limited	limited	not possible	not possible

[1] CT = Computed tomography, [2] ERT = Electrical resistivity tomography

Matrix 4: Summarized evaluation with respect to task-related criteria

The second overview characterizes the image guided techniques concerning important criteria that have to be considered for a theoretically establishment at import inspections. The evaluation is ranked in applicability of chosen methods for equal criterion from “very applicable” to “not applicable” (Table 36Table 36).

Table 36: Matrix 4: Summarized evaluation of image guided techniques with respect to task-related criteria (++ very appropriate, + appropriate, 0 neutral, - less appropriate, -- not appropriate)

Technique	Detectability	Detection accuracy	State of development	Investment costs	Costs per measurement	Availability	Time exposure
CT[1]	++	++	+	--	0	++	++
Thermography	--	--	+	0	++	++	++
Radar	(++)	(+)	+	0	+	++	0 (++)
ERT[2]	0	(+)	0	++	++	0	--
Coupled Ultrasonic	-	-	0	0	+	+	-
Non-contact ultrasonic	-	-	+	0	0	+	0

[1] CT = Computed tomography, [2] ERT = Electrical resistivity tomography

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Annexes

Table 37: Mean moisture content of wood samples (*Acer pseudoplatanus*) as a function of stem region and sealing of end faces at screening measurements

Properties		Wood moisture [%]	Standard deviation
Region	Sealing of end faces		
center	with Paraffin	71.7	16.4
	without Parafin	45.9	2.6
edge	with Paraffin	63.3	13.6
	without Parafin	34.4	4.1

Table 38: Mean moisture content of wood samples (*Acer pseudoplatanus*; with Paraffin for sealing of end faces) as a function of type of measurement

Measurement	Wood moisture [%]	Standard deviation
Thermography (preliminary test)	76.0	8.7
Thermography (long-term measurement 2)	70.9	5.7
Radar	80.3	12.3
ERT	80.0	9.3
Ultrasonic (coupled)	76.3	6.3
Ultrasonic (non-contact)	66.0	4.6

Table 39: Mean electrical resistance and difference between measurement plane 1 (MP 1; control) and measurement plane 3 (MP 3; borehole) as a function of borehole diameter at *24 electrode* measurement

Borehole diameter	Mean electrical resistance [Ωm]		
[mm]	MP 1	MP 3	Difference MP3 – MP1
2	192.72	196.55	3.82
5	178.31	197.03	18.73
10	179.29	229.06	49.77

Table 40: Mean electrical resistance and difference between measurement plane 1 (MP 1; control) and measurement plane 3 (MP 3; borehole) as a function of stem diameter class and borehole diameter class at *24 electrode* measurement

Stem diameter class	Borehole diameter [mm]	Mean electrical resistance [Ωm]		
		MP1	MP3	Difference MP3 – MP1
A	2	194.90	199.60	4.70
A	5	172.89	199.97	27.08
A	10	193.55	282.98	89.43
B	2	196.50	199.27	2.77
B	5	178.65	194.84	16.20
B	10	179.50	232.90	53.40
C	12	186.77	190.76	3.99
C	5	182.84	196.29	13.44
C	10	171.95	198.26	26.31

Table 41: Mean electrical resistance and difference between measurement plane 1 (MP 1; control) and measurement plane 3 (MP 3; borehole) as a function of borehole diameter at *12 electrode* measurement

Borehole diameter [mm]	Mean electrical resistance [Ωm]		
	MP 1	MP 3	Difference MP3 – MP1
2	944.82	985.35	40.53
5	883.91	902.91	19.01
10	894.58	1107.56	212.97

Table 42: Mean electrical resistance and difference between measurement plane 1 (MP 1; control) and measurement plane 3 (MP 3; borehole) as a function of stem diameter class and borehole diameter at *12 electrode* measurement

Stem diameter class	Borehole diameter [mm]	Mean electrical resistance [Ωm]		
		MP1	MP3	Difference MP3 – MP1
A	2	969.72	1033.24	63.53
A	5	1068.27	918.31	-149.96
A	10	991.91	1276.54	284.63
B	2	986.05	1034.57	48.51
B	5	892.05	969.62	77.57
B	10	853.99	1119.70	265.71
C	2	878.69	888.23	9.54
C	5	783.58	828.50	44.93
C	10	886.52	1010.93	124.41

Work Package 3: Development and testing of diagnostic techniques in the absence of the organism

WP Leader

Partner P5 (CRA-ABP, Agricultural Research Council - Agrobiology and Pedology Research Centre, Florence, Italy)

Participants

Partner P3 (BFW, Federal Research and Training Centre for Forests, Natural Hazards and Landscape, Department of Forest Protection, Vienna, Austria)

Partner P7 (ILVO, Institute for Agricultural and Fisheries Research, Merelbeke, Belgium)

Introduction

When exit holes are found in wooden plant material or wooden packing material, longhorned beetle larvae or pupae have often already left the galleries, and the causal agent remains uncertain. In that case, identification and confirmation of *Anoplophora* spp. symptoms are problematic. Young larvae of *Anoplophora* spp. and other Cerambycidae tunnel into the bark and feed in the layer between bark and wood. Later on, the larvae tunnel into the heart wood. As the larvae feed, solid faeces are mixed with wood shavings that also result from its feeding and so this frass consists of insect waste and sawdust. The larval frass can be collected and investigated for the presence of insect body parts. In the areas where infestations of CLB occur, it is necessary to identify what type of xylophagous are living inside host plants by avoiding the destruction of the trees with frass in the buffer zone. This is very important in particular in the cities where nature or monumental trees are present.

It is important however to ensure that the research for determination methods is not only focused on *A. chinensis* and *A. glabripennis*, as the import of other *Anoplophora* species plus other exotic cerambycid beetles and the potential danger originating from such species could be overlooked.

Objectives

1) Develop a method for the dissection of infested wooden material in the laboratory and investigation of the frass for the presence of body parts of insects.
2) Investigate a range of molecular diagnostic techniques to determine if the damage is caused by ALB or CLB in the absence of the pest.
3) Develop molecular tests for other *Anoplophora* spp

Program of work (topics)

4) Dissection of symptomatic wood in the laboratory to obtain insect frass and investigation of the collected frass for the presence of body parts of insects using stereomicroscope
5) Diagnosis/confirmation of *Anoplophora* spp. based on molecular analysis of insect body parts
6) Analysis of other *Anoplophora* species
7) Non destructive diagnosis of *Anoplophora* spp. tree colonization based on analysis of insect frass
8) Dissection of symptomatic plants and analysis of the annual ring in laboratory to date exit holes

Dissection of symptomatic wood in the laboratory to obtain insect frass and investigation of the collected frass for the presence of body parts of insects using stereomicroscope and Diagnosis/confirmation of Anoplophora spp. based on molecular analysis of insect body parts

These two topics are both performed by **Partner P7** (**ILVO**, **Hans Casteels**) and are here presented and discussed together, considering that they are two sequential topics.

Objectives

The first objective of this research was to develop an efficient dissection method of symptomatic wood in the laboratory, to obtain insect frass out of the tunnels, and microscopically investigation of the collected frass for the presence of body parts of insects using stereomicroscope.

The second objective was to investigate a range of molecular diagnostic techniques to determine if the damage is caused by ALB or CLB in the absence of the pest.

Material and methods

ILVO has investigated symptomatic plant material (trees, shrubs) as well as wooden packing material showing exit holes for the presence of the different stadia of *Anoplophora* spp. and larval frass. The infested plant material was delivered by the Belgian NPPO inspection service and arboriculture and public green employees; the wooden packing material was delivered by the Belgian NPPO.

Besides we also received a lot of infected wooden material from Italy, delivered by G. Sabbatini (Agricultural Research Council - Agrobiology and Pedology Research Centre, CRA-ABP, Firenze) and Daniel Zovi (Department of Environmental Agronomy–Entomology, University of Padova).

Programme of work

- Dissection of symptomatic wood in the laboratory to obtain insect frass and investigation of the collected frass for the presence of body parts of insects using stereomicroscope
- Diagnosis/confirmation of *Anoplophora* spp. based on molecular analysis of insect body parts

Dissection of symptomatic wood in the laboratory to obtain insect frass

There is no official method for sampling trees and wooden packing material showing exit holes. In the ILVO lab we used following method:

- Using an electric chainsaw, we obtained a piece of wooden packing material or plant material (length ± 20 cm) by cutting a cross-section approximately 10 cm on either side of the exit hole
- We then cleaved this piece along the grain using an axe or, for bigger wood samples, an electric log splitter till the larvae galleries become visible
- In cases where the tunnel continued beyond the 20-cm sample, we cut additional 20-cm pieces as above and cleaved them till the end of the tunnel is revealed

Investigated samples

- Wooden packing material with exit holes, delivered by the Belgian NPPO (7 samples), with origin China (3), South-Vietnam (3) and India (1): no insect remains were found during investigation with stereomicroscope
- *Acer* spp. (origin China) with damage symptoms: no insect remains detected
- Trees and shrubs with exit holes delivered by the Belgian NPPO and arboriculture and public green employees:
  - 4 samples of *Ficus carica* (origin Portugal): insect remains of *Clytus arietis* (Cerambycidae)
  - 1 bonsai *Fagus crenata* (origin China): head capsule and larval skins
  - 5 *Populus* spp. (Belgium): larval skins of *Sesia apiformis* (Lepidoptera: Sesiidae)
  - 1 *Acer saccharinum* (Belgium): larval skins of *Zeuzera pyrina* (Lepidoptera: Cossidae)
- Infected wooden material from Italy (± 50 samples)
  - CLB remnants collected in old larval galleries (3-5 years old) on *Acer negundo*, collected in the field in the infested site in Rome, dry stored (9 samples)
  - CLB remnants of larvae reared on artificial diet (October-November 2011), stored at –20°C (5 samples)
  - CLB remnants of larvae reared on artificial diet (September-October 2011), stored in ethanol 95% in the fridge (10 samples)
  - Samples with sawdust out of infested *Acer pseudoplatanus*, *Salix alba*, *Ulmus glabra* and several other tree hosts (15 samples)
  - Logs (empty), dry-out by oven (3 samples)
  - New samples with CLB remnants (26/10/2012), after problems with the DNA extraction from previous samples (4 samples)

Investigation of the collected frass for the presence of body parts of insects using a stereomicroscope

- Upon detection and opening of the tunnels, we removed the frass using a small spoon (when possible under the stereomicroscope)
- Frass was collected in a Petridish
- The collected material was carefully examined using a stereomicroscope (magnification 6, 8, 10, 12, 16, 20, 25 x) for the presence of insect remains
- The body parts were collected using a small forceps and placed in small recipients for further molecular analysis

Diagnosis/confirmation of Anoplophora spp. based on molecular analysis of insect body parts

Molecular analysis was carried out on the insect remains. DNA was extracted with the Roche High Pure PCR Template Preparation Kit, with the Genomic DNA from Tissue XS kit (Macherey-Nagel) or the high-throughput/low-cost extraction protocols using Chelex 100 Resin (developed by the Canadian Centre for Barcoding). Extracted DNA was analysed in PCR-sequencing.

For *Anoplophora spp.*, the mitochondrial gene Cytochrome oxidase 1 (CO1) was amplified with primers LCO1/HCO1, which are the standard primers for barcoding Arthropods. Finally, identification of the obtained sequence was done by using barcode databases such as Q-Bank (http://www.q-bank.eu) or the Barcode Of Life Data (BOLD) systems (http://www.boldsystems.org).

Results

Dissection of symptomatic wood in the laboratory to obtain insect frass

Insect frass can be collected from galleries in the wood after cutting a cross-section approximately 10 cm on either side of the exit hole with an electric chainsaw and splitting the sample with an axe

or electric log splitter as many times as necessary till the larvae galleries become visible. In cases where the tunnel continued beyond the 20-cm sample, we cut additional 20-cm pieces and cleaved them till the end of the tunnel is revealed.

Investigation of the collected frass for the presence of body parts of insects using a stereomicroscope

Once the galleries become visible a magnification of 10-16x is the optimal magnification for the detection of the body parts in the frass. To look more in detail a magnification of 20-25x can be useful.

Unfortunately we couldn't detect insect remains in the galleries of the imported wooden packing material. The insect remnants we detected in the trees and shrubs delivered by the Belgian NPPO and the arboriculture and public green employees didn't belong to *Anoplophora* species. The damage was caused by other wood-boring insects (*Clytus arietis*, *Sesia apiformis* and *Zeuzera pyrina*). As we had less or no results in the first year we looked for infested wooden material from Italy. In the Italian samples a lot of body parts were detected: head capsules, larval skins, empty pupal cases, pieces of legs and antennae, mandibula, pieces of chitin (see appendix 1).

Diagnosis/confirmation of Anoplophora spp. based on molecular analysis of insect body parts

On about 80 samples with body parts of *Anoplophora* spp. the DNA isolation was carried out with the High Pure PCR template preparation kit from Roche. The DNA extraction in the molecular lab failed, all the samples were lost.

On the new samples from Rome (Italy) with CLB remnants the protocol Strangi et al. (2012) was used (see appendix 2).

All the samples were 100% identical and sample A_ch_4 was used for megaBLAST search at NCBI's nr databases. The closest hits covered 100% of the query and were identical to the *Anoplophora chinensis* entries.

Conclusions

Insect frass can be collected from galleries in the wood by cutting a cross section on both sides of the exit hole and splitting the sample with an axe or electric log splitter as many times as necessary to open the gallery. Insect remains in the gallery (or in the collected frass) can be detected using a stereomicroscope with a magnification of 10-16 x. To look more in detail, a magnification of 20-25 x can be useful. The results of the molecular analyses were not successful in the beginning because (1) it is not always possible to find insect remains in the frass, (2) some body parts didn't belong to the genus *Anoplophora* and (3) problems with DNA extraction of the Italian samples. New Italian samples (Rome): protocol Strangi et all (2012) was used: results were OK.

Reference

A. Strangi, G. Peverieri Sabbatini & P.F. Roversi (2012). Managing outbreaks of the citrus long-horned beetle *Anoplophora chinensis* (Forster) in Europe: molecular diagnosis of plant infestation. Pest Manag Sci.(10.1002/ps.3416).

Appendix 1:Body parts detected in the frass

Other insect parts ≠ *Anoplophora* spp.

Appendix 2: *Anoplophora chinensis* (CLB) detection

Samples

1. Rome-IT-CLB on Acer-Mandibula
2. Rome-IT-CLB on Acer-Body Larve-Last Instar
3. Rome-IT-CLB on Citrus-Body Larve-Last Instar
4. Rome-IT-CLB on Acer-Body Larve-Last Instar

DNA isolation

DNA isolation was performed with the High Pure PCR template preparation kit from Roche, with a final elution step of 50µl instead of 200µl.

PCR 1: General COX1 barcode PCR

The PCR's were adapted from to Strangi et al. (DOI 10.1002/ps.3416). 5µl of extracted DNA was used as template in a first round PCR using primers LCO1490 /HCO2198 (Folmer et al., 1994) to generate a 658bp amplicon.

	COI 1
Product	1
tDNA	5
PCR buffer 10x	5
dNTP 10mM	1
P1628 100µM	1
P1629 100µM	1
FastStart Taq	0,4
UHQ water	35,6
Totaal volume	50

Cycling:

5'-94°C / 3x(94°C-30"/45°C-30"/72°C-1') / 35x (94°C-30"/51°C-1'/72°C-1') / 72°C-7'/4°C hold on a Applied Biosystem 9700 PCR cycler.

Amplicon check was performed using the Qiaxcel Advanced system from Qiagen, using a DM150 module together with Fast DNA Analysis kit:

All samples exhibited an amplicon , although the one for sample 2 was very weak.

All samples were gelextracted using a Macherey-Nägel PCR and Gel Clean-up kit with a final elution step of 30µl.

PCR 2: *Anaplophora chinensis* specific PCR

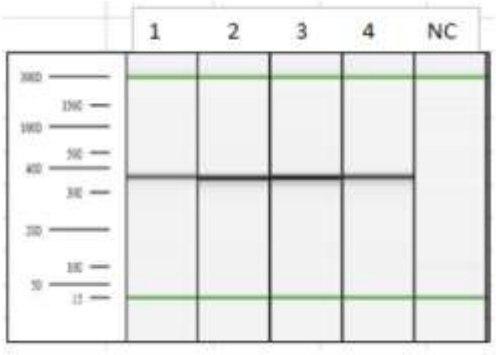
5µl of the purified products from PCR were used as template for the second round PCR, adapted from Strangi et al. with primers ChinensisF and ChinensisR to obtain a 345bp amplicon.

	COI 1
Product	1
tDNA	5
FastStart Buffer 10x	5
dNTP 10mM	2
P2497 100µM	1
P2498 100µM	1
FastStart taq 5U/µl	0,4
UHQ water	34,6
Totaal volume	50

Cycling:

5-94°C / 45x(94°C-1'/60°C-1'30''/72°C-1')/ 72°C-7' / 4°C – hold on an Applied Biosystems 9700 PCR cycler.

Amplicon check was again performed using the Qiaxcel Advanced system from Qiagen, using a DM150 module together with Fast DNA Analysis kit:



PCR sequencing

These amplicons from PCR2 were quantified using a Nanodrop (product yields between 1000-1200 ng/µl).

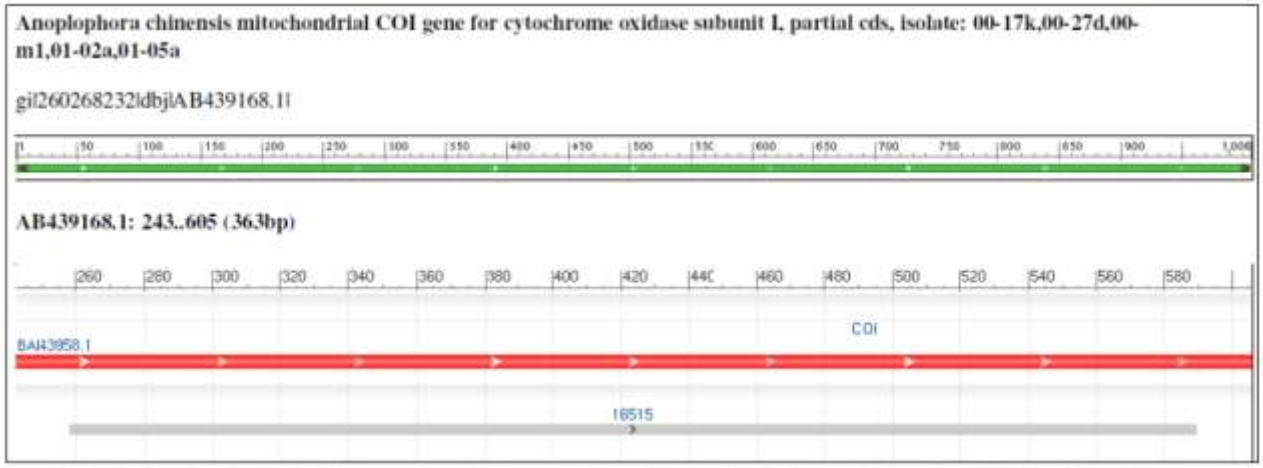
These were 10x diluted and sent to Beckman-Coulter Genomics (UK) to perform custom Sanger Sequencing reactions using the ChinensisF/R primers.

QC of the Abi files was performed with Applied Biosystems's Sequence Scanner v1.0 software.

Abi files were assembled using Bionumerics v6.6 using the batch sequence assembly plug-in.

Results

All 4 samples were 100% identical and sample A_ch_4 was used for megaBLAST search at NCBI's nr databases. The closest hits covered 100% of the query and were identical to the ***Anaplophora chinensis*** entries:



Anoplophora chinensis mitochondrial COI gene for cytochrome oxidase subunit I, partial cds, isolate: 00-17k,00-27d,00-m1,01-02a,01-05a

Sequence ID: dbj|AB439168.1| Length: 1006 Number of Matches: 1

Range 259 to 589: GenBank Graphics

Score	Expect	Identities	Gaps	Strand
612 bits(331)	6e-172	331/331(100%)	0/331(0%)	Plus/Plus

```
Query  1    TACTTCCACCTTCATTAATATTGCTACTAATAAGAAGAATTGTAGATAGAGGGGCAGGAA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  259  TACTTCCACCTTCATTAATATTGCTACTAATAAGAAGAATTGTAGATAGAGGGGCAGGAA  318

Query  61   CAGGATGAACAGTTTATCCACCATTAGCTGCTAATGTTGCACATAGAGGTTCTTCAGTTG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  319  CAGGATGAACAGTTTATCCACCATTAGCTGCTAATGTTGCACATAGAGGTTCTTCAGTTG  378

Query  121  ATTTAGCTATTTTCAGATTACATCTTGCTGGAATTTCCTCAATTTTAGGAGCAGTTAATT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  379  ATTTAGCTATTTTCAGATTACATCTTGCTGGAATTTCCTCAATTTTAGGAGCAGTTAATT  438

Query  181  TTATTACAACAGTAATTAATATACGACCTAAAGGAATAAATTTAGATCGATTACCTTTAT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  439  TTATTACAACAGTAATTAATATACGACCTAAAGGAATAAATTTAGATCGATTACCTTTAT  498

Query  241  TTGTATGAGCAGTTAAAATTACTGCTATTCTACTTCTACTTTCTTTACCAGTTCTTGCTG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  499  TTGTATGAGCAGTTAAAATTACTGCTATTCTACTTCTACTTTCTTTACCAGTTCTTGCTG  558

Query  301  GAGCAATCACAATACTTCTTACAGATCGAAA  331
            ||||||||||||||||||||||||||||||
Sbjct  559  GAGCAATCACAATACTTCTTACAGATCGAAA  589
```

Develop molecular tests for other *Anoplophora* spp.

This topic was performed **by Partner P3** (**BFW, Tomiczek U.**, **Hüttler Ch.**)

For the establishment of molecular genetically finger prints of other *Anoplophora* species than *A. glabripennis*, *A. chinensis* and *A. chinensis* form *malasiaca* by the PCR-RFLP method several adult dead beetles per species in good conditions were needed. Therefore one of the famous experts for Chinese *Cerambycidae*, Carolus Holzschuh (sub-contractor of P3), was engaged to collect such beetles in the origin country China. During the project period he provided the BFW with the following *Anoplophora* species from China:

Anoplophora macularia (from Taiwan)

Anoplophora davidis

Anoplophora beryllina

Anoplophora elegans

Anoplophora granata

Anoplophora sollii

These species were definitely determined to morphological features by the expert Carolus Holzschuh.

A. glabripennis originated from Austria*, A. chinensis* from China, and *A. chinensis* form *malasiaca* from Italy. *A. macularia* and *A. davidis* are species which are very similar to *A. glabripennis*, *A. chinensis* and *A. chinensis* form *malasiaca* due to morphological features.

A.glabripennis

A.chinensis

A.chinensis form malasiaca

Matteo Maspero, Minoprio Foundation, IT

A.macularia

A.davidis

Four other *Anoplophora* species, *A. beryllina*, *A. elegans*, *A. granata,* and *A. sollii* are morphologically very similar to each other because of slightly blue or green shining spots on the black background of the wings instead of white spots like for *A. glabripennis* and *A. chinensis*.

A.beryllina

James Connell, BFW

A.granata

James Connell, BFW

A.elegans

James Connell, BFW

A.sollii

James Connell, BFW

The basis of the PCR-RFLP method is the amplification of two different parts of the COI (Cytochrome Oxidase I) gene of the mitochondrial DNA of the beetles (or eggs, larvae, pupae) after extraction of total DNA using the DNeasy™ Tissue Kit from QIAGEN following the manufacturers' instructions of the protocol for "animal tissue". The polymerase chain reaction (PCR) – restriction fragment length polymorphism (RFLP) method creates with selected differentiating restriction endonucleases species-specific restriction patterns for various species of *Anoplophora*. The principle of the PCR-RFLP method bases on mutations of the target DNA (COI gene) happened during the long evolution history of the various species. Mutations of the COI gene DNA could create specific restrictions sites for different restriction endonucleases with the result that the same part of the COI gene can be digested with one specific restriction nucleases in case of species 1, but not in case of species 2. This phenomenon is used for distinguishing species of the same genus and sometimes also across genera. The amplification of two PCR fragments (650 bp and 920 bp, respectively) of different parts of the mitochondrial COI gene and the following digestion of each fragment with five different restriction endonucleases increase the certainty of the determination. This molecular analysis method for *Anoplophora* species was developed at the Department of Forest Protection of the Federal Research and Training Centre for Forests, Natural Hazards and Landscape (BFW) in Vienna, Austria, in cooperation with the Department of Forest Entomology, Forest Pathology and Forest Protection of the University for Applied Sciences (BOKU) in Vienna, Austria. The following pictures illustrate the different species-specific PCR-RFLP patterns obtained for the mentioned *Anoplophora* species for both PCR fragments of the COI gene. In the literature the relationship between *A. chinensis* and *A. chinensis* form *malasiaca* is often discussed. The revision of the *Anoplophora* species by Lingafelter and Hoebeke in 2002 united the former two separated species *A. chinensis* and *A. malasiaca* to one species *A. chinensis* with the sub-form *A. chinensis* form *malasiaca*. With this PCR-RFLP method *A. chinensis* and *A. chinensis* form *malasiaca* can clearly distinguished from each other and each also from *A. glabripennis*, *A. macularia* and *A. davidis* (Fig. 1, Fig. 2).

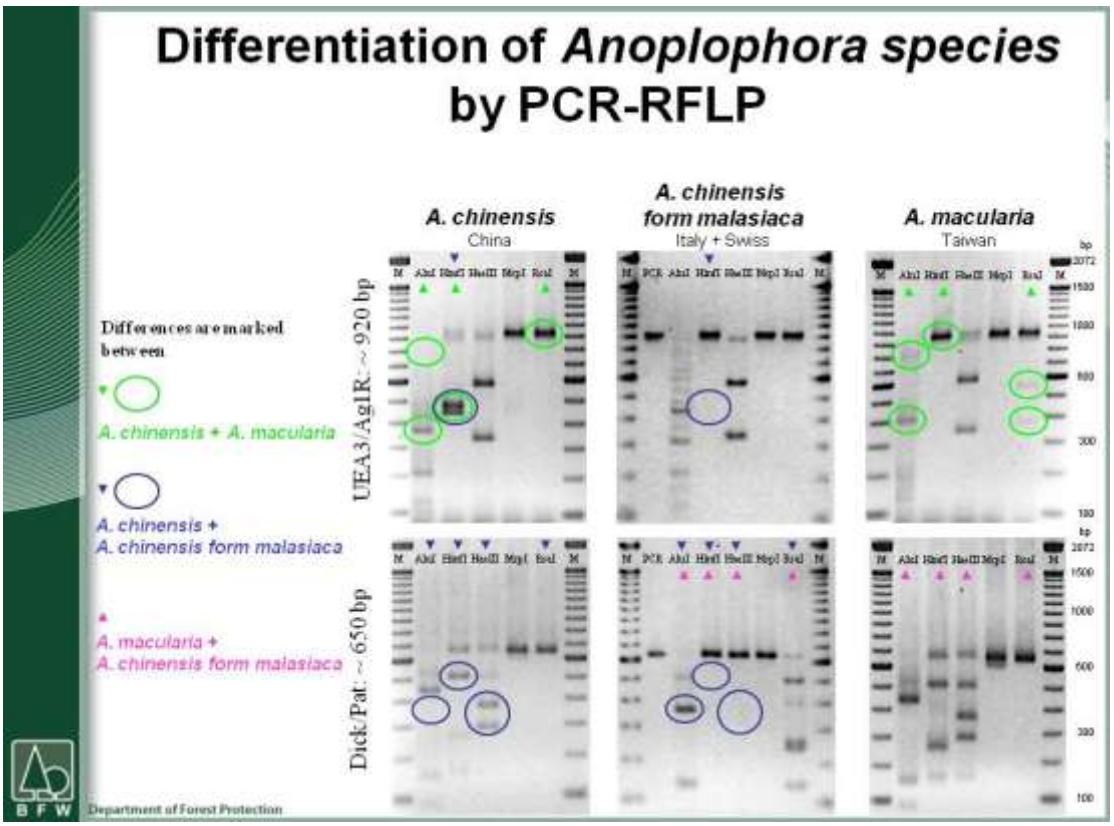


Fig. 1: Differentiation of *A. chinensis, A. chinensis* form *malasiaca* and *A. macularia* by PCR-RFLP analysis of two fragments of the COI gene.

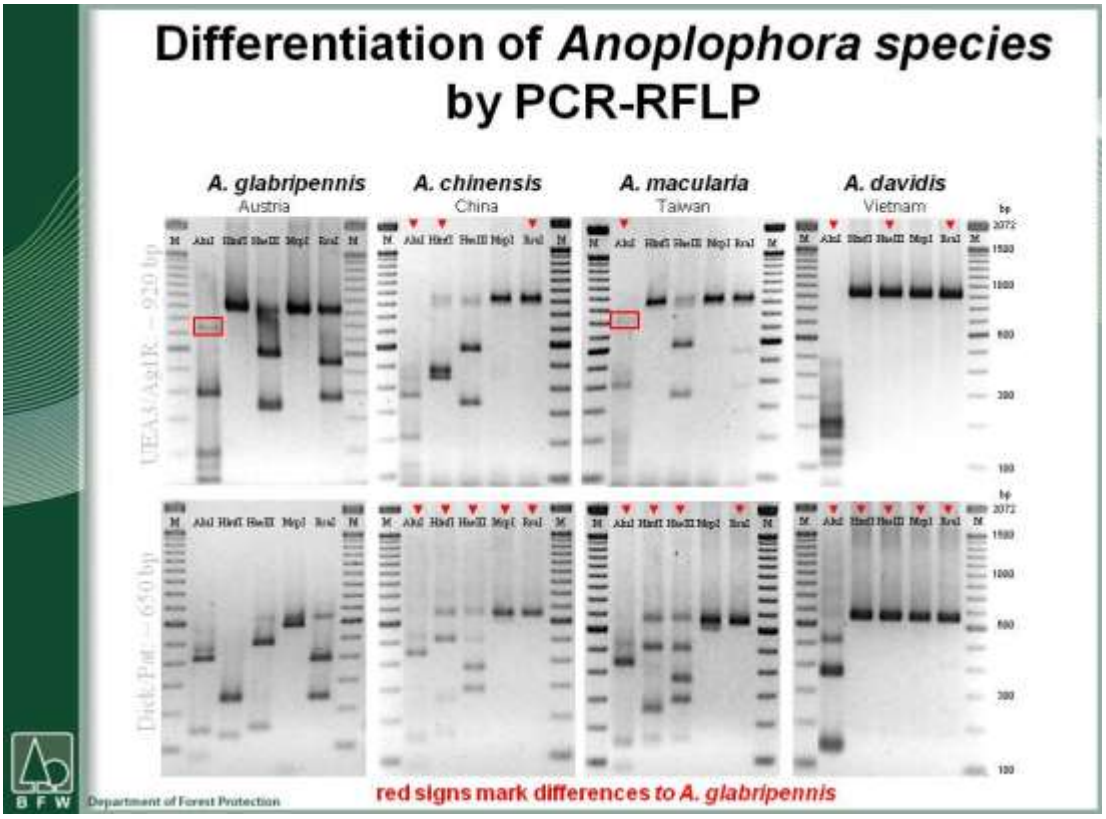


Fig. 2: Differentiation of *A. glabripennis, A. chinensis, A. macularia* and *A. davidis* by PCR-RFLP analysis of two fragments of the COI gene.

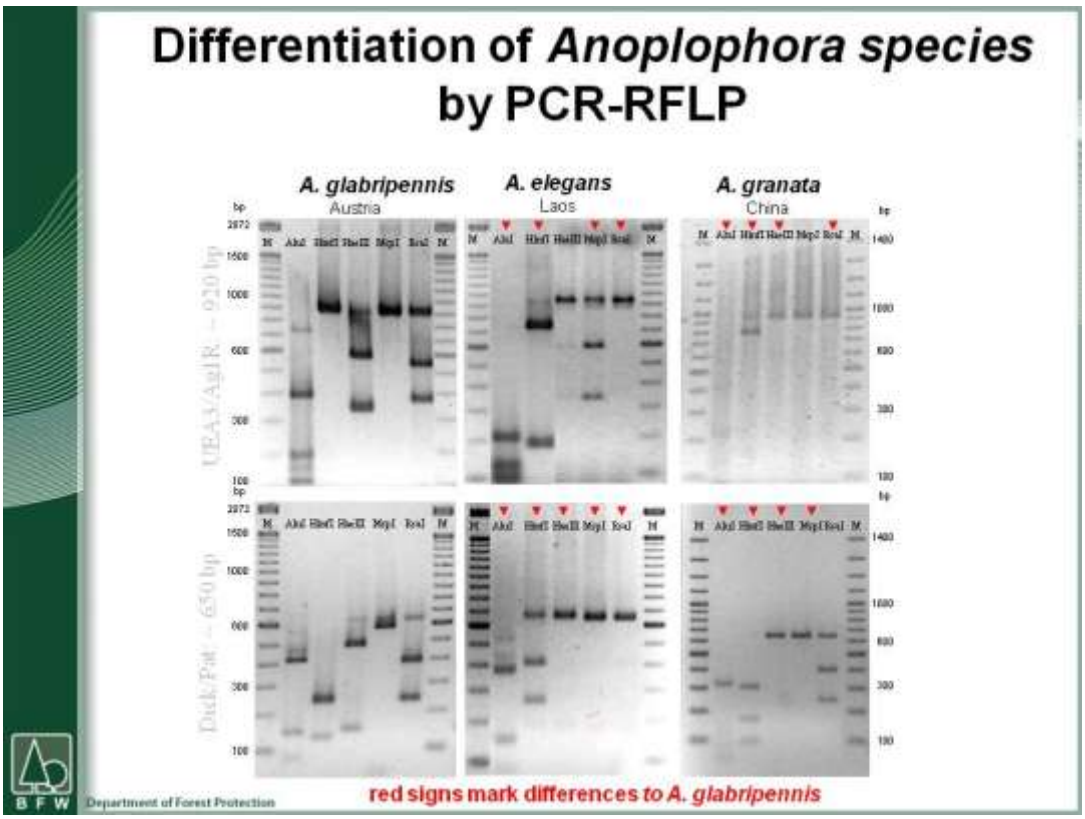


Fig. 3: Differentiation of *A. glabripennis, A. elegans* and *A. granata* by PCR-RFLP analysis of two fragments of the COI gene.

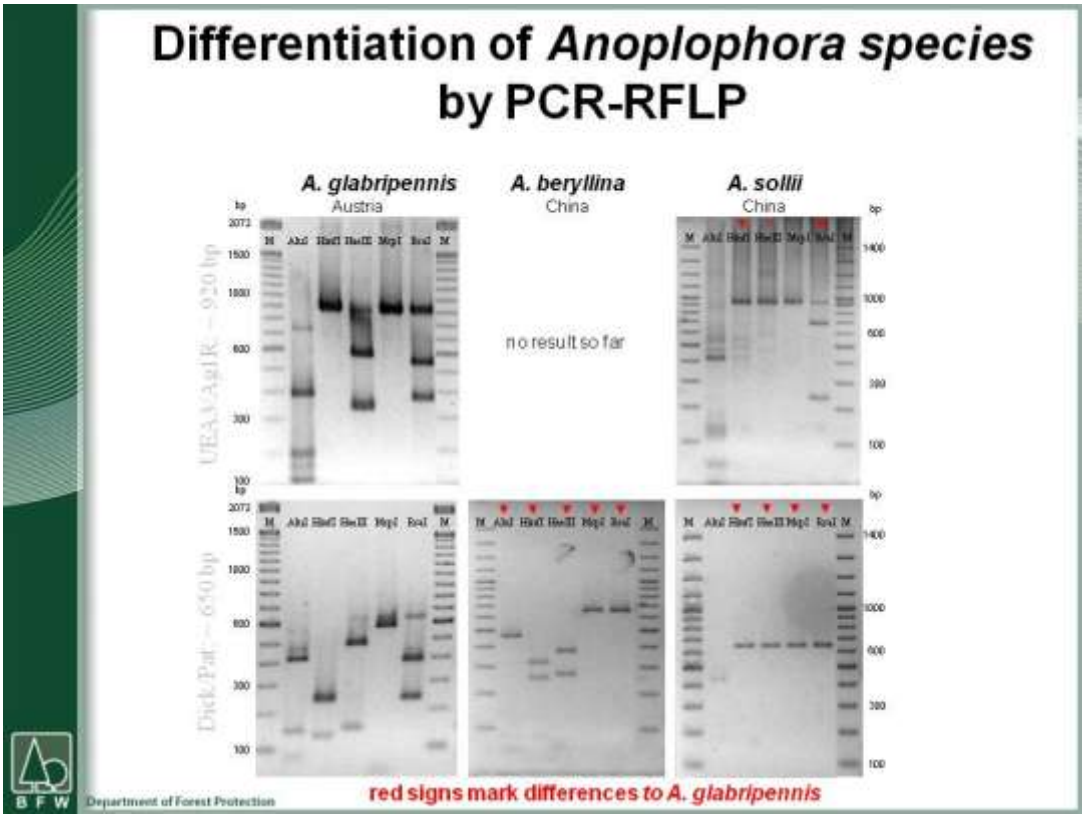


Fig. 4: Differentiation of *A. glabripennis, A. beryllina* and *A. sollii* by PCR-RFLP analysis of two fragments of the COI gene.

Legend for Fig. 1-4: bp: base pairs (unit of DNA size); *Alu*I, *Hin*fI, *Hae*III, *Rsa*I, *Msp*I: restrictions enzymes to digest PCR fragments; M: DNA size marker (100 bp ladder), Dick/Pat, UEA3/Ag1R: primer pairs for amplification of PCR fragments, PCR: undigested PCR fragment.

The Figures 3 and 4 illustrate the species-specific PCR-RFLP patterns of *A. glabripennis* in comparison with those of *A. elegans* and *A. granata* (Fig. 3) as well as with those of *A. beryllina* and *A. sollii* (Fig. 4). The larger PCR-fragment of 920 bp could not be amplified for *A. beryllina* probably due to destroyed DNA in this part of the COI gene of the reference specimens. But nevertheless the differentiation of *A. beryllina* to the other *Anoplophora* species established within this diagnostic method is possible. Additionally, the differences between *A. glabripennis* on the one hand and *A. elegans, A. granata, A. beryllina* and *A. sollii* on the other hand are obviously and clear.

For the following *Anoplophora* species species-specific finger prints are available based on the PCR-RFLP diagnostic method established at the BFW in Vienna:

- *Anoplophora chinensis*
- *Anoplophora chinensis* form *malasiaca*
- *Anoplophora glabripennis*
- *Anoplophora beryllina*
- *Anoplophora davidis*
- *Anoplophora elegans*
- *Anoplophora granata*
- *Anoplophora macularia*
- *Anoplophora sollii*

To establish other *Anoplophora* species than mentioned above definitely determined reference specimens in good physical conditions are necessary.

The PCR-RFLP method established at the BFW is based on the amplification of two different parts of the COI (Cytochrome Oxidase I) gene of the mitochondrial DNA. The two obtained PCR-fragments of 650 bp and 920 bp size, respectively, are digested each with five different restriction endonucleases to receive species-specific restriction patterns. Therefore at maximum ten different patterns per species enable the differentiation of one species from other species increasing the certainty of the determination. As the patterns of the other *Anoplophora* species prove, this method is also suitable for determination of other *Anoplophora* species than *A. chinensis* and *A. glabripennis* after establishing the species-specific patterns on the base of definitely morphologically determined reference specimens.

References

Lingafelter, S.W., Hoebeke, E.R., 2002: Revision of *Anoplophora* (Coleoptera: Cermabycidae). The Entomological Society of Washington, D.C., USA, ISBN 0-9720714-1-5.

Non destructive diagnosis of *Anoplophora* spp. tree colonization based on analysis of insect frass

This topic was performed **by Partner P5** (**CRA-ABP, Roversi P.F., Sabbatini Peverieri G., Strangi A.)**

Signs of infestation of *Anoplophora chinensis* on trees are the presence of exit holes and/or the presence of frass expelled outside from the infested plant by the larvae during their feeding activity. However, some times during phytosanitary inspections, insects cannot be collected from infested trees due to different reasons or insects may leaved the plant before inspection, therefore no molecular analysis can be performed on the insect. Moreover, the infestation signs on plants are not species specific and can be confused with other longhorned beetles which can be instead native to the European fauna. The possibility to identify the species of the insect causing the infestation on trees, based on the analysis of the frass produced by the larvae during the feeding activity can be a valid perspective, taking into consideration that frass of woodboring insects contain a large amount of wooden material but also larval faeces. These larval faeces should contain remnants of gut cells and potentially retain traces of the insect's DNA.

Material and Methods

A. chinensis was obtained from the infested site in Rome (Italy). *Morimus asper*, *Aromia moscata*, *Herophila tristis*, *Monochamus galloprovincialis, Zeuzera pyrina Paranthrene tabaniformis* were collected during entomological surveys. *A. glabripennis* were obtained from the infested site in Cornuda (Italy). An additional exotic insect, recently introduced accidentally into Europe, was also added to the list of investigated species: *Psacothea hilaris* of were obtained from laboratory-reared colonies originating from Northern Italy.

DNA was extracted using a modified CTAB–PVP protocol. Specific 24-mer primers (*ChinensisF-R*) were designed on the basis of sequences derived from *A. chinensis, A. glabripennis, H. tristis, M. asper, A. moschata, M. galloprovincialis, P. hilaris*, *Z. piryna* and *P. tabaniformis* specimens and from DNA sequences present in the nucleotide database of NCBI for *Saperda charcarias* and *Leiopus nebulosus*. Another primers pair, *LCO1490-HCO2198* and *1859F-HCO2198* (*1859F* designed starting from *C1-J-1859* and subsequently adapted on the basis of *A. chinensis*, *A. glabripennis*, *M. asper*, *H. tristis*, *A. moschata*, *M. galloprovincialis* and *Z. piryna* sequences) were used.

Larval frass of *A. chinensis* was collected from host plants infested sites of Italy (Lombardy and Rome). Frass was collected in 50-ml plastic vials from host plants using clean spoons. Within 6 h of collection from the field, samples were stored at -20°C and preserved until the start of laboratory analyses. A sample of *A. chinensis* larval frass was collected from each of the following plant species: *Acer negundo*, *Acer saccarinum, Acer pseudoplatanus, Aesculus hippocastanum*, *Corylus avellana*, *Citrus* x *sinensis*, *Platanus spp., Fagus spp., Carpinus spp., Chaenomeles spp.*; 10 samples were collected in total. Larval frass of other xylophagous insects (*M. asper*, *A. moscata*, *H. tristis*, *M. galloprovincialis*, and *Z. pyrina*) were obtained from infested plant hosts collected in the field or from artificially infested plants.

Results

DNA extractions were tested for the presence of PCR inhibitors using the *HCO2198-LCO1490* primer pair, and all samples were successfully amplified (Figure 1A). *Chinensis* primers showed the ability to selectively amplify *A. chinensis* COI fragment in PCR conditions (Figure 1B).

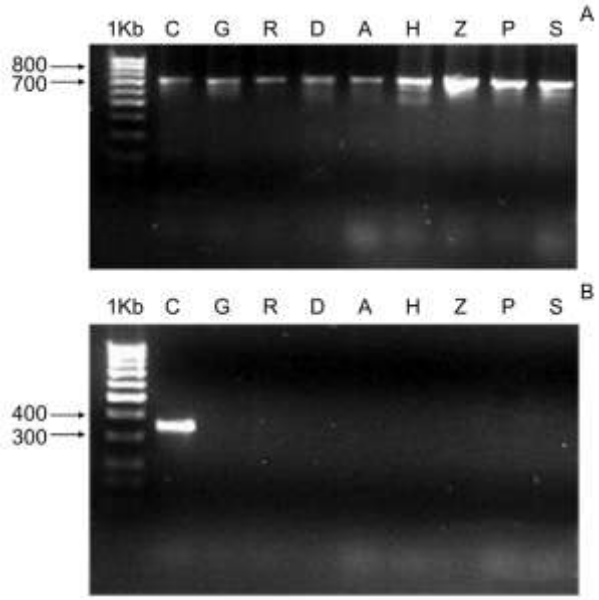


Figure 1. Specificity of the primer pair *Chinensis* tested against common xylophagous insects that may be mistaken for *A. chinensis*. (A) Amplification with the *HCO2198-LCO1490* primer pair of different insect samples. Lanes: 1Kb — DNA scale; C — *A. chinensis*, G — *A. glabripennis*, R — *M. asper*, D — *H. tristis*, A — *A. moscata*, H — *M.s galloprovincialis*, Z — *Z. pyrina*, P — *P. hilaris*, S — *P. tabaniformis*. All listed DNA samples could be amplified. (B) Amplification with the *Chinensis* primer pair

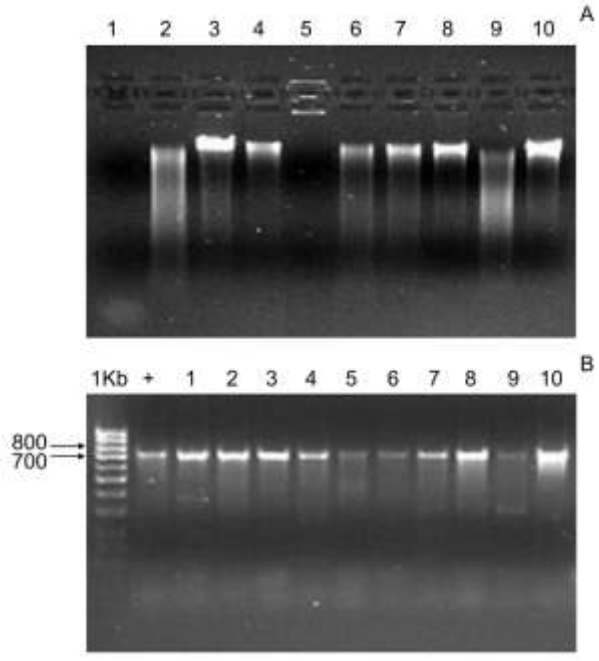


Figure 2. Evaluation of DNA extracted from frass samples collected on different plant species. (A) DNA extracted from frass. Lanes: 1 — *A. negundo*, 2 — *A.hippocastanum*, 3 — *Platanus* sp., 4 — *A.saccharinum*, 5 — *Fagus* sp., 6 — *Carpinus* sp., 7 — *C.* × *sinensis*, 8 — *C. avellana*, 9 — *Chaenomeles* spp. 10 — *A.*

pseudoplatanus. (B) Amplification with the *HCO2198-LCO1490* primer pair for the same samples as in A. 1Kb — DNA scale; + — *A. chinensis* genomic DNA as positive control

DNA samples from different trees infested by A. chinensis *showed variable shapes, from the high molecular weight single band (Figure 2A, lanes 4, 5, 7, 8, 9 and 11) to the smeared DNA (Figure 2A, lanes 3 and 10). All samples could be amplified with the HCO2198-LCO1490 primer pair (Figure 2B).*

The diagnostic protocol was tested using the frass of some xylophagous insects that may be mistaken for *A. chinensis.* Frass-extracted DNA samples can be amplified using the *HCO2198-LCO1490* primer pair (Figure 3A), confirming the absence of PCR inhibitors in DNA extractions. PCR with the *1859F-HCO2198* primer pair enabled us to conclude that all samples tested positive for the presence of insect DNA (Figure 3B). Positive results with the *Chinensis* primers were only reported in *A. chinensis* samples and not in cerambycids or lepidopterans (Figure 3C). Positive results with *1859F-HCO2198* and *Chinensis* primers were further confirmed by sequencing.

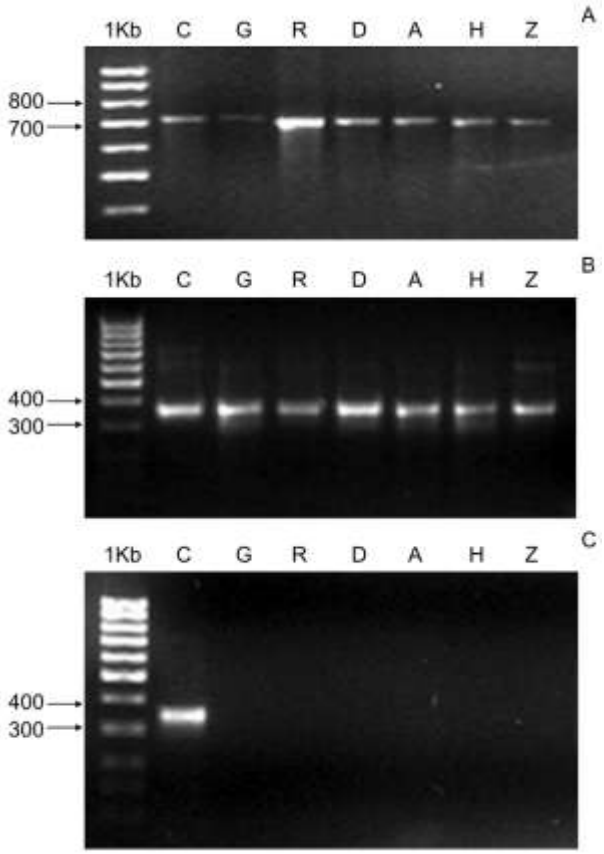


Figure 3. (A) Amplification with the *HCO2198-LCO1490* primer pair for DNA extracted from frass samples of different insects. Lanes: 1Kb — DNA scale; C — *A. chinensis*, G — *A.glabripennis*, R — *M. asper*, D — *H. tristis*, A — *A. moscata*, H — *M. galloprovincialis*, Z — *Z. pyrina*. (B) Amplification with the *1859F-HCO2198* primer pair using 5 µl of purified *HCO2198-LCO1490* fragment, confirming the presence of insect DNA in the sample. (C) Nested PCR amplification with *Chinensis* primer pair

Results show that the possibility to diagnose the presence of *A. chinensis* trough analysis of the larval frass collected in field is reliable and can build up a valid prospective for a new diagnostic tool in phytosanitary survey. Discriminative results were reported with the *ad hoc* designed *Chinensis* primers only in samples originated from *A. chinensis*.

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Dissection of symptomatic plants and analysis of the annual ring in laboratory to date exit holes

This topic was performed **by Partner P5** (**CRA-ABP, Roversi P.F., Sabbatini Peverieri G., Furlan P., G. Cortini,** and by **G. Bertini, CRA-SEL,** Agricultural Research Council, Forestry Research Centre, Arezzo, Italy)

The larvae of *Anoplophora chinensis* are woodborers, feeding in the phloem tissue and in the xylem and the adult beetles of emerge from infested plant producing a circular exit hole by chewing a thin portion of bark separating the pupal chamber burrowed in the xylem from the external environment (Figs. I, 1, 2 and 3).The presence of exit holes on trees is one of the most important diagnostic signs to detect *A. chinensis* infestation and are visible on the bark surface of infested trees for several years (Fig. II, 1). The exit hole dug by adult beetles in the wood of the infested plant can be considered an injury like any other event causing a damage to the trunk and plants respond to injuries occurring during their life cycle by many chemical and anatomical reactions; these reaction tissues remain in the wood structure. The reaction ability of the plant to the insect injury depends on many factors but the exit hole is eventually enclosed by the reaction tissue (callus) and after some years the hole is no longer visible (Figs. II, 2 and 3). However, such injuries can be recognised after many years by inspection of the wood after cross-sectioning of the tree. It is possible to date the time of occurrence of the injury using the annual growth ring method for tree age analyses, i.e. by counting backwards from the year of felling to the margins of the exit hole (if the tree is still alive at the time of felling). Dating damage caused by woodboring insects is a feasible approach in studies of an insect outbreak history. Previous studies on dating exit holes of *Anoplophora glabripennis*, a species close to *A. chinensis* in many aspects, suggested the possibility to analyse the dynamics of an infestation through the years and the pattern of infestation in a given area.

Fig. I - *A. chinensis*: a well grown larva in a gallery in the xylem of an infested tree (1); adult specimen (female) during the emergence from an infested tree (2); adult specimen (male) just emerged and walking on the stump of a tree (3).

Fig. II - *A. chinensis* exit holes: current year (1); exit holes fully closed by the plant reaction (2); comparison of two exit holes fully closed by the plant reaction (3), with clear margins of the hole (a) and with margins no longer observable due to the tangential expansion of the bark during tree growth (b).

Material and Methods

Wooden samples colonized by *A. chinensis* was obtained from the infested site in Rome., The stumps of the 11 collected trees, almost belonging all to *Acer negundo* (other plant species were *Aesculus hippocastanum* and *Ulmus umilis*) were sampled randomly, but three trees which shows old signs of infestation were also intentionally selected. Sampled trees were growing in public parks or in private gardens. Trees were still alive at the time of felling and in good vegetative condition upon visual inspection. The collection of wood samples from trees colonized by *A. chinensis* involved several difficulties due to the ethology of the females, which tend to oviposit at the base of the tree trunks near the soil surface or on shallow roots, and the behaviour of the larvae, digging galleries mainly with a downward direction in the tree trunk and in the roots. Therefore, exit holes are mainly present in the basal part of the trees and cutting trees at ground level in order to obtain the most part of the stump is a demanding task; this however can lead to an undesired lost of wooden material interested potentially by *A. chinensis* exit holes. Stumps of trees were cut at a height of 20-50 cm from the ground level with a chainsaw. The root collar was cut close to the ground line so as to collect as much of the wood as possible, whereas, the tree root system was not sampled. Collection of the wood samples required much care to preserve the entire section of the stump or at least most part of it in order to have a wide cross-section surface on which perform the annual ring analysis. Stumps were put on pallets under a roof in a shaded site under “quarantine” conditions, ensuring sufficiently dry conditions to avoid saprotrophic fungal activity that could cause wood deterioration and staining, but also avoiding excessive drying of the wood, with consequent cracks that might compromise the cross-section analysis. The entire cross-section of each collected stump was carefully inspected and all clearly visible exit holes were marked and recorded. Special attention was given to suspected old exit holes (exit holes that seem completely enclosed by callus material), which provide the best opportunity to date older exit holes and thus to obtain information on the outbreak history and introduction date of the pest into the area. The use of a petrol-powered or electric chainsaw to cross-section the stump was avoided because the chain and its vibration during cutting might cause a significant loss of woody tissue. Cross-sections were cut with an all-purpose/sabre saw and/or a hand saw, and when necessary with the aid log wedges. The stump was sectioned progressively step by step from one end to the opposite one, producing approximately 3-5 cm wide cross-sections. Both surfaces of the cross-sections were inspected for signs of fully closed exit holes not recognised from the outside.

Identified exit holes were carefully cut in the middle of their sections so as to obtain two cross-sections, each with half of the sign of the exit hole (Figs. III, 1 and 2).

Fig. III - Cross-sections of *A. negundo* trunks with *A. chinensis* exit holes partially (1) and fully closed (2) by the plant reaction, and a cross-section prepared for annual ring analysis (3).

The cross-sections were sanded with abrasives of decreasing grain size (P60, P100, P150, P240, P400), using belt, orbital and delta sanders. After sanding, the samples were cleaned with an air compressor. Each exit hole was dated by annual growth ring analysis using different magnifications under a stereomicroscope (Nikon SMZ-2B) and a dendrochronograph system (Lega) (Fig. III, 3). The annual rings of the tree were counted from the outer side (the cambium layer under the bark) to the inner side until the margins of the exit hole were reached. Since the date of felling was known and the trees were still alive at the time of felling, the age of the exit holes dug by the beetles could be determined. To minimize the possibility of counting false rings, each ring was checked by following its track on the cross-section; rings that disappeared for no visible reason or rings that did not show a typical early/late wood tissue pattern were not considered annual growth rings. In the present work, it was assumed that the tendency of tree species to present missing rings due to lack of annual growth did not apply to the infested area, given the sub-Mediterranean climate of the city of Rome. Staining products such as safranine, methyl blue and chalk/graphite powder were also used to try to improve the visibility of the annual rings in dubious cases. At times, a cutter was used to remove a thin woody tissue layer from the cross-section in an attempt to obtain a more clearly observable surface.

Results

The data on dating of *A. chinensis* exit holes of the outbreak site in Rome are shown in figure IV. In total, 44 exit holes were analyzed; most of them were recognised before the felling of the tree, while 10 exit holes were detected only after cross-sectioning of stumps because they were completely closed by the callus and no external signs were visible on the bark. All 10 of these exit holes correspond to the older holes (years 2002, 2003, 2004 and 2005). Only 4 completely closed exit holes were detected before cross-sectioning, at the time of tree felling, due to the presence of a visible circular dimple on the bark. They were dated 2005, 2007, 2009 and 2011 respectively. The rest of the exit holes were dated to 2007, 2008 and 2010. On the whole, half of the total number of exit holes were dug in 2008, the year the pest was detected in Rome. Few holes were

discovered in the years after starting the eradication program and all them were dated to be excavated in the period 2009 - 2011.

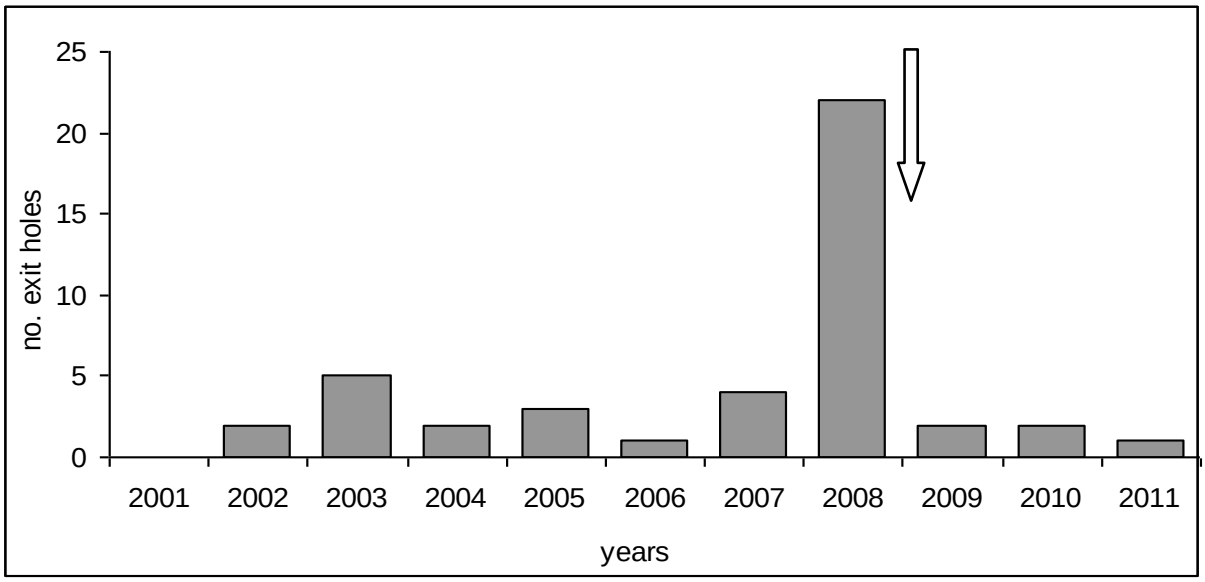

Fig. IV - Number of *A. chinensis* exit holes inspected and dated at the infested site in Rome (data referring to stumps of 11 infested trees felled in the period 2009 - 2012. The arrow indicates the year when the eradication program started.

Surveys conducted on the infested material collected in Rome showed that the first *A. chinensis* emergences from infested trees occurred in 2002. Since the species takes in Rome 1 or 2 years for the juvenile development, we can speculate that the first introduction of *A. chinensis* into the city took place in the years 2000 or 2001. The data reported in figure IV show that, six years after the emergence of the first *A. chinensis* specimens in 2002, the number of exit holes increased considerably in 2008, suggesting a clear population growth. However, the area of the infestation site in Rome did not expand considerably, taking into account that the infested site was restricted to a circular area of approximately 700 m of diameter. The infested area in Rome, where host trees in are numerous and many of them are very large, the *A. chinensis* population appears to have multiplied steadily in a limited area, exploring more or less the available susceptible trees in the area. Therefore, *A. chinensis* has shown infestation dynamics similar to those of *A. glabripennis*, whose infestations do not spread rapidly, at least when hosts are available and no adverse factors influence beetle establishment in the environment. When such conditions no longer exist, adult specimens move in search of more suitable sites.

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Additional studies performed within the WP3

Within the present WP3, the Partner P5 (CRA-ABP) could perform his activity in an additional field of study, even if it was not a specified topic in the original project. This aspect concerned the possibility to identify species of *A. chinensis* or *A. glabripennis*, or at least to exclude their involvement in plant infestations, based on morphological attributes of the larvae, by the use of classical taxonomy.

This aspect was performer by **F. Pennacchio, G. Sabbatini Peverieri, P. F. Roversi** of the **CRA-ABP,** in collaboration with **C. Jucker** of the **University of Milan**, Italy and **G. Allegro** of the **CRA – PLF**, Agricultural Research Council - Research Unit for Intensive Wood Production, Casale Monferrato, Italy.

Introduction

*A. chinensi*s, *A. glabripennis* and *Psacothea hilaris* are longhorned species native to the far eastern regions of Asia and were recently accidentally introduced into Europe. The three exotic species are harmful insects to broadleaved plant species, and much attention is being paid to prevent further introductions and spread in the European Union. Crucial for control is rapid identification of the longhorned species during phytosanitary inspections, both in entry ports and in the rest of the territory of the European Union. Taxonomic keys and descriptions of the adult morphology are available in the literature, but there are significant gaps in the taxonomy of larval morphology, and thus molecular analyses are required. During monitoring activities, a practical morphological taxonomic key would be a rapid and useful tool for species identification of the larvae and separate larvae belonging to native species to Europe.

Material and Methods

In the present study, the work by Löbl and Smetana (2010) was adopted for the taxonomic hierarchy while Švácha and Danilevsky (1986) was used for the taxonomic nomenclature of larval morphology. The biological material was prepared for analysis using methods proposed by Švácha and Danilevsky (1986): larvae were narcotized with ethyl acetate, followed by immersion in boiling water for a period of time varying from 2 - 20 seconds depending on the body size of the specimens. To avoid osmotic deformations of the body, some small holes were drilled in the cuticle with the aid of entomological needles. Prepared specimens were stored in Pampel's fluid or acid alcohol (Švácha and Danilevsky, 1986; Stehr, 1987). Most of the specimens were collected in the field during entomological surveys and prepared as described above, but some of the material was obtained from the entomological collection of the CRA-ABP in Florence.

Results

The general aspects of the larvae of *A. chinensis*, *A. glabripenni*s and *P. hilaris* are common to the family Cerambycidae (Figs. I and II): larvae are elongate, cylindrical, fleshy, cream-colored; the head is prognathous and usually retracted into the prothorax; the prothorax is always larger than the meso- and metathorax and abdomen; the pronotum is more or less partially sclerotized and often provided with a pronotal pigmented plate; the abdomen has 10 visible segments; sprialces are present on the mesothorax and on abdominal segments I-VIII; like some native European

species, *A. chinensis*, *A. glabripenni*s and *P. hilaris* have relatively large larvae when mature, up to 50 mm in body lenght or slightly more. These common general aspects make species identification of the larvae of the three exotic beetles difficult and they can be confused with some native European longhorned beetles.

THE EUROPEAN FAUNA LISTS APPROXIMATELY 680 SPECIES IN THE FAMILY CERAMBYCIDAE, GROUPED INTO 8 SUB-FAMILIES. *A. CHINENSIS*, *A GLABRIPENNIS* AND *P. HILARIS* ARE CERAMBYCID SPECIES BELONGING TO THE TRIBE MONOCHAMINI OF THE SUB-FAMILY LAMIINAE. AMONG THE EUROPEAN FAUNA, THERE ARE ABOUT 340 SPECIES LISTED IN THIS SUB-FAMILY, BUT ONLY ONE GENUS OF THE NATIVE SPECIES REPRESENTS THE TRIBE MONOCHAMINI (*MONOCHAMUS* SPP.). THE SUB-FAMILY LAMIINAE CONTAINS SOME SPECIES WITH FEATURES AND BODY SIZE SIMILAR TO THOSE OF THE THREE EXOTIC SPECIES CONSIDERED HERE. HENCE, THEY CAN LIKELY BE MISTAKEN FOR EXAMPLE FOR *MORIMUS ASPER* (SULZER) AND *LAMIA TEXTOR* L. (BOTH OF THE TRIBE LAMIINI), THE SPECIES IN THE GENUS *MONOCHAMUS* (TRIBE MONOCHAMINI), AND THE SPECIES *SAPERDA CARCHARIAS* L. (TRIBE SAPERDINI).

Taxonomic key for identification of the exotic species *A. chinensis*, *A. glabripennis* and *P. hilaris* among the Cerambycidae of the European fauna (based on last instars larvae)

1 - Legs present, 4 jointed (excluding coxa) (Fig. III)

sub-fam. **Prioninae** Latreille

sub-fam. **Parandrinae** Latreille

sub-fam. **Vesperinae** Mulsant

sub-fam. **Lepturinae** Latreille

sub-fam. **Necydalinae** Linnaeus

sub-fam. **Spondylidinae** Audinet-Serville

sub-fam. **Cerambycinae** Latreille (*pars*)

- Legs absent (sub-fam. **Cerambycinae** (*pars*) and sub-fam. **Lamiinae** Latreille) **2**

2 - Clypeus very narrow, with only slender basal arms reaching to mandibular articulations (Fig. IV). Mandibular apex and dorsal angle more or less lacking; mandible short, apically rounded, spoon-like (Fig. V) sub-fam. **Cerambycinae** Latreille

- Clypeus more or less trapezoidal, filling entire space between dorsal mandibular articulations (Fig. VI). Mandibles not rounded, with distinct apex and more or less distinct dorsal angle (Fig. VII) (sub-fam. **Lamiinae** Latreille) **3**

3 - Anal pore transverse (Fig. VIII) tribe **Lamiini** Latreille

- Anal pore triradiate (one ventral and two lateral rays) (Fig. IX); the ventral ray can be shorter in some species (Figs. X and XI) **4**

4 - Pronotal shield and dorsal ambulatory ampullae with dark spinule visible under a low magnification (Figs. XII and XIII) tribe **Saperdini** Mulsant

- Pronotal shield and dorsal ambulatory ampullae with very minute spinule visible under high magnification. In some tribes (Lamiini, Monochamini, *etc*.) the pronotal shield under low magnification appears as a dark uniform plate, provided with small depigmented rounded areas, more or less joined (Figs. XIV, XV and XVI). Dorsal ambulatory ampullae with different features (Figs. XVII, XVIII and XIX) and never provided with visible spinule under low magnification. In some tribes a distinct pronotal shield is lacking (Fig. XX) **5**

5 - Dorsal ambulatory ampullae granular, build up by small granules in distinct transvere rows or in elongate oval clusters formed by large joined granules (Figs. XVII and XVIII) **6**

- Dorsal ambulatory ampullae not granular, but with small spinule (Fig. XIX)

tribe **Acanthocinini** Blanchard

6 - Dorsal ambulatory ampullae medially with large granules in 4 distinct transverse rows (Fig. XVII). Body size of the last instars larvae, generally more than 40 mm (tribe **Monochamini** Gistel) **7**

- Dorsal ambulatory ampullae with different aspect, granules in less than 4 rows or in elongated oval clusters formed by large joined granules (Fig. XVIII). Last instars larvae smaller than 35 mm

For example: tribe **Pteropliini** Thomson

tribe **Acanthoderini** Thomson

tribe **Mesosini** Mulsant

and other tribes not Monochamini

7 - Abdominal epipleurum of the segments III-IX protuberant (Fig. XXI). Anal pore with the ventral ray distinctly shorter than the two rays (Figs. X and XI) **8**

- Abdominal epipleurum protuberant only on the segments VII-IX. Anal pore with the ventral and two lateral rays of the same length; in some cases, the ventral ray is slightly shorter (Fig. IX)

9

8 - Abdominal segments provided, laterally from the dorsal ambulatory ampullae to the epipleurum, with a number of setae (generally more than 100 for each side), some of them being up to 3 - 4 times as long as the major diameter of the corresponding abdominal spiracle (Fig. XXII). Setae of abdominal segments IX and X numerous and very long (Fig. X). Pleural tubercles with 2 small sclerotized dots (Fig. XXIV). Species developing on conifers of the genera *Pinus, Picea* and *Abies* genus ***Monochamus*** Dejean

- Abdominal segments provided, laterally from the dorsal ambulatory ampullae to the epipleurum, with a smaller number of setae (generally less than 60 for each side), with some of them shorter then approximately 2 times the major diameter of the corresponding abdominal spiracle (Fig. XXIII). Setae of abdominal segments IX and X less numerous and short (Fig. XI). Pleural tubercles without sclerotized dots (Fig. XXV)

Psacothea hilaris **(**Pascoe)

9 - Although, the species separation through larval mor -phology can be quite difficult, the following diagnostic aspects can be proposed:

- Pronotal shield uniformly pigmented, with the exception of small rounded depigmented areas. A very distinct pigmented band is present anterior to the pronotal shield (Fig. XIV)

Anoplophora chinensis (Förster)

- Pronotal shield with non-uniform pigmentation, darker on the anterior part, fading gradually posteriorly. Anterior to the pronotal shield, the band is less observable due to less pigmentation (Fig. XV) ***Anoplophora glabripennis*** (Motschulsky)

Further notes on larval morphological differences among the exotic species *A. chinensis, A. glabripennis, P. hilaris* and other longhorned beetles of the tribe Lamiini native to Europe.

There are additional characters in the larval morphology which can be considered in the identification of the exotic species *A. chinesis*, *A. glabripennis* and *P. hilaris* in cases when the body parts of the larvae are not entirely observable or are missing, *i.e.* if parts of the larval body are lost during extraction from the infested plants. However, for species identification in these cases, at least the whole anterior part of the larvae up to abdominal segment III must be available for observation. In such cases, where the anal pore structure is not observable, the key proposed above can be used to sequentially separate all species of the tribes different from Lamiini and the three exotic species, since the considered diagnostic characters after step 3 of the key concern the anterior part of the larval body. Once all other tribes can be exlcuded, following characters can be used to separate the European species of the tribe Lamiini and the species *A. chinesis*, *A. glabripennis* and *P. hilaris*. Native European species of the tribe Lamiini belong to 5 genera 1:

1 AN ADDITIONAL GENUS IS *TAENIOTES* SERVILLE, BUT IT IS REPRESENTED BY ONLY ONE SPECIES, *T. SCALATUS* (GMELIN), PRESENT IN EUROPE EXCLUSIVELY IN THE AZORES ARCHIPELAGO.

Morimus (only one species*2*: *M. asper* with several sub-species), *Lamia* (only one species: *L. textor*), *Herophila, Dorcadion* and *Neodorcadion*. Species belonging to these genera can be separated from *A. chinesis*, *A. glabripennis* and *P. hilaris* by the aspects described below. Regarding *Anoplophora* spp., the pigmentation of the lateral parts of the head capsule (pleurostoma and gena) is much more expanded posteriorly in larvae of *M. asper* (Fig. XXVI) than in *Anoplophora* spp. (Fig. XXVII). The pleural tubercles in *Anoplophora* spp. are provided with 2 – 3 (some times 4) setae (Fig. XXVIII), while in *M. asper* there are generally 5 - 8 (Fig. XXIX). Moreover, in *M. asper* the setae on the lateral parts of the abdominal segments (from the ambulatory ampullae to the epiplerum) are always more numerous (approximately 120), with several well developed setae with a length at least twice that of the major diameter of the spiracles and with a number of much shorter setae, while in *Anoplophora* spp. there are generally not more than 50 setae. The larvae of *L. textor* presents a higher number of setae than *Anoplophora* spp. on the lateral parts of the abdominal segments, and the epipleurum of the abdominal segments is distinctly protuberant in segments III-IX, while in *Anoplophora* spp. only in segments VII-IX.

In a similar way, misunderstandings can occur among *P. hilaris* and species of the tribe Lamiini. In *M. asper* the epiplerum is protuberant starting from abdominal segment VII, while in *P. hilaris* starting from segment III. In *M. asper* there are two sclerotized dots on the epipleural tubercles (Fig. XXIX), which are absent in *P. hilaris* (Fig. XXV). Moreover, in *M. asper* the number of setae present on the area between the dorsal ambulatory ampullae and the epileurum is higher than in *P. hilaris*. Differences between *L. textor* and *P. hilaris* are that in the former the two sclerotized dots on the epipleural tubercles are present, and laterally the number of setae between the dorsal ambulatory ampullae and the epipleurum is higher in *L. textor*.

All three exotic species considered here can be separated from the other European species of the tribe Lamiini, *i.e.* the genera *Herophila*, *Dorcadion* and *Neodorcadion*, since those species are in general much more smaller than *Anoplophora* spp. and *P. hilaris* and the last instars larvae are generally less than 35mm in body length. Moreover, the genera *Dorcadion* and *Neodorcadion* include species which develop only in roots of herbaceous plants.

2 A SECOND SPECIES OF THE GENUS *MORIMUS* IS *M. ORIENTALIS* REITTER, BUT ITS NATURAL AREA IN EUROPE IS RESTRICTED TO THE EUROPEAN PART OF TURKEY.

Fig. I - Larvae of Lamiinae: *Anoplophora chinensis* (Förster), lateral view.

Fig. II - Larvae of Lamiinae: *Anoplophora chinensis* (Förster), dorsal view.

Fig. III – Larvae of Prioninae: legs in *Ergates faber* (L.).

Fig. V – Larvae of Cerambycinae: *Plagionotus arcuatus* (L.), the arrow indicate the apical margin of the mandibles.

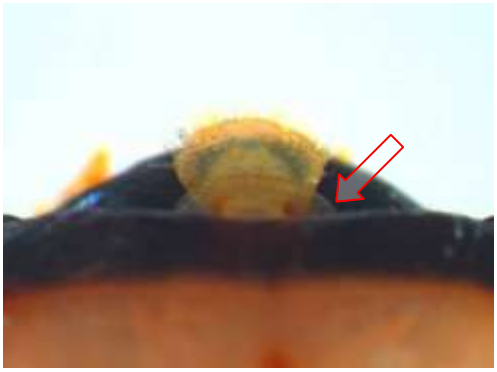

Fig. IV – Larvae of Cerambycinae: *Plagionotus arcuatus* (L.), the arrow indicate the clypeus.

Fig. IX – Larvae of Lamiinae: *Anoplophora chinensis* (Förster), anal pore.

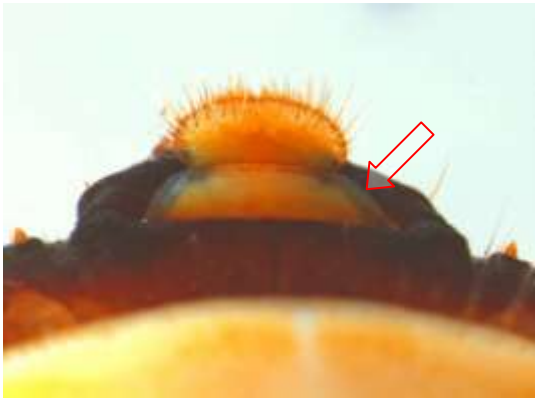

Fig. VI – Larvae of Cerambycinae: *Monochamus galloprovincialis* Olivier, the arrow indicate the clypeus.

Fig. XI – Larvae of Lamiinae: *Psacothea hilaris* (Pascoe), anal pore.

Fig. VII – Larvae of Lamiinae: *Aegomorphus clavipes* (Schrank), the arrow indicate the apex of the mandibles.

Fig. VIII – Larvae of Lamiinae: *Morimus asper* (Sulzer), anal pore.

Fig. X – Larvae of Lamiinae: *Monochamus galloprovincialis* Olivier, anal pore.

Fig. XV – Larvae of Lamiinae: *Anoplophora glabripennis* (Motschulsky), pronotal shield.

Fig. XII – Larvae of Lamiinae: *Saperda carcharias* L., pronotal shield.

Fig. XVII – Larvae of Lamiinae: *Anoplophora glabripennis* (Motschulsky), dorsal ambulatory ampullae of the I abdominal segment.

Fig. XIII – Larvae of Lamiinae: *Saperda carcharias* L., dorsal ambulatory ampullae of the II abdominal segment.

Fig. XIV – Larvae of Lamiinae: *Anoplophora chinensis* (Förster), pronotal shield.

Fig. XVI – Larvae of Lamiinae: *Morimus asper* (Sulzer), pronotal shield.

Fig. XIX – Larvae of Lamiinae: *Acanthocinus griseus* (Fabricius), dorsal ambulatory ampullae of the IV abdominal segment.

Fig. XVIII – Larvae of Lamiinae: *Acantoderes clavipes* (Schrank), dorsal ambulatory ampullae of the I-III abdominal segments.

Fig. XX – Larvae of Lamiinae: *Aegomorphus clavipes* (Schrank), pronotum without a distinctive shield.

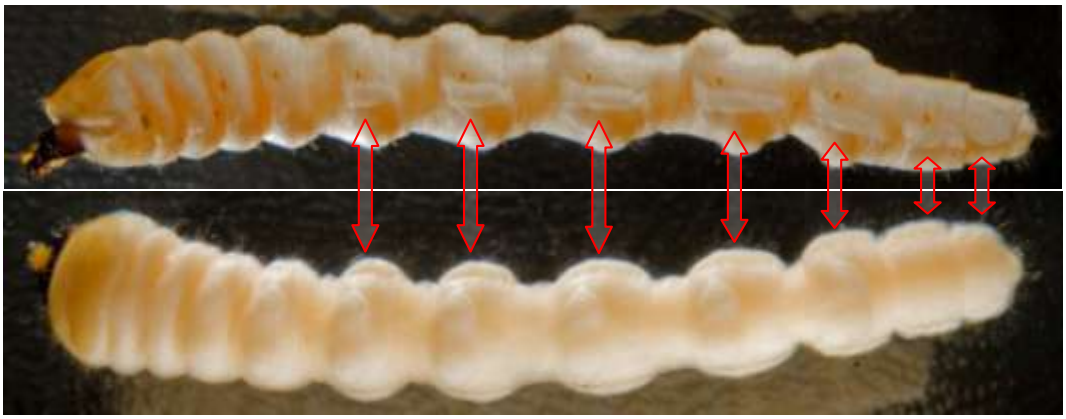

Fig. XXI – Larvae of Lamiinae: *Monochamus galloprovincialis* Olivier, lateral and dorsal view, protuberant epipleurum of the III - IX abdominal segments (arrows).

Fig. XXII – Larvae of Lamiinae: *Monochamus galloprovincialis* Olivier, epipleurum of the III and IV abdominal segment.

Fig. XXVI – Larvae of Lamiinae: *Morimus asper* (Sulzer), the arrow indicate the backwards expansion of the pigmentation of the pleurostoma and gena.

Fig. XXIV – Larvae of Lamiinae: *Monochamus galloprovincialis* Olivier, pleural tubercles of the I abdominal segment.

Fig. XXIII – Larvae of Lamiinae: *Psacothea hilaris* (Pascoe), epipleurum of the III and IV abdominal segment.

Fig. XXV – Larvae of Lamiinae: *Psacothea hilaris* (Pascoe), pleural tubercles of the I abdominal segment.

Fig. XXVIII – Larvae of Lamiinae: *Anoplophora glabripennis* (Motschulsky), pleural tubercles of the I abdominal segment

Fig. XXVII – Larvae of Lamiinae: *Anoplophora glabripennis* (Motschulsky), the arrow indicate the pigmentation not expanded backwards of pleurostoma and gena.

Fig. XXIX –. Larvae of Lamiinae: *Morimus asper* (Sulzer), pleural tubercles of the I abdominal segment

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Pennacchio F., Sabbatini Peverieri G., Jucker C., Allegro G., Roversi P.F., 2012. A key for the identification of larvae of *Anoplophora chinensis*, *Anoplophora glabripennis* and *Psacothea hilaris* (Coleoptera Cerambycidae Lamiinae) in Europe. REDIA XCV: 57-65.

Work Package 4: Understanding the potential for dispersal at outbreak sites

Objectives

1. Investigate if *A. glabripennis* is able to complete its development on fruit trees in Europe
2. Establish whether conifers are suitable host trees for *A. chinensis*
3. Consider the impact of host density and other environmental variables on the dispersal and potential spread of *Anoplophora* species
4. Determine the susceptibility of the most important *Citrus* spp. to CLB

Methods and Results

Review of known dispersal behaviour

Activities came from **P9 (Hans Peter Ravn, University of Copenhagen, DK) in co-operation with the University of Padova (IT), Massimo Faccoli and Riccardo Favaro,** who carried out a study on the correlation between distance and probability of attack from *Anoplophora glabripennis* (ALB).

The study was based on data of trees infested and uninfested with *Anoplophora glabripennis* in the area of Cornuda, Veneto (NE-Italy), during the period of 2009 to 2012. It was shown that the spreading distance is related directly to the probability of ALB-attack – so, for example the probability of an ALB-attack is 2.25% being 300 meters away from an attacked tree, it is 1.48% at 500 meters distance to an attacked tree and it is lower than 0.01% at 2000 meters distance. The analysis of data is still in progress.

Dispersal of *Anoplophora* spp. is also influenced by climatic conditions. **P9 (Antoon Loomans & Roel Potting, Netherlands Food and Consumer Product Safety Authority, Wageningen, NL)** estimated the lifecycles of *Anoplophora* species using an accumulated Degree Day Model.

Calculating degree days (DD) - see WP6 for more details - shows that on average it takes 3 years for ALB to develop under Dutch conditions. However, when the population is split up in those larvae that develop fast (10% of the ALB population; DD-10%: Keena & Moore, 2010) calculation of DD results in generation time of 2 years or less. On the other hand, it takes 6 years for 99% of the ALB population to complete its development.

Monitoring of fruit trees for the occurrence of ALB

Information about additional unknown host plants of *A. glabripennis* was one of the activities from **P3 (U. Hoyer-Tomiczek, G. Sauseng, P. Menschhorn, Ch. Hüttler, M. Brandstetter: BFW, Department of Forest Protection, Vienna, AT).**

In the infested area of ALB in Upper Austria, Braunau/Inn, monitoring of all infested trees - including fruit trees – was carried out in public and private ownership by visual inspections from the ground and by tree climbers for typical damage symptoms (exit holes, saw dust, oviposition scars, larval galleries) and the use of detection dogs.

A tree register map of the infestation area in Austria (Braunau/Inn) developed by the BFW provided data for studies on the spreading structure of infestation and on host-plant preferences of ALB.

In the infested area of ALB in Upper Austria, Braunau/Inn, since June 2009 no infested trees or ALB beetles were found. Despite of the fact that a high number of fruit trees are present within the infested area in Braunau, during all 11 years of intensive monitoring never a fruit tree could be detected being infested by ALB, independent of the distance to other infested trees. Among the in total 201 ALB infested trees in Braunau, the following tree genera are attacked in decreasing order of frequency of infestation: *Acer*, *Betula*, *Salix*, *Aesculus* / *Fagus*, *Fraxinus* / *Alnus, Populus* / *Platanus*. The numbers of infested *Aesculus* and *Fagus* found are the same as well as for *Fraxinus* and *Alnus* as well as for *Populus* and *Platanus*, respectively.

In July 2012 a new ALB infestation area was detected in Austria, in Oberaichet/St. Georgen, also located in Upper Austria. This new ALB infestation established independently from that one in Braunau and was detected at the store of a stone importer during an inspection of wood packaging material. Within a 500 m radius all (more than 600) deciduous host trees including fruit trees and some shrub species were preventively cut, examined (also with detection dogs) and chipped. Only within the closest 100 m radius five infested trees were found: one *Aesculus hippocastaneum*, one *Fagus sylvatica*, one *Salix alba* with larval stages. On the *Aesculus* tree also one single exit hole was detected. Additionally one *Acer platanoides* and one *Prunus avium* were found having dead oviposition marks and starting but empty larval galleries. This is the first and single proof that *Prunus* sp. was attacked by ALB in Austria. So far *Prunus* was known as host of *A. glabripennis* in more southern countries like Italy.

Testing of selected fruit trees as suitable hosts for ALB and CLB under controlled conditions

The testing of fruit trees as suitable host for *Anoplophora glabripennis* (ALB) was the **activity of P4 (Christa Lethmayer, AGES, Institute for Sustainable Plant Protection, Vienna, AT)** with **support from P3 (BFW, Department of Forest Protection, Vienna, AT)**. Glasshouse trials with apple trees were carried out under quarantine conditions in Vienna (Austria).

Materials and Methods

The trial was carried out in a quarantine cabin in the greenhouse under controlled climatic conditions (temperature: + 25°C, relative humidity: 60%, 16 hours daylight, 8 hours darkness). It was started in June 2011 and ended in November 2012.

Following parameters were evaluated quantitatively and/or qualitatively during this investigation:

(1) survival of ALB-beetles (→ maturation feeding, duration of life time)
(2) oviposition (→ occurrence of oviposition scars and eggs)
(3) larval activity (→occurrence of sawdust and larvae)
(4) exit holes (→occurrence of adult beetles)

Within this quarantine cabin 6 cages (h = 185 cm, l = 90 cm, b = 75 cm) were placed: 5 cages with apple (*Malus domestica*, cultivar: Golden Delicious) as 5 replicates and 1 cage with maple (*Acer platanoides*) as control (see Fig. 1).

Fig. 1: Quarantine cabin with 6 cages

Each cage contained one potted tree (h = ~ 150 cm, Ø = ~ 3 cm to 6 cm at the bottom), 2 cut branches (1 thin branch: l = ~ 50 cm, Ø = ~ 7 - 10 cm and 1 thick branch: l = ~ 50 cm (apple) and 80 cm (maple), Ø = ~ 13 - 15 cm) and 2 flasks with fresh maple leaves (*Acer platanoides*, *A*. *platanus* and/or *A*. *campestre*) as food plants which were changed regularly every 2 to 3 days.

In total 100 adult ALB-beetles (50 couples) were released into the 6 cages on 3 releasing dates due to the availability of hatched beetles: 01.06.2011 (36 beetles), 17.06.2011 (44 beetles) and 18.07.2011 (19 beetles) resulting in 12 - 20 beetles in every cage during the trial.

The ALB-beetles were provided from P3. They collected ALB-infested wood pieces from Italy, region Veneto, Cornuda and Maser, during the period of January to December 2011. This was enabled by the support of the Plant Protection Service of region Veneto, especially by Dr. Marco Vettorazzo, and the University of Padua, especially by Dr. Massimo Faccoli. Afterwards these infested wood pieces were stored at BFW in Vienna (Austria), Department of Forest Protection, under quarantine conditions to allow emergence of adult beetles. The first beetle emerged on May 23rd 2011, the last on August 26th 2011. In total 137 beetles emerged, 54% (74) males and 46% (63) females. In the first part of the emergence period mainly males emerged and with 3-4 weeks delay the amount of emerging females increased.

The survival of beetles was observed every 2 to 3 days from the first release in the beginning of June 2011 until the death of the last beetle with counting all living and dead beetles. Oviposition scars were counted on living trees and cut branches. Larval activity was indicated by the presence of sawdust which was observed in regular intervals, too. Furtheron, stems of the trees and branches have been investigated after exit holes and larval galleries were opened in order to find larvae.

Results

(1) Survival of ALB-beetles

Maturation feeding of beetles has been observed on branches of food plants and living trees (maple and apple) and on leaves of the trees especially on leaves of the living maple tree (see Fig. 2).

Fig. 2: Maturation feeding of ALB-beetles

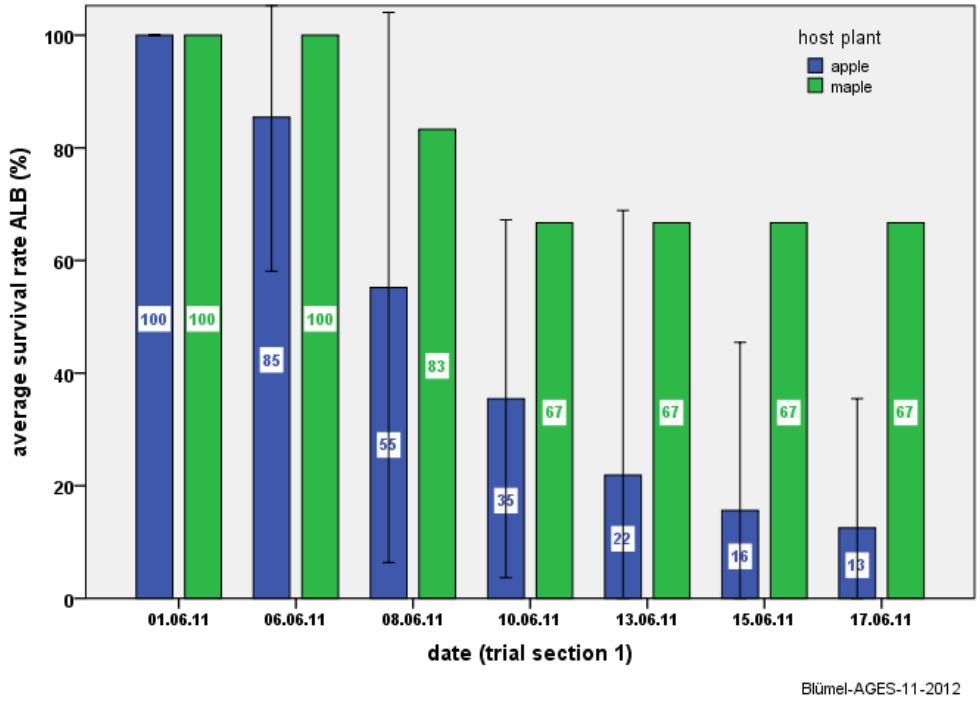


Fig. 3: Average survival rate of 36 ALB-beetles (in %) for the 1st releasing date on 01.06.2011.

In Fig. 3, 4 and 5 the average survival rate of ALB-beetles is shown for the 3 releasing dates, each time starting with 100% survival of beetles. After 16 days only 13 % of the beetles survived on apple trees (average of 4 trees) whereas 67 % of beetles survived on the maple tree (Fig. 2).

The results for the 2nd releasing date on 17.06.2011 (44 new beetles added) were obtained after 4 weeks. Again the survival rate of beetles on the maple tree (40 %) was higher than on the apple trees (average of 5 trees) with 15 % (Fig. 3).

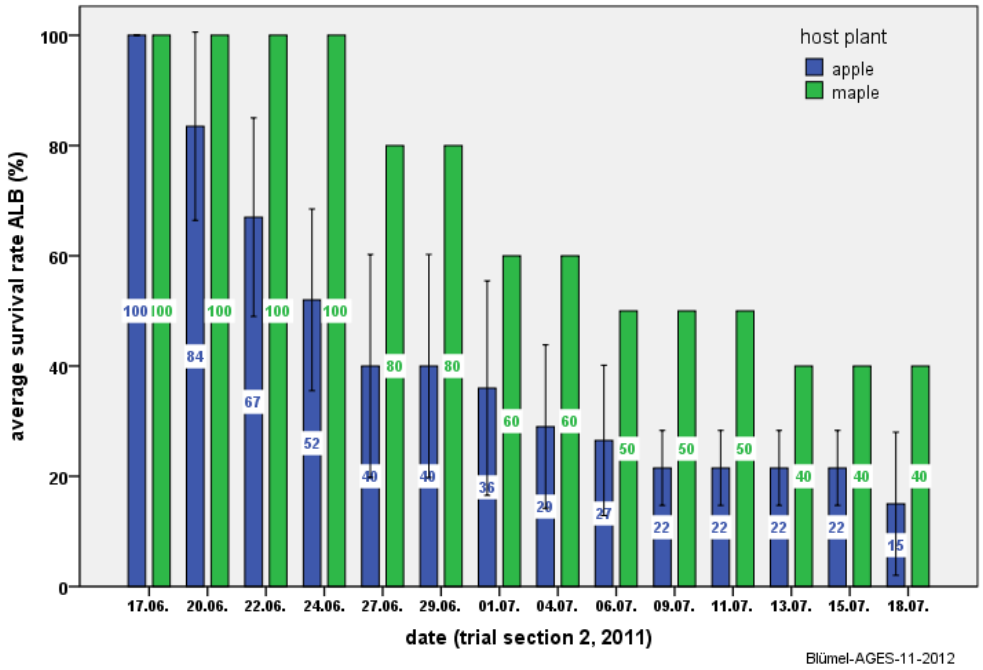


Fig. 4: Average survival rate of 44 ALB-beetles (in %) for the 2nd releasing date on 17.06.2011.

The survival rate for the 3rd releasing date on 18.07.2011 (19 new beetles added) was evaluated after 6 weeks respectively in the end of August 2011. The duration life time of beetles in maple was again longer (average survival rate of 17 %) compared to apple trees (average of 5 trees) with 5 % which represented the presence of 1 single beetle (Fig. 4). Until the first week of September 2011 all beetles died except 1 male in cage 2 with an apple tree. This beetle survived until the end of September 2011.

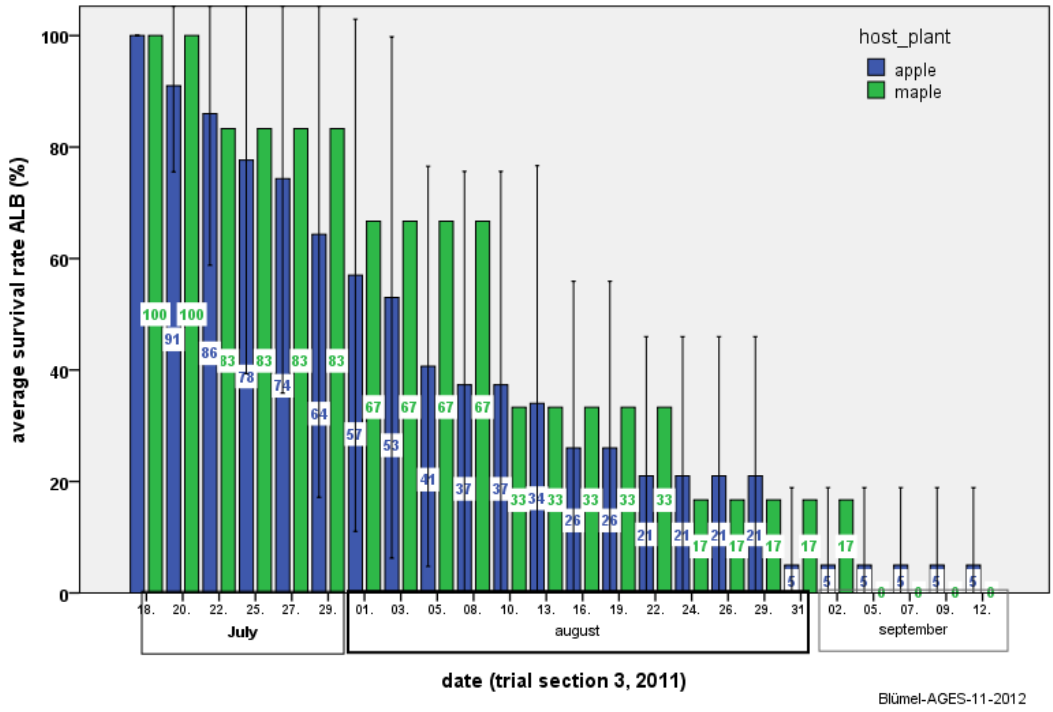


Fig. 5: Average survival rate of 19 ALB-beetles (in %) for the 3rd releasing date on 18.07.2011.

The survival of adult ALB-beetles respectively their life time period was significantly longer in the cage with maple than in the cages with apple trees.

(2) Oviposition

Copulation and egg laying of females have been observed in cages with apple trees and maple tree (Fig. 6 and 7).

Fig. 6: Copulation of beetles

Fig. 7: Oviposition of female

The highest number of oviposition scars (Fig. 10) was found on maple both on living tree and on cut maple branches (see Fig. 8 and Fig. 9)

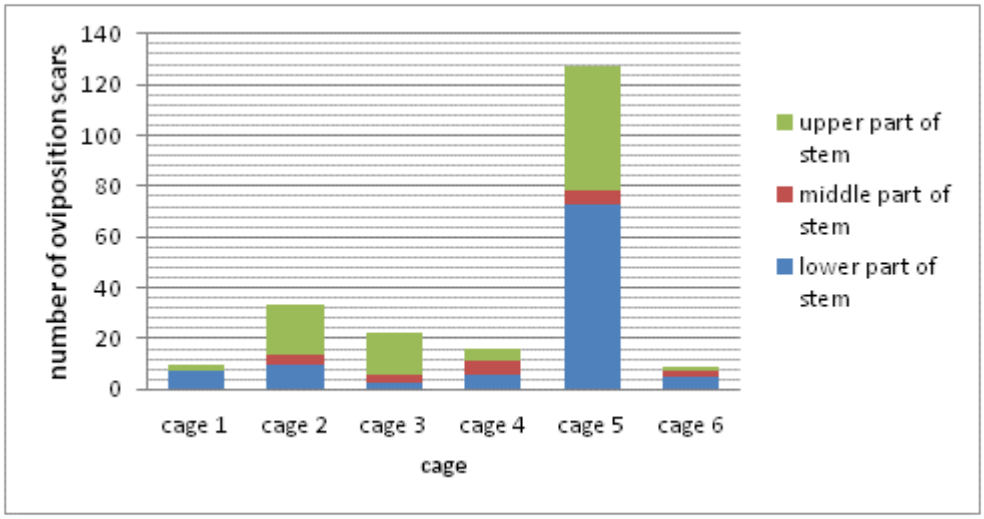


Fig. 8: Number of oviposition scars on living trees (cage 1-4, 6: apple, cage 5: maple)

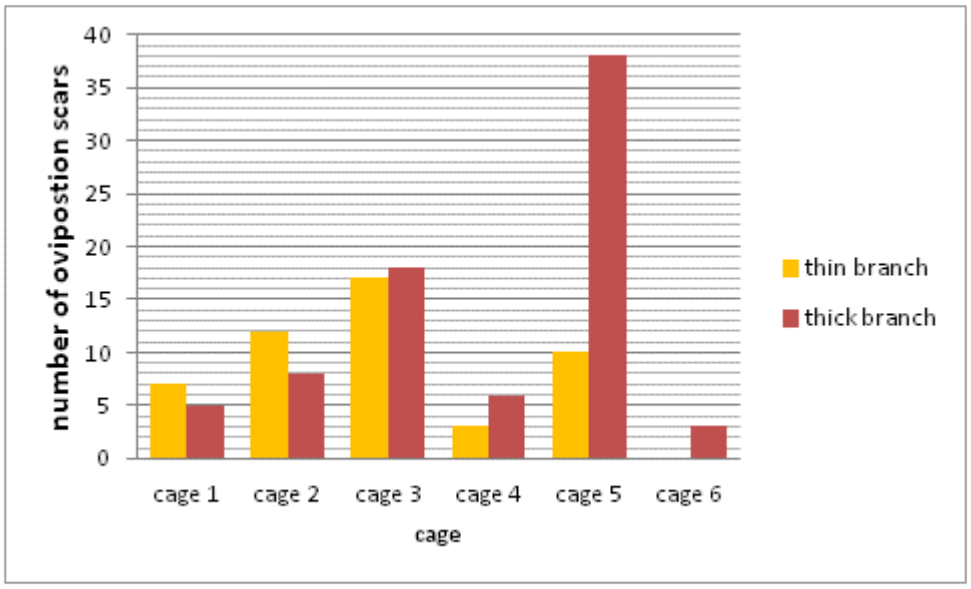


Fig. 9: Number of oviposition scars on cut branches (cage 1-4, 6: apple, cage 5: maple)

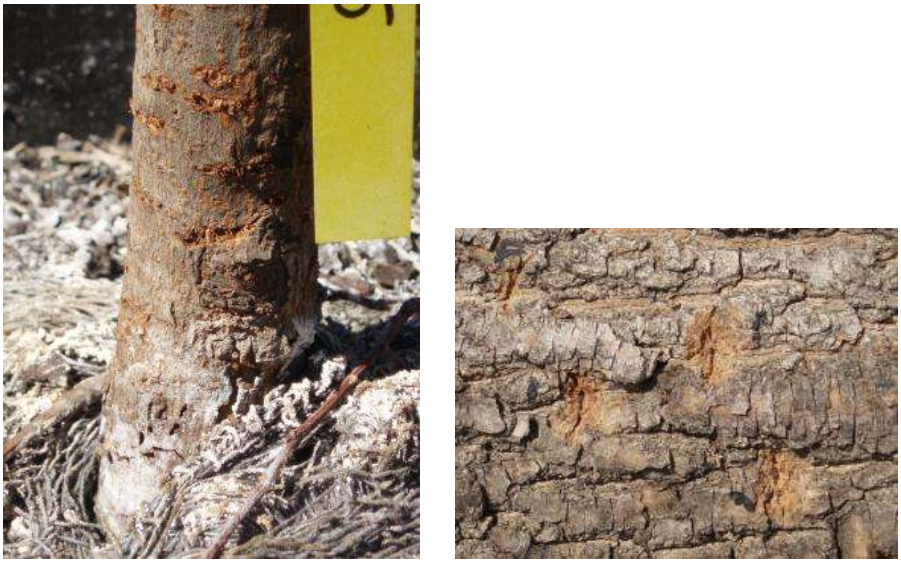
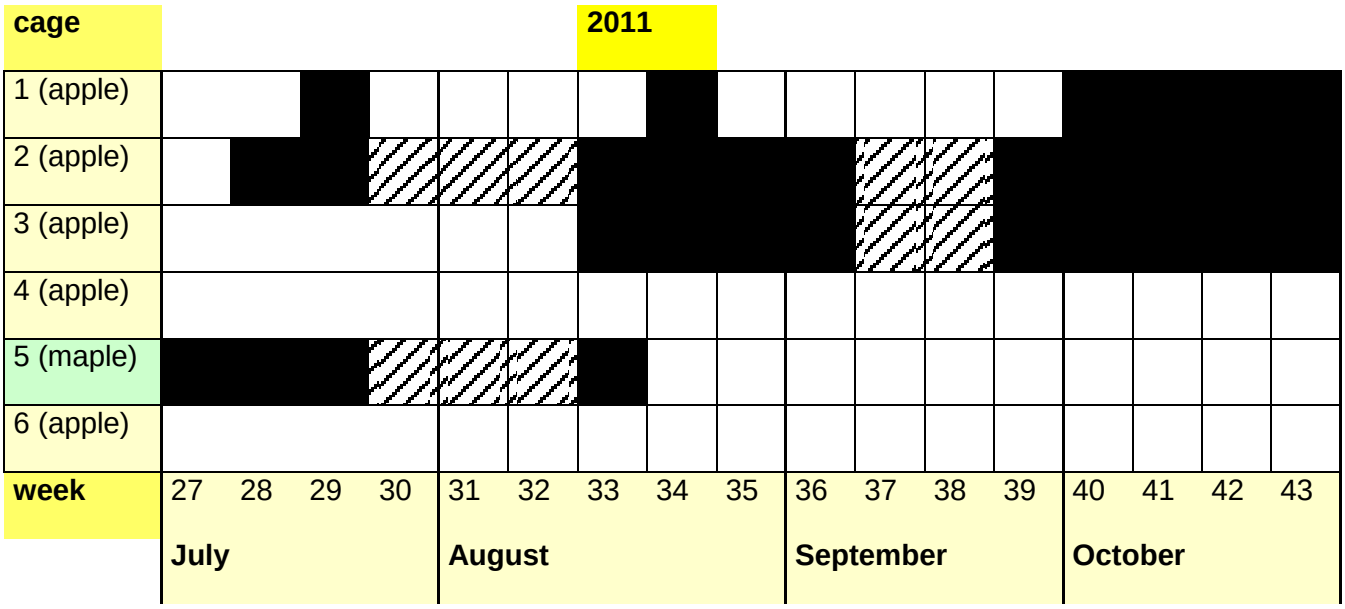


Fig. 10: Oviposition scars on living tree and cut branch

(3) Larval activity

Observation of sawdust indicated larval activity. Sawdust from **living trees** was found on the soil of maple tree (cage 5) since 08.07.2011 and in cage/apple tree 1, 2 since mid of July 2011 and in cage 3 since August; no sawdust was seen from the apple trees in cage 4 and 6 (Fig. 11). Larval activity in the maple tree was stopped in mid of August 2011 likely due to the death of the maple tree whereas sawdust from the apple trees was still observed for several months, in cage 3 until November 2011 and in cages 1 and 2 until January 2012.



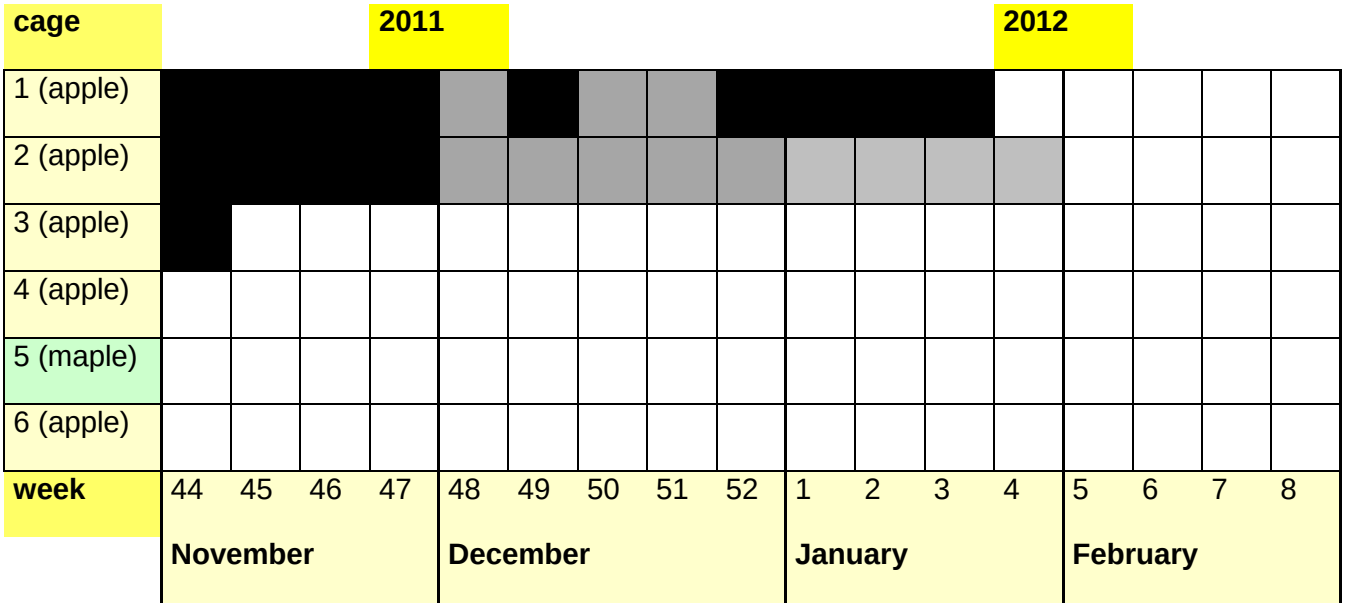


Fig. 11: Occurrence of sawdust from living apple and maple trees during July 2011 to February 2012 (□… no sawdust, ▒ … several sawdust-chips, ■... plenty of sawdust, ▨… presumption of sawdust; red numbers indicate that no data were available for this period).

Sawdust from **cut branches** was visible (evaluation after about 7 weeks on 18.07.2011) on the thin apple branches in cage 1, 2 and 3, on the thick apple branches in cage 3 and 6 and on the thick maple branch (cage 5) whereas no sawdust was under the thin maple branch. Sawdust was observed for about 4 weeks (evaluation on 16.08.2011).

After opening the bark from living apple and maple trees frass and sawdust from larval activity (Fig. 12) was found and also **larval galleries**.

In the galleries of the apple trees in total 3 **larvae** were found in cage 1, 2 and 3. In tree 1 of cage 1: 1 larvae (13 mm long + dried up, in 108 cm height), in tree 2 of cage 2: 1 larvae (10 mm long, dried up) (Fig. 13) and in tree 3 in cage 3: 1 larvae (4 mm long, dried up). In the maple tree no larvae was detectable.

Fig. 13: Dried up larvae from an apple tree (cage 2)

Fig. 12: Frass and sawdust from larval activity in apple trees

(4) Exit holes

During investigation of the infested trees 1 adult female (17 mm long) was found already dead in tree 1 of cage 1 sticking in its exit hole (Fig.14). This exit hole was found only in 18 cm height from the ground in the stem.

Fig. 14: Dead ALB-female in apple tree 1

Testing of conifers as suitable hosts for CLB

The testing of conifers and fruit trees as suitable host for *Anoplophora chinensis* (CLB) was performed by the **subcontractor of P5,** CRA-ABP (= Agricultural Research Council - Agrobiology and Pedology Research Centre), IT: **Costanza Jucker, Daniela Lupi and Mario Colombo (University of Milan, Department of Food, Environmental and Nutritional Sciences, IT).**

Methods

In order to acquire additional data on the susceptibility of different plants species to *Anoplophora chinensis* attacks in Italy, a field trial was conducted in a quarantine structure in Lombardy, within the infested area (Fig. 1).

In particular, the adult feeding, oviposition and the presence of larval trophic activity were tested on the following 8 species:

- *Acer saccharinum*
- *Citrus reticulata*
- *Citrus x sinensis*
- *Citrus x limon*
- *Cryptomeria japonica aritaki*
- *Pinus sylvestris viridus compacta*
- *Populus x euramericana*
- *Taxus baccata*

For each species 5 potted plants with a diameter of 5-8 cm were tested in a no-choice experiment. The plants height varied with the host species. *Acer saccharinum* was used as control.

Fig 1: Quarantine structure

Fig 2: Individually caged plant

Starting from the end of June, each plant was infested with adults captured during the surveys conducted in the infested area in Lombardy. Each host plant was infested with a couple of adults; after the adult insertion, plants were individually caged (Fig. 2). When no clear signs of *A. chinensis* attack were visible, plants were re-infested. Every week each plant was observed looking for adult feeding, oviposition scars and larval trophic activity.

Results

Adults feeding

Adults feeding was observed on almost all the host species, with some preferences. In fact, while on *Acer saccharinum* and *Citrus reticulata* the adults fed on all the experimented plants, on some other species the alimentary activity was seen only on some plants (Fig. 3). On *Pinus sylvestris* no sign of feeding was present.

Adults fed of the tender bark of the small branches or on the trunk (Fig. 4, 5, 6).

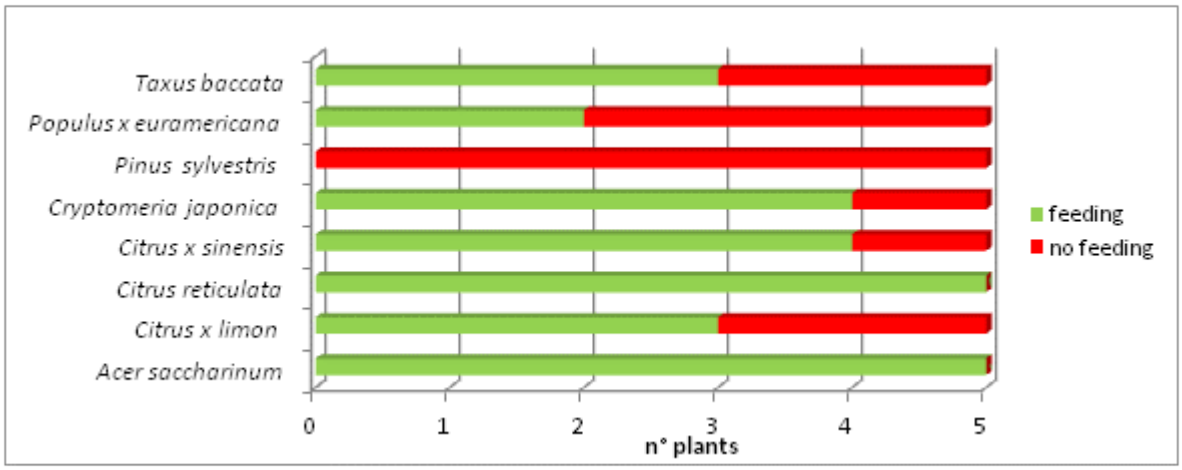


Fig 3: Adults feeding activity on different host plants.

Fig. 4: Adult feeding on *Cryptomeria japonica*

Fig. 5: Adult feeding on *Taxus baccata*

Fig. 6: Adult feeding on *Citrus* spp.

Oviposition

Oviposition scars were observed on 7 species; in fact only on *Citrus x limon* no signs of deposition was present (Fig. 7). All *Acer saccharinum* and *Cryptomeria japonica* plants showed oviposition scars, with a high number on maples, while on the other species not on

all the plants they were present. In particular, only one sample of *Pinus sylvestris* had oviposition scars.

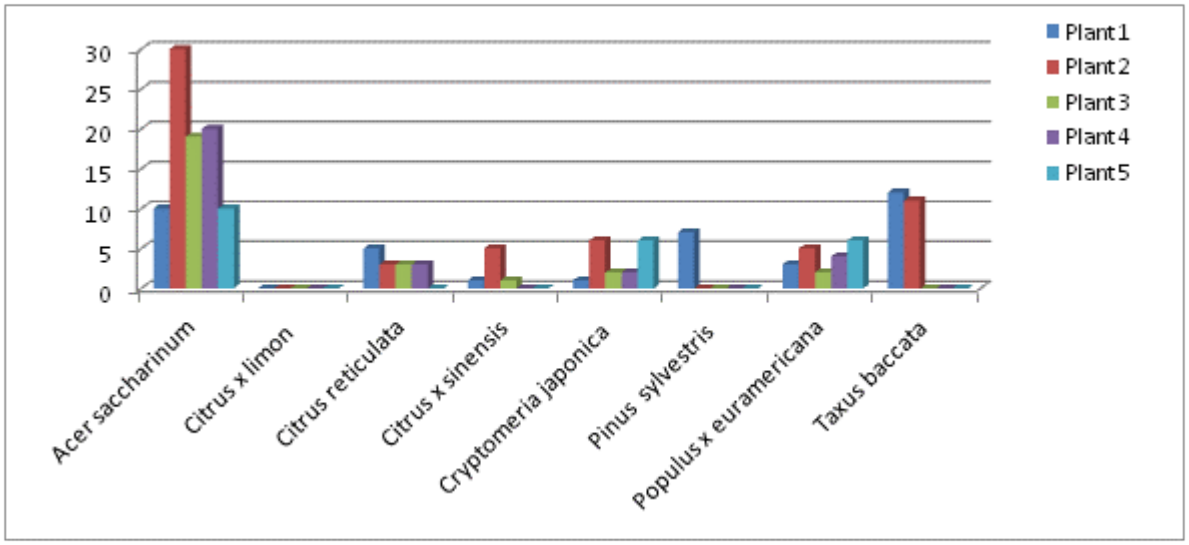


Fig. 7: Number of oviposition scars observed on the host plants.

All the signs were present on the basal portion of the trunk (Fig. 8), except on maple and poplar where they were distributed also on the upper part.

Fig. 8: Oviposition scars on *Taxus baccata*

Larval activity

Larval activity was registered as presence of sawdust at the base of the infested plant. Its presence was observed on 5 species (Fig. 9, 10, 11). Although oviposition scars were observed on 7 species, on 2 of them (*Taxus baccata* and *Pinus sylvestris*) no larval activity had followed.

Moreover, on *Acer saccharinum* the sawdust was present an all the plants, while on the other host plants larval presence was noticed only on some of them.

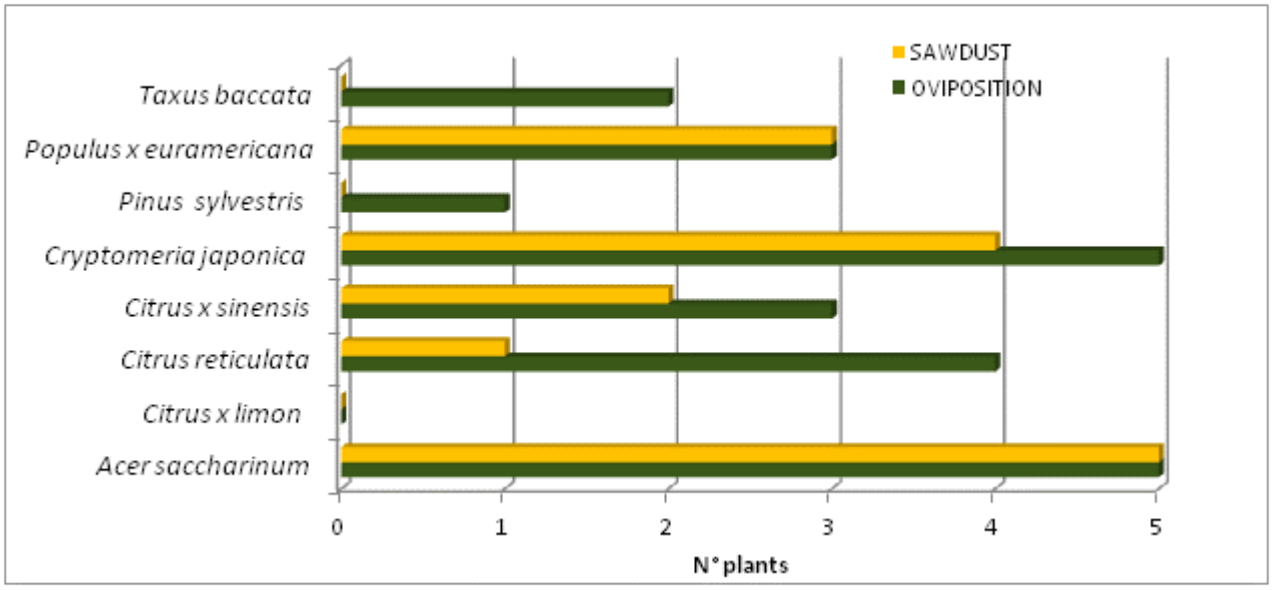


Fig. 9: Presence of oviposition scars and sawdust on the host plants.

Fig. 10: Sawdust on *Populus* x *euramericana*

Fig. 11: Sawdust on *Cryptomeria japonica*

Summary and discussion

Monitoring activities in European areas with *Anoplophora*-infestation and specific host plants-tests with both *Anoplophora* species revealed interesting new results about additional new host plants.

In Austria the deciduous tree species *Fagus* sp., *Fraxinus* sp. and *Alnus* sp. are new hosts of *A. glabripennis* which had not been known as host plants so far in Europe and partially also in the rest of the world. Alder (*Alnus* sp.) could also be an additional host where only oviposition scars and young larvae of *A. glabripennis* have been observed but no further development of them due to cutting of these trees (further development of these young larvae in the cut branches failed under quarantine laboratory conditions of BFW). An ALB-infestation of fruit trees has never been observed during a monitoring period of 11 years in Upper Austria with exception of one finding in a new infestation area in 2012 where one *Prunus avium* tree showed oviposition scars and starting larval galleries, but no larvae.

Specific testing of apple trees (*Malus domestica*, cultivar: Golden Delicious) showed that this fruit tree is a suitable host-plant for complete development of *Anoplophora glabripennis* (ALB) under controlled conditions, but it is not proved for natural field conditions. The complete development of ALB is even possible in trees with stems of only 3 cm diameter. There is a potential risk in apple production (Golden Delicious) in Europe in case of ALB-infestation - especially if there is a „lack“ of „better“ hosts.

The Italian testing of conifers and deciduous trees including *Citrus* species as suitable hosts for *A*. *chinensis* (CLB) showed clearly its polyphagy and adaptation to many tree species. In fact, it was possible to observe within the field trial that adults fed on all tested *Citrus* species (*C*. x *sinensis*, *C*. *reticulata* and *C*. x *limon*), on *Populus x euramericana* and *Acer saccharinum* and also on conifers (*Taxus baccata*, *Cryptomeria japonica aritaki*, but not on *Pinus sylvestris*), oviposition scars only were not present on *Citrus x limon* and larval activity was present on 5 of 7 tested species with signs of oviposition (*Populus x euramericana, Cryptomeria japonica aritaki*, *Citrus x sinensis*, *Citrus reticulata* and *Acer saccharinum*). Due to the start of the trials in 2012 it is only possible to verify the full development of CLB on these host plants in the next future, maybe in summer 2013 or 2014.

It can be concluded that deliverable **D4.1 (Information about additional unknown host plants of *A. glabripennis* and *A. chinensis* in Europe, especially if fruit trees are suitable hosts for ALB and coniferous trees for CLB)** was fulfilled during this project.

Concerning the achievement of deliverable **D4.2 (Review of the current knowledge on the dispersal behaviour of *Anoplophora* species; modelling the dispersal capacity of *Anoplophora* species in relation to biotic and abiotic conditions at an outbreak site)** it can be stated that it was partially fulfilled.

Preliminary results of an Italian study which was based on data of an infestation area in NE-Italy showed that it is unlikely that ALB will spread much farther than 2000 meters according with previously reported dispersal distance (Smith et al., 2001; 2004).

The Dutch team investigated the influence on dispersal of *Anoplophora* species by climatic conditions and estimated their lifecycles using an accumulated Degree Day Model. Thus, the development of *Anoplophora* beetles under Dutch conditions will take on average 3 years.

Main Conclusions

The obtained results and knowledge about new suitable host plants of *Anoplophora* species should be of high importance for monitoring and eradication measures in every infestation area in Europe and have to be considered when taking decisions on the surveys or eradication programs.

Papers, other publications and dissemination activities done

Oral presentation about preliminary results at the Austrian Plant Protection meeting (Österreichische Pflanzenschutztage 2011) in St. Pölten, 30.11.2011:

Lethmayer, C. & Hoyer-Tomiczek, U.: Asiatischer Laubholzbockkäfer (*Anoplophora glabripennis*) – auch eine Gefahr für den Apfelanbau in Österreich?

(= Asian long-horn beetle (*Anoplophora glabripennis*) – also a danger for the Austrian apple production?)

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Smith, M.T; Bancroft, J.; Li, G.; Gao, R. & Teale, S. (2001): Dispersal of *Anoplophora glabripennis* (Cerambycidae). - Environmental Entomology 30(6): 1036-1040.

Smith, M.T.; Tobin, P.C.; Bancroft, J.; Li, G & Gao, R. (2004): Dispersal and Spatiotemporal Dynamics of Asian Longhorned Beetle (Coleoptera: Cerambycidae) in China. - Environmental Entomology 33(2): 435-442.

Planned publication:

Favaro, R.; Wichmann, L.; Faccoli, M. & Ravn, HP. (2013: An invasion of Asian Longhorned Beetle (*Anaplophora glabripennis*) in Northern Italy (in prep).

Work Package 5: Investigating the potential efficacy of different management practices

Review of different management practices

Debbie Collins, Fera, UK

Summary

A desk study was undertaken to collate and interpret the current knowledge on the potential for controlling/managing infestations of *Anoplophora* spp (longhorned beetles). A review of the available information aimed to provide information on current management practices, identify relevant knowledge gaps, evaluate the potential of a range of chemical / treatment methods and evaluate the potential for utilisation of biological control.

There is a wealth of information on the variety of techniques that have been investigated to prevent, control and eradicate *Anoplophora* infestations, including cultural (e.g. siviculture, tree management, pest resistant clones), chemical (e.g. systemic, contact, fumigation), physical (e.g. heat, irradiation, exclusion) and biological (e.g. fungi, nematodes, bacteria, parasitoids) measures. Those that have received the greatest attention and which show the greatest potential for use in a practical situation, have been considered in this review. Eradication is the goal for all infestations, however an integrated approach is essential for that goal to be achieved. The main strategies that have been vital to achieving eradication of *Anoplophora* infestations include tree removal and chipping, the use of protective insecticides, and public involvement.

Further research should investigate maximising the efficacy of existing control measures and investigating some of the more novel control measures, such as effects on the beetles' metabolism. The use of fungal bands may offer the most promising use of biological control agents, with efficacy enhanced by combining with an attractant or pheromone. The potential exists to augment eradication efforts by combining different methodologies. Other areas to consider may include pesticide rotation to reduce the risk of pesticide resistance and the use of alternatives methods for the treatment of wood packaging.

Background

Insects belonging to the genus *Anoplophora* are wood-boring, longhorned beetles in the family Cerambycidae and are native to China, Japan, Korea and other Asian countries (Lingafelter and Hoebeke, 2002). The Asian longhorned beetle (ALB) (*Anoplophora glabripennis*) and the citrus longhorned beetle (CLB) (*Anoplophora chinensis*) are considered pests of great concern worldwide and are ranked as high risk quarantine pests in many countries (MacLeod et al., 2002). ALB and CLB pose an economic and ecological threat to horticulture, forestry and woodland trees in Europe with CLB also posing a risk to citrus production in the Mediterranean (Eyre and Giltrap, 2010). They are considered as

serious threats to urban and natural forests and as a result, both species are listed in the EU Plant Health Directive (2000/29/EC, 2002/36/EC) as harmful organisms whose introduction into, and spread within, all Member States is banned. In the EU emergency measures against CLB have been in place since November 2008 (2008/840/EC) due to the many interceptions and findings of CLB on *Acer* spp. imported from China and Japan (http://eur-lex.europa.eu/).

In Europe and North America, most ALB individuals have been accidentally introduced through international trade in wood packaging materials such as crating, pallets or packing blocks which are often constructed of raw wood material originating in Asia (Hérard et al, 2005). Most CLB individuals have been introduced in imported live woody plants, such as bonsais and nursery stocks, from China and Japan (Eyre et al., 2010). The pests often remained undetected until the goods have reached their final destination. The movement of firewood e.g. when used for camping or as a fuel source, can also serve as a vector in the transport of non-native species (Tobin et al., 2010).

In Europe, ALB and CLB have been found in 15 urban sites where they are considered as serious threats to urban and natural forests and are subject to eradication efforts (Hérard and Roques, 2009). In the U.K., sporadic interceptions of CLB have extended back as early as 1921, however since 2000, several breeding populations have been detected in Italy, France and The Netherlands (Eyre et al. 2010; Van der Gaag et al., 2010). Breeding populations of ALB have been discovered in Austria, France, Germany and Italy and as of November 2010, in The Netherlands (Hérard and Roques, 2009; EPPO, 2010).

ALB and CLB are highly polyphagous with dozens of broadleaved tree species from several families being reported as hosts in Asia, Europe and North America (Hérard et al, 2005; Pan, 2005). The most common trees infested by ALB include those in the genera *Acer*, *Betula*, *Populus*, *Salix* and *Ulmus* whereas the host range of CLB is broader and may include those in the genus *Corylus*, *Carpinus* and *Alnus* (Haack et al., 2010; Hérard et al., 2005).

The beetles attack either live, healthy or weakened and stressed trees, ranging in size from bonsais to mature trees. The larval stage is the most damaging as it feeds internally on the vascular systems of the roots, trunk, branches or twigs of its hosts. In the early stages of development the early instars feed in the phloem-cambium region and then bore into the xylem region in later instars. The resulting tunnels weaken the tree and may, over several years, kill it. Secondary infection may also occur as a result of pathogens entering through feeding wounds or exit holes. The adults cause more limited damage by feeding externally on foliage and bark before becoming sexually mature.

The lifecycle combines concealed immature stages, flying adults and a tendency to lay small numbers of eggs in several trees, thus making detection and prevention of spread difficult to achieve. Approximately 90% of their lifecycle is spent within trees as larvae and pupae, with the remaining 10% spent as a free-living adults, residing mainly in the mid to upper tree canopy. These beetles develop slowly and the larval stages may be present in trees for long periods (1-2 years in its native range; at least 2 years in northern Europe) (EPPO, 1999; Lingafelter and Hoebeke, 2002). Adults also emerge from wood asynchronously, over many months and can be long-lived (Hajek and Bauer, 2009). Detection is therefore often difficult until the tree shows signs of stress.

Wood-boring insects are difficult to control with conventional insecticides due to the cryptic behaviour of the larvae and pupae being protected within the tree. Once the adults emerge, they typically disperse high into tree canopies to feed making it difficult to reach all the adult beetle feeding or resting sites when cover sprays are applied to tree canopies. The favoured application method is through either trunk or soil injections. Systemic uptake can, however, result in an uneven distribution of low insecticide concentrations within trees (Poland et al 2006b). Systemic insecticides also travel up the tree through the xylem resulting in limited efficacy against phloem feeders such as the early instars.

Due to the lack of optimal control methods for wood boring beetles, infested trees, once detected, are often removed, chipped and burned either to eradicate an invasive pest or to reduce the population and remove hazardous trees. In forests, however, all infested trees cannot be removed necessitating the development of alternative control methods (Hajek and Bauer, 2009). The removal of infested trees has, however, been successful in containing the spread of the beetle.

Current eradication and preventative measures include quarantines, surveys, detection and destruction of infested trees and protecting un-infested trees with trunk or soil injections with systemic insecticides. Populations of ALB in North America are relatively low, but the US and Canadian governments have been willing to support costly eradication campaigns. In the US, the total eradication costs up to 30 September 2009 have been $US398 million (Warren et al., 2009) with >21,000 and >25,000 infested, at risk and potential host trees removed in the US and Canada respectively (USDA-APHIS; Natural Resources Canada). Total eradication is a long and costly process. In 2008, ALB was considered successfully eradicated in Chicago after 10 years at a cost of $US63 million (Warren et al., 2009). In the EU, as of December 2008, ALB and CLB control costs exceeded 3.3 million Euros (Hérard and Roques, 2009).

Pest management of *Anoplophora* spp. has focussed on preventing introductions through quarantine measures, eradication by control measures and forest management. Eradication is the goal for all infestations, however an integrated approach is essential for that goal to be achieved. There is a wealth of information on the variety of techniques that have been used to prevent, control and eradicate *Anoplophora* infestations (Haack et al., 2010). Many studies have been conducted in China, however, the relevant literature is poorly reported outside China because much of it is published in Chinese in journals that are not readily available. However Pan (2005) and Hu et al. (2009) have provided excellent reviews detailing the various studies undertaken in China which have included afforestation models, altering the shelterbelt structure and composition, the use of bait trees, using pest resistant varieties and clones and chemical and biocontrol applications.

It does appear, however, that there is no universal panacea on the effectiveness of the different techniques in various situations which is imperative for the development and implementation of effective pest management strategies. A desk study was undertaken to collate and interpret the current knowledge on the potential for controlling/managing the adults and larvae of *Anoplophora* spp.

The aims of this review were to:

1. Review the available information on management practices and attempt to fill relevant gaps.

2. Evaluate the potential of a range of chemical / treatment methods.

3. Evaluate the potential for utilisation of biological control and/or enhancement of natural enemies.

Databases and websites resourced

A thorough search of the published and grey literature provided the information detailed in this review. For a full list of the databases resourced, see Appendix 1.

Details on individual control techniques applied to growing trees and processed wood are described below, with the advantages and disadvantages of each method summarised in tables 1 and 2 respectively.

Cultural control

The use of cultural control methods and ecological forest management have been extensively used in China and are important approaches for maintaining populations of ALB below the economic threshold density (Hu et al., 2009; Pan, 2005). Forest management and diversification are, however, long term management techniques.

Tree planting

Planting forests with different tree species including non-host and "resistant" species (e.g. *Fraxinus* spp., *Quercus* spp., *Alnus* spp.) and trap trees (e.g *Populus* spp., *Acer* spp.) can regulate the structure of the forest, restrict the spread of ALB and protect high value trees (in Pan, 2005). Trap trees are used to lure ALB to lay eggs which are then destroyed. The recommended ratio between resistant tree, non-host tree and trap tree is 45-50% to 45-50% to 5-10%. However, trees that are resistant to ALB are often slow-growing and of less commercial value than preferred hosts and therefore an optimal economic and ecological forest design is required. This approach is limited in that it cannot be applied to areas already heavily infested by ALB (Hu et al., 2009).

By careful tree selection and tending, planted trees can grow faster and have greater vigour and tolerance which in turn results in the rotation of the tree being shortened and the chance of being damaged by ALB reduced (Pan, 2005). The annual growth should be more than 3cm in diameter which causes the eggs and larvae to be crushed by the fast growing trees. Some trees e.g. *Populus* spp., can also be harvested early, although cutting away the trunk and pruning can reduce the quality of the wood.

Grafting is one of the main methods that has been used for tree regeneration and ALB control in north-west China (Pan, 2005). Chinese white poplars have been grafted onto the original poplar stumps making use of the cut stump and reducing the costs of afforestation. Chinese white poplars grow very fast and have good resistance to ALB.

Some species and clones of poplar have been found to be highly resistant (81%) to ALB (Pan, 2005). By selecting and introducing specific resistant species and clones, poplar plantations can be established where ALB damage is reduced. Some species with coarse and keratose bark are also effectively resistant to ALB (Pan, 2005).

Tree management

Tree management is an important tool used to eradicate and restrict the spread of *Anoplophora* spp. Measures include sanitation cutting and felling, pruning, the removal of weak dead wood and the destruction of infested trees. The removal and destruction of infested trees is an integral part of *Anoplophora* eradication programmes. Initially all infested trees were cut down, chipped and burned soon after discovery of an infestation. However, chipping alone was found to be sufficient in killing nearly all life stages within the tree, thereby eliminating the need for burning (Wang et al., 2000). However, with CLB, 90% of the population is below ground level and therefore the roots must be removed to a depth of 40 cm (Hérard et al., 2005). The removal and destruction of infested trees is essential to reduce the chance of secondary infestations. As well as reducing *Anoplophora* populations, effective tree management also improves the condition and health of trees which may reduce subsequent infestations. The use of tree management for the control of *Anoplophora* infestations may be difficult to achieve in large forested areas but is feasible on a small scale.

Chemical control

A wide variety of insecticides have been evaluated against *Anoplophora* infestations in China, with application methods including placing insecticide impregnated sticks into larval sites, blocking larval holes with insecticide impregnated mud and direct spraying of adults (Pan, 2005). However the most widely used methods employ the injection of systemic insecticides into the trunk or soil.

Systemic Insecticides

Systemic insecticides are injected into the trunk or soil around trees and the compounds are translocated throughout the tree. Systemic applications target adult beetles that feed on midribs of leaves, leaf petioles and the bark of twigs and the larvae developing within the tree.

Poland et al. (2006a) found both azadirachtin and imidaclorprid to be effective against ALB in the laboratory. The highest larval mortality of 60% was achieved when fed with artificial diet treated with 50ppm azadirachtin and 160ppm imidacloprid. Complete mortality of adult ALB was achieved when fed twigs treated with 150ppm imidacloprid after 13 days (Poland et

al., 2006a). Both compounds also exhibited strong antifeedant effects. Subsequent field studies in China using azadirachtin, emamectin benzoate, imidacloprid and thiacloprid found imidacloprid produced the highest and most consistent mortality levels of ALB throughout the study although it was not completely effective (Poland et al., 2006b).

A range of neonicotinoids have been evaluated in the laboratory as potential systemic insecticides against ALB (Wang et al., 2003; 2005). Imidacloprid was shown to have most promise (Wang et al., 2003).

The neonictoinoid insecticide, imidacloprid, is the only insecticide used to treat at-risk trees in the USDA eradication program (Warren et al., 2009). It is applied systemically by injection into the soil or trunk, although current USDA ALB eradication programmes primarily use soil injection methods. Tree injections allow sufficient levels of imidacloprid to control ALB within 1-3 weeks whereas soil injections require 2-3 months (Warren et al., 2009). In the U.K., imidacloprid is registered for use on ornamental plants and hardy nursery stocks as a granular or spray application, but not for use in forestry (https://secure.fera.defra.gov.uk/liaison/secure/).

Systemic uptake can however, result in uneven distribution of low insecticide concentrations within trees leading to insects being exposed to sub-lethal doses (Poland, et al., 2006b). Imidacloprid concentrations in leaves steadily increase during the growing season with lower levels in the outer bark, phloem and fine roots, indicating that translocation occurs mainly in the xylem (Mota-Sanchez et al., 2009). This results in limited efficacy against phloem feeders, such as the early instars of ALB (Hajek and Bauer, 2009). Low levels are detected in the leaves in the following season after treatment (Mota-Sanchez et al., 2009), with repeat treatments required. Imidacloprid also acts as an antifeedant and sub-lethal doses could lead to increased adult dispersal (Warren et al., 2009). There are also concerns regarding the effect of imidaclorprid on non-target organisms, particularly bees.

Although the use of systemic insecticides can significantly increase larval and adult mortality, they are not 100% effective and should therefore only be used as one tool in a comprehensive management programme (Poland et al, 2006b). The continued reliance one type of insecticide with a specific mode of action may also lead to increased tolerance and resistance and a rotation of pesticides would be advisable. Alternative pesticides being considered by the ALB eradication programme in the U.S. include the avermectin, emamectin benzoate and two other neonicotinoids, clothianidin and dinotefuran (Warren et al., 2009). Emamectin benzoate has a higher toxicity to most non-target organisms when compared to neonicotinoids, and clothianidin and dinotefuran have similar characteristics to imidacloprid, so would have similar concerns (Warren et al., 2009).

Contact insecticides

The most widely adopted method for controlling ALB in China consists of spraying the pyrethroid cypermethrin in the canopies of host trees to kill adults (Hu et al., 2009). However it is difficult to reach all adult feeding or resting sites. Another method employs coating the trunks or branches of trees with a band of cypermethrin which takes advantage of the adults'

habit to walk along trunks after emergence and when searching for mates or moving between feeding and oviposition sites, thereby becoming contaminated with the insecticide.

Wang et al., (2003) evaluated a range of pesticides when applied to twigs to control adult ALB, including bifenthrin, deltamethrin, permethrin, acephate, chlorpyrifos, lindane, bendiocarb and fipronil. Complete mortality was recorded after two days, apart from with bifenthrin (0.02 ml/l) however mortality of the other compounds dropped when adults were exposed to twigs treated 2 weeks previously, indicating little residual effect (Wang et al., 2003).

A contact breaking microcapsule formulation of cypermethrin has aimed to prolong efficacy and reduce the environmental impact of pesticide treatments and has shown promising results with 90% beetle mortality, 38 days after treatment (Tang and Xia, 2000).

Lambda-cyhalothrin has been found to be almost 100% effective against ALB and is a useful detection measures and beetles that contact the insecticide are knocked down and fall from the trees (Peabody and Flores, 2006). An encapsulated formulation of lambda-cyhalothrin (Demand CS) provided 100% mortality of ALB for 90 days when applied to bands at 450mg a.i./L and 99% population control for 58 days when sprayed onto *Acer* trees at 300mg a.i./L (Smith et al., 2006).

In Italy, trunks and crowns of CLB infested and non-infested host trees have been sprayed with deltamethrin in Italy, three times a year (Van der Gaag, 2007). In France ALB infested trees were sprayed twice with bifenthrin to kill emerging adults during the summer (Hérard et al., 2005). In the U.K., contact insecticides that are registered for use in forests and on shrubs and trees include cypermethrin (α and λ), deltamethrin, bifenthrin, diflubenzuron and chlorpyrifos (https://secure.fera.defra.gov.uk/liaison/secure/).

Fumigants

Fumigation treatments are generally used to treat solid wood packaging materials (SWPM) but aluminium / zinc phosphide has been used in China to treat trees by inserting into galleries and filling with mud to fumigate larvae (Pan, 2005).

The use of pressure treatments with wood preservatives (ammoniacal copper quaternary compound, disodium octaborate tetrahydrate or a mixture of 30ppm imidacloprid and 300ppm 2-n-octyl-4-isothiazolone-3-one) have been evaluated against the new house borer (*Arhopalus productus*) for the treatment of SWPM (Schauwecker and Morrell, 2008). Larvae survived the initial treatment process, but no adults emerged (Schauwecker and Morrell, 2008).

The use of sulfuryl fluoride (SF) as a quarantine treatment for ALB and other wood borers has been assessed by Barak et al. (2006a,b; 2010). SF treatments at a dose of 104g/m^3 and temperature of 15.6^{o}C and above, and an achieved Ct product of 1095g-h/m^3 or above are recommended for the treatment of regulated wood packaging infested with ALB larvae and pupae (Barak et al., 2006a,b).

Higher doses of SF are required against *Agrilus planipennis* (emerald ash borer) (Barak et al., 2010). Completely effective CT dosages for eggs in chambers and larvae within ash logs were 3723 and 6072 g-h/m^3 after 24 and 48 hours exposure respectively at 15.6^oC, and 3172 and 4210 g-h/m^3 after 24 and 48 hours exposure respectively at 21.1^oC (Barak et al., 2010). Larvae within logs required higher doses than naked eggs which may have been due to reduced penetration of the fumigant through high density wood (Barak et al., 2010).

All the larval stages of ALB were completely killed after 6 hours exposure to 11 g/m^3 ethanedinitrile at 21-25^oC (Dowsett and Ren, 2007). At lower temperatures Ct products increased but were comparably lower that those of methyl bromide and carbonyl sulphide (Ren et al., 2006). In comparison with methyl bromide, sulfuryl fluoride and carbonyl sulphide, ethanedinitrile has high efficacy at high relative humidity and high CO_2 concentrations (Ren et al., 2006). The recommended application rate to timber and logs for export is 50g/m^3 for 6 hours, where commodity should be greater than 15^oC (Ryan et al., 2006).

Fumigation of pinewood blocks (10cm x 10cm x 30cm) with SF, phosphine and ethanedinitrile found that penetration was achieved to all parts of the blocks, but the speed and extent of penetration were different, with an even concentration most rapidly achieved with phosphine and ethanedinitrile (Ren et al., 2010). Use of these fumigants may prove useful in the EU where the use of methyl bromide for the treatment of wood packaging materials is prohibited.

Chemosterilants

Chemosterilants are chemical compounds that arrest or adversely affect reproductive capacity. Two chemosterilants, referred to as CSI and CSII, were sprayed onto adults of ALB, with CSII found to provide the best sterile effects in the laboratory, with a 72% reduction in the numbers of progeny in the field (Tang et al., 2001, 2005). The activity of chemosterilants may not be restricted to the target pest and may affect non-target organisms, however, combining with pest specific pheromones or attractants would ensure that only the target pest is affected (Smith et al., 1964).

Physical control

Various physical methods have been used in Asia to control ALB, including the collection of adults by hand, hammering egg sites, inserting wires into galleries to kill larvae and blocking oviposition sites and holes with cement (Hu et al., 2009; Pan, 2005). Although these manual techniques may be useful in bringing communities together and effective in maintaining ALB populations below threshold levels in urban areas, they are expensive and time and labour intensive (Hu et al., 2009) . Most physical control methods have been developed to treat wood products.

Heat

It is known that oviposition and egg hatch in ALB is prevented at ≥ 35°C (Keena, 2006) and that high temperatures can be used to control pest populations. The 2002 FAO International Standard for Phytosanitary Measures (ISPM) no. 15 requires that solid wood packaging material (SWPM) be fumigated or heat treated prior to export. Heat treatments of wood packaging require a minimum wood core temperature of 56°C for a minimum of 30 minutes. However a higher temperature of 65°C for 30 minutes was required to treat logs infested with the emerald ash borer, *Agrilus planipennis*, (Nzokou et al., 2008).

Microwave

Blocks of poplar wood (4x4x4 inch) artificially infested with ALB larvae and pupae were treated with 2.45GHz microwave energy irradiation and reached 60°C within 0.5 to 5 minutes of irradiation (Fleming et al., 2003). Preliminary results showed that between 30 seconds and 3 minutes of irradiation at 900 watts were lethal to larvae and pupae of ALB and may provide an alternative to fumigation, heat or preservative treatments (Fleming et al., 2003).

Microwave treatments were not as effective as kiln treatments for the treatment of logs infested with *A. planipennis* (Nzokou et al., 2008).

Irradiation

Irradiation has been used as a method of sterilising adult ALB (Chen and Xie, 2004; Li et al., 2006; Lu et al., 2001). Control was achieved by irradiating adults with γ-rays at a dose of 1.5-1.75 Gy/min, 7-10 days after emergence (Li et al., 2006; Lu et al., 2001). Field tests showed that the population could be suppressed with a release ratio of 5:1, however a major drawback is the problem of mass artificial rearing of the species in order to provide sufficient numbers of insects to be irradiated (Lu et al., 2001). Irradiation was fatal at a dosage of 0.776kGy (Chen and Xie, 2004).

Irradiation of third to fifth instar larvae of ALB with 20-140 Gy, resulted in prolonged developmental periods with the fifth instar the most resistant (Wang et al., 2006). Irradiation at a dose of 60 Gy also prevented the mature larvae pupating and was suggested as the minimum effective dose for quarantine treatment of ALB larvae in logs and wood packing materials (Wang et al., 2006).

Vacuum

Low pressure vacuum creates a controlled atmosphere and desiccating environment which is lethal to a range of wood borers with mortality occurring when percentage body weight loss exceeds 40% (Chen et al., 2005; 2008). The lethal vacuum time varies with species with 51 hours required at 20mmHg at 20°C for ALB larvae, 45 hours for *A. planipennis* larvae and 66 hours for *Hylotrupes bajulus* (old house borer) larvae, when assessed in the laboratory (Chen et al., 2005; 2008). Low pressure vacuum also killed ALB eggs and pupae (Chen et al., 2008). Temperature, pressure and relative humidity affect desiccation rates with slower

desiccation occurring with decreasing temperature, increasing pressure and increasing wood moisture (Chen et al., 2008).

Electric currents

The application of a high voltage electrical current to poplar trees was effective in killing first to third instar larvae of *A. nobili* and trees infested with ALB (Pan, 2005). The application may also show promise for treating wood products as part of quarantine measures.

Mechanical exclusion

Various methods have been assessed to prevent oviposition by CLB on citrus trees, including the spraying bottoms of trunks with whitewash containing fenitrothion (15%), loosely covering the bottoms of trunks with sticky cardboard, covering bottoms of trunks with fishing net (2cm mesh) and covering bottoms of trunks with fine wire mesh (6 openings/cm) and piling up soil around base (Adachi and Korenaga 1989; Adachi, 1990).

The use of the fine wire mesh was the most effective method in preventing oviposition (Adachi and Korenaga 1989). Efficacy was further enhanced by piling up soil around the base of the trunk between the ground and the net (Adachi, 1990). The base of trunks is the favoured oviposition site for CLB, therefore by physically preventing egg laying to this area, infestation can be reduced (Adachi, 1990). Although this method may be effectively utilised in restricted areas such as citrus groves, the practicality of its use in natural woodland may be reduced, although it may prove useful when applied to susceptible host trees in urban areas.

Ultraviolet light

The development of a push-pull strategy combining yellow lighting with UV kill traps was effective against another Cerambycid, *Arhopalus ferus* (Pawson and Watt, 2009). This species was strongly attracted to ultra violet light and light trapping may be useful in areas where wood is processed.

Biological control

There are many natural enemies of longhorned beetles, including predators, parasitoids and pathogens.

Fungi

A comprehensive review on the development and use of entomopathogenic fungi for the control of wood boring beetles is given by Hajek and Bauer (2009). Entomopathogenic fungi in the genera *Beauveria, Isaria* (=*Paecilomyces*) and *Metarhizium* have been isolated from larvae and adults of ALB in China (Shimazu et al., 2002) and North America (Poland et al., 2003). Twenty isolates of four species of entomopathogenic fungi (*Beauveria bassiana,*

Beauveria brongniartii, Isaria farinose and Metarhizium anisopliae) have been found to be pathogenic to adults of ALB (Dubois et al., 2008). The *B. brongniartii* and *M. anisopliae* isolates killed ALB adults in the quickest time (survival times for 50% of the beetles (ST_{50}) = 5 days) (Dubois et al., 2008).

The development of ALB control using fungi, has focused on the use of non-woven fiber bands impregnated with fungal cultures as fungal sprays applied to trees have proved less effective (Dubois et al., 2004a, b). This application technology was originally developed in Japan for the control of native cerambycids attacking orchards (Higuchi et al., 1997). It is generally targeted towards the adults, as the location of the larvae within the tree makes contact with the fungi difficult to achieve and fungal virulence has been shown to be lower against larvae (Shimazu et al., 2002). However, as well as mortality effects against adults, entomopathogenic fungi have also been shown to have indirect effects by affecting female fitness by decreasing female longevity, decreasing oviposition before death and through horizontal transmission to offspring (Hajek et al., 2008).

The fungal bands are wrapped around trunks and branches when adults reach adulthood and start to emerge from trees. As the adults walk over the bands they are contaminated with conidia. Optimal exposure occurs during the pre-ovipositional period when infection can prevent or reduce oviposition (Hajek et al., 2008). Bands inoculated with isolates of *B. brongniartii* and *M. anisopliae* have proved effective against adult ALB in laboratory and field experiments (Dubois et al., 2004a, b; Hajek et al., 2006, 2008). Bands have maintained virulence in the field for at least 112 days and can also be stored at 5^{o}C for >1year and still remain active in the field for at least one month (Hajek and Bauer, 2009; Higuchi et al., 1997; Shanley and Hajek, 2008; Shanley et al., 2009).

The efficacy of the fungal bands does, however, rely on the adults coming into direct contact with the fungi. Development of an attractant to increase conidial uptake would therefore enhance efficacy. ALB males produce a pheromone that consists of a blend of two dialkyl ethers (Zhang et al, 2002) which attracts females, especially virgins although they do not seem to be involved in sex recognition (Nehme et al., 2009; Zhang et al, 2002). A long-range female pheromone from virgin ALB beetles was found to increase male trap catch (Wickham and Teale, 2009). A contact sex pheromone from female CLB was found to be comprised of three gomodalactones (Mori, 2007; Yasui et al., 2007). Several species of cerambycids also use plant compounds rather than long-range pheromones as mediators for mate location (Nehme et al., 2009). The use of male-produced pheromone components and plant volatiles (linalool, (Z)-3-hexen-1-ol, linalool oxide, *trans*-caryophyllene and *trans*-pinocarveol) were found to significantly increase trap catches of females compared to control traps and traps baited with pheromone alone (Nehme et al., 2010). Therefore incorporation of such attractants into fungal bands may increase conidial uptake.

The use of fungal bands may prove useful in protecting valuable urban/suburban trees in conjunction with other eradication procedures and has been successfully used in a forest situation on a large scale in China, against another cerambycid *Monochamus alternatus* (Li et al., 2007). The numbers of bands required per tree or area would need to be optimised to ensure maximum efficacy. Environmental conditions are also known to affect conidia viability in the field, with high temperatures (>30^{o}C) and low humidities (<90%) reducing efficacy (Dubois et al., 2004a). Also exposure to full sunlight drastically decreases the half-life of

entomopathogenic fungi (Khetan, 2001). Improving the fungal formulation to reduce environmental degradation and maintain viability over long periods should be investigated.

Synergism between *Metarhizium brunneum* and imidacloprid was demonstrated against adult ALB with a decrease in survival time when used in conjunction compared to when the treatments were used alone (Russell et al., 2010). This is an important consideration as the potential exists to augment ALB eradication efforts by combining imidacloprid tree injections with fungal bands.

Further development of *B. brongniartii* in the U.S.A. was suspended as it was unclear whether the species was native to North America (Dubois et al., 2008) however isolates of *B. brongniartii* are registered as microbial control agents in Europe, Asia and South America for other uses. Virulence of fungi against larvae is, however, reduced (Dubois et al., 2008; Shimazu et al., 2002) although this use may not be appropriate due to their location within the wood.

Nematodes

Entomopathogenic nematodes of the families Steinernematidae and Heterorhabditae have the ability to locate and kill insects in cryptic environments and can target insects beneath tree bark and in larval chambers making them potentially suitable control agents (Begley, 1990; Solter et al., 2001; Qin et al., 1988). The presence of nematodes in dead ALB removed from infested trees and field studies using nematodes in China (Qin et al., 1988) suggest that the environment in larval galleries is suitable for nematode development and host seeking.

A range of nematode isolates including *Steinernema feltiae*, *S. glaseri*, *S. riobrave*, S. *carpocapsae* and *Heterorahabditis marelata*, *H. bacteriophora* and *H. indica* have been assessed against ALB larvae in the laboratory and field (Fallon et al., 2004; Qin et al., 1988; Solter et al., 2001), with *S. feltiae*, *S. carpocapsae* and *H. marelata* the most effective (Fallon et al., 2004; Solter et al., 2001).

Larval resistance to nematode infection increases as larvae mature with first instars the most susceptible and seventh instars the most resistant (Fallon et al., 2004). Later instars have a more robust cuticle which may affect penetration by the nematodes although the important entry route through spiracles, would not be affected by the robustness of the cuticle. There does not appear to be a strong host immune response (Solter et al., 2001).

Injection into oviposition sites or larval tunnels may be the most effective application method for *Steinernerma* species, particularly *S. feltiae* due to its attraction to the host, host seeking capability, a relatively low lethal time and activity at lower temperatures (Fallon et al., 2004). However the practicality of treating infested trees would need to be determined and other methods may need to be evaluated. The impact of the environmental conditions on field released nematodes would be significant as seasonal conditions change and more information is required as to efficacy.

Parasitoids

The larvae of *Dastarcus helophoroides* (Fairmaire) (Coleoptera: Bothrideridae) are ectoparasites of late instar larvae, pupae and young adults of ALB in its native China (Wang et al., 1996). Eggs are laid on the outer surface of the bark near host entrance holes or larval tunnel walls and once hatched, the first instar larvae actively move to locate and parasitize their hosts. It has been found to parasitize and kill and much as 60% of ALB, with one late-instar ALB larva able to support the development of 10-35 *D. helophoroides* larvae, with death occurring within 10 days (Qin and Rao, 1988). Studies have assessed its efficacy as a biocontrol agent and determined that (R)-(+) limonene is an important kairomone in host location (Qin and Gao, 1988; Wei et al., 2008). Field applications of limonene may therefore enhance the efficacy of *D. helophoroides* as a biocontrol agent. An artificial diet has also been developed for *D. helophoroides* (Ogura et al., 1999) which may allow for mass rearing of the parasitoid for use in biocontrol programmes.

Aprostocetus fukati (Miwa and Sonen) and *A. anoplophorae* (Delvare) (Hymenoptea: Eulopidae) are egg parasitoids of CLB. *Aprostocetus anoplophorae* was recovered from CLB specimens in Italy and is believed to be of Asian origin and most likely accidentally imported into Italy along with its host (Delavare et al., 2004). It is quite specific to CLB and is now strongly established in most CLB infestations in Italy. A larval parasitoid, *Ontsira anoplophorae* (Hymenoptera: Braconidae) has been found associated with CLB and is considered to be a potential biological control agent for several species of *Anoplophora* (Smith, 2000).

The exploration, identification and evaluation of natural enemies in China and Far East has been limited, therefore there may be an abundance of as yet undiscovered natural enemies. This may be particularly true in forested areas of northeast China where ALB is said to be endemic as a result of natural control (Smith, 1999). Biological control is likely to focus on the egg and early larval stages of ALB as they represent the more vulnerable stages in the life cycle (Smith, 2000). A successful biocontrol programme will rely heavily on the integration of several control measures. Successful biocontrol may lie in the development of new associations between natural enemies and ALB or from the introduction of exotic natural enemies from China.

In Italy, surveys found eight native Hymenopteran species using early instar CLB larvae as hosts: *Sclerodermus* sp. (Bethylidae), *Spathius erythrocephalus* (Braconidae), *Calosota agrili* and *Eupelmus aloysii* (Eupelmidae), *Eurytoma melanoneura* and *E. morio* (Eurytomidae) and *Cleonymus brevis* and *Trigonoderus princeps* (Pteromalidae) (Herard et al., 2007). Experimental field tests using ALB larvae recovered seven of the above eight species with *S. erythrocephalus* and *T. princeps* the most frequent (Herard et al., 2007). Although *S. erythrocephalus* and *T. princeps* are robust and effective parasitoids, their broad host preference among xylem-inhabiting beetles probably eliminates them from consideration as biocontrol agents (Herard et al., 2007).

Parasitic mites in the genus *Tetrapolipus* have been reported from *Anoplophora lucipor* (Husband, 2008).

Predators

Woodpeckers that predate on larvae contribute to the natural control of ALB in China and are encouraged to nest in susceptible woodland, where they are reported to reduce populations by 30 to 80% (Pan, 2005).

The common house spider *Achaeranea tepidariorum*, and some ant species have also been shown to predate on longhorned beetles (Morewood et al., 2003; Smith, 1999).

Viruses

An ALB specific virus has been discovered (Peabody and Flores, 2006).

Bacteria

Bacillus thuringiensis var. *tenebrionis* and *B. thuringiensis* toxins were assayed against larval and adult ALB (D'Amico et al., 2004). In vitro tests indicated that Cry 1b toxin may be effective in vivo, however, no significant effects were shown on larvae and adults (D'Amico et al., 2004). It was suggested that ALB midgut chemistry may be incompatible with toxin activation or mode of action (D'Amico et al., 2004). For most wood boring beetles the most effective deployment of *Bt* may require expression of their Cry toxin genes in transgenic trees (Hajek and Bauer, 2009). However, another *B. thuringiensis* strain, Bt886, isolated from the dead body of a *Tribolium* sp., caused 60% mortality and significant delay in growth when fed to ALB larvae (Chen et al., 2004).

A bacterium isolated from the egg of ALB, was identified as *Serratia marcescens* and was to be lethal to the early larval instars (Deng et al., 2008). Larval mortality ranged from 16.1% to 80.6% following inoculation with $2.0x10^{7}$ to $7.8x10^{10}$ to of the bacterial suspension (Deng et al., 2008). In a field experiment, wood trunks were sprayed with the bacterial suspension and adult pairs were confined to the trunk for oviposition. After two months larval mortality was found to be only 5.3% (Deng et al., 2008).

Microsporidia

Microsporidia have been isolated from the epithelial cells in the midgut of ALB larvae collected in China and are thought to be most related to the *Endoreticulatis-Eterocyozoan* taxonomic group (Bauer et al., 2003). Zhang et al. (2003) also described *Nosema glabripennis* from ALB which was capable of vertical dissemination. Microsporidia typically increase host mortality, prolong developmental period and reduce adult longevity and fecundity (Bauer et al., 2003).

Potential for Biological control

A successful biocontrol programme may lie in the development of new associations between natural enemies and the pest or from the introduction of exotic natural enemies from their native range. Whatever strategy is employed, its effectiveness will rely heavily on the integration of several control measures.

Natural rates of ALB increase and spread appear to be relatively low, both of which tend to improve the potential success of natural enemies to regulate pest populations (Smith, 1999). However, the exploration, collection and identification of natural enemies of ALB and CLB has been limited, and of those identified few have received thorough investigation and have not yet been fully developed for use as biological control agents. Therefore there may be an abundance of as yet undiscovered natural enemies. This may be particularly true in forested areas of northeast China where ALB is said to be endemic as a result of natural control (Smith, 1999).

As fairly recently introduced exotic pests, the potential natural enemies of *Anoplophora* spp. may not have had the time to develop the close associations required to be considered effective (Smith, 1999). The rich tree species diversity of areas where ALB is found in the U.S. is thought to provide a potential source of natural enemy fauna, whether from the development of new associations between native enemies and ALB or from the introduction of exotic natural enemies from China (Smith, 1999). The identification of native natural enemies of species whose hosts are similar/related to ALB (e.g. genetically, ecologically and behaviourally) may indicate that they are more able to adapt to different hosts. The tree species richness should also minimise the probability that ALB will develop mechanisms to overcome any natural occurring tree defences.

The natural enemy that shows considerable promise in biological control is the cylindrical bark beetle, *D. longulus*. In Chinese locations where *D. longulus* is established in relatively high numbers, ALB is said to be under natural control (Pan, 2005). It was under investigation for future potential introduction in the U.S (Smith, 1999). The use of fungal bands may offer the most promising use of biological control agents. However efficacy relies on the adult beetles coming into contact with the band to pick up the fungal conidia and viability may be affected by environmental conditions. Combining with an attractant or pheromone may enhance efficacy.

There are numerous advantages to using microbial control agents, particularly for wood-boring beetles. Due to a lack of optimal control methods, infested trees, once detected, are removed and destroyed either to destroy an existing infestation or remove potential susceptible hosts. The removal of valuable urban and suburban trees can be avoided by individual treatment and the use of microbials. Also the removal of all infested trees in a forest may not be possible so alternative control methods are required (Hajek and Bauer, 2009).

The development of methods for the microbial control of *Anoplophora* spp. are constrained by the difficulties in controlling wood boring beetles. However the levels of control provided by pathogens (often not 100% immediate mortality) would be appropriate because it takes many beetles to kill individual trees over extended periods (Hajek and Bauer, 2009). The risk to non-target organisms should also be considered.

Quarantine measures

The potential of solid wood packaging material (SWPM) to harbour non native insects led to the development of the 2002 FAO International Standard for Phytosanitary Measures (ISPM)

no. 15 (FAO, 2002). SWPM is a common pathway by which ALB has been transported beyond its native range. Approved measures for the treatment of wood packaging are heat treatment (minimum wood core temperature of 56^{o}C for a minimum of 30 minutes) and fumigation with methyl bromide (minimum temperature should not be <10^{o}C and minimum exposure time should be 16 hours e.g. 14g/m^{3} for 16 hours at 21^{o}C or above). The requirements for SWPM entering the EU came into force on the 1st March 2005 (Directive 2004/102/EC) and are based on ISPM no. 15. However as of 19 March 2010, following an EU Commission Decision (2008/753/EC) of non-inclusion of methyl bromide in Annex I of 91/414, methyl bromide treatment of SWPM is illegal throughout the EU with heat the only approved treatment for SWPM.

However, it must be considered that there is still the potential for insects to colonise and develop in wood after heat and fumigation treatments as there is no residual effect (Haack and Petrice, 2009). Other methods being considered for approval (when appropriate data becomes available) under ISPM no.15 include, but are not limited to the fumigants, phosphine, sulfuryl fluoride, carbonyl sulphide, chemical pressure impregnation processes using high pressure/vacuum, double vacuum, hot and cold open tank, and sap displacement and irradiation with gamma radiation, x-rays, microwaves, infrared, electron beam treatments and controlled atmospheres (FAO, 2002).

The movement of firewood e.g. when used for camping or as a fuel source can also serve as a vector in the transport of non-native species and the regulation of such movement would be challenging (Tobin et al., 2010). As of April 2011, Directive D-11-01 will be enforced by the Canadian Food Inspection Agency specifying the phytosanitary requirements for plants for planting and fresh decorative branches that are considered hosts of *Anoplophora* spp (CFIA, 2011).

Novel methods

Wood boring insects require gut microbes to aid in wood degradation and digestion with bacteria in the gut of ALB responsible for the breakdown of lignin (Bugg et al., 2010; Schloss et al., 2004). Larvae that fed on wood from a resistant tree host showed suppressed gut cellulose activity (Geib et al., 2009; 2010). Understanding the role of the gut microflora and enzymes involved in cellulose degradation could lead to novel control measures that disrupt the insects' metabolism and development.

Protein inhibitors act by binding directly with the proteinases in the insect gut, thereby reducing the insect's digestive capacity and causing a reduction in the availability of amino acids necessary for growth and development, or by interfering with important biochemical or physiological processes, such as proteolytic activation of enzymes or moulting. Li et al. (2010) identified the gene for a cysteine protease inhibitor and cloned it into bacterial vectors. The engineered bacteria was tested against ALB larvae and found to produce 46% mortality (Li et al., 2010). The potential of this technique is being further investigated.

Ice nucleating active (INA) bacteria increase an insect's susceptibility to low temperatures and are thought to promote the formation of ice causing freezing throughout the insect

(Fields, 1993). Sun et al., (1997) applied INA bacteria to the larvae of ALB and found that the mean freezing temperatures were increased by 7^{o}C and 5.6^{o}C when sprayed and fed to the larvae respectively compared to controls. There is potential for this method to reduce populations of overwintering larvae.

Proteins extracted from entomopathogenic fungi have been found to be highly toxic against the larvae of another wood borer, the Japanese pine sawyer (*Monochamus alternatus*) (Li et al., 2005) and this may have potential against *Anoplophora* spp.

Pheromones and host attractants of *Anoplophora* spp. are poorly understood, so allelochemical lures are inefficient or unavailable. ALB have been found to produce oviposition detererrent pheromones which are comprised of four components, although the chemical composition is unknown (Lund, 2003). The use of such pheromones in a push-pull strategy with an attractant, could be used as part of an integrated control strategy (Lund, 2003). Sex pheromones have also been identified which if used in conjunction with other methods e.g. trapping or fungal bands could enhance efficacy.

It is known that some tree species are more resistant to insect attack than others. Host tree resistance to ALB may be due to physical mechanisms, such as abundant sap flow or due to the chemical composition of the tree which may include compounds that are toxic or which interfere with normal growth and development of the beetle or which have repellent properties (Morewood et al., 2004; Tang et al., 1999). Tang et al, (1999) found that compounds from *Ailanthus altissma* (tree of Heaven) were repellent to adult ALB. The identification and manipulation of such compounds have the potential to be used to protect more vulnerable trees (Morewood et al., 2004). Repellents will not kill insects and may allow for other trees to be attacked. To increase efficacy, repellents should be used in combination with other control measures (Tang et al, 1999).

Impact of pesticides on non-target organisms

Imidacloprid is the only insecticide used to treat at-risk trees in the ALB USDA eradication programme and is applied by soil or trunk injection, although soil injection is the primary treatment method (Warren et al., 2009). Imidacloprid comes from a group of insecticides, the neonicotinoids, which target and block acetylcholine receptor pathways and inhibit functioning of the nervous system. Neonicotinoids are more selective against insects than mammals and therefore possess high insect toxicity and low mammalian toxicity. Application of imidacloprid to treat individual trees entails very high dosages of insecticide per unit area, placed as point-sources near the base of trees (Cowles, 2009). In Austria horse chestnut trees died after tree injection with imidacloprid thought to be due to phytotoxic effects (Van der Gaag, 2007). As a systemic insecticide, imidacloprid must have sufficient water solubility to be taken up by fine roots and later be transported through the tree (Cowles, 2009). However, this water solubility could allow leaching to groundwater although its high organic binding capacity should prevent in leaching through highly organic soils in forests. Soil injection of insecticides is not possible in the Netherlands due to the high ground water level (Van der Gaag, 2007). To minimise the risks of environmental contamination, a combination of optimum dosing of trees and pesticide formulation selection should be utilised (Cowles,

2009). The mobility of imidacloprid in soil could be considerably reduced by the use of controlled release formulations (Pradas et al., 1999).

Imidacloprid poses the largest risk to honeybees which exhibit a significant susceptibility at a wide range of doses (Warren et al., 2009). The method and timing of applications (e.g. not during periods of high bee activity) should be taken into consideration when applying imidacloprid in order to reduce potential effects. The USDA-APHIS produced a questions and answers fact sheet regarding the use of imidacloprid for the control of ALB and its potential effect on bees (APHIS, 2009). It is generally considered that although harmful to bees at certain concentrations (higher than expected to occur under realistic exposure scenarios), its use within an ALB infestation programme does not pose a significant risk to bees or other pollinators. Current research is underway to investigate specifically how the treatment of trees with imidacloprid relates to the health of bees.

Risks to other non target organisms are variable depending on taxa but is generally considered as low although care should be taken to reduce exposure to aquatic invertebrates (Warren et al., 2009). Because of the small area treated, impacts to sensitive species will be localised and not widespread (Warren et al., 2009).

Kreutzweiser et al., (2008, 2009) assessed the effects of imidacloprid concentrations in the fallen leaves from imidacloprid-treated maple trees on non-target decomposer organisms. Imidacloprid in maple leaves at realistic field concentrations (3-11mg kg^{-1}) did not affect the survival of aquatic leaf-shredding insects or litter dwelling earthworms. However adverse sub-lethal effects were detected with reduced feeding rates, decrease in leaf decomposition, weight loss in earthworms and significant inhibition of aquatic and terrestrial microbial decomposition activity (Kreutzweiser et al., 2008, 2009). Also the leaching of imidacloprid from soil applications into water bodies is likely to be 10 times more toxic to leaf shredding insects than foliar concentrations (Kreutzweiser et al., 2007). The use of imidacloprid as a systemic insecticide will pose less risk of harm to non-target aquatic decomposers if applied as trunk injections than when applied as soil injections (Kreutzweiser et al., 2007).

The extent to which leaves from imidacloprid-treated trees will pose a risk of harm to non-target decomposer invertebrates and litter breakdown processes will depend on the level of exposure (Kreutzweiser et al., 2009). However this potential risk of harm should be considered in the larger context of the ecological risk if large areas of trees are not treated and become infested and die or are cut down as part of a pest eradication strategy (Kreutzweiser et al., 2009). Alternatives to imidacloprid are being considered not only due to the effects on bees but also the potential for resistance. A rotation of chemicals with different methods of activity should be considered.

Environmental contamination may also occur from the use of fungal bands (Shanley and Hajek, 2008) and this should also be considered in relation to the effects on non-target organisms. Shanley and Hajek (2008) found that viable conidia of *M. anisopliae* were mostly contained on the fungal bands and nearby tree bark, which would minimise the impacts on non-target arthropods. A significant correlation was found between rainfall and the occurrence of conidia on bark indicating that rain may be an important mechanism for conididal movement (Shanley and Hajek, 2008).

Eradication programmes

Eradication is the goal for all *Anoplophora* infestations. There is no one tool that can be used to eradicate infestations, but a combination of several management strategies, used in an appropriate manner, can effectively contain and eliminate the problem. Infestations can be highly variable therefore it is difficult for general guidelines to be proposed (Van der Gaag et al., 2010). The key to any strategy is to determine the source of the infestation, the outer edges of the infestation and approximate size, as soon as possible (Van der Gaag et al., 2010). This information can then be used to decide on tree removal, establishment of a buffer zone and surveying.

In the U.S., removal of infested or at-risk host material reduces ALB populations and is the most important mechanism for eradication the pest (Warren et al., 2009). Since the beetle spends most of its time within the tree, removal and destruction of host material by chipping inhibits completion of its development. Application of imidacloprid through trunk or soil injections protects at-risk trees from infestation and kills adults feeding on treated leaves and twigs and young larvae feeding in the phloem area. Public involvement in the detection and reporting of potential pest problems is also integral to the eradication campaign.

In North America, eradication programmes have included:

- Quarantine zones - established around infested areas to prevent accidental spread.
- Cutting, chipping and burning of infested trees – all infested trees are removed, chipped in place and the chips are burned. The stumps are ground to below soil level.
- Insecticide treatments – treating of non-infested and potential host trees with trunk injections of imidacloprid within 200-800m radius of infested trees.
- Extensive surveys – all host trees within a distance from an infested area are surveyed. Infested areas are re-surveyed at least once a year for 3-5 years after the last beetle or infested tree is found.
- Shipping restrictions – the use of SWPM no. 15 are regulated for adequate treatment methods at certain ports.
- Public awareness and education.

The three strategies that were vital to the eradication of ALB in Chicago, Illinois and Hudson County in the U.S. were: tree removal and chipping, use of protective insecticides, and public involvement (Warrant et al., 2009). In April 2011, the USDA treated 96,000 non-infested ALB host trees in Massachusetts with trunk injections of imidacloprid. All trees showing signs of ALB infestation were removed and destroyed. Public meetings were held to inform citizens about the treatments and property owners were contacted within the treatment areas to obtain signed treatment releases. Property owners were encouraged to support the treatment of host trees (USDA news release (9 March 2011).

The EU Commission Decision 2008/840/EC requires that where an infestation of CLB is discovered a buffer zone of at least 2km should be demarcated around the infested area. Official measures to be taken inside demarcated areas include at least the felling and destruction of infested trees and trees showing signs of infestation, including the roots and intensive monitoring of CLB in infested and buffer zones, annually on host plants at appropriate times.

In Europe, contingency plans for dealing with *Anoplophora* outbreaks include:

- Identification of pest species
- Import inspections / ban on plant movement.
- Surveys for early detection – all host trees are monitored with 1km area of risk location.
- Trace-backward/forward surveys
- Quarantine boundaries – resurveyed twice a year for two years.
- Monitoring
- Cutting down and destruction of aerial portions of infested trees.
- Removal and destruction of infested trees – preferably during cool part of day before beetles are active. Burn or chip at location.
- Stump removal - with ALB stump does not need to be removed; with CLB uprooting is needed (up to 40cm). Herbicide treatment to kill the stumps or by covering with wire mesh, soil or concrete.
- Removal of all host trees within a 150-400m radius.
- Spraying of pesticides. E.g. trunks and crowns of infested and non-infested host trees are sprayed with deltamethrin in Italy, three times a year.
- Early communication with stake holders and members of public.
- Replanting – with host trees within quarantine area to act as trap trees.
- Shipping restrictions – the use of SWPM no. 15 are regulated for adequate treatment methods at certain ports.

Conclusions

Pest management of *Anoplophora* spp. has focussed on preventing introductions through quarantine measures, eradication by control measures and future protection by forest management. Eradication is the goal for all infestations, however an integrated approach is essential for that goal to be achieved. There is a wealth of information on the variety of techniques that have been investigated to prevent, control and eradicate *Anoplophora* infestations, all of which could not be included in this review, however those that have received the greatest attention and those which show the greatest potential for use in a practical situation, have been considered.

No one control method is completely effective. Cultural control methods have been effectively used in China but they are long term management techniques and require time before the effects become evident. The removal and destruction of trees effectively contains the spread of the pest but is difficult to achieve on a large scale, affects the environmental landscape and causes public concern. Direct control with chemical or microbial insecticides is problematic because adults are mobile and long-lived and the immature stages are protected with the tree. Applications of systemic insecticides are effectively used in eradication programmes, but they are not completely effective, distribution within trees may be variable and there are issues regarding effects on non-target organisms. The use of contact sprays to treat tree canopies, target the adult stage but it is difficult to achieve complete coverage of all feeding and resting sites. The use of biocontrol agents can specifically target all life stages and are suited to the pests' biology, but they are not completely effective and may be affected by environmental conditions and require specialised application techniques. The use of fungal bands may offer the most promising use of biological control agents. However efficacy relies on the adult beetles coming into

contact with the band to pick up the fungal conidia and viability may be affected by environmental conditions. Combining with an attractant or pheromone may enhance efficacy.

Many research investigations on ALB and CLB have been hampered by a lack of test insects and replication. Although the development of an artificial diet has greatly reduced the developmental time (Keena, 2005; Dubois et al., 2002), laboratory cultures are expensive and labour intensive to maintain. Because of the length of time required for development and the need to change the artificial diet occasionally and provide freshly cut wood for oviposition, it is very expensive to rear ALB. It is estimated that rearing ALB in North America costs at least US$21 per beetle (Keena, 2005). By using test insects collected in the field, the age and physiological condition of the insects is not known, aspects which may affect the efficacy of potential control measures.

Further research should may be investigate some of the more novel control measures, such as effects on the beetles' metabolism, however these would have long term development objectives. Combining fungal bands with pheromones or attractants or fungi with insecticides should be further investigated as the potential exists to augment eradication efforts by combining different methodologies. Other areas to consider may include pesticide rotation to reduce the risk of pesticide resistance and the use of alternatives methods, such as low temperatures or controlled atmospheres for the treatment of wood packaging.

The main strategies that have been vital to achieving eradication of *Anoplophora* infestations include tree removal and chipping, the use of protective insecticides, and public involvement. The success of any eradication programme is dependant on the availability of sufficient funding, clear lines of authority among the agencies involved in the eradication efforts, susceptibility of the target pest to control, availability of measures to prevent reinvasion and early detection of the pest after establishment (Haack et al., 2010).

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Table 1: Control measures applied to growing trees

Control measure	Brief description	Advantages	Disadvantages	Reference
Cultural control				
Planting of resistant clones / species	Planting of insect resistant clones of poplars / tolerant tree species.	• Resistant clones found to be highly resistant (81%) to ALB. • Increases clonal diversity.	• Shortage and cost of resistant trees. • Limited practice.	Qin et al. (1985) Qin et al.(1996) In Pan (2005)
Planting of a mixed tree forest	Plant forests with non-host, "resistant" and trap tree species (45-50%:45-50:5-10% ratio).	• Regulates structure of forest. • Trap trees restrict spread of ALB. • Enhances ability to withstand ALB outbreaks.	• Susceptible trees are of higher value. • Shortage and cost of resistant trees. • Long term management required.	In Pan (2005)
Cultivating fast-growing timber forests.	Selection and tending to make trees grow faster and healthier. The annual growth should be more than 3cm in diameter (eggs and larvae are crushed by fast growing trees).	• Rotation of the tree is shortened and the chance of being damaged by ALB is reduced. • Improves tree vigour and tolerance.	• Reduced wood quality. • Limited practice.	In Pan (2005)
Planting trap trees	Trap trees e.g. *Acer* spp. which are preferred by ALB are planted to lure ALB to lay eggs which are then destroyed. Ratio is 5% trap trees to 15% main trees.	• Protects high value trees. • Easy to use and popular method in China. • Effectively reduces local ALB population density.		In Pan (2005)

Grafting	Chinese white poplars are grafted onto original poplar stumps.	• Makes use of the cut stump and reduces costs of afforestation. • Chinese white poplars grow very fast and have good resistance to ALB.		In Pan (2005)
Effective management e.g. sanitation cutting, pruning, removal and destruction of infested trees.	Cut down infested trees. Remove weak, dead and dying wood and ensure proper disposal. Cut upper trunk to renew growth. Prune twigs to prevent adults laying eggs.	• Improves condition of standing forest. • Reduces ALB population density.	• Limited practice. • May only be feasible on small scale.	In Pan (2005)
Removal of infested trees	Cut down, chip and burn infested trees.	• Widespread eradication technique. • Successfully contains spread.	• Root removal required with CLB. • Trees are removed.	In Pan (2005)
Chemical control				
Trunk injection	Imidacloprid	• Allows sufficient levels of imidacloprid to control ALB within 1-3 weeks. • Persistent for several months. • Low toxicity to mammals.	• Repeat injections required. • May increase fungal infections through wounds. • Sub-lethal doses could lead to increased adult dispersal. • Distribution of imidacloprid within trees is variable. • Large larvae may not be exposed to	Warren et al., (2009) Poland et al., (2006b)

			lethal doses. • Imidacloprid is highly toxic to bees. • Potential for resistance	
Soil Injection	Imidacloprid	• Used in USDA eradication programme. • Low toxicity to mammals	• Insecticides may leach into ground water. • Not suitable where there is high ground water levels. • Sub-lethal doses could lead to increased adult dispersal. • Distribution of imidacloprid within trees is variable • Requires 2-3 months to reach effective levels compared to 1-3 weeks with tree injection • Imidacloprid is highly toxic to bees. • Potential for resistance • Large larvae may not be exposed to lethal doses.	Warren et al., (2009)
Contact insecticides	Treatment of tree canopy with cypermethrin.	• Widely adopted method in China	• Difficult to reach all adult beetle feeding or resting sites	Hu et al (2009)
	Microcapsule formulation of cypermethin applied as bands around tree.	• Prolonged efficacy. • Resistance to environmental factors. • Reduced environmental impact.	• Difficult to apply on a large scale.	Tang & Xia (2000)
	Deltamethrin, permethrin, acephate,	• 100% mortality of ALB adults after 2 days	• Little residual effect	Wang et al (2003)

	chlorpyrifos, lindane, bendiocarb and fipronil applied to twigs.			
Physical control				
Prevention of oviposition	Used against CLB in citrus groves, methods used around base of trunk: Whitewash (with 15% fenitrothion). Sticky cardboard Fishing net (2cm mesh) Fine wire mesh (6 openings/cm) with soil piled up around base of trunk.	• Wire mesh with soil piled around base of trunk was most effective. • May be effective around susceptible host trees in urban environments.	• White wash not effective. • Cardboard caused bark to rot. • Adults able to lay eggs through net. • May be difficult to apply on a large scale. • ALB lays eggs on upper trunk and main branches.	Adachi & Korenaga (1989) Adachi (1990)
Manual	Hand collection of adults, hammering egg sites, use of wires, blocking holes.	• Effective in maintaining below threshold levels. • Useful in urban areas.	• Expensive, time and labour intensive	Hu et al (2009); Pan (2005)
Sterile insect technique	Adults irradiated with 1.5 Gy/min, 7-10 days after emergence	• Field population suppressed.	• Problems with mass rearing.	Lu et al., 2001
Biological control				
Parasitoids				
Dastarcus helophoroides (=D. longulus) (Coleoptera:	Larval/pupal parasitoid of ALB.	• Can kill 60% of ALB. • One ALB larva can support 30 parasitoids.	• Native to China	Qin & Rao (1988) Ogura et al.,

Bothrideridae).		• Kills ALB within 10 days. • Where established in high numbers, ALB is under natural control in China. • Artificial diet developed. • Potential for further development.		(1999).
Aprostocetus fukutai; A. anoplophorae (Hymenoptera: Eulophidae).	Egg parasitoids of CLB.	• Highly specific to CLB • High rates of parasitism.	• Specific to CLB	Delavare et al (2004) Herard et al. (2007)
Ontsira anoplophorae (Hymenoptera: Braconidae).	Larval ectoparasitoid of CLB.			Smith (2000)
Spathius erythrocephalus (Hymenoptera: Braconidae).	Egg parasitoid.	• Attacks ALB and CLB.		Herard et al. (2007)
Scleroderma guani; S. sichuanensis (Hymenoptera: Bethlidae).	Ectoparasitoid	• Promising results with ALB and CLB.		In Pan (2005)
Bacteria				
Bacillus thuringiensis var. *tenebrionis* and *B. thuringiensis* toxins	In vivo and in vitro tests assayed against larval and adult ALB.	• Limited in vitro effects.	• No significant effect with in vivo tests.	D'Amico et al. (2004).
Nematodes				
Steinernema feltiae, S. carpocapsae, H. marelata	Lab. and field studies against ALB and CLB.	• Infective to larvae, pupae and adults • Actively locates host within larval tunnels.	• Older larvae more resistant • Environmental conditions affect efficacy.	Fallon et al. (2004); Solter et al. 2001; Qin et al. (1988) In

			• Application techniques may be limiting.	Pan (2005).
Fungi				
Beauveria bassiana, B. brongniartii, Metarhizium anisopliae, Isaria farinosa	Adult ALB submerged in conidia suspensions (10^7 conidia /ml) for 15s.	• All pathogenic to adults. • *B. brongniartii* & *M. anisopliae* killed in quickest time (ST_{50}=5 days). • Isolates of *B. bassiana*, *B. brongniartii* & *M. anisopliae* registered in Europe.	• Low replication and low numbers of beetles used. • Isolates of *I. farinosa* not registered in Europe	Dubois et al. (2008)
Beauveria bassiana, B. brongniartii, Metarhizium anisopliae, Paecilomyces spp.	Larvae and adult ALB submerged in conidia suspensions for 30s.	• *B. brongniartii* isolate most promising against adults (LC_{50}=6.2x10^4/ml)	• Lower virulence against larvae.	Shimazu et al., (2002)
Fungal bands	Band impregnated with *B. brongniartii, B. bassiana & Metarhizium anisopliae* most effective. Lab and field studies with adult ALB.	• Potential to create epidemics. • Mortality effects against adults • Sub-lethal effects on reproduction. • Can be stored and remain effective for long periods. • Environmental conditions e.g. rainfall can move conidia.	• Low replication and low numbers of beetles used. • Lower virulence against larvae. • Relies on adults coming in contact with fungi. • Environmental conditions affect conidia viability • UV reduces efficacy. • Potential effects on non-target arthropods	Dubois et al. 2004a,b; Hajek et al., 2008; Shanley & Hajek, 2008. Shanley et al., 2009.

Table 2: Control measures applied to processed wood.

Control measure	Brief description	Advantages	Disadvantages	Reference
Chemical control				
Pressure treatment with wood preservatives	Pine boards infested with new house borer (*Arhopalus productus*). Treated with ammoniacal copper quaternary compound, disodium octaborate tetrahydrate or a mixture of 30ppm imidaclorpid and 300ppm 2-n-octyl-4-isothiazolone-3-one. Assessed over 2 years.	• Easy to treat with conventional wood preservatives. • Adult emergence prevented.	• Larvae survived in treated materials. • Periodic surfacing of larvae could allow movement to other wood.	Schauwecker & Morrell (2008)
Sulfuryl fluoride (SF)	Ash logs infested with *Agilus planipennis* (emerald ash borer). 100% control with CT dosages of 3723 (24h) & 6072 (48h) g-h/m^3 at 15.6^{o}C; 3172 (24h) & 4210 g-h/m^3 at 21.1^{o}C. Poplar timbers infested with ALB. 104g/m^3 SF at >15.6^{o}C and CT product of >1095g-h/m^3 is	• Effective under commercial fumigation conditions. • Effective alternative to MeBr. • Lower doses required for ALB compared to *A. planipennis*.	• High density wood reduces fumigant penetration. • Embedded larvae need higher doses than eggs under bark. • Higher doses required for embedded larvae due to reduced fumigant penetration. • Cold acclimated larvae require higher CT product.	Barak et al., (2010) Barak et al., (2006a)

	recommended for larvae and pupae.			
Ethanedinitrile	Killed all larval stages of ALB after 6 hours exposure to 11 mgL^{-1} at 20-25^{o}C.	• High toxicity • Lower CT products compared to MeBr and SF. • Penetrates through wood faster than MeBr and carbonyl sulphide.		Dowsett and Ren (2007) Ren et al., (2006).
Physical control				
Microwave irradiation	30 seconds and 3 minutes of irradiation at 900 watts was lethal to ALB larvae and pupae respectively in poplar blocks	• Low levels of microwave irradiation required for eradication.	• Small number of test insects	Fleming et al., (2003)
Low pressure vacuum	ALB larvae inserted into wood blocks. Held at 20mmHg at 20^{o}C; 10mmHg at 30^{o}C. Estimated lethal time = 51 hours at 16.6 - 21.6% m.c.	• Effective against eggs, larvae and pupae.	• Temperature, pressure and relative humidity affect dessication rate. • Commercial scale testing required.	Chen et al., (2008)
Heat	Miniumum core temperature of 56^{o}C for a minimum of 30 minutes.	• ISPM standard no. 15.	• No residual protection.	FAO (2002)

Appendix 1 – Databases resourced

Websites:

- Canadian Food Inspection Agency (www.inspection.gc.ca)
- EUROPA European Union (ww.ec.europa.eu/)
- European and Mediterranean plant protection organisation (www.eppo.org/)
- Food and Agriculture Organisation (www.fao.org/)
- Forestry Commission (www.forestry.gov.uk/)
- Natural Resources Canada (www.nrcan.gc.ca)
- United States Department of Agriculture - Animal and Plant inspection Service (www.aphis.usda.gov/)

Literature databases:

AGRICOLA (AGRICultural OnLine Access) is an extensive bibliographic database (maintained since 1970) consisting of records for literature citations of journal articles, monographs, theses, patents, translations, microforms, audiovisuals, software, and technical reports.

AGRIS International is the international information system for agricultural sciences and technology. The AGRIS International database serves as a comprehensive inventory of worldwide agricultural literature which reflects research results, food production, and rural development to help users identify problems involved in all aspects of world food supply.

BIOSIS Previews® contains citations from *Biological Abstracts®* (BA), and *Biological Abstracts/Reports, Reviews, and Meetings®* (BA/RRM) (formerly *BioResearch Index®*), the major publications of BIOSIS®. *Biological Abstracts* includes approximately 350,000 accounts of original research yearly from nearly 5,000 primary journal and monograph titles. *Biological Abstracts/RRM* includes an additional 200,000+ citations a year from meeting abstracts, reviews, books, book chapters, notes, letters, and selected reports.

CAB Abstracts is a comprehensive file of applied life science information (from 1972) containing all records in the 44 abstract journals published by CAB International (CABI), plus many more records which appear online only. CABI has long been recognized as a leading scientific information service in agriculture and related sciences.

CSA Life Sciences Abstracts contains abstracts and bibliographic citations from recent worldwide research literature in major areas of biology, medicine, biochemistry, biotechnology, genetics, immunology, ecology, and microbiology, and some aspects of agriculture and veterinary science.

Elsevier Biobase (1994-2011).

Embase (1974-2011).

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Prospects for use of biological control agents against *Anoplophora spp* in Europe [an updated version of the biocontrol section of the main report]

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Abstract

The longhorned beetle species *Anoplophora chinensis* and *Anoplophora glabripennis* have emerged in the last two decades as a risk to urban and woodland trees in Europe and there have been several outbreaks of these beetles in Europe. This review summarises the literature on biocontrol of *Anoplophora spp.* and discusses which are the strongest candidates for use in Europe. Some of the methods below could be useful for control, but are unlikely to be instrumental in achieving eradication. Below is a summary of the findings:

- **Entomopathogenic fungi:** Strong candidate as a biopesticide as fungal infection results in high mortality rates and has already been developed into a commercial product in Japan for *Anoplophora* control. *Beauvaria bassiana,* is already authorised for use as a biopesticide in the UK.
- **Parasitic nematodes:** Another strong candidate for use as a biopesticide due to high mortality rates and effective application methods have already been developed. Two nematode species, *S. feltiae* and *S. carpocapsae,* are also au for use in the UK and other parts of the EU.
- **Parasitoids:** Several parasitoid species (e.g. *Dastarcus helophoroides*) have been shown to be effective biological control agents in China but their specificity would need to be investigated further before use in Europe. Some native European parasitoid species (e.g. *Spathius erythrocephalus*) have potential to be used as a biocontrol.
- **Predators:** Two woodpecker species (*Dendrocopos major* and *Picus canus*) have been shown to be effective at controlling *A. glabripennis* numbers in Chinese forests and are also native to Europe.
- **Pathogenic bacteria:** Are currently not a strong prospect for use in biocontrol with no study yet advancing to field trials.

Introduction

The tree pests *Anoplophora chinensis* Forster (Coleoptera: Cerambycidae) and *Anoplophora glabripennis* Motschulsky (Coleoptera: Cerambycidae) are native species of China and Korea, and also Japan in the case of *A. chinensis* [19]. There have been several outbreaks of these two species in Europe with breeding populations of *A. chinensis* found in Italy, France, Netherlands and Croatia [5, 26, 48, 70, 71] and *A. glabripennis* breeding populations being found in Austria, Netherlands, France, Germany, Italy and UK [1, 2, 4, 26, 28, 30, 38, 39, 47, 61, 68]. In these countries the pests are controlled through physical methods with restrictions on the movement of trees and wood and potentially-infected trees being cut down and chipped

or burnt. Injecting imidacloprid into the tree trunks or into the soil at the base of trunks has been shown to be an effective method of preventing infestation by *Anoplophora* in the USA, but is not effective at targeting older larvae or pupae in the sapwood so is unsuitable for use in treating infested trees. Furthermore these uses are not approved in the EU. The identification of biological control methods that could target beetles at these stages would therefore be a useful tool in controlling outbreaks of both species. These methods could include both biological control agents which are self-replicating after release, such as parasitic insects, and biopesticides that are applied in a similar manner to conventional insecticides. However, as the juvenile stages of wood boring insects develop within wood they have natural protection from most non-specialist control agents. It is important to distinguish between methods used for control (reducing or eliminating the economic or environmental impact) and eradication, some of the methods used for control may not be sufficiently effective for eradication campaigns. Current research on biological controls has focused on five areas and these are: entomopathogenic fungi, parasitic nematodes, parasitoid species, entomopathogenic bacteria and predators.

Entomopathogenic fungi

Certain fungal species can infect insects and so a number of studies looked to identify such species for *Anoplophora.* Six species of fungi were found that are pathogenic to *Anoplophora* of which three species *Beauveria bassiana* (Hypocreales: Corducipitaceae), *Beauveria brongniartii* (Hypocreales: Corducipitaceae) and *Metarhizium brunneum* (formally *M. anisopilae*) (Hypocreales: Clavicipitaceae) can infect both *A. chinensis* and *A. glabripennis* [3, 12, 59, 64, 69]. Of the other three species two *Isaria farinose* (Hypocreales: Clavicipitaceae) and *Nosema glabripennis* (Dissociodihaplophasida: Nosematidae) have been shown to infect *A. glabripennis* [14, 84] and one species *Beauveria tenella* (Hypocreales: Corducipitaceae) infects *A. chinensis* [37] [3, 37, 59, 69]. Differences in experimental design make it difficult to compare the pathogenicity of these species in terms of mortality rate and lifespan of infected beetles but several studies on *A. glabripennis* compared the effectiveness of multiple species and found that *B. bassiana, M. brunneum* and *B. brongniartii* were the most pathogenic [12-14]. *I. farinose* and *N. glabripennis* both had significantly lower mortality rates than other fungal species so may not be effective as control agents [14, 84]. The strain used can also have an effect on pathogenicity as a study looking at the effect of different *M. brunneum* strains found that the mortality rate varied between 40 % and 97 % [3]. Most studies on fungal species as a biocontrol agent have used adult beetles and very few have looked at the effect on fungi on larvae or eggs. Of the two that looked at other life stages, one reported that larvae were unaffected by *B. brongniartii* infection and the other reported that the *M. brunneum* can be transferred vertically from mother to offspring and affects them at both the egg and larval stages [23, 64]. The latter study's findings would suggest that as well as a decrease in the lifespan of the adults the number of viable young will also decrease and thus further reduce the population of *Anoplophora.* This reduction is further enhanced by the finding that infected females have a reduced rate of oviposition leading to a further reduction in the number of offspring [22, 23].

A number of studies have investigated the most effective method to use fungi as a biocpesticide .Three methods were tested including impregnating non-woven or polyurethane bands with fungal conidia and then attaching the band onto trees, spraying the

conidia directly onto trees and mixing conidia in with a paste and applying this to trees [29, 32, 37, 81]. The use of the fungus in a paste did not lead to any infections of *A. chinensis* and so can be discounted as a delivery method [32]. Of the other two methods, the use of bands was considered more effective as although initial infection rates were similar they decreased more rapidly for spraying than bands [12, 81]. The bands work by infecting adult beetles that walk over them and so must be placed in positions where a large number of beetles will cross them in order to maximize the number of adults infected. *A. chinensis* oviposition holes are generally made at the base of host trees, and so if the bands are placed below the first branch joint all newly emerging adults should be become infected if they move up the tree into the canopy to feed [37, 69]. *A. glabripennis* ovipositions are found higher up the trunk or on main scaffold branches so the bands must also be placed higher up the tree in order to infect the emerging adults [22]. During a study using bands impregnated with *B. brongniartii* to control *A. chinensis* the mortality rate decreased from 84-100 % to 55-73 % if only half the trees were banded [69]. This means that all trees within an infected area must be banded in order to maximize the proportion of adults infected. In terms of the longevity of these bands two field studies have estimated that after 90 to 112 days the number of conidia per band is enough to achieve the LC50 of the adult beetles and so the bands should still be effective [20, 63]. This means that the bands could be used throughout an entire growing season without needing to be replaced. This fungal band treatment has been developed into a commercial product (Biolisa Kamikiri) in Japan using *B. brongniartii* as the chosen fungal pathogen [22, 29].

Conidia can spread from bands along tree trunk or branch or to trees up to 50m away through the action of wind, rainfall and also by the beetles themselves [62, 83]. This would result in infections of beetles that do not themselves cross the bands but may also result in environmental contamination by the fungus. It has also been found that fungal infections can be transferred during mating where it was found that an infected beetle could transfer this infection to its partner, particularly if the infected beetle is the male [21, 55]. The effectiveness of entomopathogenic fungi as a control can also be enhanced by using it in conjunction with chemical control methods as a study found that when used together *M. brunneum* and the insecticide imidacloprid caused a greater reduction in survival time of *A. glabripennis* adults than either treatment method used individually [60]. It is thought that this increase is caused by the insecticide reducing the adults' movement speed and so increasing the chance of infection by the fungus. This combined control method does however have a drawback in that the rate of sporulation of the fungus is reduced so could reduce the longevity of the fungal band.

Pathogenic bacteria

Several bacterial species have been tested for use as a biocontrol agent of *A. glabripennis* but with limited success. One candidate, *Bacillus thuringiensis* Berliner var. *tenebrionis* (Bacillales: Bacillaceae), failed to infect *A. glabripennis* adults or larvae despite in vitro tests suggesting that it could infect the mid-gut of the insects [6]. One of the toxins from *B. thuringiensis,* Cry3Aa, has however been developed into a potential biological insecticide by the addition of a protein fragment to the toxin that can bind to the Cx cellulase enzyme in the larval mid-gut [18]. This resulted in increased mortality rates of larvae fed on the modified toxin, although a suitable delivery mechanism would be required for this insecticide. Another bacterial species, *Serratia marcescens* (Enterobacteriales: Enterobacteriaceae), was shown

to be able to infect larvae at the 1st and 2nd instar stage in laboratory tests, although the mortality rate was highly variable (16 % to 81 %) [9, 10]. The mortality rate decreased to 5 % during field trials suggesting that it may not be suitable as a biocontrol agent unless this rate can be increased. Another study looked at bacteria that can increase ice nucleation rates and found that if *A. glabripennis* larvae were fed or sprayed with these bacteria the temperature at which the larvae froze and died increased to 7.04 °C and 5.62 °C for the respective treatment [67]. So far only a laboratory trial has been completed so the effectiveness of this technique in the field is unknown.

Parasitic nematodes

Currently three nematodes species have been shown to parasitize *A. chinensis* and they are *Steinernema feltiae* Filipjev (Rhabditida: Steinernematidae), *Steinernema carpocapsae* Weiser (Rhabditida: Steinernematidae) (also known as *Neoaplectana carpocapsae*) and a *Priostonchus sp.* (Rhabditida: Diplogastridae) that has not yet been fully identified [31, 35, 36, 41, 59]. Two of these species, *S. feltiae* and *S. carpocapsae,* also parasitize *A. glabripennis* [15, 46, 57]. There are also three other nematode species that are parasites of *A. glabripennis* and they are *Steinernema bibionis* Bovien (Rhabditida: Steinernematidae), *Heterorhabditis marelatus* Liu et Berry (Rhabditida: Heterorhabditidae) and another uncharacterised *Heterorhabditis sp* (Rhabditida: Heterorhabditidae) [45, 57, 66]. Unlike entomopathogenic fungi, nematodes can infect larvae pupae and adult beetles so can be used to target multiple life stages, although mortality rates are higher when they parasitize early stage larvae [15, 33, 66]. Of the six species of nematode *Priostonchus sp.* was difficult to maintain in culture so is unlikely to be a good candidate for a biocontrol and the *Heterorhabditis sp.* must first be fully characterised [41]. The best candidates as biopesticides are *S. feltiae* and *S. carpocapsae* and they have been studied the most. The effectiveness of using nematodes for controlling *Anoplophora* depends on the application method. Several application methods were tested on *A. chinensis* and are listed in Table 37 along with the mortality rate for each treatment. Of these spraying directly into or using material to cover or block larval tunnels were the most effective. The mortality rate of nematode treatment can also be affected by whether the trunk or branch is shaded or in direct sunlight as it was found that sunlight has a deleterious effect on mortality rates for two of the methods tested [36]. This effect is lessened if the treatment method itself provides some protection from desiccation as the decrease in mortality rate was lower for nematodes applied as compost rather sprayed directly onto tree trunks or branches (Table 37).

Table 37: Different application methods for parasitic nematodes

Delivery method	Species	Mortality rate (%)	Reference
Plugging the larval tunnel with sponge coated with nematodes	*S. feltiae*	91	[33]
Bark compost containing nematodes	*S. feltiae*	77 (Shade) 59 (Direct sunlight)	[36]
Nematodes sprayed onto bark	*S. feltiae*	77 (Shade) 25 (Direct sunlight)	[36]
Covering larval tunnel with gauze coated with nematodes	*S. carpocapsae*	90	[35]

Delivery method	Species	Mortality rate (%)	Reference
Nematodes sprayed onto bark	*S. carpocapsae*	60	(35)
Sprayed directly into larval tunnels	*S. carpocapsae*	86 *	(46)

The mortality rates shown are for *A. chinensis* except for the one labelled with* which is for *A. glabripennis*

Parasitoids

There are a number of insect species that can parasitize *A. glabripennis* and *A. chinensis*. The best studied of these is *Dastarcus helophoroides* Fairmaire (Coleoptera: Bothrideridae), which is a parasite of a number of longhorned beetle species including both *A. glabripennis* and *A. chinensis* in Japan and China (52, 58, 65, 86). Most research has focused on its parasitisation of *A. glabripennis. D. helophoroides* larvae can parasitize late instar larvae and young adults of *A. glabripennis* and the adults have also been shown to predate *A. glabripennis* larvae (53, 58, 79). Each year female *D. helophoroides* can lay enough eggs to parasitize ten to twelve *A. glabripennis* larvae of which 50-70 % will die as a result of this parasitisation (43). Field trials have shown a decrease in *A. glabripennis* populations after release of *D. helophoroides,* including areas outside the original release area which is likely due to the movement of the adults as they are strong fliers (44, 77, 78). *D. helophoroides* can be reared on an artificial diet allowing for sufficient numbers to be produced for biocontrol purposes (54, 73). A release strategy that has been shown to be effective is to place eggs at the entrance of *A. glabripennis* larval tunnels and this resulted in an 85-90 % reduction in the *A. glabripennis* population (44). As *D. helophoroides* can be reared in captivity and causes a significant decrease in the *A. glabripennis* population it would therefore be a good candidate for a biocontrol. However, it has been shown that rates of parasitisation vary with tree species as larval frass, which is used by *D. helophoroides* to detect larvae, from different tree species elicited varying levels of response from *D. helophoroides* adults (76). This means that *D. helophoroides* may not be effective at treating *A. glabripennis* infestations on certain tree species, for example the North American *Acer negundo* (Sapindales: Sapindaceae). The region from which the *D. helophoroides* originated is also important for their use as a biocontrol as the preferred host insect varies between regions and so parasitisation rates of *A. glabripennis* vary (75).Beetles from Guangdong province in China showed the highest rates of parasitisation and so would be most suitable as a biocontrol.

A previously uncharacterised parasitoid of *A. chinensis*, *Aprostocetus anoplophorae* Delvare (Hymenoptera: Eulophidae), was identified in Italy in 2002 (8, 27). Despite so far only being identified in Italy it is suspected to be a non-native species and instead most likely arrived when *A. chinensis* eggs were brought into Italy on bonsai trees from Japan. The parasitoid appears to be highly specific to *A. chinensis* as it was unable to parasitize the native Italian native longhorn beetle *Saperda carcharias* Linnaeus (Coleoptera: Cerambycidae) and in trials to test its ability to parasitize *A. glabripennis* only one parasitized egg was identified (25, 27). *A. anoplophorae* does not however show any specificity in terms of the host plant (27). There are two generations of *Aprostocetus anoplophorae* per year in Italy with the first emerging during July/August and the second in September and so it can infest *A. chinensis* eggs throughout the entire summer period (27). Levels of parasitisation of *A. chinensis* eggs vary between 21 % to 72 % (27, 50).The parasitoid is also socially gregarious making it suitable

for rearing for biocontrol. Another species from the *Aprostocetus* genus, *Aprostocetus fukutai* Miwa et Sonan (Hymenoptera: Eulophidae) has also been shown to parasitize *A. chinensis* eggs [24]. This species is found in China and has a parasitisation rate of around 8 % to 59 %.

Amongst native European parasitic species of xylophagous insects there are a number that have been identified that can parasitize *A. chinensis* and these are: *Spathius erythrocephalus* Wesmael (Hymenoptera: Braconidae), *Eurytoma melanoneura* Walker (Hymenoptera: Eurytomidae), *Calosota vernalis* Curtis (Hymenoptera: Eupelmidae), *Cleonymus brevis* Boucek (Hymenoptera: Pteromalidae, Cleonyminae), *Trigonoderus princeps* Westwood (Hymenoptera: Pteromalidae, Pteromalinae), *and Sclerodermus sp.* (Hymenoptera: Bethylidae) [25, 49]. Of these species *Spathius erythrocephalus* and *Trigonoderus princeps,* an egg and larval parasitoid respectively, were the most prevalent parasites of *A. chinensis* in Italy and *S. erythrocephalus* was also found to parasitize *A. glabripennis* [25, 51]. *Sclerodermus spp.* vary in their effectiveness and require dry conditions so would only be useful in controlling *A. chinensis* larvae in dry or dead wood such as wood packaging material [25]. They have however, also been shown to infest *A. glabripennis* larvae so can be used against both species.

There are several parasitoids found in countries where *A. chinensis* is a native species. A bethylid wasp species *Scleroderma sichuanensis* Xiao (Hymenoptera: Bethylidae) has been shown to parasitize *A. chinensis* larvae in China with rates of parasitism varying between 5-44 % [11, 34]. There are several species from the *Ontsira* genus that parasitize *A. chinensis* of which one is *Ontsira anoplophorae* Kusigemati et Hashimoto (Hymenoptera: Braconidae), however little is known about this insect other than it is an ectoparasite and is a native of Japan [40].

Predators

The woodpecker species *Dendrocopos major* Beicki (Piciformis: Picidae) and *Picus canus* Gmelin (Piciformis: Picidae) are predators of *A. glabripennis* larvae in China but have not been reported to predate *A. chinensis*, possibly due to the larvae being found nearer the base of the tree making them unsuitable prey [16, 87]. In field studies to assess the rate of predation of *A. glabripennis* by woodpeckers the reduction in the beetle's population varied between 31 % and 79 % with an increase in predation during the breeding season [16, 80, 85, 87]. A single pair of woodpeckers were shown to be able to control the *A. glabripennis* population of an area of forest of around 92 to 111 hectares making them effective control agents [80]. Efforts to encourage woodpeckers to move into forested areas have found that mixed tree planting and the conservation of deadwood is effective [42]. The characteristics of nest holes have also been identified in order to produce suitable artificial nest holes for woodpeckers [72]. Currently only one predator of *A. chinensis* has been identified and that is the formicid *Oecophylla smaragdina* Fabricius (Hymenoptera: Formicidae) which preys on larvae [82]. It has been shown that in areas where it was present insecticides were not required to control *A. chinensis* in orchards.

Prospects for biocontrol agents in Europe

Most research on the biocontrol of *A. glabripennis* and *A. chinensis*, with the exception of some parasitoid research, has been carried out in Asia and the US. This means that before these methods could used in Europe they must be first assessed for their suitability in case they themselves become an invasive species or have a deleterious effect on native European wildlife. For example *D. helophoroides* has been successfully used to control *A. glabripennis* in China in field trials and analysis of its preferred climate would suggest it should be capable of surviving in European climates [7, 44, 77, 78]. However, as well as *Anoplophora spp., D. helophoroides* also parasitizes other xylophagous beetles including *Massicus raddei* Blessig (Coleoptera: Cerambycidae), *Monochamus alternatus* Hope (Coleoptera: Cerambycidae), *Apriona germari* Hope (Coleoptera: Cerambycidae), *Apriona swainsoni* Hope (Coleoptera: Cerambycidae) and *Batocera horsfieldi* Hope (Coleoptera: Cerambycidae) [17, 56, 58, 74]. This lack of host specificity may mean it could target native European longhorned beetles or other xylophagous insects so this would need to be assessed before it could be considered for biocontrol purposes. *Aprostocetus anoplophorae* is another non-native parasitoid in Europe but there is a breeding population already in Italy and is highly specific to *A. chinensis* making it a better candidate for use in Europe [8, 25, 27]. There are two European native species of parasitoids, *Spathius erythrocephalus* and *Trigonoderus princeps*, which were shown to be effective parasites of *A. chinensis* and also *A. glabripennis* in the case of *S. erythrocephalus*. These are probably the best candidates for use in Europe although research would be required into their rearing in captivity and best application method.

The biocontrol prospects for predators are more promising as both woodpecker species that predate on *A. glabripennis* are native in Europe and would only require measures to encourage their establishment in area of infestation such as artificial nest boxes and changes in management of woodland to allow more deadwood and more diversity of tree species [42, 72]. Entomopathogenic fungi and parasitic nematodes also have potential as research in both fields has identified species that are native to Europe and are effective in controlling *Anoplophora spp..* For instance in the UK the fungus *Beauveria bassiana* and nematodes *Steinernema feltiae* and *S. carpocapsae* are all authorised for use as biopesticides. The best delivery mechanism for control of *Anoplophora* using both fungi and nematodes has also been determined and in the case of entomopathogenic fungi has also been commercialised [22, 29]. This would make it easier to adapt the currently licensed treatments for use against *A. glabripennis* and *A. chinensis.*

In conclusion, biological control agents have not formed a significant part of eradication campaigns for *Anoplophora* in North America or Europe, but have been used for control purposes in Asia. Entomopathogenic fungi and nematodes have the potential to be used as alternatives to conventional insecticides, but research to date has not indicated that their efficacy levels are sufficient to replace either tree removal or prophylactic treatments with imidacloprid. The use of bands impregnated with entomopathogenic fungi could also be readily integrated with other management techniques. Further research would be needed to provide the necessary evidence to support releases of a specific parasitoid such as *Aprostocetus anoplophorae* in locations outside of Lombardy where it has already become established.

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Work Package 6: Investigating the biology in relevant EU climatic conditions

Summary

- The rate of development of *Anoplophora* is restricted by temperature in the more northerly locations of its native and exotic range
- Models indicate that day degrees are likely to be the limiting factor for *Anoplophora* in northern Europe
- The minimum temperature for the development of the juvenile stages of both *Anoplophora chinensis* and *Anoplophora glabripennis* is around 10-13°C and the optimum temperatures for adults are around 23-24°C.
- By taking account of the natural variability in the rate of development in ALB larvae, on average it would take 3 years for complete development in the Netherlands, but 10% of a population could develop in 2 years, but it would take 6 years for 99% of a population to complete its development.
- Using simple application of day degrees, CLB and ALB are estimated to be able to complete their development in Denmark in 2, but with greater probability in 3 years.
- Larvae developing in tree branches or trunks exposed to sunshine could potentially develop around 1.9 times as fast as those in trunks or branches that are continuously shaded.

The relationship between climate and the population development of *Anoplophora chinensis* and *Anoplophora glabripennis* (D6.1)

Dominic Eyre, Fera, UK

The development rate of woodboring beetles

Although, some longhorn beetle larvae remain in the richer sapwood of their hosts, most bore deeper into the heartwood and sometimes reach the pith. The larval food is the carbohydrates and proteins and cellulose found within wood vessels. The nutritional value of this diet can be very low, and so larvae may take many years to develop. In contrast, the adult stage of the life cycle is generally short, a period of weeks rather than months or years. The length of the lifecycle is therefore largely determined by the time taken for the larvae to develop.

The lifecycle of some longhorn beetles can be completed in a year, but it can very much longer. A lifecycle of 45 years has been documented for a species of North American longhorn in yellow pine (Evans, 1975).

Hylotrupes bajulus (L.) is a cosmopolitan pest of timber and its lifecycle varies around the world. In Canada, the lifecycle is usually from 3 to 6 years, but can be as short as 2 years under optimal conditions (*c*. 30°C, relative humidity of 80-90%, wood moisture content 10-12%) but may extend to more than 10 years in unfavourable conditions (Campbell *et al.*, 1989). In Europe, the life cycle is generally three or four years. In South Africa it can be a fast as one year, but can also be 2-4 years depending on the host (Duffy, 1957). Thus, the speed of development of many species of longhorn beetles is determined by host quality and environmental conditions.

Time to complete a generation

Voltinism may vary as a function of local climatic conditions, and a significant correlation has been established with latitude. It has been estimated that overall in China about 80% of *A. glabripennis* can complete their development within 1 year and <20% require 2 years Li & Wu (1993). However, the time to complete one generation may vary among populations in a single area, depending on the type of host in which the larvae develop.

Table 1: Reported generation times for *Anoplophora beetles*

Species	Years per generation	Location	Reference
*A. glabripennis**	1	Taiwan	Li & Wu (1993)
*A. glabripennis**	1-2	East of China, Hebei, Liaoling, Shangxi	
*A. glabripennis**	2	Beijing, Ningxia and Inner Mongolia	
*A. glabripennis (nobilis)**	2 in Shanxi	Shanxi	
A. glabripennis (nobilis)	2	Shanxi, Junan	
A. glabripennis (nobilis)	One or two years	Liaoning, Shangdong, Henan and Jiangsu Provinces (≈ 32° to 42° north)	Hua et al. (1992)
A. glabripennis	90% in one year	Shangdong	
A. glabripennis	98% in one year, 2% two years	Jiangsu	
A. glabripennis	85.7% in one year, 14.3% in two years	Yinan County, Shangdong province	Gao et al (1997)
A. glabripennis	Generally 1 occasionally 2	Worcester, MA, USA	Pers. comm., Alan Sawyer, Aphis
A. glabripennis	One generation in 1.5 to 2 years	Austria	Hoyer (2003)
Anoplophora chinensis	One year	China	Lieu (1945)
Anoplophora chinensis	Generally one/year in 1989-92; 15% 2 generations in 3 years	Ouhai County, Zheijiang Province, China	Xu Qi (1997)
Anoplophora chinensis	One to two years	Laboratory in Japan with fluctuating temperatures	Adachi (1994)
Anoplophora chinensis	Two to three years	Japan	Duffy (1968)

* = *A. glabripennis* and *A. nobilis* are now recognised as being the same species (Tang & Zheng, 2004).

Thresholds for development

Working at seven constant temperatures (5, 10, 15, 20, 25, 30, and 35°C), Keena (2006) reported the adult survival, reproduction, and egg hatch of *A. glabripennis* from two US populations, one from Chicago and one from New York. Nonlinear regressions were used to estimate the temperature optimum and thresholds for each life history parameter. Results are summarized in the table below:

Table 2: Lower temperature threshold (minimum), optimum temperatures and upper temperature threshold (maximum) for *Anoplophora glabripennis* life history parameters (Source: Keena, 2006)

Feature	Minimum temperature threshold (°C)	Optimum temperature (°C)	Maximum temperature threshold (°C)
Adult survival: ♀	-3	18	39
♂	-2		38
Fecundity: New York	11	23	34
Chicago	14	24	35
Oviposition	10	24	35
Egg hatch	10	23 - 29	32

Building on Keena (2006), Keena & Moore (2010) conducted experiments to examine the effect of temperature on *A. glabripennis* development at eight constant temperatures of 5, 10, 15, 20, 25, 30, 35 and 40°C. The temperature threshold for development of each life stage was derived from the rate of development at each temperature. For lower instars, the threshold is reported as approximately 10°C but for older instars it is between 12°C and 13°C. For temperatures between 10 and 30°C the researchers report a linear relationship between temperature and rate of development. Above 30°C the rate of development slowed. The lethal upper temperature was between 35 and 40°C. The authors point out that almost all development of *A. glabripennis* occurs in the heartwood of hosts. As such the temperature experienced by larvae and pupae can be very different from the ambient air temperature recorded at meteorological recording stations. The internal temperature of a tree is proportional to factors such as air temperature, the location of a tree, the depth of wood, wood density, solar exposure, seasonality and wind speed. At night the stored thermal radiation keeps wood at a higher temperature than ambient air temperature. Differences in air temperature and that in the phloem can be 10°C or higher, although perhaps more typical would be a difference of 2°C. Thus if developing a model based on air temperature, the authors suggest that modellers add at least 2°C to air temperature.

Gao et al. (1997) found that more *A. glabripennis* larvae survived from eclosion until mid-October when the rainfall and temperature were high in Yinan County, Shangdong Province, China.

In Gangu county, Gansu province 284 trees were examined to record where *A. glabripennis* had laid its eggs (Zhou *et al.*, 1984 – *A. nobilis*). The highest proportion of eggs (40%) were laid on the west side of the trunk, 27% on the east side and 13% on the north [and presumably 20% on the south side]. Zhou et al (1984) concluded that this distribution may be due to environmental temperature.

Degree-days to complete a generation ALB

Under field conditions, 1,264 degree-days (DD) above a threshold of 13.4°C are required to complete development of ***A. glabripennis*** (Yang *et al*., 2000). Under laboratory conditions, the accumulated DD and the lower development temperature threshold for egg, first and second instar larvae were estimated as 250 DD at 10.2°C, 160 DD at 11.7°C and 232 DD at 11.4°C, respectively (Zhang *et al.,* 1995).

Adachi (1994) studied the development of ***A. chinensis*** at constant and fluctuating temperatures. The development from egg to the final larval stage was found to require 1357 degree-days (DD) above a threshold of 11.2°C. Adding to this figure, the degree days required for pupation (209 DD) gives a total of *c.* 1570DD until beetles emerge from their host. At a constant temperature of 20°C, 57% of adults completed their development and emerged as adults during the period from 306 to 704 days after oviposition (Adachi,1994). Post emergence, *A. chinensis* are not sexually mature for a further 10 days at a mean temperature of *c.* 20°C (86DD), (Adachi, 1988) and peak egg laying is not until 21 days later at a mean temperature of around 23°C (248 DD). Therefore, the total number of degree days between egg laying and the date on which the maximum eggs are laid by a female developing from this egg is around 1900DD.

Time taken for eggs to hatch (days)

A. glabripennis : 11-14 (Shanxi), 12-17 (Shandong) & 7-13 (Shangxi);

A. nobilis: 20 (Gansu), 23 (Gansu), 180 (Gansu) Li & Wu (1993).

Overwintering stage

A. glabripennis are reported to overwinter in the larval, pupal and occasionally egg stage (Li & Wu, 1993).

Time of year for adult activity and the presence of different life stages

Table 3: The month of adult emergence of *Anoplophora* sp. beetles

Species	Adult emergence month	Location	Reference
*A. glabripennis**	Mid May – Mid September in Anhui	Anhui	Li & Wu (1993)
*A. glabripennis**	Early June – Early October	Beijing	
*A. glabripennis**	Mid June – Early October	Hubei	
*A. glabripennis (nobilis)**	Mid July – late August	Shanxi	
*A. glabripennis (nobilis)**	Mid April – Late May	Hubei	

* = *A. glabripennis* and *A. nobilis* are now recognised as being the same species

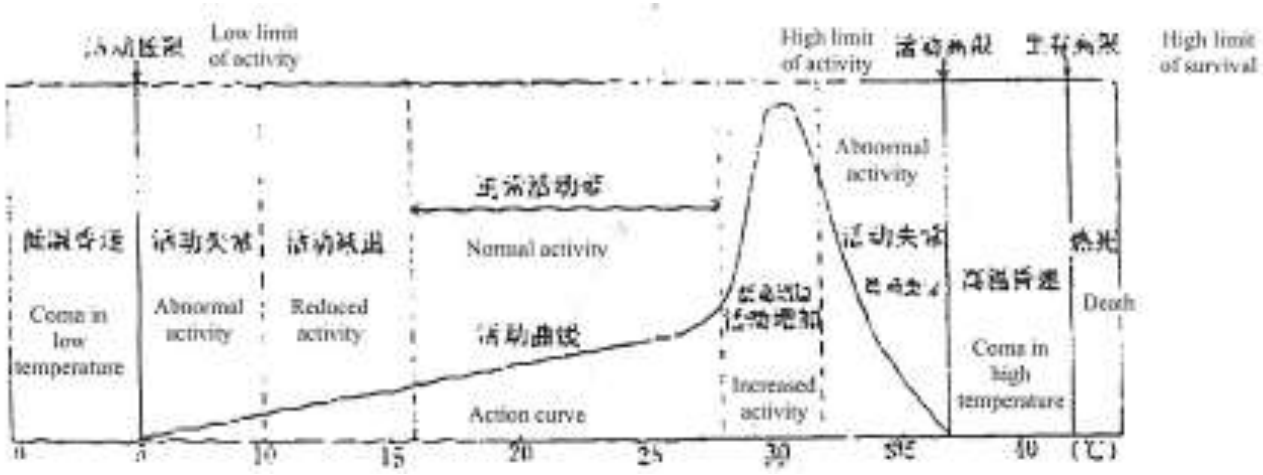
Gao & Zheng (1998) found that the lifespan of *A. glabripennis* adults was longer for those feeding on *Acer negundo* than those feeding on *P. X euramericana* cv 'I-69'. The lifespan of adult of *A. glabripennis*, amounts of feeding and oviposition was positively correlated to the sugar content of the host species. He & Huang (1993) found that the number of eggs laid per female *A. glabripennis* was greatest for *Acer negundo* (32), followed by *Salix matsudana* (24.0), *A. truncatum* (23.3) and *P. Canadensis* (20.5).

Zhao et al (1993) surveyed *A. glabripennis* in Changzhi prefecture of Shanxi province, the best time to look for five stages of the beetle were as follows: eggs –late October, early stage larvae (bark stage) – late April, later larval (sapwood stage) – late May, pupae – early June and adults – late August.

Adult activity

In Ganugu County, Ganusu Province, China, *A. glabripennis* emerged during July and August when temperatures were high, peak emergence generally occurred just prior to and during the hottest weeks (Zhou *et al.*, 1984 – *A. nobilis*). The impact of temperature on the adult activity of *Anoplophora glabripennis* was recorded by holding adults at each temperature for half an hour and then observing the beetles for half an hour (Figure 1).

Figure 1: Correlation



Cold tolerance

Cerambycids are generally freeze-avoiding with low supercooling points, as opposed to chrysomelids which freeze at high temperatures and are tolerant to freezing. The different strategies between these two families could be due their different diets, cerambycids feeding on dry wood with a low water content, causing a restrictive water balance, whereas chrysomelids feed on leaves with high water content (Zachariassen et al., 2008).

Larvae develop through several instars and most individuals overwinter as larvae which are freeze tolerant, e.g. US laboratory trials showed female larvae could recover from being kept at -40^{o}C for 24 hours and go on to develop, mature and successfully reproduce, when retuned to temperatures above 12^{o}C (Roden *et al*., 2008).

The prepupal stage (average 21.8 days) is followed by a pupal stage (average 19.6 days).

Gaps identified in current knowledge

1) *A. glabripennis* have been reported to oviposit more on a particular side of trees (Zhou, 1994). It is not known whether this behavior is also exhibited in other parts of China or in invasive populations. It is not known whether *A. chinensis* exhibits this behavior.

Comment: It would be relatively simple to record the orientation of oviposition holes at outbreak sites for *A. glabripennis* and possibly *A. chinensis.*

2) The relationship between air temperature as recorded by meteorological stations and temperature inside trees is poorly understood.

Comment: A greater understanding could aid in making predictions of where *Anoplophora* might survive and when emergence of adults might be expected in a particular year.

3) Impact of drought on the activity of Anoplophora larvae

4) The behavioural response of *Anoplophora* adults to high temperatures

5) The development rate of *Anoplophora* in very climates with relatively cool summers such as parts of northern Europe and the impact of such conditions of fecundity etc.

6) Impact of rain on behavior of *Anoplophora* adults

7) There is relatively little temperature response data available for *Anoplophora* larvae especially for *A. chinensis*

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Measuring the differences between air temperature and the temperature experienced by *Anoplophora* sp. larvae (D6.2)

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Introduction

Anoplophora glabripennis Motschulsky (Coleoptera: Cerambycidae) and *Anoplophora chinensis* Forster (Coleoptera: Cerambycidae) are wood boring insects native to China and Korea and are considered a threat to European urban and woodland trees through the damage that they cause [15]. These two species are considered a threat as there have been outbreaks of both species in mainland Europe and also an outbreak of *A. glabripennis* in Kent in the UK [5, 6, 9, 10, 16-18, 22, 23, 27, 28, 32, 36-38] This demonstrates that the European climate is suitable for the establishment of breeding populations of these species and this is supported, in the case of *A. glabripennis,* by climate modelling using CLIMEX that also suggests the climate is suitable [25]. However, the CLIMEX study only considers the climate over a large geographic area and therefore cannot take into account effects of the micro-climate on *A. glabripennis* and *A. chinensis* development. Microclimate can have a significant impact on whether a pest species can survive in a specific location. In the case of the *Anoplophora* species one of the most important microclimates is that of the inside of the tree trunk or branch.

Temperature within the bole of a tree

Temperature is one of the most important factors in determining if an insect species can establish in an area outside its normal range. Most studies use air temperature and this data is easily available from weather stations. However, care must be taken about conclusions on whether a species can survive in a given location as it may not take into account the microclimate that the insect would experience. For instance the leaves and bark of plants typically tend to be warmer than the air temperature [4]. This disparity in temperature between air and the microenvironment is particularly true for wood boring insects such as *A. chinensis* and *A. glabripennis* where the larvae reside within the sap and heartwood of the tree. A number of studies have investigated under bark temperatures, usually in regard to the physiology of the tree itself. These studies found that temperature through a horizontal cross-section of the trunk was anisotropic with the side facing the sun showing large fluctuations in temperature whereas the side facing away from the sun had smaller fluctuations in temperature [12, 13, 33, 35]. This difference is reflected in the preference of African longhorned beetle species which tend to prefer sides that receive direct sunlight [14]. There is also a difference in tree bole temperature in regard to height with points further up the bole having a higher mean temperatures than points lower down [35]. This is most likely caused by the lower parts of the trunk being more shaded. The effect of direct sunlight can also be seen by the fact that fluctuations in temperature are larger in winter than summer due to the lack of a canopy during the winter months [13]. Stress can also affect tree temperature as in a study looking at the susceptibility of trees to *Sirex* infestation it was found that stressed and weakened trees had a higher temperature than healthy ones [19]. The paper suggested that this higher temperature was due to the trees having a smaller canopy and not being able to

control their temperature as well through transpiration. These weakened trees were also susceptible to *Sirex* infestation, which resulted in a further increase in temperature due to a further reduction in transpiration. These studies would all suggest that tree temperature must be considered when looking at the suitability of areas for *Anoplophora* species.

Day degree models

Degree days are used in biology as a measure of the development of poikilothermic organisms such as insects in regard to temperature. They can consequently be used to determine if the climate of a particular region is suitable for an insect to complete its life cycle. There are a number of different methods and models for measuring degree days of which there are two categories linear and on-linear [26]. All methods follow the same basic principle of calculating the number of degrees that the temperature exceeds a minimum threshold and remains lower that a maximum threshold during a certain period of time. The minimum threshold being the minimum temperature required for the development and maximum threshold being the temperature at which it is too warm for development to occur and are here on described as the lower and upper developmental thresholds respectively. These thresholds vary between species and it is important to acquire accurate values as minor differences can result in significant changes to the degree days [4]. The main difference between the two types of degree day calculations is in regard to development [26]. The rate of development of an insect species is not constant but varies with temperature [7, 11, 30, 34]. When the temperature rises above the lower developmental threshold the rate of development increases from zero before levelling out when the optimum temperature range is reached. The rate of development then decreases when the temperature increases above the optimum and reaches zero at the upper developmental threshold. Non-linear models account for this change when producing day degree values [26, 29]. Linear methods by contrast assume constant rate of development and therefore that the temperature range experience by the insect is within that of the optimum range [8]. This assumption can therefore lead to inaccuracies, particularly for extreme temperatures and so if accuracy is required non-linear models are preferable [26, 31]. However, non-linear models consequently require far more information regarding the effect of temperature on the development of an insect and so for species where little information is known, linear models provide a suitable alternative to non-linear models.

Due to their greater complexity there are a large number of non-linear degree day models for a variety of different insects. For example CLIMEX has previously been used to look at the suitability of the European climate to *Anoplophora glabripennis* [25]. There are however far fewer linear models due to their greater simplicity. There are three main types of linear model average: sigmoidal and triangulation [2, 3, 24]. Of these average is the most simplistic as it assumes that the graph area

Aim of study

The aim of this study was to compare the degree days accumulated within a tree trunk and compare it to the number of degree days as measured by air temperature. This was achieved by calculating the yearly accumulation of degree days using a linear method. As sunlight has a large effect on tree temperature the study also looked at the difference in degree days between trees in the open and those within a stand to how this may affect *A. chinensis* and *A. glabripennis.* There are also a number of different linear methods for calculating day degree so the study also looked at which is the most accurate method.

Methods

Site location

Temperature data was collected from six trees on the Food and environment research agency Sand Hutton site for the entire 2012 calendar year. The trees were numbered one to six. The trees were selected in two groups. The trunk of three of the trees (one, two and five), was exposed to sunlight and the trunk of the other three (three, four and six) was shaded from direct sunlight at least of part of the day by the presence of other trees. All six trees were *Betula pendula* (Fagales: Betulaceae), silver birch.

Temperature measurement

Temperature data was recorded using Gemini Tinytag Plus 2 temperature loggers and Gemini Thermistor PB-5005-0M6 probes. For trees 1 to 5 the TGP 4020 model of Tinytag Plus 2 loggers were used and the TGP4510 model was used for tree 6. A different model was used for tree 6 as this model can take dual measurements and was used to measure the air temperature as well as the under bark temperature. Holes were drilled in the south facing side approximately 20 cm from the base of the tree and to a depth of around 44 mm. A 3mm drill bit was used for the first 38-40 mm and then a 2.5 mm drill bit was used. The probes were placed in holes and then the holes were then sealed with silicon door sealant. The diameter of the tree bole where the probe was inserted is recorded in the table below:

Tree Number	**Exposed/ Shady location**	**Height of logger temp. Probe above ground (cm)**	**Diameter of tree at height of temp. Probe (cm)**	**Diameter (cm) of tree at breast height (137cm)**	**Circumf. of trees at probe height (cm)**	**Circumf. of trees at breast height (cm)**
1	Exposed	18	16	11	49	34
2	Exposed	18	27	18	84	57
3	Shaded	23	24	17	74	54
4	Shaded	20	14	10	44	32
5	Exposed	15	18	12	56.5	37
6	Shaded	21	18	13	56	41

Table 1: Dimensions of trees used in the study

The data loggers were placed in wooden nest boxes attached to the base of the tree. For the tree 6 logger as the air temperature was being measured by an inbuilt probe in the logger, a hole was cut in the side of the bird box and covered with a fabric mesh to allow air flow past this sensor. Loggers were initially set to record hourly temperature readings but this was changed to half hourly readings on 26/01/2012 for the loggers attached to trees 1, 2 and 3 and 13/06/2012 for the loggers attached to trees 4, 5 and 6. Data from the loggers was downloaded periodically and converted from Tinytag format (TTD) to comma separated value (CSV) format using the Tinytag software. This allowed the data to be manipulated in Microsoft excel.

Data analysis:

The data from the six trees and the air temperature were analysed using Microsoft excel. For each data set the mean and average and extreme maximum and minimum temperatures were found for each month, season and the entire year. The number of degree days above 10°C was calculated for each tree and the air temperature. 10°C is the lower developmental threshold of *A. glabripennis* [20, 21]. No upper developmental threshold was used as *A. glabripennis'* upper developmental threshold is 35°C and none of the trees or the air went above this temperature [20, 21]. A linear model was used to calculate the degree days as currently there is no non-linear model for *Anoplophora* species that can be used to look at the microclimate. The linear model used calculated the change in degree days in hourly or half hourly time intervals from the area under a graph of temperature against time that is above 10°C or 11.6°C. A daily day degree value was then produced from the sum of these hourly/half hourly values. To calculate the area under the graph, as the graph is sigmoidal, triangulation was used. Any negative degree day values were corrected to zero before the total degree days were calculated. The degree days calculated in this manor were then compared against several methods that estimate day degrees from daily minimum and maximum temperatures. All the degree day methods are listed below in Figure 1 along with the formulas used.

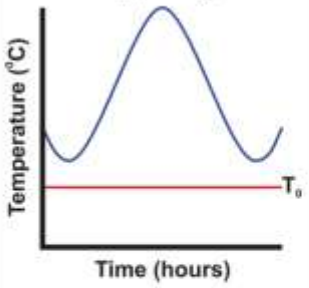
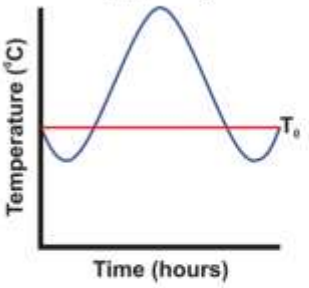
	$T_{min} > T_0$	$T_{min} < T_0$
		
Area under graph	$\Delta t\left[\frac{(T_a-T_0)+(T_b-T_0)}{2}\right]$	-
Average	$\frac{T_{max}+T_{min}-T_0}{2}$	$\frac{T_{max}+T_0-T_0}{2}$
Double sine	$\frac{T_{max1}+T_{min1}-T_0}{2} + \frac{T_{max2}+T_{min2}-T_0}{2}$	$\frac{1}{\pi}\left[\left(\frac{T_{max1}+T_{min1}-T_0}{2}\right).\left(\frac{\pi}{2}-\theta_1\right)+\left(\cos(\theta_1).\alpha_1\right)\right] + \frac{1}{\pi}\left[\left(\frac{T_{max2}+T_{min2}-T_0}{2}\right).\left(\frac{\pi}{2}-\theta_2\right)+\left(\cos(\theta_2).\alpha_2\right)\right]$
Double triangulation	$\frac{6(T_{max1}+T_{min1}-(2.T_0))}{24} + \frac{6(T_{max2}+T_{min2}-(2.T_0))}{24}$	$\frac{(6(T_{max1}+T_{min1})^2)/(T_{max1}-T_{min1})}{24} + \frac{(6(T_{max2}+T_{min2})^2)/(T_{max2}-T_{min2})}{24}$
Single sine	$\frac{T_{max}+T_{min}-T_0}{2}$	$\frac{1}{\pi}\left[\left(\frac{T_{max}+T_{min}-T_0}{2}\right).\left(\frac{\pi}{2}-\theta\right)+\left(\cos(\theta).\alpha\right)\right]$
Single triangulation	$\frac{6(T_{max}+T_{min}-(2.T_0))}{12}$	$\frac{(6(T_{max}+T_{min})^2)/(T_{max}-T_{min})}{12}$

Figure 1: Formula for calculating degree days

For each method of calculating degree days the formula is given for when the T_{min} is lower than the T_0 and when it is higher than the T_0. The area under the graph formula is the same in both instances. All the formula bar the area under the graph calculate the degree days for an entire day whereas the time interval for the area under the graph formula is variable. The symbols used in the formula are defined in Table 2.

Symbol	Definition	Symbol	Definition
θ	$\sin^{-1}(\underline{(T0-0.5(Tmax+Tmin))}$ α	**Tmin**	Minimum temperature
		T0	Lower developmental threshold
α	$\underline{(Tmax-Tmin)}$ 2	**Ta**	First time point temperature
		Tb	Second time point temperature
Tmax	Maximum temperature	**t**	Time

Table 2: Definitions of symbols used in calculating degree days

Results

Temperature analysis

Analysis of the temperature data for 2012 shows several differences between trees in the open and those in the shade. When considering the yearly distribution of temperatures there is a clear difference in the upper quartile with open trees having a far greater variation in temperature than shaded trees (Figure 2). The maximum values are consequently lower for shaded trees and are in fact similar to that of the air temperature. There is variation in the range of the lower quartile but this does not correlate with the whether the tree was shaded or open. The median and interquartile range of trees in the open are similar to each other but there is more variation for those in the shade. The fact that the upper but not the lower quartile shows a difference between open and shaded trees is most likely due to the fact that these higher temperatures mostly occur in summer when the tree canopy is present. The canopy would therefore provide shade and reduce the amount of sunlight reaching the tree bole. In winter when there are no leaves more sunlight

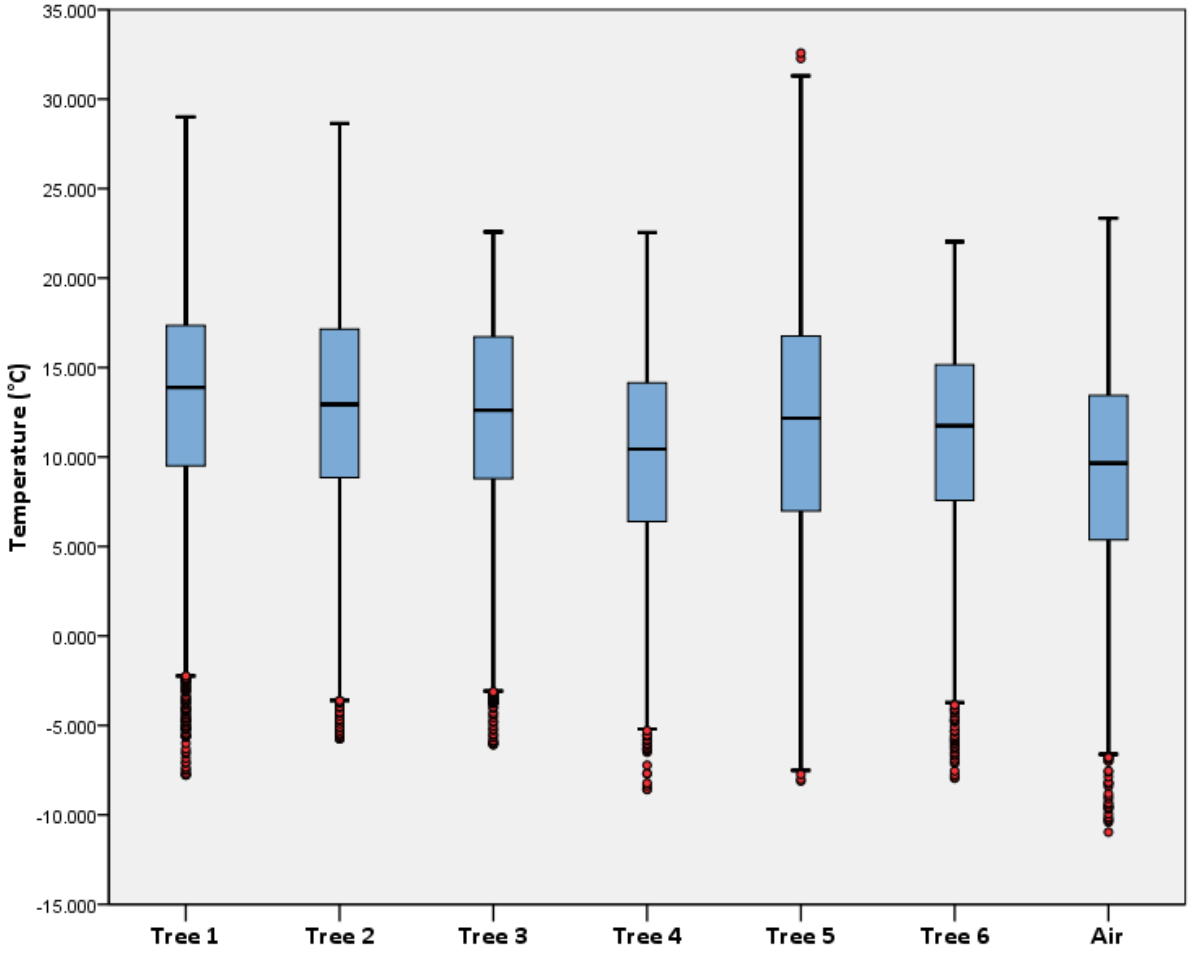


Figure 2: Variation in temperature in 2012

Cumulative degree days

Degree days were calculated from temperature data from all six trees and the air. All the trees have a higher number of degree days per year than the air, meaning that any degree day analysis for *A. glabripennis* or other wood boring insects using air temperature would be inaccurate (Figure 3 and Table). For instance if oviposition occurred on the 1st January and the number of degree days for 50% of the adults to emerge was 600 then using tree 1 data the adults would emerge on the 9th July whereas using air data the adults would emerge on the 1st October, which is a difference of 83 days. There is also a difference between shaded and exposed trees with the latter having a higher number of cumulative degree days (Figure 3 and Table). The most likely cause of this difference will be sunlight as previous research showed that sunlight has a large effect on tree temperature, although this was usually in relation to the south and north sides a tree rather than exposed or shaded trees [13, 33, 35]. However, other environmental variables could also affect tree temperature such as wind as the shaded trees would also be protected from the wind to a greater extent that the exposed trees. This difference between exposed and shaded trees would the exposed trees more suitable as hosts for *A. glabripennis* as their larvae would have a longer period in which development can occur. There is greater variation in the cumulative degree days between shaded trees than open trees with a maximum difference of 333 compared to 125 for exposed trees. The most likely cause of this greater variation is that the amount of shade given by the canopy is likely to vary thus the shaded trees will receive different levels of solar radiation.

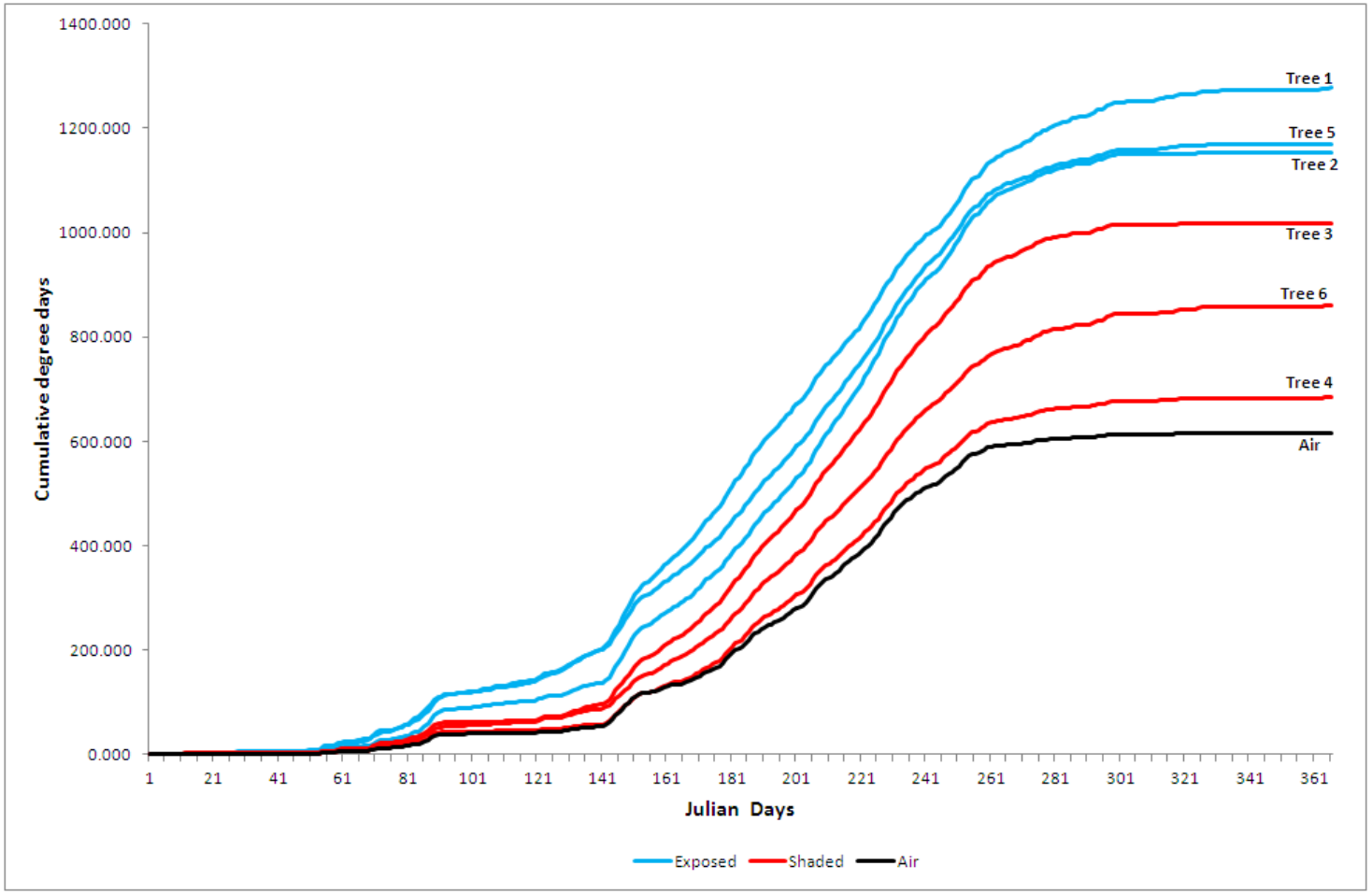


Figure 3: Cumulative degree days during 2012

Line graph showing the accumulation of degree days from 1st January 2012 to 31st December 2012 for trees in the shade or in the open and air.

Logger	Shaded/open	Cumulative degree days
Tree 1	Exposed	1278
Tree 2	Exposed	1153
Tree 3	Shaded	1018
Tree 4	Shaded	685
Tree 5	Exposed	1170
Tree 6	Shaded	861
Air	-	617

Table 3: Cumulative degree days for 2012

Table of cumulative degree days for each tree and the air temperature. Table also states whether the tree is exposed or shaded.

When the daily degree days are considered they show several differences between the data sets. For instance exposed trees differ to a far greater extent from shaded trees during days when the degree days are higher, for example between days 145-150 (Figure 4). This is similar to what is observed for temperature where the main differences between exposed and shaded trees occurred at high temperatures. Daily degree days also highlight the importance of the microclimate of each tree as, although for the total degree days all three exposed trees have a higher number of degree days than shaded, on certain days some of the shaded trees can have more degree days than exposed trees. For example on day 275 the degree days for trees 1, 2 and 5 were 4.66, 3.58 and 3.65 respectively whereas for the shaded trees (3, 4 and 6) they were 4.32, 2.97 and 4.06 respectively. The daily day degrees can also be lower than that of the air for shaded trees.

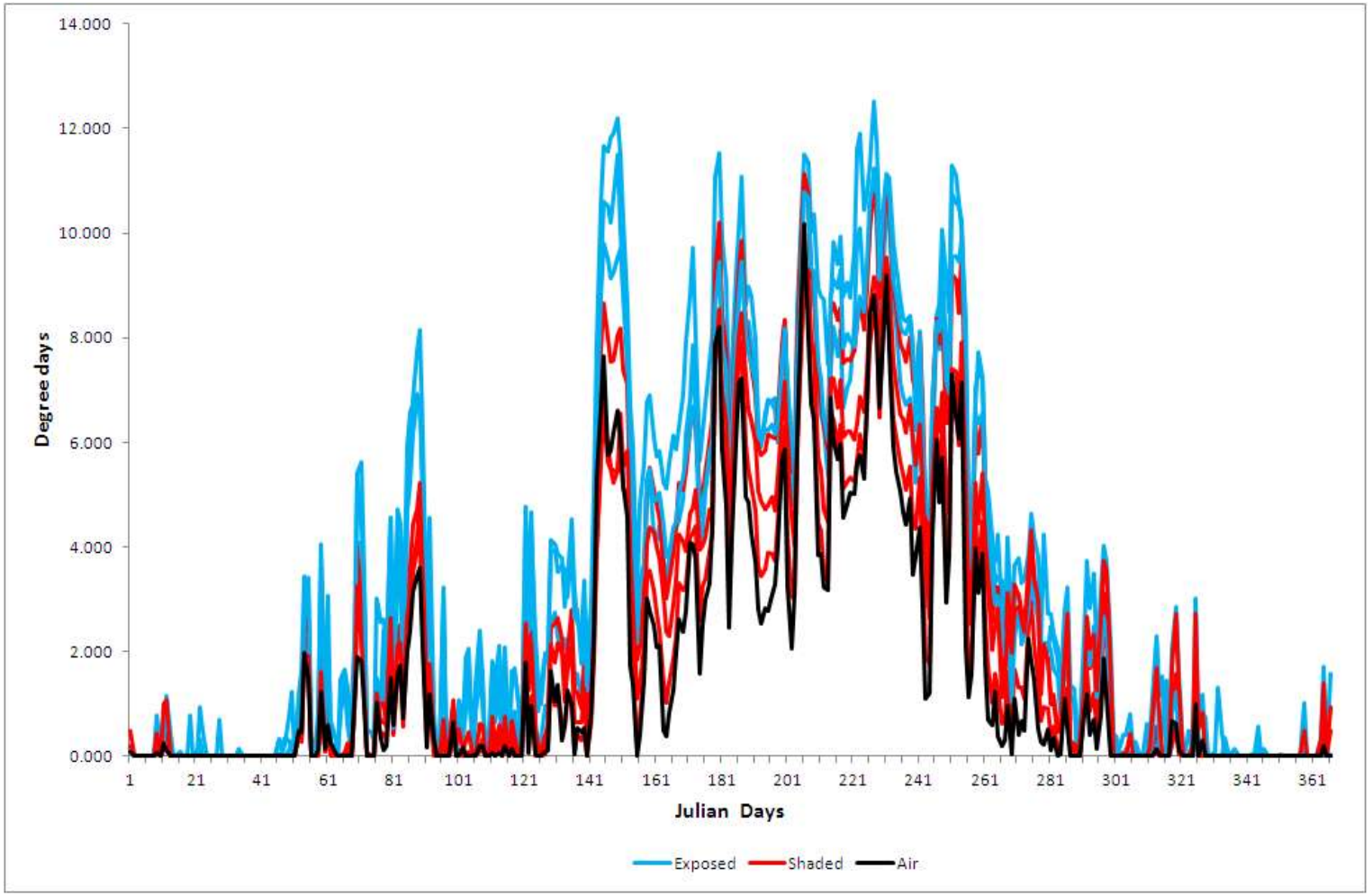


Figure 4: Number of degree days accumulated per day for 2012

Line graph showing the number of degree days for each day from 1st January 2012 to 31st December 2012. Due to the lines overlapping the individual trees are not labelled but it is shown whether the line is for air, tree in the open or a shaded tree.

Comparison of degree day models

Conclusions

The variability in both the cumulative and daily day degrees demonstrates that although general assumptions can be made regarding the day degrees of a tree depending on whether it is shaded or exposed, the specific microclimate of the tree can have a significant effect. This would have an effect on any predictions made regarding the development of *A. glabripennis.* Returning to the previously used example of oviposition occurring on the 1st January and 600 degree days being required for 50% of adults to emerge then this would occur on different days. This would therefore result in a relatively large window in which emergence could occur. However, if exposed and shaded trees are considered separately then the windows are smaller and do not overlap. This again demonstrates that treating exposed and shaded trees separately is important.

Tree	Date when 50% adults emerge by
1	9th July
2	27th July
3	5th August
4	8th September
5	21st July
6	19th August
Air	1st October

Table 4: Date on which 50% of A. glabripennis adults would emerge

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Estimates of *Anoplophora* lifecycles using an weighed accumulated Degree Day Model –

Potting RPJ & Loomans AJM (NVWA, NL) (D6.3)

To estimate the developmental times and life-cycle period for *Anoplophora* species under Dutch conditions we calculated the degree days above the lower temperature threshold in a Degree Day Model. We used meteorological data for the period starting from 1-8-2000 until 29-09-2011, i.c. Lelystad, the Netherlands. Calculations on degree-days above the lower temperature threshold were made for each immature stage, and for 10-50-90 and 99% of the larvae completing its development (Keena & Moore 2010; fast developers: DD10%, median population: DD50%, DD90%, including slow developers: DD99%). Because ALB larvae develop in galleries inside wood, temperatures are buffered, we made a correction in surplus of the ambient air temperature of 2^0C.

Calculating degree days shows that on average it takes 3 years for ALB to develop under Dutch conditions. However, when the population is split up in those larvae that develop fast (10% of the ALB population; DD-10%: Keena & Moore 2010) calculation of DD results in generation time of 2 years or less. On the other hand, it takes 6 years for 99% of the ALB population to complete its development.

Year	Emergence 10%	Lifecycle	Emergence 50%	Lifecycle	Emergence 90%	Lifecycle	Emergence 99%	Lifecycle
2000	1-8-2000		1-8-2000		1-8-2000		1-8-2000	
2001								
2002	16-7-2002	2						
2003			11-6-2003	3				
2004	7-7-2004	2			19-8-2004	4		
2005								
2006	3-7-2006	2	4-5-2006	3			18-7-2006	6
2007								
2008	12-5-2008	2	16-8-2008	2	1-8-2008	4		
2009								
2010	30-4-2010	2						
2011			1-8-2011	3				
Lifecycle		**2**		**3**		**4**		**6**

Table 1 - Calculation of development times and lifecycle of *Anoplophora glabripennis* under Dutch climatic conditions from 2000 -2011, using a Degree Day model.

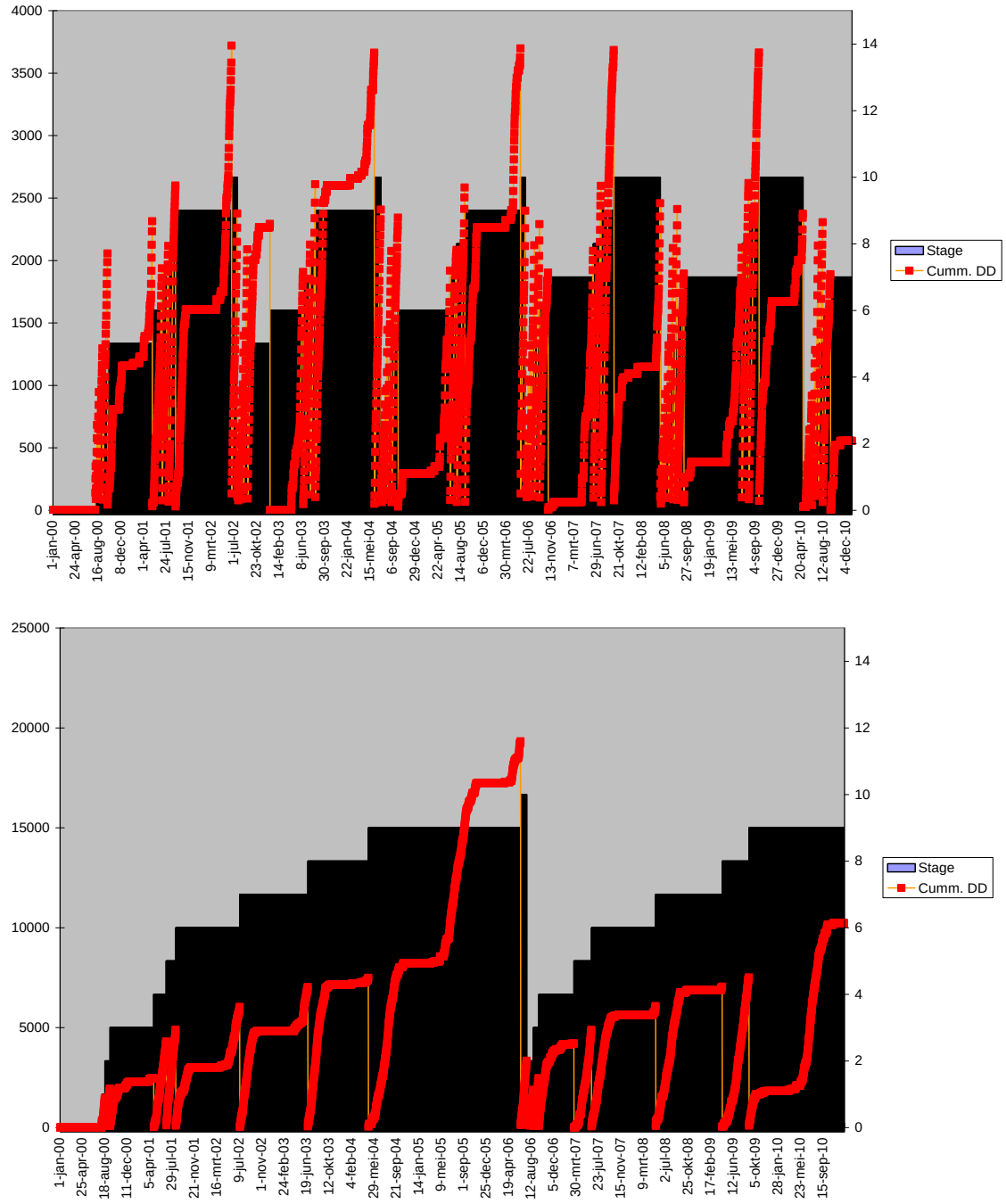

Figure 1 – Day Degree calculations using Keena & Moore (2008) developmental results for *Anoplophora glabripennis* under Dutch climatic conditions. Calculated for the first 10% (left) and right for 99% (right) of the population to develop until maturity. (y = daydegrees, date since 2000)

Reference

Keena, M.A.; Moore, P.M. 2010. Effects of temperature on Anoplophora glabripennis (Coleoptera: Cerambycidae) larvae and pupae. Environmental Entomology. 39(4): 1323-1335.

Potential for establishment of *Anoplophora chinensis* CLB and *A. glabripennis* ALB in Denmark (D6.3)

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In this report an assessment will be made of the day degree requirements for development of the two species of *Anoplophora* and whether or not these species can be expected to establish in Denmark. It has been inspired by the document by Eyre (2011).

Day degrees (DD) required by each species for development from egg to adult

Information on day degrees requirements can be useful for assessing the probability that the species will be able to complete its development from egg to adult in different areas.

Anoplophora chinensis CLB

In a laboratory study of the development of CLB at constant temperatures and in fluctuating temperatures (diel and seasonal) Adachi 1994 found that development from egg to the 7th larval stage (L7) requires 1357 DD above 11.2°C, a result obtained at constant temperature. The numbers of larval stages were 7-9 or 11-15 in individuals with one or 2 year life cycles, respectively. The pupal stage lasts 23.8 days at 20°C, corresponding to 209 DD. Thus it would seem that total development from egg to adult requires somewhat more than 1565 DD above 11.2°C, maybe 1900 or 2000 DD.

Adachi (1988) reports a preoviposition period of 86 DD.

A study of climate modelling the value 1550 DD above 7°C was used for CLB (Robinet et al. 2012).

Anoplophora glabripennis ALB

In the field 1264 DD above 13.4°C are required for completion of a generation in *A. glabripennis* (Yang et al. 2000, referred by Hu et al. 2009). Results for selected stages are: egg stage: 239 DD above 11°C (Keena 2006); egg, L1 and L2: 640 DD above 11°C ((Zhang et al. 1995, referred by Hu et al. 2009).

Keena and Moore (2010) studied the development of juveniles under many different constant temperature regimes, including periods of chilling to induce pupation. The complex data are difficult to break down to a single result, but from table 7 it seems that development of larvae plus pupae requires approximately 2800 DD above thresholds of 9-13°C, with higher temperature thresholds in late larval instars.

Thus, development from egg to adult under constant temperature requires 3040 DD above approx. 11°C.

Furthermore ALB has a preoviposition period of 10-15 days (Smith et al. 2002) which gives an additional 100 DD.

MacLoed et al. (2012) conducted climate modelling of ALB using 1450 DD above 10°C.

General remarks regarding both DD calculations:

Data obtained at constant temperatures in the laboratory generally differ from results in fluctuating temperatures and they represent a simplification of a complex situation. Thus DD can only give an indication of the actual situation, and other factors must be considered as well.

In both species a certain proportion of the population is able to complete their development at constant temperatures, whereas the rest need to be chilled before pupation. Other factors e.g. the host species, wood moisture content and larval weight affect how many larval stages an individual goes through. In addition, fluctuating temperatures during seasons and specific temperature requirements also influence when pupation is initiated. These aspects also all affect the development time of a generation and will thus have great influence on the number of required DD.

ALB and CLB live within wood as juveniles. Temperatures in wood differ somewhat from those of the ambient air. Wood in standing trunks exposed to the sun can be up to 14°C higher than the maximum air temperature of the same day (Haarløv and Petersen 1952), depending on exposure to sun/shade, wind, location in the tree etc. Investigations in the UK showed that the DD above 10°C accumulated over a year in a trunk exposed to the sun are 35% higher than the ambient air, and in the shade 14% lower (results presented by D. Eyre 2012). Calculations of which temperature regimes the insects experience are further challenged by the fact that young and old larvae live at different depths in the wood and thus experience different temperatures. Keena and Moore (2010) suggest the addition of 2°C to the air temperature to compensate for the buffering effect of trees, which though it is an arbitrary factor may be reasonable in the absence of better models.

Development in Denmark

Day degrees have been calculated for Flakkebjerg in Denmark for the years 2007-2011 (Table 1).

Table 1. Day degrees (DD) 2007 – 2011 from Flakkebjerg, Denmark, above threshold temperatures of 11 and 9°C, respectively. Based on AU Dept. of Agroecology, climate database, mean daily temperatures.

	2007	2008	2009	2010	2011	Average
DD >11°C	650	665	706	606	689	
DD >9°C	988	1002	1030	897	1037	991±50

With 9°C as threshold temperature, corresponding to an addition of 2°C to compensate for the buffering effect of the wood (Keena and Moore 2010), total development of CLB will take close to 2 years in Denmark (with 1900 DD). Individuals from eggs laid late in summer may have a 3 year-development. Total development of ALB will take 3 years in Denmark. This conclusion presupposes that development of *Anoplophora* can be longer than the 1-2 years' development time that is reported in many studies (Adachi 1994, Keena and Moore 2012). If the DD values used in MacLeod et al. (2012) and Robinet et al. (2012) are applied, development of both species will take 2 years in Denmark.

A few observations indicate development times of more than three years (CLB in Denmark 2011 (C. Scheel pers. comm.), CLB in the Netherlands 2007 (Gaag et al. 2008)), but they have not been verified. As these insect species may eventually kill their host, the breeding material can be expected to dry out with time, and maybe become unsuitable for them. However, they will be large larvae and pupae during the third year, and may be able to survive in wood with lower moisture content.

Other cerambycids attacking living, newly felled or stressed trees have very long development times:

- *Morimus funereus* (larvae for 3-4 years, live in physiologically debilitated trees or newly felled trees (Ivanovic et al. 2002),
- *Goes debilis* (3-4 years, maybe longer in northern regions, live in living oak trees and lead to death of small branches (Solomon 1977))
- *Styloxus bicolor* (3 years, live in branches of *Juniper* spp., the final larval stages enter the trunk (Itami & Craig 1989)).

Climate modelling of establishment of *Anoplophora* spp. in Northern Europe, with focus on Denmark

For an evaluation of the potential for establishment and development of invasive species CLIMEX modelling is applied, based on a range of climatic requirements for the species and meteorological data in the area of origin and the areas in danger. During this process an Ecoclimatic Index (EI, which ranges between 0 and 100) is calculated (Sutherst et al. 1999). The outcome of the calculations depends on the parameters that are used. An EI>0 indicates that establishment is possible. Several studies have used this approach.

The EI in the area in China where ALB infestations are most severe is 32.1 ± 6.9 (range 4-54). In other areas in China the EI is 12.3 ± 5.3. Large areas of Northern Europe (north of the Alps) have EIs of 30-40. In these areas several cases of established ALB occur (Austria, Germany, UK). In Denmark the EIs range between 30 and 50. The authors conclude that ALB likely to be able to establish in Northern Europe including Denmark, because the EI values are very similar to those in parts of China where the impact of the beetle is most severe (MacLeod et al. 2002).

In a more recent study on ALB MacLeod et al. 2012 reported an EI for eastern Denmark of 16-30 and for western Denmark 6-15 using 1450 DD above 10°C. Areas in China where ALB occurs have EIs of 16 and higher. The generation time was estimated to be 3 years in most of Denmark except the south-eastern areas where the generation time was 2 years.

Robinet et al. 2012 reported an EI for eastern Denmark of 16-25 and western Denmark 1-15 for CLB using 1550 DD above 7°C.

In a report on CLIMEX modelling of the potential distribution of ALB in Sweden now and in a situation affected by future climate changes, on all maps Denmark has EIs of 22.8 to 30. The same EI is found when two other global climate models are used. According to this publication EIs of 20-25 mean that the conditions for survival and development are good (Ahrné 2012).

Bidinger (2012) has calculated where ALB can live based on the climate in i) its area of origin, and ii) areas where it is invasive. Denmark is on the border between the unsuitable area and areas with low suitability. If future climate changes are taken into account Denmark is within the area with low suitability.

A risk assessment of CLB for Norway (Sundheim et al. 2012) shows the probability for establishment of this species in Europe. In this report the probability for Denmark is above average (a simple niche model based on three temperature parameters). This report estimates that CLB will be able to survive through winters in the coastal areas of Southern Norway.

Conclusion

- Based on simple application of day degrees (DD) CLB and ALB are estimated to be able to complete their development in Denmark maybe in 2, but with greater probability in 3 years.
- Climate modelling suggests that establishment and development of both species in Denmark is possible. Generation time for ALB was estimated to be 2 to 3 years.
- These conclusions are based on the assumption that ALB and CLB are able to survive as juveniles for more than the 2 years that has been considered the case until now. Several unconfirmed reports exist of development times of 3 years or more.

Acknowledgements

I thank Dominic Eyre for useful comments on this report.

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Implications of internal tree temperatures on the potential rate of development of *Anoplophora glabripennis* in Europe (6.3, D6.4)

Dominic Eyre, Fera

Over the course of 2012, the number of day degrees in excess of 10°C as measured by air temperature was 617. The number of day degrees recorded over 10°C recorded in the trees in exposed locations was 1278, 1153 and 1170, with a mean of 1200. Therefore, the number of day degrees accumulated in an exposed tree is 1.945 times greater than air temperature. Working on the assumption that this difference would be applicable to all host trees the two maps below represent theoretical development rates based on air temperature and internal tree temperature.

A CLIMEX model for *Anoplophora glabripennis* was developed. The initial parameter set used to develop this model came from a 2004 model developed by the Dutch plant health service (de Boer, 2004). This model was modified in response to newer data. Location data from was mapped using location data in the following sources Lingafelter & Hobeke (2004), Gao et al. (1997), Wang (2003) and Haack et al. (2010). In addition the model made use of laboratory data especially Keena (2006) and Keena and Moore (2010). Some of the useful data and observations from these studies were:

- The optimum temperature for adult fecundity is 23/24°C (Keena, 2006)
- Normal activity of *Anoplophora nobilis* is 16-28°C (Zhou et al, 1984)
- the maximum % of females that produced progeny peaked at 25°C (Keena, 2006)
- *A. glabripennis* has been found to stay in shaded areas when the temperature exceeds 29°C (Xiao, 1980)
- Humidity is thought to be important for *A. glabripennis* adults (Keena, 2006)
- At temperatures of ≤15°C development of *A. glabripennis* larvae was slow or did not occur (Keena & Moore, 2010)
- Mortality was lowest at 25°C and increased above and below that (Keena & Moore, 2010)
- The minimum threshold for development of *A. glabripennis* larvae and pupae varies by stage between approx. 9°C to 13°C
- The mean supercooling points (^{o}C ± S.E.) for ALB larvae is 25.8 ± 1.1 ^{o}C (Roden et al, 2009)
- At 20°C most larvae were able to proceed to pupation without a chill period or exposure to high temperatures(Keena & Moore, 2010)
- Only a few larvae at 25°C and no larvae at 30°C were able to proceed to pupation without a chill period (Keena & Moore, 2010)
- The lower temperature for development for all instars seems to be between 9°C and 13°C (Keena & Moore, 2010)
- The lower temperature for development for the first and second instars is 11.7°C and 11.4°C respectively (Zhang et al, 1995).

Table showing the parameters of the Climex model

Environmental factor	CLIMEX Parameter	Abbreviation	value
Temperature	Limiting low temperature	DV0	10
	Lower optimal temperature	DV1	22
	Higher optimal temperature	DV2	27
	Limiting higher temperature	DV3	30
	Degree days for complete life cycle	PDD	1450
Moisture	Limiting low moisture	SM0	0.02
	Lower optimal moisture	SM1	0.08
	Higher optimal moisture	SM2	1.5
	Limiting higher moisture	SM3	1.75
Heat Stress	Temperature threshold	TTHS	31
	Temperature rate	THHS	0.002
	Degree-day threshold	DTHS	40
	Degree-day rate	DHHS	1
Wet Stress	Threshold	SMWS	1.8
	Rate	HWS	0.001
Interactions			
Hot-dry stress	Temperature threshold		28
	Moisture threshold		0.07
	Stress rate		0.08
Hot-wet stress	Temperature threshold		27
	Moisture threshold		1.5
	Stress rate		0.1

Fig 1: Predicted development rate of ALB in Europe base on the CLIMEX model and the presumption that the ALB larvae experience air temperature*

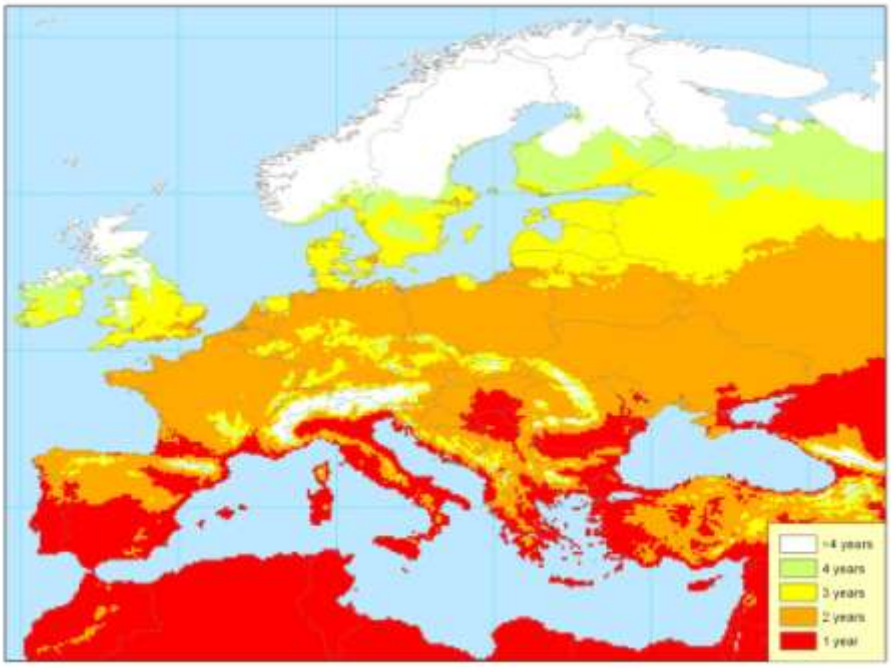
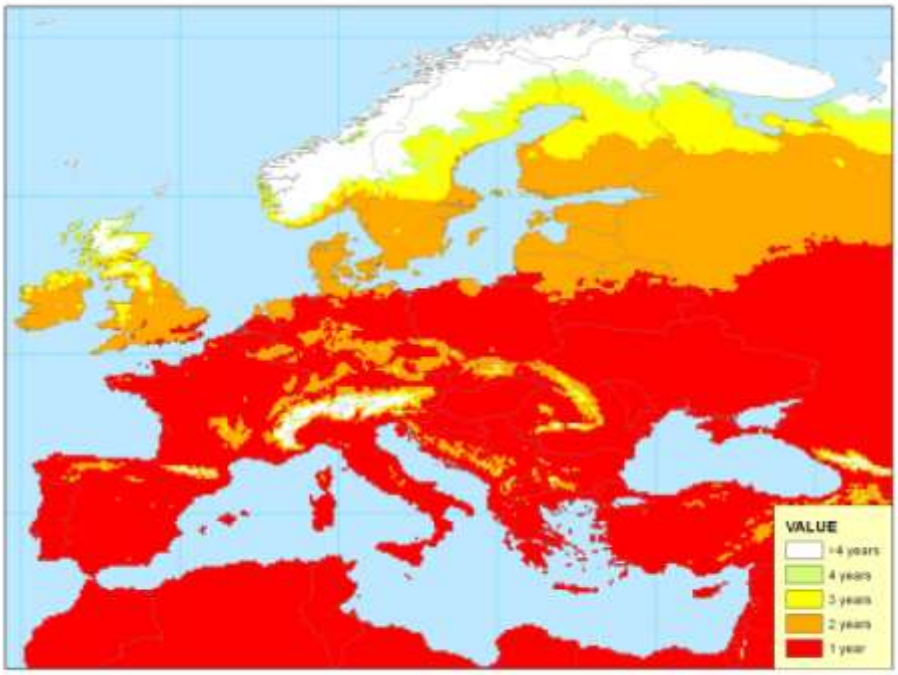


Fig 2. Theoretical development rate of ALB larvae that develop on the south side of trees in Europe*



The data used in the above two maps is derived from New et al. (2002)

Discussion

This study has shown the potential significance of microclimate in the development rate of longhorn beetles. Larvae developing in shady locations are likely to develop at rates similar to those predicted by air temperature, however larvae developing in branches or trunks that are regularly exposed to direct sunlight could develop as quickly as shown in Figure 2 above. Based on air temperature, ALB could only complete a life cycle within 2 years in a small part of southern England, whereas beetles developing in locations exposed to the sun could develop within 2 years in all except some upland parts of England.

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Work Package 7: Development of contingency plans / best practice manual