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Special issue on

Anoplophora Chinensis & Anoplophora Glabripennis: new tools for predicting, detecting and fighting.

How to save our forests and our urban green spaces

Milan, 9-11 May 2012

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In memory of Jacki, first Anoplophora sniffer dog.

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Lombardy Region experience to support the prediction and detection strategies

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Abstract

Since 2000, Lombardy Region is involved in fighting *Anoplophora chinensis,* an alien insect introduced form Asia, very dangerous for trees and for green heritage in general. This study shows the results and considerations came out from a long experience of monitoring and fighting carried out by Lombardy Phytosanitary Service in order to contain this quarantine pest.

Introduction

Anoplophora chinensis (Forster), found for the first time in Europe in 2000 in the province of Milano (Italy), is one of the most potentially damaging Invasive Aliens Species ever introduced to Lombardy.

This insect is causing serious damage to the urban ecosystem, showing a capacity to infest plants in an excellent state of health.

In the initial stage of the battle against *A. chinensis,* there were many difficulties, due primarily to our insufficient knowledge of the insect's life cycle, its ethological behavior in a new environment, its preferred tree and its ability to colonize new areas.

The fight against *A. chinensis* is particularly difficult, so we must optimize the human and financial resources employed in eradication efforts; the data collected in Lombardy and illustrated in this study are an important informational tool for those who, in the future, may find themselves facing a new outbreaks.

In any case, we underscore that all the measures utilized to eradicate or contain infestations of *A. chinensis* must comply with the indications contained in the EU Emergency Decisions (2008/840/CE, abrogated by 2012/138/CE).

The data presented are the result of the processing of data collected during the work performed in the Demarcated Area in Lombardy, which covers about 400 km^2 in 4 provinces, including over 70 municipalities.

The study addresses different aspects considered important in defining an effective strategy against infestation.

The most vulnerable host species are indicated, and the varying degrees of vulnerability within the same genus, as well as the insect's ability to conclude its life cycle in the various host species and their relative vulnerability ranking.

Knowing the distance, for the instances of infestation occurring outdoors, within which the total extent of the infestation should be contained, was considered an essential point with the ultimate goal of eradicating *A. chinensis*.

Unpublished data on the movements of adult *A. chinensis* beetles (Adachi, 1990) show that individuals of this species can, on rare occasions, move as far as 2 km from the point in which they are released. However, data published by the same author also show that most adults moved less than 50 m, with a maximum recorded dispersal distance of 160 m. Other authors confirm that adults remain close to the tree from which they emerge, and that newly infested plants tend to be close to exit holes (Hérard *et al.*, 2005 and 2006). Greater dispersal distances are found for *A. chinensis* in the presence of high-density areas of infestation.

The studies mentioned above generally refer to techniques involving the marking, release and recapture of a sample of specimens; little is instead known concerning the dispersal capacities of entire populations of the species in question.

Accurate demarcation of the survey area is essential, because if the area is too small, cases of infestation will be missed, while on the other hand, if it is too large, monitoring would require excessive effort.

Materials and methods

Item-1: Analysis of data collected during the survey of *A. chinensis* in heavily infested areas

The data used originate from 12 municipalities, each of which had at least 100 infested plants, for the year in which the greatest degree of infestation was found.

The 12 most intensely infested areas were purposely chosen in order to maximize statistical significance. The total number of infested plants analyzed was 6831, corresponding to about 70% of the total number of infested plants found in the provinces of Milan and Varese. This is the first study, on the international level, to take into consideration such a high number of infested plants. It is also the first study to include all the plants present in a large area and make a distinction between each single species, rather than just symptomatic and asymptomatic plants. All the plants checked were geo-referenced. The analysis concerns only plant species for which there was a confirmed *Anoplophora chinensis* infestation in the areas surveyed and this was not an exception.

Item-2: Outbreak areas - maximum distance between cases of infestation

Three different geostatic methods were used to define the optimal monitoring areas.

The maximum distances between cases of infestation were calculated for both urban and agricultural environments, using 7 geo-referenced study areas in 5 municipalities with high levels of infestation.

The dynamics of the development of the infestation, with particular reference to the macro-hot spot of the infested area, in the provinces of Milano and Varese, were also studied in terms of providing feedback regarding the effectiveness of the plant health measures applied.

Method 1

Calculations were made by grouping together the points of infestation in homogenous areas, then by creating a buffer zone with a radius of 100 m, using the Arcview software. Subsequently, the same software was applied to calculate the maximum distance between homogenous areas (Figure 1).

Method 2

Calculations are based on the maximum distance between specimens with exit holes and specimens showing only signs of larval activity (Figure 2).

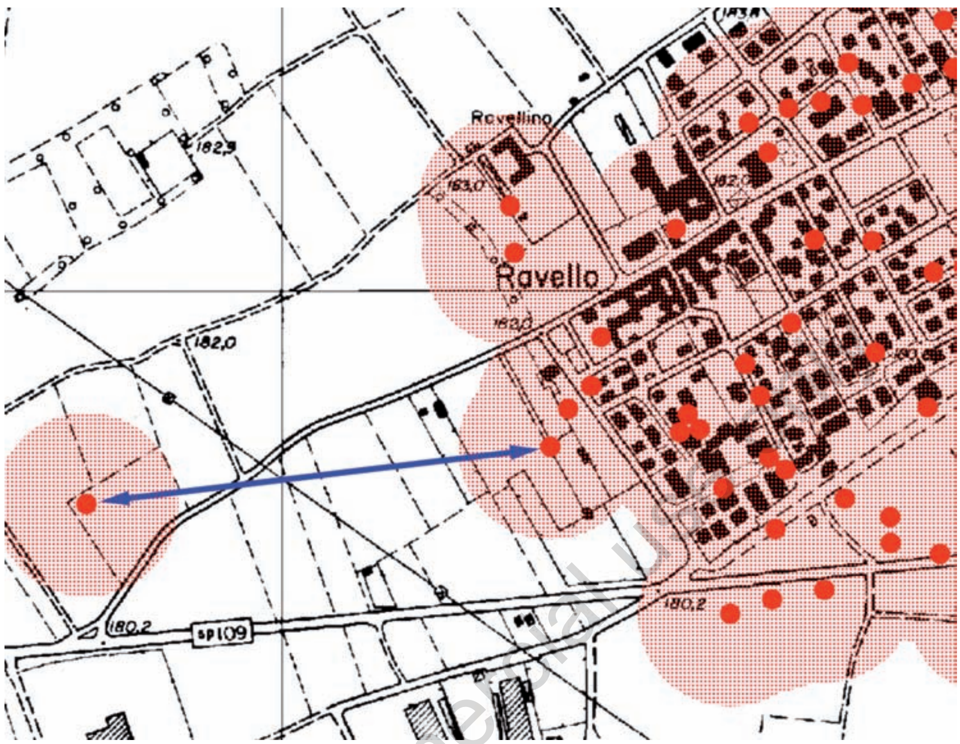

Figure 1. Method 1.

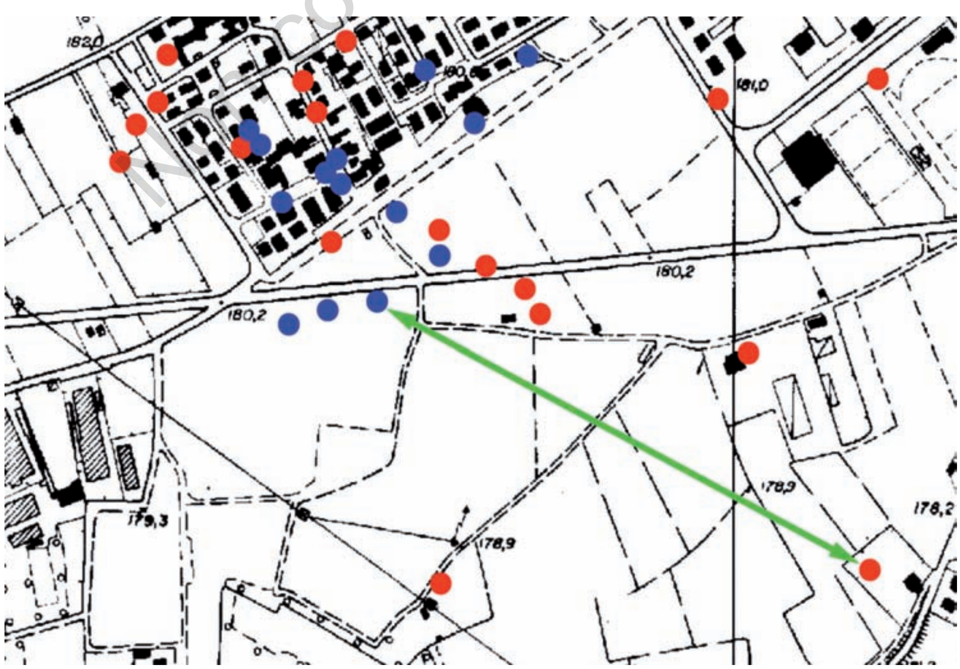

Figure 2. Method 2 (infested plants with larval frass in red, with exit-holes in blue).

Method 3

In a situation where the goal is the eradication of citrus longhorn beetle (CLB), it is not as important to know how far an individual beetle can move as it is to be able to calculate the distance within which it is possible to comprise the total infestation, starting from the most peripheral cases.

The maximum distance between cases of infestation was calculated by:

- importing of 6515 geo-referenced points collected in 2007-2010 period into the Lombardy Region cartography
- creating of 1000 m radius buffer around each infested tree, in order to create the detection chart of the *main macro-outbreaks*;
- creating 200 m radius buffer around each infested tree, in order to make the detection chart of the *micro-outbreaks*, inside every *main macro-outbreak*.

Then, the distance between the micro-outbreaks and the single isolated plants outside of these areas was calculated (Figure 3).

Figure 3. Method 3.

Results

Item-1: Analysis of data collected during the survey of A. chinensis in heavily infested areas

The analysis of the data shows that in Lombardy the genera *Acer pseudoplatanus, Corylus* spp., *Betula* spp., *Acer saccharinum* and *Carpinus* spp., account for about 80% of the total infested plants by *A. chinensis.* The data also confirm that the genus *Prunus spp.* is generally not infested, with the exception of *Prunus laurocerasus* (Figure 4).

The percentage of specimens infested for each species is linked to both the number of specimens and the species' vulnerability to the insect, and can usually be approximated by the ratio between the cases

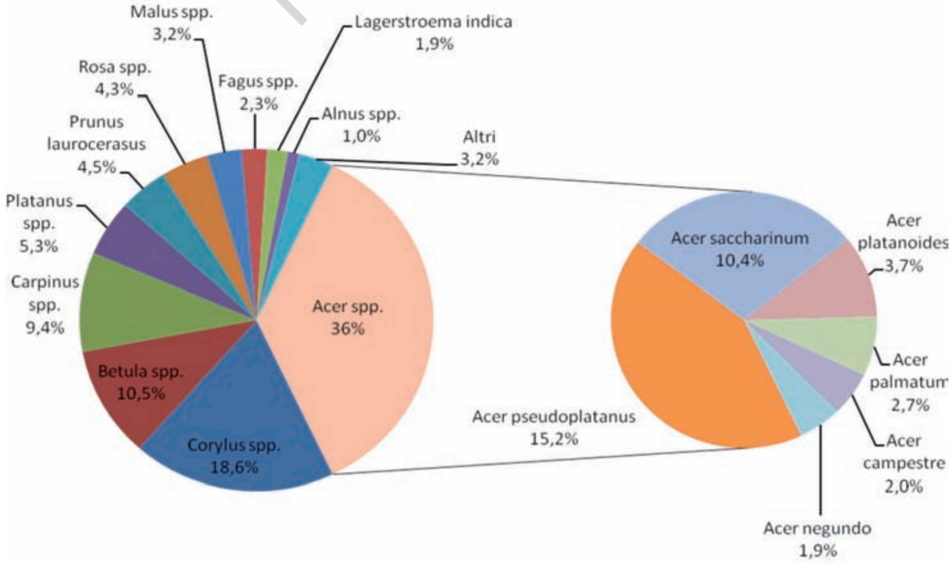

Figure 4. Percentage distribution of infested plants among plant species.

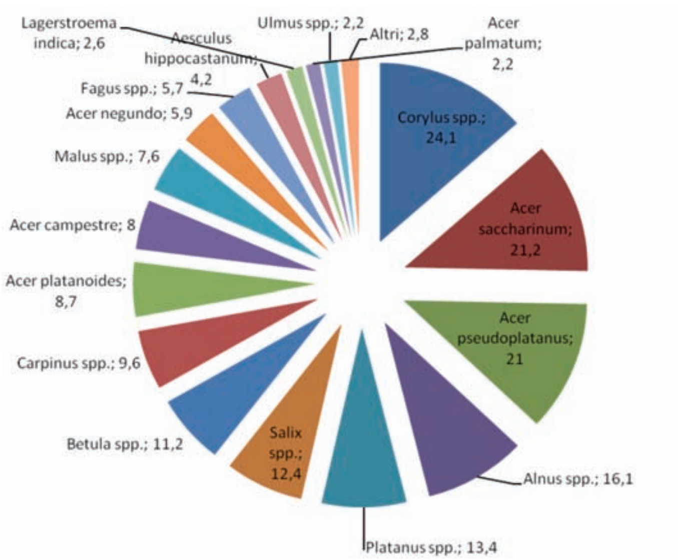

Figure 5. Ratio between the number of infested plants and the total number of plants present for each species.

of infestation found and the total number of plants present (Figure 5).

Analysing the percentage distributions of plants surveyed showing exit holes, we note that a group of these species frequently produce adults of *A. chinensis.* These are *Acer pseudoplatanus*, *Corylus* spp., *Betula* spp. and *Acer saccharinum*; a second group is composed of *Carpinus* spp., *Platanus* spp., *Prunus laurocerasus*, *Rosa* spp., *Acer platanoides* and *Acer palmatum*. Other plant species have relatively low percentages of specimens with exit holes (Figure 6).

The ratio between the number of plants with exit holes and the total number of plants present for each species provides a measure of the vulnerability of each species to this pest, in this case referred to the capacity of *Anoplophora chinensis* to complete its development to the point of producing adult specimens (Figure 7).

However, the percentage distribution of the number of frass found on the plant species surveyed shows that the major larval activity is detected on plants of *Corylus* and *Acer* genera (Figure 8).

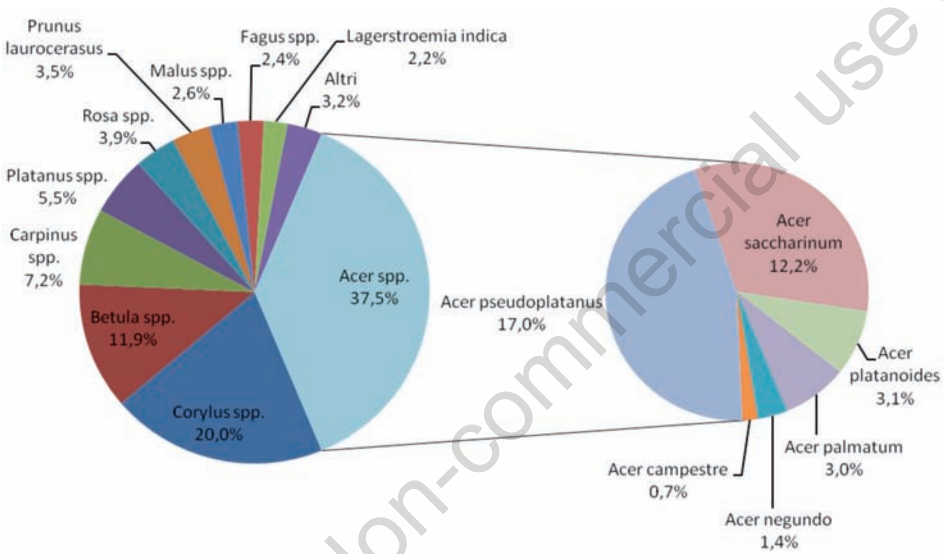

Figure 6. Percentage of plants with exit holes by species.

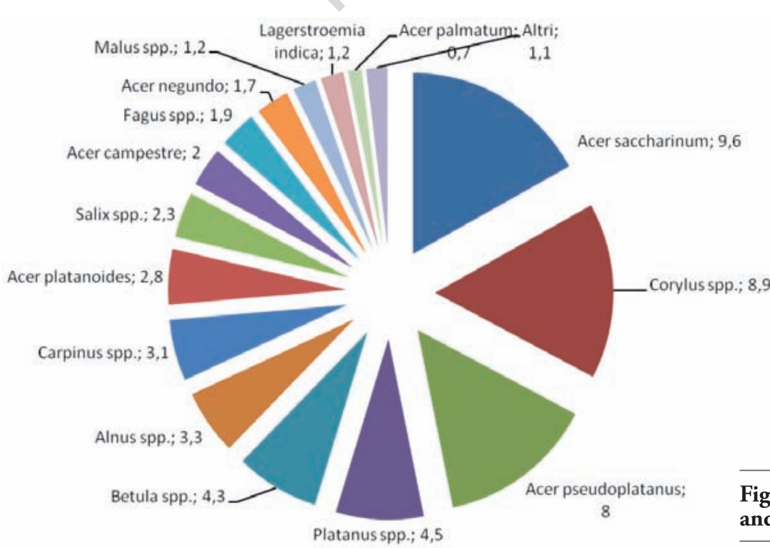

Figure 7. Ratio between the number of plants with exit holes and the total number of plants present for each species.

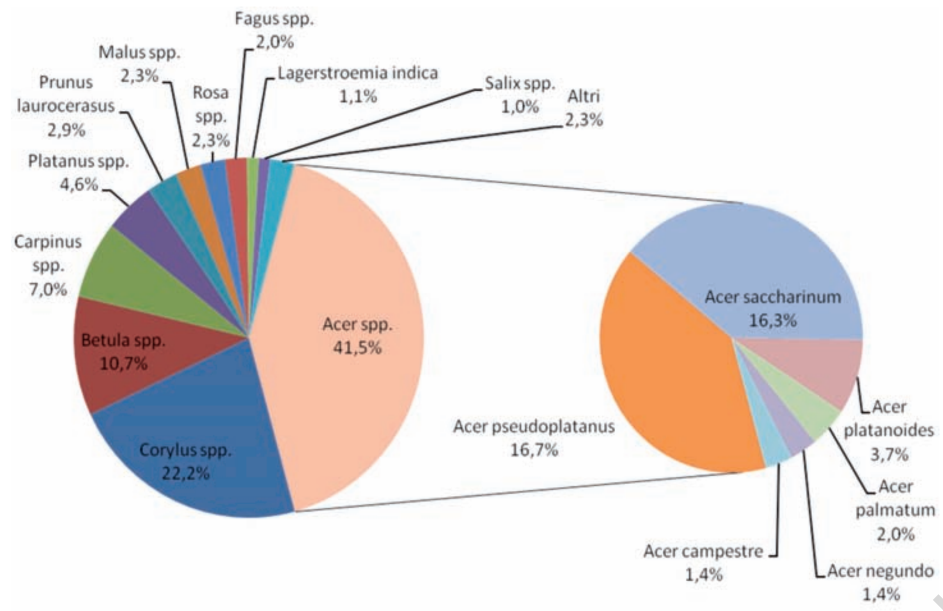

Figure 8. Percentage distribution of the number of frass per plant species.

Item-2: Outbreak areas - maximum distance between cases of infestation

The results of first two methods are collected in the Table 1 below.
Table 2 shows the results about the third method.

Table 1. Maximum distances in the study areas (Methods 1 and 2).

Municipality	Year	Method 1 Maximum distance (m) - urban	Method 1 Maximum distance (m) - agricultural	Method 2 Maximum distance (m) - urban	Method 2 Maximum distance (m) - agricultural
Nerviano	2008	327	630	326	353
Nerviano	2009	458	614	651	708
Parabiago	2009	370	322	600	683
Pogliano	2008	730	470	275	751
Pogliano	2009	615	350	629	294
Casorezzo	2009	594	699	366	939
Ossona	2009	228	362	301	910
Mean		475	492	450	663

Table 2. Results of Method 3.

Distance between points of infestation (m)	Cases (n)	Cases (%)
<200	6336	97.3
<400	6463	99.2
<600	6484	99.5
>600	6515	100

Discussion and conclusions

A statistical analysis of the 12 cases studied shows that the genus *Acer* spp., *Corylus* spp., *Betula* spp., *Carpinus* spp. and *Fagus spp.* are those preferred by the insect and from which it has the highest rate of adults emergence.

These plants are at greatest risk and should be checked with particular attention.

The study also confirms that the genus *Prunus* spp. is generally not infested, with the exception of *Prunus laurocerasus.*

This geostatic study (Methods 1 and 2) demonstrates that all new infestations of *Anoplophora chinensis* can be found within a radius of 500 m in an urban environment and within a radius of 663m in an agricultural environment.

The beetle is not a particularly good flier, and in fact, the distance within which it is possible to find nearly all of the infestation (99.2%) is equal to just 400 m (Method 3).

An evaluation of the dynamics of the infestation in the Demarcated Area, backed also by the results of the predictive model, reveals a strong contraction in infestations by *A. chinensis,* in confirmation of the adequacy and quality of the measures applied in Lombardy.

The quality of the results obtained using a predictive model, known as the Removal Method, to estimate residual infestation could also represent a valuable tool for planning anti-infestation actions.

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First data on the dispersal and potential spread of *Anoplophora* spp.

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Abstract

Anoplophora glabripennis and *Anoplophora chinensis* are very polyphagous wood boring cerambycid beetles which are native to Asia, mainly occurring in China, Korea and Japan. Due to international trade, they were introduced to new areas – not only to other Asian countries but also to other continents such as North America and Europe. The supreme aim of National Plant Protection Organizations (NPPOs) is to avoid further distribution and damage; therefore, knowing the potential *natural* dispersal at outbreak sites of these beetles is pivotal for developing effective controlling and eradication management strategies. At present, there is a knowledge gap in the host plant preferences and dispersal capacity of *Anoplophora* adults. First data for studies on the spatial and temporal spreading of infestation and on host-plant preferences of *Anoplophora* species were provided from existing infestation areas.

Results

These studies showed that environmental factors as climatic conditions (temperature, wind velocity and direction), availability of host plants, beetles' density and own ability of movement (through flight) are the main influencing factors on the dispersal behaviour and capacity of *Anoplophora* species. Nevertheless, it is necessary to gain more insights into the behaviour and spread of *Anoplophora*.

Diversified landscape elements like variation in size and arrangement of tree species seem to have strong effects on *A. glabripennis* dispersal. Here, the distance of the preferred host plants, the size of the trees and the tree species itself play an important role. Up to now, the host plant spectrum of *A. glabripennis* is still not fully clear, especially its role in different eco-climatic zones with different host tree species where varying host plant preferences seem to exist. Hence, poplars (*Populus* spp.) and willows (*Salix* spp.) are the major hosts in China, maple (*Acer* spp.) and horsechestnut trees (*Aesculus hippocastanum*) in North America and *Acer*-species in Europe. There are many other amenity and fruit trees as hosts in China. With regard to this, it is not clear whether they are also relevant hosts for *A. glabripennis* in Europe, though it would be important to know especially for fruit production. On the contrary, it is clear that *A. chinensis* is a danger for fruit production, especially for Citrus species. Yet, we do not know whether conifer plants too are relevant hosts for *A. chinensis*.

Within the frame of the EUPHRESCO project ANOPLORISK, three assays have shed more light on the dispersal potential of *A. glabripennis*: i) data from infestation areas in USA and Europe were sampled and analysed; ii) glasshouse trials with *Malus domesticus* as possible suitable host for *A. glabripennis* were carried out under quarantine conditions; and iii) specific investigations about the influence of environmental variables have been studied in the Italian infestation area.

So far, it could be assumed that natural dispersal of most beetles appear to be only very local (mainly neighbour tree attack) and over short distances (less than 400 m) and seldom up to a maximum of 2600 m.

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Import-inspections, surveys, detection and eradication of the longhorn beetles *Anoplophora chinensis* and *A. glabripennis* in The Netherlands

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Introduction

Since 1980 The Netherlands has regularly intercepted the citrus longhorn beetle *Anoplophora chinensis* on consignments of bonsais and plants for planting coming from China and Japan. Two local outbreaks have been detected since 2007 on trees and shrubs outside nurseries. The first one was found in Westland (a greenhouse area) in December 2007 and the second one in Boskoop (a tree nursery centre) in November 2009, about 30 km apart. In 2010 another outbreak, which was related to packaging material, was found in the city of Almere.

Materials and methods

Measures were taken in line with the Commission Decision 2008/840/EC, including the demarcation of the infested zone and a buffer zone with a radius of 1 km. Upon first detection, all trunks of trees and shrubs were examined for presence of *A. chinensis* by cutting the lowest (1 m) part of the trunk into pieces of about 10-15 cm. All exit holes were found on the lowest 40 cm of the trunk. To eradicate these outbreaks, in both areas all woody deciduous host plants were removed, examined and destroyed within a radius of 100 m around infested trees and surveys were performed in an area of 1 km radius. In both areas, all host plants present within a radius of 200-300 m were removed as well. Upon the uprooting of the trees, the age of exit holes was determined.

In Westland, about 110 trees and 1400 shrubs were removed including several known host plant taxa such as *Acer* spp. (~25 trees), *Aesculus hippocastaneum* (7 trees), *Salix* spp. (17 trees) and about 40 *Rosa* shrubs. Following removal and examination of all broadleaved trees and undergrowth bushes within the first 100 m, 28 larvae and 24 exit holes in total were detected in 11 shrubs and trees (7 *Acer*, 1 *Coryllus*, 2 *Cornus* and 1 *Crataegus*). During this action, all infestations were found within a 35-m distance from the nursery and no infested plants or plants with symptoms (exit holes, frass, T-shaped cracks in the bark, discolouration of stems, signs of feeding on twigs) were found.

In the Boskoop area, 7 exit holes were detected in 2 old dead stumps of *Acer palmatum* and 1 exit fresh hole and 2 full-grown *Anoplophora larvae* in a *Carpinus* tree at the nursery entrance. All neighbouring plants were removed and checked for the presence of *A. chinensis* and a similar procedure and method were followed to survey the demarcation zone as used in Westland. In total, 344 nurseries, 6700 private gardens and all trees on 1275 ha were inspected. Moreover, 316 trees, 809 m of hedgerow, 241 large shrubs (1.5 m or higher), 1291 small shrubs (smaller than 1.5 m) present in private and public areas, and about 5000 plants from 2 tree nurseries were removed and destroyed. No signs of the pest were found in any of the plants removed in the 100 m zone, nor during the surveys in the 100-200 m zone.

In The Netherlands, wood packaging material is inspected on a regular basis at the ports of entry, *e.g.* harbour, import locations, etc. *Anoplophora glabripennis*, the Asian longhorn beetle, was first intercepted from wood packaging material in July 2008. In November 2010, a first infestation was found on native host plants in an industrial area in the city of Almere. This infestation was related to pallets used for transport of industrial machinery. Upon first detection, surveys were performed in concentric zones of 100, 500 and 1000 m around outbreak using destructive sampling and tree-climber inspections. In the first 100 m, all deciduous trees and shrubs were cut, examined and destroyed. In total, 100 trees and shrubs were examined in detail: they were cut at the ground level, and the upper part – trunk and branches – was cut into pieces of 40-50 cm, inspected on for longhorn symptoms on the outside, their bark was peeled off and the wood was cut into small pieces. In case of atypical infested trees, the complete tree was uprooted and examined. Ten trees (1 *Salix*, 9 *Acer*) were found infested within an area of 70 m distance. In total, 60 exit holes (1 *Salix*, 59 *Acer*) were found, as well as 11 (2 live, 9 dead) *A. glabripennis* adults, 7 *Anoplophora larvae* and 600 oviposition pits (feeding scars). No eggs, larvae or scars were found dating from 2010. Analysis of the age of the exit-holes and scars on infested trees allowed us to date longhorn outbreaks with high precision.

Results

In both Westland and Boskoop no *Anoplophora* infestations have been found further away than 30-40 m from the original source of infestation. In the follow-up surveys, no signs of the pest have been found until now. Surveys will be performed until 2013 at least.

Furthermore, investigations on exit-holes and scars in the Almere area showed that almost all exit-holes were formed between 2005 and 2010 and most oviposition pits between 2004 and 2007. All symptoms were formed at end of the growing season. Surveys will continue until 2014.

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The implementation of the International Standard for Phytosanitary Measures n.15 in Italy: the value of FITOK mark

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Introduction

ConLegno is a private consortium, non-profit organisation, sponsored by the Italian National Association of Industry, Commerce and Small-Medium Enterprises relating to the timber industry and involved in wood interests: from raw material to finished products (packaging but also structural wood).

The consortium's mission is to promote the many uses of wood: the main tool used is the mark of quality and reliability of the product/service.

The *Technical Committee* (composed of representatives elected by the member Companies) manages and promotes ConLegno's marks.

The International Standard for Phytosanitary Measures (ISPM n.15) is an instrument for *regulating wood packaging material in international trade*.

This standard is applied to wood packaging which accounts for approximately 80% of the packaging used in transnational shipments.

The ISPM n.15 has two objectives: i) to safeguard forests worldwide from harmful organisms originating in other countries; and ii) to minimise the obstacles to free trade.

The standard is applied to wood packaging exported outside the EU, it prescribes the phytosanitary treatments that can be used [only heat treatment (HT) in Europe] and the mark to be used, but it does not describe how it should applied and checked by the National Plant Protection Organisations (NPPOs) of the various countries (management methods).

Discussion and conclusions

In Italy, the Ministry of Agriculture and Forestry Policies has recognised ConLegno – Wood & Cork Services Consortium – as the subject manager for the International Plant Protection Convention/Food and Agriculture Organization (IPPC/FAO) mark (Decree N. 175/2005) (Figure 1). The same Decree approved the *Regulations for the use of FITOK Phytosanitary Mark* document (Figure 2).

Thanks to the FITOK implemented system, there is an important additional benefit: the phytosanitary traceability references have resulted in ConLegno securing a policy with an important international insurance group to cover part of the costs related to cases of international disputes. All FITOK packagings are insured by ConLegno.

With the revision of ISPM N.15 in 2009, ConLegno upgraded its *FITOK Regulation* and shared with the competent Ministry the correct procedures for the reused/repaired FITOK packaging.

For more information visit this website:
http://www.conlegno.org/tool/home.php?s=0,1,9,227,897

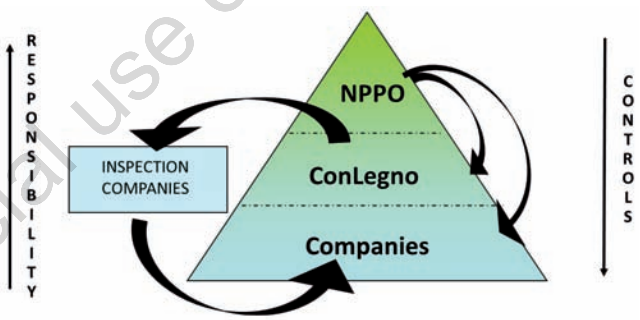

Figure 1. Implentation of the International Standard for Phytosanitary Measures n.15 in Italy.

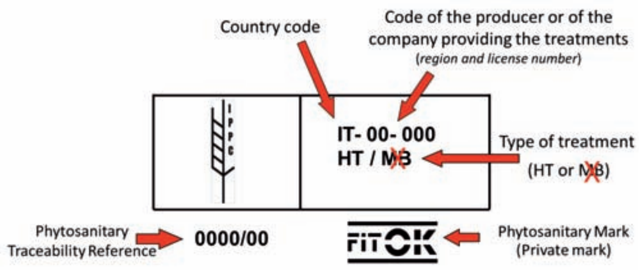

Figure 2. Italian mark for the management of the International Standard for Phytosanitary Measures n.15.

Sniffer dogs to find *Anoplophora* spp. infested plants

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Abstract

In 2009, the *Bundesforschungszentrum für Wald* (BFW) (Department of Forest Protection of the Federal Research and Training Centre for Forests, Natural Hazards and Landscape) in Vienna developed a new non-destructive method for detecting the Asian longhorn beetle (ALB) Motschulsky and the citrus longhorn beetle (CLB) by using dogs. These ALB/CLB detection dogs are able to find all stages of development in standing or imported plants and wood packaging material in different environments. Currently, these four Austrian detection dogs – the first worldwide – work all over Europe.

Introduction

The global trade leads to infestations by the Asian longhorn beetle (ALB) *Anoplophora glabripennis* Motschulsky and the citrus longhorn beetle (CLB) *Anoplophora chinensis* Forster in many European countries. The main problem within an infestation area is to detect infested trees before the beetles emerge and infest new trees. Also, during the inspections of imported plants or wood packaging material, visual control is very time consuming, deficient and needs destructive sampling. In spring 2009, this difficulty drove the BFW in Vienna to develop a new non-destructive detection method for ALB and CLB by using dogs (Figures 1-9).

Materials and methods

The four Austrian detection dogs were trained by their dog handlers to detect ALB and CLB in imported plants, wood packaging material and in standing trees up to 6 m-high and also in the roots under the soil. The training of the dogs is done by reward response with playing or food.

Figure 1. Investigation by Jackson of plants with stem protection in a nursery within the infested area of Braunau/Upper Austria.

Figure 2. Jolly investigated imported plants in a nursery standing separately in a hall area.

Figure 3. Inspection of the opened crates from the surface by Andor.

Figure 4. Investigation of 1000 plants in a *line up* by Jackson.

Figure 5. Checking of trees by Andor in a natural park of Milan.

Figure 6. Investigation of water accompanying trees by Jackson.

Figure 7. Andor investigated the willow stump.

Results

Since 2010 the Austrian detection dog teams worked in Austria in the ALB infestation area, in nurseries, ports and at stone importers. They visited The Netherlands, Italy, Croatia, Switzerland, and Germany to investigate trees in infestation areas of ALB and CLB, as well as imported plants in nurseries and wood packaging material in ports and at stone importers.

The plant protection service of The Netherlands requested the detection dog teams to investigate a consignment of 40,000 young *Acer* seedlings imported from China for the presence of CLB. Within three days the dogs checked 15,000 plants and found five plants infested by CLB.

In the years 2010 and 2011, the Austrian detection dog teams visited five times the CLB infestation areas in Lombardy Region, Italy. They visited places and parks in Milan, Legnano and Assago, agricultural areas in the province of Brescia (Gussago and Montichiari), with known CLB infestations. In one area, the work of the dogs was exacerbated by water conducting ditches because the investigation work of the dogs had to be carried out from the water side. In all locations the

Figure 8. ALB ovipositions on the willow stump in early November.

dogs were able to detect additional infested trees which have not been recognised by the monitoring people before.

In Venetian Region (Italy), the detection dogs were used for monitoring ALB in the infestation area of Cornuda and Maser in 2010 and 2011. In total, 13 places of interest were investigated and in eight of them the dogs indicated so far unknown infested trees.

In Croatia, an infestation of CLB was detected in 2007 in a nursery in Turanj, Dalmatia, on imported plants from China. 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 and around the nursery. One *Lagerstroemia* plant was proved positive by the dogs. The investigation of the whole plant resulted in one CLB larva at the base of the crown.

In August 2011, a new ALB infestation in Switzerland was detected in the small village of Brünisried (Canton Fribourg). The dogs could found additional ALB infested trees. In several ports of Basel along the river Rhine, the detection dogs investigated wood packing material imported from Asian countries and found a stone consignment from Vietnam heavily infested by powder post beetles (*Sinoxylon* spp.) in spite of treatment with Methyl bromide according to the ISPM15 stamp.

In the ALB infestation area in Neukirchen (Bavaria, Germany), the detection dogs indicated fresh oviposition sites and eggs on a stump of a large, already felt *Salix* tree.

The two Austrian dog handlers Gabriele Sauseng and Ute Hoyer-Tomiczek developed in 2011 a training programme for dog handlers and their dogs for detection of ALB/CLB. In 2011, five additional dogs and their dog handlers from Germany and Austria, mostly inspectors of the Plant Protection Services, completed the training courses successfully. In 2012, four teams from Switzerland and two further teams from Austria passed this education programme.

Discussion and conclusions

Due to the experiences of the last three years, it can be confirmed that specifically trained detection dogs can detect ALB or CLB in imported plants, wood packaging material, and standing trees in infestation areas reliably. The monitoring fields can be natural or urban environment, ports, airports, packing centres, stone importers, nurseries or other companies at destination.

This work was partially financed by the EUPHRESCO project ANOPLORISK.

Figure 9. Checking of wood packaging material of stones from various Asian countries in a harbour of Basel with the detection dogs.

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Use of the electronic nose for the detection of *Anoplophora chinensis* (Forster) on standing trees: preliminary results

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Abstract

Anoplophora chinensis and *Anoplophora glabripennis* represent a serious danger for tree heritage because they attack dozens of tree species. For this reason, it is very important to monitor and early detect the infestation. Contextually to traditional techniques and monitoring programmes carried out in Lombardy infected areas, a three-year trial has been initiated, with the support of Lombardy Region, in order to evaluate the effectiveness of the diagnosis of the electronic nose in identifying trees infested by *A. chinensis*.

Introduction

The presence of two harmful species of insects of Asiatic origin – *Anoplophora chinensis* and *Anoplophora glabripennis* – has been detected in Italy for some years. The larvae of *A.chinensis* dig tunnels inside the base of the trunk and in the roots, while those of the congener *glabripennis* develop in the aerial part of the stem and branches. Their activity obviously involves a reduction in the stability of the tree leading to his death. The disease spreads quickly and currently the only way to contain it seems to be the early detection of infected plants and their removal. In order to improve the detection method, an experimental programme of diagnosis using an electronic nose has been developed with the partnership of Lombardy Region.

Materials and methods

The device

The portable electronic nose 3 (PEN3) (Figure 1) is a very compact device (255×190×92 mm), light-weighing (2.1 kg), and a portable olfactory system. It consists of an array of 10 different doped semi-conductive metaloxide gas sensors (MOS) positioned into a very small chamber with a volume of only 1.8 mL.

Sampling

The sampling has been carried out by sucking the telluric air in correspondence to the selected subjects (Figure 2). To suck the air, a pump sucking the air from inside and releasing it outside has been employed. To collect the telluric air a bag of *Nalophan*® (Kalle GmbH, Wiesbaden, Germany) has been employed instead. The samples thus obtained have been stored at room temperature for a max. period of 12 h prior to instrumental analysis.

Selection of sample trees

The first preliminary test has been done by sampling the telluric air of 17 trees situated in the green area of Bosco in Città (Milan, Italy), 8 of these were symptomatic, 5 with diagnosis to be confirmed and 4

Figure 1. Portable electronic nose 3 used for analysis.

Figure 2. Sampling of telluric air.

asymptomatic (Figure 3). In Canegrate (Varese, Italy), the sampling was carried out on 20 subjects [18 infected stumps (Figure 4) and 2 healthy trees located in a green area facing to the felling site].

Figure 3. Sampling of trees situated in Bosco in Città (Milano, Italy).

Figure 4. Sampling of stumps in Canegrate (Varese, Italy).

Results

Regarding the trees coming from Bosco in Città, the sensor analysis showed that the group of healthy trees was marked by high values of the sensors no. 1, 3, 5, and 10 (Figure 5). Within the asymptomatic group, the presence of *Anoplophora* was not certain on subject no. 1316, according to visual analysis. The felling of this subject enabled to verify the absence of infection at the stump. Regarding the other subjects, it did not seem to be a strong characterisation of olfactory fingerprints and of the sensors involved in the analysis. We can suppose that there was high variability among the infected subjects, which was caused by the progress of the disease, considering that asymptomatic subjects did not have such dispersion.

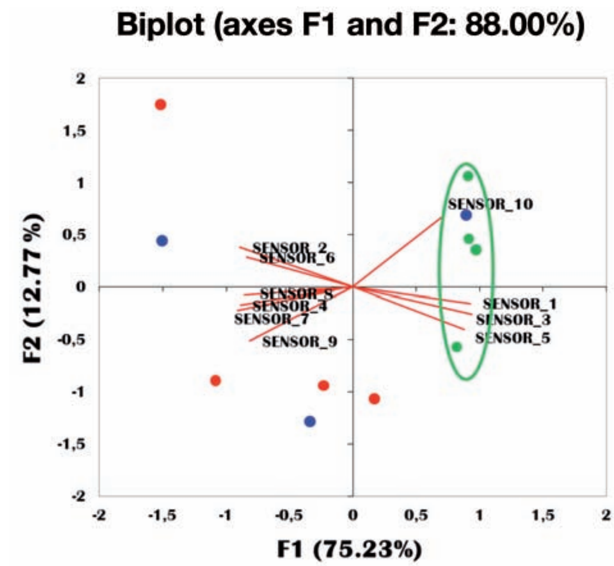

Figure 5. Sensors contribute to explain the variability of the data: the group of asymptomatic trees show more uniform olfactory fingerprints (grouped in the green circle) also marked by higher values of sensors 1, 3, 5, and 10.

The analysis of the data obtained from the samples taken in Canegrate did not show a typical olfactory impression of the symptomatic sample against the asymptomatic one; however, it must be pointed out that the asymptomatic sample in this case was very small (only two trees rooted in a neighbouring area separated from the rest of the sampled trees). In this case, the type of sampling performed on fresh cut stumps (for the symptomatic sample) and on standing trees (for the asymptomatic sample) was also added to the essential variability determined from the soil and from the site of rooting.

Discussion and conclusions

From the revision of the data obtained during this first survey we can state that the sample of trees examinated in the area of Bosco in Città has shown a discrimination between the asymptomatic subjects (grouped in a well defined cluster) and other subjects. An interesting outcome regards the sensors more involved in characterising the volatile fingerprints of the asymptomatic trees. As far as the sample of trees in the site of Canegrate is concerned, results do not seem to show a discriminated cluster between symptomatic and asymptomatic trees. However, it the small sample (2 subjects) and the variability of the sampled material (fresh cut stumps for the symptomatic sample and standing trees for the asymptomatic one) should also be considered.

The preliminary results obtained from these tests must be supported by further research in order to explore the potentiality of this technique by increasing the sample size in terms of quantity and botanic variety and by evaluating the results in different seasons of the year.

Prevention plan ensuring the protection of the PlantaRegina district: stepped-up surveillance for early detection of *Anoplophora chinensis*

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Abstract

The Plant Health Service of Lombardy Region, which has been involved in fighting *Anoplophora chinensis* for many years, developed a plan to implement a stepped-up surveillance for the early detection of this pest in the most important nursery areas (number of farms and business amount was considered). Method, results and aim of this prevention plan are illustrated below.

Introduction

Anoplophora chinensis is an Asian beetle which has been accidentally introduced into Europe several times since the late 90s, via the importing of Bonsai plants and seedlings or young plants of the genus *Acer* (maples) intended for planting. The Plantaregina district area, a zone with many nurseries in Canneto sull'Oglio, extends for more than 50,000 ha between the provinces of Mantua and Cremona and hosts the biggest concentration of commercial nurseries in Lombardy. The area businesses specialise in the cultivation of full-size deciduous ornamental trees. Each year, almost 3 million plants of the species most vulnerable to *A. chinensis* are grown in open fields. To protect this district and ensure its economic well-being, the Plant Health Service has designed and implemented a stepped-up surveillance system, in line with the Food and Agriculture Organization/International Standards for Phytosanitary Measures (FAO/ISPM) international standards.

Materials and methods

The plan of reinforced surveillance includes the following steps: i) planning; ii) control of urban green spaces (Figure 1); iii) surveillance of the territory; iv) control of high-risk points; v) control of high-risk fields.

The planning stage is basic and is based on the preparation of the requisite cartography to be used as an operational tool, and on involving the nursery operators in the district and others in the area so that they participate in the planned actions. The urban green spaces were inspected using the methods already utilised in the demarcated areas, but a new methodology was devised for the enhanced territorial surveillance, as there were no established guidelines to follow. We drew up a map with a buffer zone of 2 km radius around all the areas dedicated to nursery cultivation. Then, we overlaid a grid of 500 m on each side, leading to the creation of 2156 cells, subsequently classified on the base of the risk to plant health and identified by different colors. From 1 to 4 key points were defined for each cell, depending on the risk to plant health. Using a GPS system, more than 3450 key points were located and mapped out, for a total of 11,233 plants. In addition, 16 nurseries were classified as high risk sources, according to the type of plants they produced and the

Figure 1. Inspection in urban green areas.

Figure 2. Inspection in nurseries.

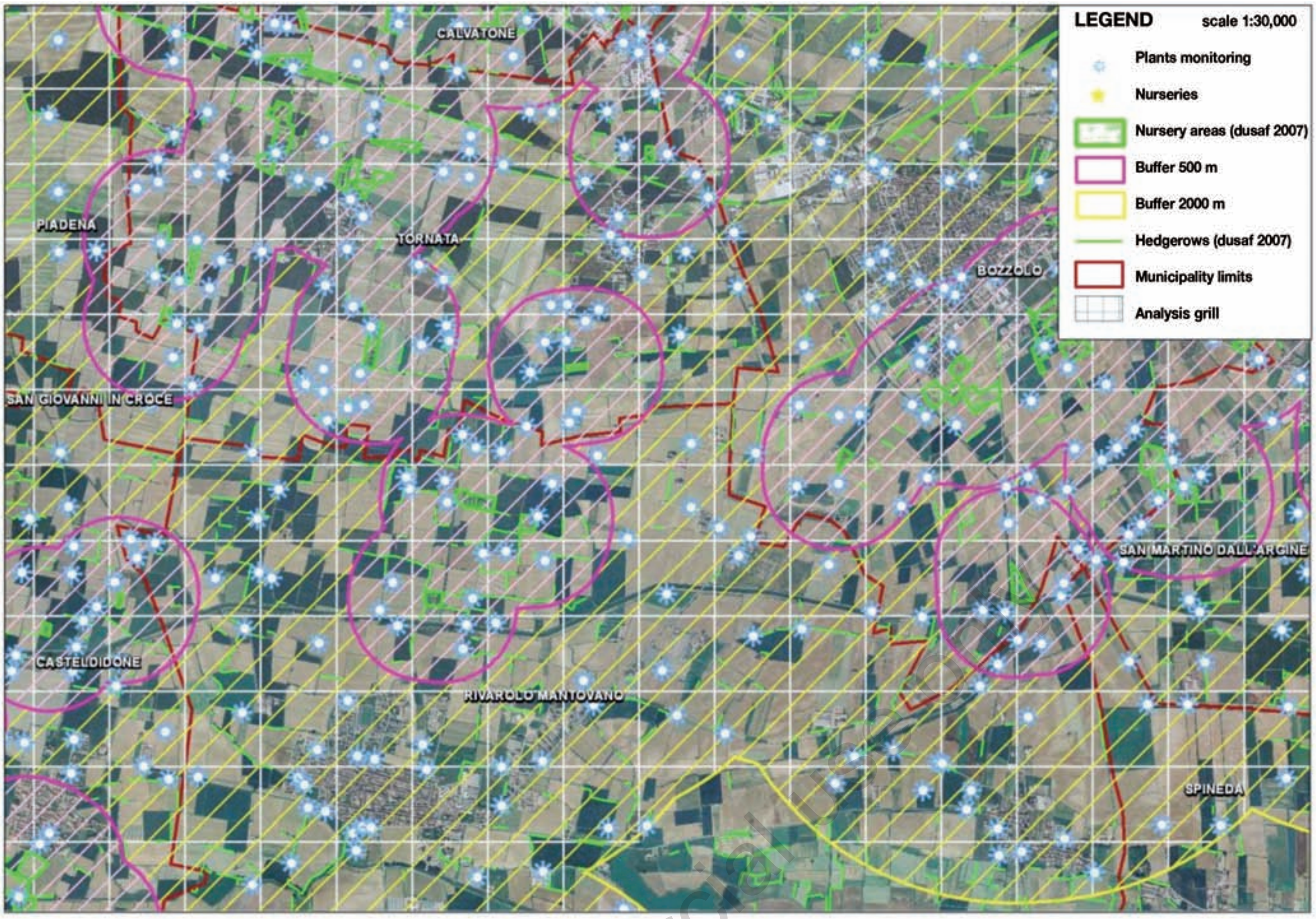

Figure 3. Sentinel points map.

origin of their supplies (Figure 2). In these cases, not only were the plants being cultivated controlled, but also a buffer zone with a radius of 100 m around the company headquarters. Lastly, controls were conducted in fields containing the plant species considered most vulnerable, based on their age and origin.

Results

Reinforced survey in the Plantaregina distict area took 140 workdays and the monitoring of the Plant Health Service: i) 6223 host plants in urban green areas of 31 municipalities; ii) 5746 host plants inside the buffer zone of 54,000 ha; iii) 82,275 host plants in 15 nursery fields for the amount of 3450 detections points (Figure 3). So far, the stepped-up surveillance has not resulted in the discovery of plants with symptoms of infestation and has confirmed the area's pest-free status based on FAO/ISPM standards.

Discussion and conclusions

In the event of accidental introduction of infested plants by *A. chinensis* into the district, the early detection system defined by the plan allows for: i) carrying out an effective eradication action to eliminate the pest organism; ii) applying an exception to the demarcation procedure indicated in EU decision 138/2012/CE; iii) reducing the costs associated with the application of the official measures; iv) reducing the obligations that producers must fulfill; v) avoiding a sales ban on products cultivated in the district.

Dendrochronology as a tool for dating *Anoplophora* spp. outbreaks

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Introduction

Dendrochronology, the study of tree rings, is a high-precision tool to date outbreaks of xylobiont insects such as the invasive Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky), and citrus longhorned beetle, *Anoplophora chinensis* (Forster). Dendrochronology enables dating of the exact year and provides an estimate of the season of insect-induced wounds such as oviposition pits (only for *Anoplophora glabripennis*) or exit holes. This information can be used to support eradication measures and understand population dynamics of *Anoplophora* spp.

Materials and methods

Anoplophora beetles leave traces in the wood of living trees during various stages in their lifecycles: after hatching, by forming exit-holes, by laying eggs and by maturation feeding. These activities lead to wounding of the cambium zone – the interface between the bark and sapwood of trees. Every time the cambial zone is wounded, the tree responds with a series of wound reactions to secure the vital function of its xylem and phloem for transport and storage of water and assimilates. An instant reaction to wounding is the dying-off of the directly affected cambium cells.

Hereafter, a barrier zone closing the wound to the outside is formed. This barrier zone comprises traumatic parenchyma cells *callus*, wound-periderm and wound xylem and the wound remains visible as a scar within a particular tree ring. By dating the tree rings containing these specific scars, the exact year of wounding can be determined. An indication of the season of wounding can be obtained by studying the position of the scar within the tree ring.

Since 2008 we have studied wound reactions in trees infected by *Anoplophora* and in experimentally wounded trees as well as seasonal growth dynamics of host species, *e.g. Acer palmatum,* to enhance the precision of dating.

Results

Our research shows that dating precision is limited due to highly variable intra-annual growth. Yet, we are able to distinguish three periods, *i.e.* outside growing season, growing season itself, and the very end of growing season. Only with knowledge on intra-annual tree growth, a further indication can be obtained whether the wounds originated during the first half or second half of the growing season.

Discussion and conclusions

Based on these studies, we dated outbreaks with high precision and reconstructed the population dynamics of *Anoplophora* spp. in The Netherlands. We observed that *A. glabripennis* (Almere outbreak) in The Netherlands tends to form exit-holes and oviposition pits late in the season, whereas *A. chinensis* appears much earlier. These findings could contribute to answering the question: why was *A. glabripennis* naturally declining in population size during an outbreak in Almere (The Netherlands) over time? On the other hand, dating exit-holes made by *A. chinensis* in imported *Acer* trees, contributes to determine the exact location where adult beetles have emerged and take appropriate measures.

A new perspective for *Anoplophora chinensis* (Forster) (Coleoptera Cerambycidae) infested plant diagnosis by molecular analysis of larval frass

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Introduction

Anoplophora chinensis (Forster) (Coleoptera Cerambycidae) is a poliphagous longhorned beetle native to the Eastern Asia which develops on a number of broadleaf plants of an extreme wide range of ages and dimensions: from potted plants and bonsais to old and monumental specimens of forest, urban, fruit and ornamental trees and shrubs. Larvae are woodborers, feeding on the phloem and xylem of infested plants, causing disruption of vascular tissue and structural weakness which can lead to plant death. *Anoplophora chinensis* was accidentally introduced into the EU, it is considered a severe pest and is subject to strong phytosanitary regulations to prevent further introductions and spread. Eradication measures have been put in place to curb the existing outbreaks. Monitoring the presence of this longhorned beetle is mandatory for all countries in the EU. Signs of its infestation on trees are the presence of exit holes and/or frass expelled outside from the infested plant by the larvae during their feeding activity. However, these signs are not species-specific and can be confused with other longhorned beetles, which, instead, can be native to the EU fauna. Therefore, to correctly identify the pest it is necessary to obtain the specimens of the insect. Sometimes, this task can be quite difficult and demanding: adults can be occasionally collected in the environment, but are present only for few months during the vegetative period, while juveniles need to be extracted from the plant tissues. Therefore, a non-invasive diagnostic approach should be useful where an infestation of *A. chinensis* is suspected but need to be confirmed.

Materials and methods

The possibility to identify the species of the insect by analysing the frass produced by the larvae during the feeding activity – which in some cases can be present in large amount at the bases of infested trees – can be a valid non-invasive diagnostic tool during phytosanitary inspections. Frass of woodboring insects contain a large amount of wooden material and larval faeces. We present here a protocol based on polymerase chain reaction (PCR) amplification using DNA extracted from faecal pellets present in the frass samples, collected in the field on infested plants. The diagnostic protocol was tested against frass produced by larvae of some common xylophagous insects which can may be mistaken for *A. chinensis*. DNA samples extracted from frass were successfully amplified using primer pair HCO2198-LCO1490. The presence of insect DNA was assessed using the *1859F-HCO2198* primer pair (Figure 1). Discriminative results were reported with the *ad hoc* designed *Chinensis* primers only in samples originated from *A. chinensis*.

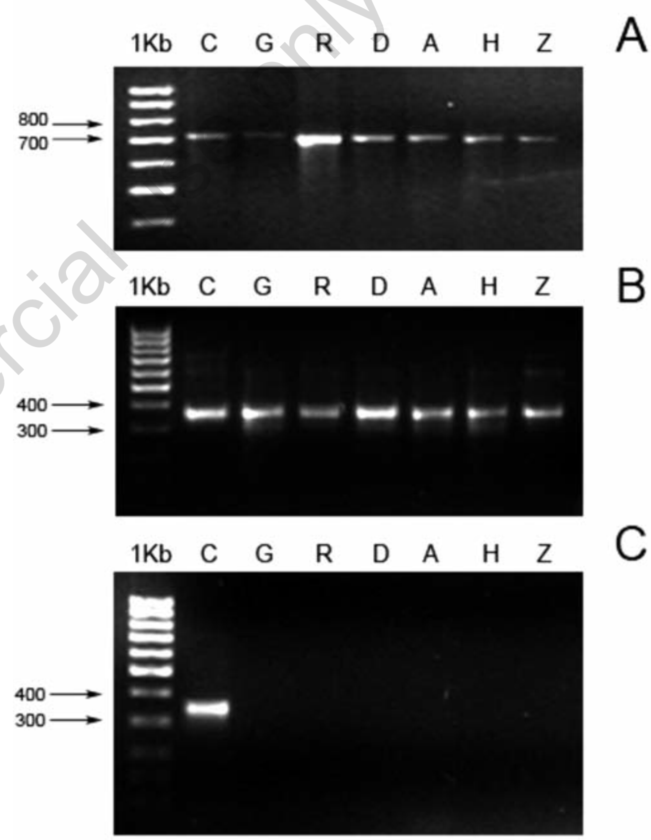

Figure 1. Primers *Chinensis* retain their specificity also in presence of non-target DNA. (A) Amplification with HCO2198-LCO1490 primer pair of DNA extracted from frass samples of different insects. Lanes: C, A. chinensis; G, A. glabripennis; R, M. asper; D, H. tristis; A, A. moscata; H, M. galloprovincialis; Z, Z. pyrina. (B) Amplification with 1859F-HCO2198 primer pair using 5 μL of purified HCO2198-LCO1490 fragment confirming the presence of insect DNA inside the sample. (C) Nested polymerase chain reaction amplification with *Chinensis* primer pair using 5 μL of purified HCO2198-LCO1490 fragment. Only sample derived from *A. chinensis* frass was able to amplify (Strangi *et al.*, in press).

Results and conclusions

Results show that the possibility to diagnose the presence of *A. chinensis* through 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.

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Development of a trapping system for Asian longhorned beetle using semiochemicals

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Introduction

Anoplophora glabripennis (Motschulsky) (Coleoptera: Cerambycidae), commonly known as the Asian longhorned beetle, is an invasive wood-boring pest causing considerable economic losses both in the USA and abroad. Early detection of introduced populations can reduce management costs by focusing survey efforts and limiting tree cutting.

Materials and methods

Monitoring *A. glabripennis* using semiochemical-baited traps was tested in China in 2007 and 2008. We conducted trapping experiments in the greater Worcester area in Massachusetts (MA) for three years (2009-2011) to improve monitoring efficacy under USA field conditions and assess efficacy of the lures. In 2009, a total of 105 Intercept^{TM} traps were deployed in Worcester, MA, distributed into 7 lure treatments: i) control with no lure; ii) *A. glabripennis* male-produced pheromone at 10 µg/day; iii) 0.1mg/day or iv) 1 mg/day; v) the male-produced pheromone at 10 µg/day with linalool, linalool oxide, *trans*-caryophyllene, *cis*-3-hexen-1-ol and *trans*-pinocarveol at 0.1 mg/day each; vi) the male-produced pheromone at 10 µg/day with linalool, linalool oxide, *trans*-caryophyllene, *cis*-3-hexen-1-ol and 3-carene at 0.1 mg/day each; and vii) linalool, linalool oxide, *trans*-caryophyllene, *cis*-3-hexen-1-ol and 3-carene at 0.1 mg/day each without any pheromone added. A total of 9 beetles were caught. All were females. Five out of 9 were caught in traps baited with lure v above. A survey of exit holes in host trees within a 30 m radius of each of the traps was performed. On average, distance to the closest infested host was around 80 m. In 2010, 40 traps were deployed in 9 sites in the quarantine zone in MA. Ten traps were left as control while the rest was baited with either male-produced pheromone with linalool, linalool oxide, *trans*-caryophyllene, and *cis*-3-hexen-1-ol or lure v used in 2009. Four female beetles were caught in traps baited with lure v, while none was caught in the remaining traps. In 2011, 500 traps were deployed in 10 radial lines starting from the centre of the quarantine zone toward the borders. Trapping lines covered Worcester, Holden, Boylston, West Boylston and Shrewsbury. Traps were set 100 m apart. Fifty traps were unbaited, while the rest was baited with either lure A (alcohol and aldehyde components of the male produced pheromone combined with linalool, linalool oxide, *trans*-caryophyllene, *cis*-3-hexen-1-ol in new dispensers); lure B (same as lure A without linalool oxide); lure C (same as lure A with high release rate of the alcohol and aldehyde); or lure D (alcohol:aldehyde 50:50 in same dispenser, combined with linalool, linalool oxide, *trans*-caryophyllene, *cis*-3-hexen-1-ol in dispensers stored from 2010). Work is ongoing to calculate distances between traps and the closest infested trees. A total of 23 beetles were caught, including 21 females and 2 males. Eight out of 23 beetles were caught in lure B. The remaining lures caught 6 beetles in lure A, 4 in lure C and 5 in lure D. No beetles were found in control unbaited traps.

Results

During the 3 trapping seasons, communication was ongoing throughout the summer with USDA-APHIS ALB cooperative eradication programme to inform them of trap catches as soon as they occur. Few of the remarkable results included new findings of infested trees in Dodge Park (Worcester) that were missed by survey and expanding the survey zone when beetles were caught in 2011 in areas previously unsurveyed. Future work will focus on optimising emitters to obtain desired release rates and to evaluate different ratios of plant volatiles.

Discussion and conclusions

Our results showed that these monitoring traps can be useful tools to guide survey and detect existing populations, but not to determine the population density. Monitoring traps decrease management cost of this invasive beetle by focusing survey work on specific areas and allowing early detection and removal of infested trees before beetles can spread to more hosts.

First findings of *Anoplophora glabripennis* in Switzerland

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Introduction

This presentation reports the first findings on the Asian longhorn beetle (ALB) in Switzerland and the subsequent measures aiming at eradicating this quarantine pest.

Results

The first alert about the species arose in summer 2011 because beetles were found in Weil am Rhein (Germany), near the Swiss port in Basel. However, despite intensive surveys, no beetles or traces of an ALB infestation were detected on Swiss territory. Nonetheless, surveys of trees around the Swiss port are upheld and import controls of granite stones have been intensified. The first living beetles on Swiss territory were reported from a member of the public in September 2011 in Brunisried (Canton Fribourg), in a rural area. Survey measures started to find and delimit the infestation. Oviposition sites were subsequently found on a single *Acer* tree and a nearby hedgerow with small *Acer* trees near the first finding of the beetles. A focal (200 m) and a buffer zone (2000 m) were established and surveys intensified. Three more beetles were found, but no exit wholes or further infested hosts could be detected. The infested tree and hedgerow were both cut and the wood was burned. Intensive surveys will continue in the forthcoming years. In October 2011, sniffer dogs were used to inspect potential host trees around the infestation site. The dogs were also working in the port of Basel, with no findings of ALB. The dogs will return to both sites in May 2012. In addition, the dogs will also survey an area in Eastern Switzerland (Canton Thurgau), where in October and December 2011 dead ALB specimen had been found on a construction site. While the interception in Thurgau could successfully be retraced to a storage site of granite stones in Weil am Rhein, it is as yet unclear if and how the Brunisried infestation is related to the interceptions in Weil am Rhein.

Discussion and conclusions

The collaboration between the national and regional authorities is well functioning. While regional authorities are in charge of the survey measures, the Swiss National Plant Protection Organisation has launched a public awareness campaign (press releases, flyer and Internet site) and is collaborating with experts and regional authorities to develop a strategy on how to mitigate the risk of further ALB introductions and to efficiently manage ALB outbreaks.

Potential candidates for biological control of the Asian longhorned beetle (*Anoplophora glabripennis*) and the citrus longhorned beetle (*Anoplophora chinensis*) in Italy

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Abstract

The Asian longhorned beetle (ALB) *Anoplophora glabripennis* (Motschulsky), and the citrus longhorned beetle (CLB) *Anoplophora chinensis* Forster (Coleoptera, Cerambycidae) are polyphagous xylophages native to Eastern Asia where they cause serious damage to many deciduous trees, mainly the genera *Populus*, *Acer* and *Salix*. CLB is also a major pest of citrus in Japan and China. The Asian longhorned beetle is established in North America and both pests are established in Europe where they are considered as serious threats to the urban and natural forests, and are subject to eradication. In conjunction with the eradication programmes, biological control studies were initiated in order to find, identify, and evaluate parasitoids that might successfully control ALB and CLB. Recently, a survey was made in Italy to find possible new associations between ALB or CLB and natural enemies from the European fauna.

Results

Seven species of Hymenopteran idiobiont ectoparasitoids attacked early stage larvae of ALB and CLB: *Spathius erythrocephalus* Wesmael (Hym.: Braconidae), *Trigonoderus princeps* (Westwood), *Cleonymus brevis* Boucek (Hym.: Pteromalidae), *Eurytoma melanoneura* Walker, *Eurytoma morio* Boheman (Hym.: Eurytomidae), *Calosota agrili* Nikol'skaya, *Eupelmus aloysii* Russo (Hym.: Eupelmidae) and *Sclerodermus* sp. (Hym.: Bethylidae). Each of these parasitoids was already known from several species of xylophagous hosts in Europe. *S. erythrocephalus* and *T. princeps* were the parasitoids most frequently observed on CLB and ALB in Italy. Laboratory rearing techniques for both species and their major life history traits and host attack behaviour are presented. Although both parasitoids possess several attributes of good biological control agents, they are not being considered for release against either invasive pests because of their polyphagy. No parasitoid was found in eggs of ALB in Italy. In contrast, eggs of CLB were often parasitised by *Aprostocetus anoplophorae* Delvare (Hym.: Eulophidae), a parasitoid new to science. The egg parasitoid proved to be CLB-specific in Italy. Very likely the parasitoid is native to Eastern Asia and was accidentally introduced via host eggs deposited within bark of plants prior to their export to Italy. In Northern Italy, the egg parasitoid is well established in the centre of the major CLB infestation around Parabiago, where it was retrieved every year since 2002 through 2012.

Discussion and conclusions

Results indicate that *A. anoplophorae* may be the most promising biological control against CLB: its impact was locally very high (72% parasitism in 2008 in Canegrate, Italy) and the host and its parasitoid have a high degree of developmental synchronicity. A study of its geographical distribution throughout the CLB infestations in Lombardy Region has shown that the egg parasitoid is still absent outside Parabiago area. Studies to finalise a laboratory rearing technique for *A. anoplophorae* and to determine suitable conditions for its obligatory diapause are in progress. Inoculative releases of the parasitoid in the CLB infestations where it does not yet occur are planned in Italy.

Summary of 2008-2011 trials on the possibility of controlling *Anoplophora chinensis* with pesticides

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Abstract

Chemicals of the class of neonicotinoids and pyrethroids were tested in 2008-2011 to verify their efficacy in preventive (against adults) or curative (against larvae) treatments of the trunk. Some neonicotinoids demonstrated a good curative efficacy, while the best efficacy against adults occurred with lambda cyhalothrin as capsule suspension (CS) formulation.

Introduction

In 2005-2007, 18 trials were conducted in field, involving more than 600 plants. The purpose was to find the possibility to control the *A. chinensis* with simple and economic treatments. A lot of pesticides were applied as trunk treatments. Neonicotinoids resulted the best products to guarantee efficacy against larvae.

In 2008-2011, studies with any pesticides continued with two objectives: i) evaluating the curative efficacy (against larvae); and ii) evaluating the preventive efficacy (against adults).

Materials and methods

Some trials were conducted on small plants in pots and other on mature trees in field. In total, about 300 plants were treated. The treatments have always interested the basal part of the trunk and have been made either by spraying or brushing. The volume used in the treatments was always of 350 mL/m^2 of trunk surface. The trials on potted plants were set to saccharin maples (*Acer saccharinum*) in 2008, 2010 and 2011 and on webbed maples (*Acer palmatum*) in 2009. The plants had an average circumference of the trunk, measured at 20 cm from ground, of 5 cm and about 2-m high. Each plant was been locked in a cage consisting of a network of aluminium.

The trials on field plants were made on plants of saccharin maples (*Acer saccharinum*) about ten year-old, with a natural infestation of *A. chinensis* in 2009 and artificially infested in 2010, by fitting the adults of *A. chinensis* in cages fixed to the trunk.

The plants in the trials for evaluating the curative efficacy were treated when larvae were present. Some months after, the trunks and roots of all plants were dissected and the live larvae per plant were counted. The plants in the trial for the evaluation of the preventive efficacy were treated before the placing of adults in the cages. Were then counted the number of live and dead insects every few hours.

Results

Results are summarised in Tables 1 and 2. These three years of trials confirmed what we saw in the previous 3 years of experimentation: thiamethoxam, at the rate of 100 g s.a./hL used in treatments on the trunk with 350 mL/m^2 of trunk surface, can guarantee good results as a curative product against larvae present in shallower areas of the trunk.

Even when the mortality of 100% was reached later, all individuals placed on plants treated with demand CS died already 24 h after placement. Even in a very limited contact situation (in practice, the contact surface should be higher) the product showed an excellent preventive efficacy and good duration of action, until about 20 days after treatment. Karate Zeon showed to have the same efficacy and duration of action.

Table 1. Curative trials on maple. Number of live larvae per plant.

Product	a.i.	Rates (mL,g p.c./hL)	Rates (mL,g s.a./hL)	Treatment	Trial number 08-FM-02a	08-FM-02b	09-PC-01	10-FM-01
Untreated check					2.4 ab	2.2 a	2.08 a	1.5 a
Laser	Spinosad 480 g/L	100	48	Trunk	3.6 a	2.2 a		
Actara 25 WG	Thiamethoxam 25%	200	50	Trunk	0.2 b	1.2 a		
Actara 25 WG	Thiamethoxam 25%	400	100	Trunk			0,5 b	0 b
Actara 25 WG	Thiamethoxam 25%	400	100	Trunk+roots			0.58 b	
Dantop 50 WG	Clothianidin 50%	150	75	Trunk				0 b
Demand CS	Lambda-cyhalothrin100 g/L	450	45	Trunk				0.2 b

a.i., active ingredient; CS, capsule suspension.

Table 2. Preventive trials on maple. Percent of mortality of the adults positioning after the trunk treatment.

Product	a.i.	Rates (mL,g p.c./hL)	Rates (mL,g s.a./hL)	Hours after insect positioning	Trial number 09-FM-03	10-FM-02	11-FM01a	
Untreated check				8	0	0	0	0
				24		0	0	0
				48		0	25	0
				72		0	25	0
				168		0		0
				216				0
Demand CS	Lambda-cyhalothrin 100 g/L	450	45	8	100	25	0	
				24		25	25	
				48		25	100	
				72		25	100	
				168		100		
Karate Zeon 1.5	Lambda-cyhalothrin 15 g/L	3000	45	8			25	0
				24			25	0
				48			75	0
				72			100	17
				96				50
				120				67
				216				100
Insect positioning: days after treatments					11	16	19	28
Lengh of trunk treated (cm)					80	20	30	

a.i., active ingredient; CS, capsule suspension.

Discussion and conclusions

Interpreting the results of the trials, it seems possible to suggest a schedule of treatments for the control of *A. chinensis*: i) first treatment with Karate Zeon CS 1.5 to the appearance of the first adults (usually in Lombardy Region, in late May-early June); ii) a second treatment after about 20 days with Karate Zeon CS + Actara 25 WG (or other neonicotinoids, such Dantop 50 WG) to join a curative action to any egg-lay escaped the first treatment.

These two actions should cover 90% of the outputs of adults, and a third treatment after other 20 days may be needed.

These indications are valid on maple, *i.e.* the species which we have enough experience on.

Anoplophora chinensis control in a city park

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Abstract

An outbreak of *Anoplophora chinensis* was find in the summer of 2006 in the city of Milan, very near to one of the most important city park. Monitoring showed that some infested plants were also inside the park. In order to front this particular and delicate situation, a special and experimental control plan, alternative to cutting plants, was developed.

Introduction

Anoplophora chinensis is a quarantine pest and it was accidentally introduced (probably with infested plants of Asian origin) in 2000 in a town close to Milan, in Lombardy Region, Northern Italy.

Since 2001, the Plant Protection Service of Lombardy Region has implemented a monitoring to detect the spread of the insect in its territory through a specific survey of the infested area and a general surveillance by a communication campaign directed to municipalities technicians and landscaping professionals.

In the summer of 2006 some plants of the genus *Acer* attached by *A. chinensis* were found in the yard of an industrial building in Milan, next to *Parco delle Cave* (Figure 1), one of the main city parks. A technical committee was quickly established and began a close collaboration with the Plant Protection Service, the Municipality of Milan and the *Center for the Urban Forest,* which manages the park. With the support of phytosanitary inspectors and researchers of Minoprio Foundation, a specific survey has been carried out inside the park in the surrounding public green: more than 100 infested trees were found inside the park in a grove of *Acer pseudoplatanus*, while only a few infested plants were found in public green areas.

Figure 1. ***Parco delle cave.***

In order to find a way to control private and public green areas, the whole task of monitoring private gardens was assigned directly to ERSAF (*Ente Regionale per i Servizi all'Agricoltura e alle Foreste*; Regional Service for Agriculture and Forest).

The cutting phase of symptomatic and neighbouring plants, *i.e.* the only instrument effective in controlling the insect until now, has attracted much concern especially when the same situation is repeated in subsequent years with the discovery of new plants infested also in the portion of the park so called *Boscoincittà*. The technicians of the park were mainly worried about eliminating hundreds of plants, subtracting a portion of the forest to its users, and reducing the presence of some native species (maple, hornbeam and hazel) due to the ban on planting of susceptible plants, with the consequent impoverishment of biodiversity. The Plant Protection Service, instead, proposed strict phytosanitary measures to reduce the risk of spread of the insect using the principles of caution. To accommodate the needs of both parties without lack of compliance, an insect control plan including the use of chemical means was finally developed in consultation.

Materials and methods

The infested area was divided into 19 parcels (for a total of 35,000 m^2) in 2010 and into 25 parcels (for a total of 143,000 m^2) in 2011 for trials. Every parcel was then mapped by GIS.

For each of them was selected a minimum of host species, preferring plants of 10 cm of diameter of trunk and with good structure and health (Figure 2). The other sensitive plants inside the lots were cutted down and their stumps eradicated. Trials consisted of: i) treating selected plants every 3 weeks with lambda-cyhalothrin principle, distributed on trunks up to 1-m high and emerging roots; ii) monitoring plants from June to September in order to search for adults, larval frass, exit holes, oviposition signs; iii) recording data indicating the presence of Anoplophora; iv) collecting data concerning adults (sex, vitality).

Results

The presence of adults and larval frass was considered as a parameter for trials' evaluation: adults number decreased during the trials (42 adults in 2010 and 3 in 2011); in 2010, only 8 plants showed larval frass, in 2011 no plants showed this symptoms (Tables 1 and 2).

Figure 2. Marked plants.

Table 1. Infested plants in 2010.

Species	Parcel	Exit holes	Larval frass	Adults
Acer campestre	M	0	1	0
Acer campestre	O	1	0	1
Acer pseudoplatanus	O	3	2	4
Acer campestre	O	1	0	1
Carpinus betulus	O	0	1	0
Malus sylvestris	N	0	2	0
Acer platanoides	O	4	0	4
Carpinus betulus	S1	1	0	1
Acer pseudoplatanus	N	3	1	0
Acer pseudoplatanus	O	4	1	0
Acer pseudoplatanus	O	5	0	2
Acer pseudoplatanus	F	5	2	1
Acer pseudoplatanus	N	2	0	0
Acer pseudoplatanus	N	2	1	0
Acer pseudoplatanus	N	1	0	0
Acer pseudoplatanus	L	1	0	0
Total		33	11	14
Total symptomatic plants		13	8	7

Table 2. Infested plants in 2011.

Species	Parcel	Exit holes	Larval frass	Adults
Acer pseudoplatanus	N	2	0	1
Acer campestre	N	0	0	1
Acer campestre	O	1	0	1
Acer campestre	O	1	0	0
Total		4	0	3
Total symptomatic plants		3	0	3

Data about exit-holes are difficult to be interpreted, considering also that life-cycle of Anoplophora *chinensis* often lasts 2 years (33 exit-holes from 13 trees in 2010, 4 exit-holes in 2011).

Results on monitoring infested area from 2009 to 2011 proved also that *A. chinensis* mostly prefers *Acer* and *Corylus* genera; on the other hand, *Quercus* and *Fraxinus,* which are highly spread in the park, are not appreciated by this coleoptera.

Moreover, monitoring showed that *Anoplophora chinensis* is able to make oviposition and to complete its life-cycle also on plants with very little trunk diameter (up to 2 cm).

Discussion and conclusions

The results of these tests with a chemical product showed that an integrated approach to contain *A. chinensis* is possible, as required by the new European decision 2012/138/UE, simultaneously preserving the environment biodiversity and the public fruition of the green areas.

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Control strategies of *Anoplophora chinensis* in an area of considerable artistic and archaeological value in Rome

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Abstract

The outbreak of *Anoplophora chinensis* - citrus longhorned beetle - in Rome municipality regards an area of considerable historical and archaeological value. Thus, the peculiar features of this area led to take into account phytosanitary measures. These measures, alternative to mechanical removal or distruction of infested trees and stumps, were found in order to ensure both effective phytosanitary actions on infested plants and preserve the archaeological heritage of this area.

Introduction

In 2008, *Anoplophora chinensis* was detected for the first time in Rome. The green areas subject to statutory notices for the infested plant presence, were represented by six historic private gardens, close to the *Appia Antica* archaeological park, and one public park next to the *Thermae of Caracalla*.

One of the private gardens, where infested plants were revealed, pertains to a historic Roman *Villa*, called *Horti Galateae*, which was the residence of the painter and sculptor Giulio Aristide Sartorio in the late nineteenth century.

Also, the entire infested area, carefully monitored by the Plant Protection Service, is rich in historical sites such as *Thermae of Caracalla*, the *Columbarium* of *Pomponio Hylas* and the *Casina of Bessarione Cardinal*. Often in these sites, secular specimens of trees susceptible to the parasite were detected.

Materials and methods

In order to eradicate the pest in an area of considerable artistic and archaeological value, the Plant Protection Service of Lazio Region in some cases has carried out alternative eradication measures being as effective as the measures established by European dispositions.

These cases were represented by infested stumps not mechanically removable because they were close to Roman walls (Figure 1) or because their mechanical removal could cause damage to the historic gardens where the infested plants were present. In the Figures below, it is possible to see some infested stumps neutralised by using wire mesh (Figures 2-4) because of their location on a sloping ground that made them not-mechanically removable.

In addition, for five *Platanus* spp. – three infested by the pest and two not infested but close to the first ones – the Plant Protection Service of Lazio Region has realised the trunk and stump coverage using wire mesh (Figures 5-8).

These plants are secular specimens detected in the private garden pertaining to the historic Roman *Villa* called *Horti Galateae*. Before the application of alternative eradication measures, a careful inspection of these plants was carried out also on branches located at about 15 m above the ground.

Figure 1. Infested stump close to a Roman wall covered by wire mesh and concrete.

Figure 2. Technicians laying down wire mesh.

Figure 3. Wire mesh covering an infested area.

Figure 4. Edging of an infested area before covering with wire mesh.

To monitor the efficiency and efficacy of these alternative measures, the inspectors of the Plant Protection Service have carried out weekly inspections of these plants along all insect flight period. Several cases of adult insects trapped under the metal mesh used to neutralise the infested stumps were detected (Figure 9).

Results

These alternative measures were found equally effective to mechanical removal. Indeed, in 2008, a total of 39 plants infested were detected; in 2010, 8 infested plants were found; in 2011, 2 flowerbeds of *Chaenomeles* spp., 2 flowerbeds of *Rosa* spp. and 2 *Acer* spp. infested by the pest were found; and finally in 2012, no infested plant was detected (Table 1).

Figure 5. First covering using mosquito polyethylene net.

Figure 6. Second covering using wire mesh properly secured and overlapped.

Figure 7. Excavation for undergrounding the wire mesh used to cover the root system of *Platanus* spp.

Figure 8. Wire mesh covering trunks and soil.

Figure 9. Adult insect trapped under the wire mesh.

Table 1. Results of the analysis.

Species	SP	IP
Acer spp.	16	32
Aesculus hippocastanum	7	6
Corylus avellana	0	1
Ulmus spp.	36	0
Total 2009	59	39
Ulmus spp.	5	1
Aesculus spp.	0	1
Corylus spp.	0	1
Platanus spp.	2	4
Acer spp.	1	1
Citrus spp.	1	0
Total 2010	9	8
Chaenomeles spp.	2 flowerbeds	
Rosa spp.	2 flowerbeds	
Acer spp.	1	1
Total 2011	1	1+4 flowerbeds

SP, sensitive plans; IP, infested plants.

Discussion and conclusions

Analysing the number of infested plants detected from the first discovery to the present day (Table 1), we can state that the surveillance and eradication measures applied by the Plant Protection Service of Lazio Region were actually effective for achieving full eradication of outbreak found in Rome in 2008. Measures have taken into account, in addition to the European provisions, even the landscape and the archaeological constraints to which the infested area was subject.

Anoplophora glabripennis outbreak management in Veneto Region, Italy

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Abstract

This study was aimed to evaluate the *Anoplophora glabripennis* outbreak management in Veneto Region, Italy. Since the first discovery, a programme of measures designed to eradicate this pest was immediately launched thanks to the effective collaboration between various public agencies at different levels of expertise. The interventions to date implemented proved to be capable of likely reducing the presence of the parasite within the demarcated area and to avoid the spread outside of it.

Introduction

In June 2009, an outbreak of *Anoplophora glabripennis* Motschulsky (Coleoptera: Cerambycidae) [Asian lonhgorned beetle (ALB)] was detected for the first time in Veneto Region (municipality of Cornuda, Treviso province, North-East of Italy).

Immediately, an intensive and specific monitoring was began in the municipality of Cornuda, checking all the susceptible trees located in public and private areas to establish a demarcated area (periodically updated).

Following the detection of this harmful organism, Veneto Region has established emergency measures (Ordinance n° 137 of 22 July 2009 issued by regional President of Veneto Region *Required measures to manage and to eradicate Asian Longhorned Beetle (*Anoplophora glabripennis*) within the Veneto Region*).

Attention was drawn to this pest by its introduction into Veneto region, where a major eradication plan is underway.

Materials and methods

The eradication plan implemented by the Plant Protection Organization (PPO) of Veneto Region and its co-operators, hinges on various elements: i) check all tree species which are potential ALB hosts within 2 km from each infested tree (PPO Veneto Region); ii) delimiting infestations imposing quarantine, and implementing control measures within the delimitate zone; iii) cut and chip all infested trees [Forest Service of Veneto (FSV) Region]; iv) make an inventory of all tree species potential ALB hosts (geographic position, species, ownership); v) make the public aware of the situation.

Early detection and rapid response are necessary to get good results in the eradication efforts; local partners participated in monitoring and eradication (synergy).

Results

All plants found (or suspected to be) infested by ALB during monitoring activities, both in public and private areas, were identified, georeferenced and assigned to cut by monitoring teams.

Given the low propensity recognised in the literature to adults of ALB to abandon the plants where they were born for new colonisation, if not molested (for example by the vibrations of chainsaws), and observing on average their possible presence from the end of May to early November, it was decided not to proceed with the cuts during this period, yet concentrating them, along with the destruction of waste wood, in the period from December to April of each year.

The cuts were carried out by the technical staff of FSV.

The waste wood, before being submitted to chipping, was amassed in a fenced storage area, equally under the supervision of FSV. The material was subsequently chipped by a contracted company, that carried the final product to an incinerator. The used chipping machine originated a product of such dimensions as not to allow the survival and development of the larval forms (according to a study conducted by the University of Padua).

The plants cut down so far were 1400 total, of which 1066 infested and 334 high-risk.

Discussion and conclusions

Efforts to eradicate the *A. glabripennis* infestation in Veneto Region, as an integral component of the eradication programme, are giving some first positive results. However, caution must be maintained at all levels.

The effectiveness of the eradication plan and the difficulties in applying appropriate measures aiming at eradicating the harmful organisms and monitoring through appropriate inspections, are still under discussion.

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Fighting *Anoplophora chinensis* in a wooded habitat. The Gussago case

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Abstract

During the 2009 surveillance activit, signs of *A. chinensis* were observed in San Rocco: a wooden area mostly populated by groups of trees of the oak-ornbeam type, in the northern part of Gussago (Figure 1). *A. chinensis* was strongly fought by the removal of more than an hectare of woods. During works, the creation of service paths and roots systems removal caused a significant alteration of the hillside. The site was restored with seeding and plantation. Since then, fewer signs of *Anoplophora* presence were found.

Introduction

Gussago is a municipality close to northern hills leading up to Prealpi. In the northern part of the village, there are wooded hills mainly populated by trees of the oak-ornbeam type, consisting mostly of *Ostrya carpinifolia*, *Fraxinus ornus*, *Acer campestre*, *Quercus pubescens* and *Quercus petraea*.

During the 2009 surveillance activity in San Rocco – a partly wooded hill located east of the residential area of Gussago – signs of *A. chinensis* (frass and exit holes) were observed in *A. campestre* and *O. carpinifolia.* The infested portion of the hillside, with North-West exposure, was characterised by old terraces from 3 to 5 m high, still evident and clearly visible. In order to calibrate the phytosanitary measures, the observations were repeated during the same winter when 34 infested plants were observed: 20 *Acer* spp., 14 *Ostrya* spp. and 1 *Corylus* sp.

Materials and methods

Eradication activities began in March 2010. The wide area between San Rocco and other *Anoplophora*-free forested areas, permitted the application of strong eradication measures: 1136 plants were cut down and more than an hectare of woods was removed. The felled trees were: *Ostrya* spp., *Acer* spp., *Corylus* spp., *Crataegus monogyna*. The spared species were: *Quercus* spp., *F. ornus* and *Celtis australis*. During the activities, a significant alteration of the hillside due to the creation of service paths and roots system removal was caused.

After the removal of infected plants, the site was restored: to prevent landslides, a jute netting was positioned along an almost 90 m slope. The surface was seeded with a hydroseeding technique and trees were planted with backing poles and shelters, throughout the entire site. Over than 3000 bushes and saplings of the following species have been replanted: *C. australis, F. ornus, Quercus* spp., *Coronilla emerus, Cornus* spp., and *Euonymus europaeus*.

During that year, maintenance works, including periodic mowing and replacement/elimination of withered plants were performed.

Results

During the 2010 surveillance season, other 12 infested plants (11 *Ostrya* spp., 1 *Acer* sp.) were found in San Rocco – on the South-West side – and were promptly removed.

In 2011, only a single infested tree (*O. carpinifolia*) was found on the South-West slope. No more symptoms of infestation were found on the North-West slope.

Figure 1. San Rocco wooden area.

Anoplophora chinensis in Lombardy Region: description of survey methodology and quality management

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Introduction

Part of the *Anoplophora chinensis* eradication plan in Lombardy Region consists of survey activities to detect and delimit infestation and locate host trees with signs of attack to be removed. Annually, from July to December, an area of 40,000 ha is monitored by 20 trained inspectors of the Regional Plant Protection Service (ERSAF), involving 72 municipalities in the provinces of Milan, Varese, Como and Brescia.

Methodology

The monitoring is carried out in accordance with the Lombardy Region Decree n. 4379 - 27/04/2010 (*Nuove misure regionali di controllo ed eradicazione di* Anoplophora chinensis *(Forster) in Lombardia*) and the Decision 2008/840/EC, and it is based on intensive surveys of all the species suitable to attack, whitin the demarcated area (infested and buffer zone) established around each infestation.

The infested zone is where host trees are located and all the susceptible host trees are inspected in both public green areas and private gardens. The buffer zone includes a radius of at least 2000 m beyond the boundary of the infested area: in the first 500 m beyond the boundary, all the potential host trees are inspected as well as the infested zone; in the rest of the area (1500 m.), all the potential host trees are checked out only in the public green areas and selected at random in the private ones (Figure 1).

Before the survey, municipalities and public are informed about the monitoring activities (meetings and leaflets), in order to improve and maintain public support. The survey consists of visual inspection of all the potential host plants, in order to locate all trees with signs of injury, mainly detectable in the lower trunk, root collar region and exposed root (larval feeding frass, adult circular exit holes, and feeding bark damages on young branches). Fieldwork data are then entered into a database and used for GIS application and statistical analysis to plan the following surveys and pest management (Figures 2 and 3).

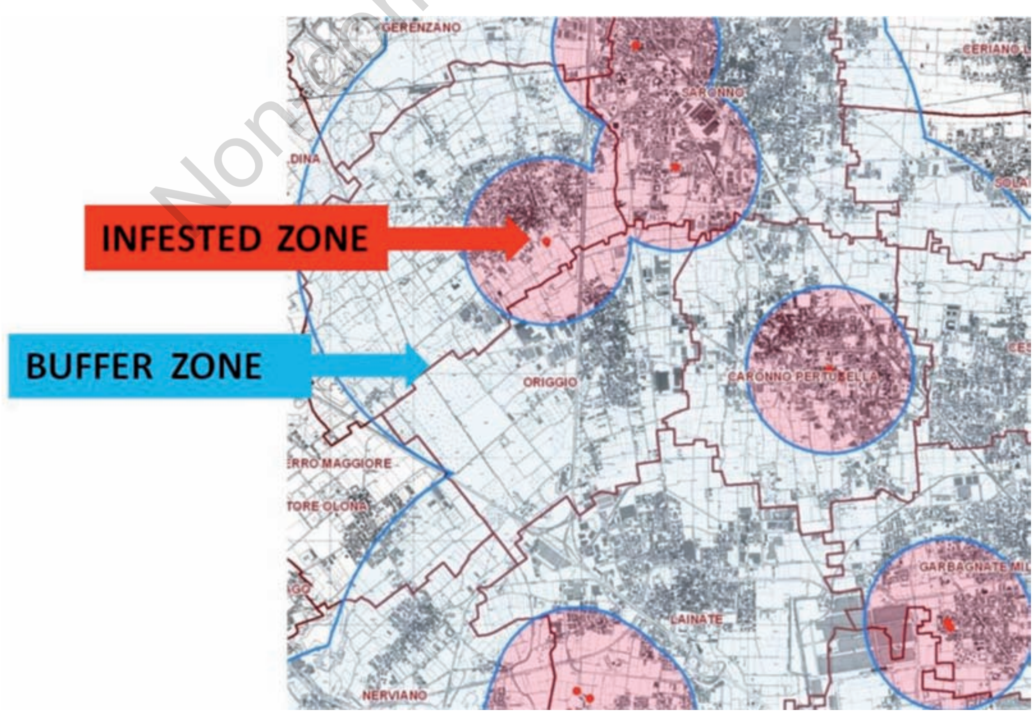

Figure1. Example of the infested zone and buffer zone detected.

Figure 2. Hand-held computer with GPS.

Figure 3. Visual inspection and recording data.

Results

Annually, each inspector checks out an average of 20,000 trees and 12,000 linear m of hedges. Referring to Milan and Varese provinces, the average number of plants checked out in the infested zone by each technician per day in the period 2008-2010 was quite constant, resulting in 163 plants.

Discussion and conclusions

The survey of the demarcated areas is a fundamental step in the Lombardy programme of containment and eradication of *Anoplophora chinensis*. The collected data are very useful to develop predictive models to estimate residual infestation; check distances between infestation sites; evaluate the effectiveness of fellings by comparison of pre-/post- cut infestation maps; and identify the most commonly infested tree species among the potential host plants.

Model assist deepens *Anoplophora* spp. biology and spreading under relevant climatic conditions

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Introduction

The climate mapping software Climex has been used to predict the range of potential establishment for *A. chinensis* and *A. Glabripennis*.

Materials and methods

In the context of the ANOPLORISK research (Risk Management for the EU listed *Anoplophora* spp., *A. chinensis* and *A. glabripennis*), studies are being conducted to gather more and broader information on: i) whether larval development could be prolonged over more than two seasons and what restricts the distribution in the region of origin; ii) how much the local/regional profile of meteo variables could affect the short and long distance movement of *Anoplophora*.

As outline and items of work, an improvement of the CLIMEX model for the distribution of *A. glabripennis* in Europe will be performed.

The environment that *Anoplophora* larvae inhabit during their development (within trees), insulated from the air temperature as measured by weather stations, will be studied. At present, no attempt has been made to incorporate this insulating effect on the development rate and modelisation of *Anoplophora* spp. Loggers have been put inside tree trunks at Fera to monitor the internal temperature.

Results and conclusions

The outbreaks of both *A. chinensis* and *A. glabripennis* in Europe and recent laboratory studies have led to a greater understanding of the relationship between temperature and the development of *Anoplophora*. The results from temperature loggers will be analysed in order to refine and understand day degree models.

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

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Introduction

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

Materials and methods

In summer 2009, 46 trees belonging to 4 genera (*Acer*, *Ulmus*, *Betula* and *Aesculus*) infested by ALB were randomly sampled from the whole of the infested area, which extended over an urban area of about 2x2 km in the village of Cornuda (Treviso) (45° 49' 56" N 12° 0' 19" E). The coordinates of each sampled tree were recorded by GPS and their position mapped. Infested trees were cut and a number of branch sections bearing exit holes variable according to the infestation density was cut from each host tree and taken to the laboratory. Each section was then cut orthogonally to the branch axis in the centre of the exit hole. The resulting surfaces were smoothed by sandpaper and the tissue layers around the hole were dated by analysis of the growth rings. It was also noted whether the first tissue layer around the edge of the exit hole was of early- or late-wood type, to indicate the timing of the ALB adult emergence.

Results and discussions

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

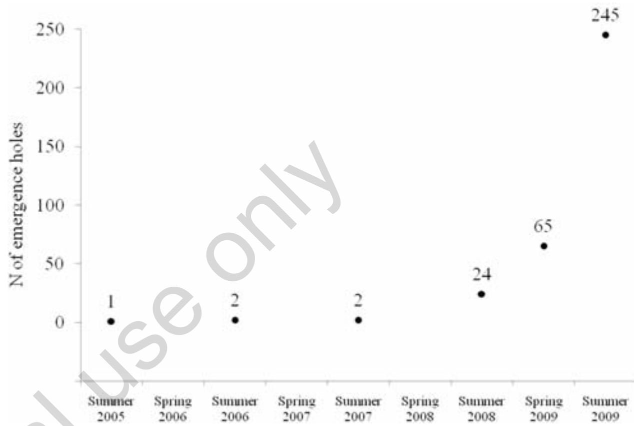

Figure 1. Estimated age of the analysed exit holes.

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An overview of the ongoing research on *Anoplophora chinensis* in Lombardy Region. A special focus on biological control studies and use of sentinel trees technique

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Introduction

The citrus longhorned beetle (CLB) *Anoplophora chinensis* (Forster) (form malasiaca) (Coleoptera, Cerambycidae) was recorded for the first time in Northern Italy in 2001. It is a pest of many broadleaf tree species and it is considered as a serious threat to the urban and natural environments. The insect is originating from eastern Asian.

Ten years of eradication efforts have aimed to contain the invasive woodborer, and several research projects were developed to better understand the biology of the pest, and assess varied potential control techniques. New funding by the Lombardy Region, running 2011 through 2013, will permit us to conduct additional studies that may have a very positive impact on the suppression of the CLB populations.

Results

In the major infestation around Parabiago, a Eulophid gregarious egg parasitoid, specific to *A. chinensis* was discovered. It was new for science and was described and named *Aprostocetus anoplophorae* by Delvare *et al.* (2004). Because of its high potential for biological control of CLB, it was thought it would be very beneficial to introduce it in the other CLB infestations where it had not occurred yet. Field experiments to finalise a suitable release technique of the egg parasitoid were planned.

In spring 2012, 90 maples (*Acer pseudoplatanus*) were planted in 6 CLB-infested sites (15 trees per site). In September 2012, 5 trees received CLB eggs from females caged in wire mesh screen sleeve cages around the trunk. Then, the CLB eggs were exposed to Aprostocetus females, so that the CLB eggs get parasitised. The weather conditions during September should have induced diapause of the parasitoid larvae in the host eggs.

At several dates in June and July 2013, we will use the 10 remaining trees of each plot to create a local population of freshly laid CLB eggs as candidates for parasitization by the Aprostocetus individuals emerging from the 5 trees infested in September 2012. To get this, we will place CLB females in wire mesh sleeve cages on the trees. Each cohort of CLB eggs will be suitable hosts during at least 10 days for the parasitoid adults emerging from the 5 trees. In this way, we will determine if *Aprostocetus* successfully goes through diapause in the experimental conditions, and if the potential for its establishment is high.

The sentinel trees method is based on the use of highly attractive tree species (*e.g. Acer saccharinum*) to attract and kill the pest in areas where infestations are known to currently harbor or previously harbored beetles or infested trees. This includes areas that are difficult to monitor. Simply stated, the primary objective is to attract beetles and kill them by spraying an insecticide on the trunk. The killing agent would simply be applied to the lower 50cm of the trunk where adult CLB lay eggs. The objective would be to kill beetles prior to oviposition. Killing beetles that are attracted to and land on the sentinel trees gives this strategy its name, *Attract-and-Kill*. In two sites in Brescia province (Montichiari and Gussago), rows of 50 maples (*Acer saccharinum*) were planted for that purpose. Treatments and data collection will be made in summer 2013.

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Public awareness: a crucial point for a successful eradication campaign against the longhorned beetles *Anoplophora chinensis* and *A. glabripennis*

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Introduction

The two longhorned beetles, *Anoplophora chinensis* and *A. glabripennis* are native to Asia and worldwide considered as some of the most dangerous woodborers able to colonise new environments. Spreading through living trees (the first), and also through wood packaging materials (WPM) (the second one), many times their infestations are undetected for years and years, allowing them to spread to new areas and reach levels of population that are difficult to be eradicated. Such examples of this evolution are well known in European countries as well as in North America.

In Italy, in Lombardy Region, *A. chinensis* was first detected in 2001 and over a decade, the Lombardy Plant Protection Service (PPS) has developed a good level of experience to try to contain this xilophagous pest.

Thanks to all these efforts, the Lombardy PPS's staff realised that the key point for a successful and effectiveness eradication program, is the public awareness. In order to increase it, since the beginning of the infestation, Lombardy Region implemented a strong campaign of information through various media to give the public a better knowledge of the extent of the problem.

Results

Thanks to an increasing level of information among citizens, in 2006 a new infestation of CLB was detected in Brescia province, almost 150 km far from the historic outbreak in Milan, at Parabiago.

One year later, during the surveys carried out for *A. chinensis* in Milan province and thanks to the same information campaign, in 2007 the first record of *A. glabripennis* in Italy was occurred.

Municipalities concerned by the pest were part of this education effort. Leaflets and posters on *A. chinensis* were distributed and a very unique campaign of awareness was held in Milan subway, with almost 200 posters in 82 station points (Figures 1 and 2). On the poster, a big and defined picture of the beetle as well as an image of a stump with numerous exit holes was showed. Each year, starting from 2008, posters were fixed on the walls of the subway stations for one month, in the flying period of the beetle, from June through July.

A voice-mail and a mail box were also activated by ERSAF and a person in charge was employed for receiving the contacts.

In 2008, 500 contacts were received and the number increased during the following years: 1069 in 2009 and 1447 in 2010.

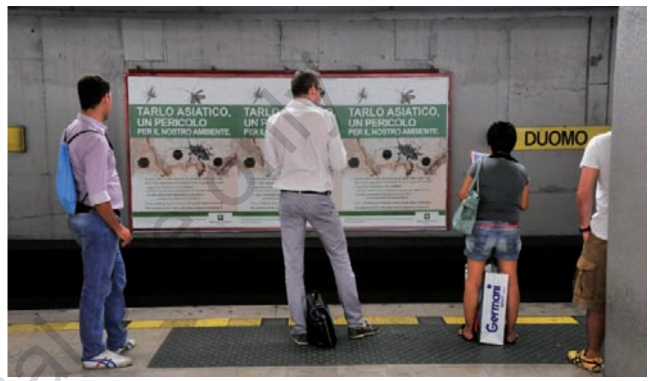

Figure 1.

Figure 2.

Meetings with technicians of the municipalities and citizens were organised, press articles were released in newspapers and magazines, radio and TV spots were broadcasted, and articles were published on scientific journals.

As for establishing the number of areas where the beetle is able to establish, both in private gardens within a urban area and in the countryside all around the infested municipalities, and early detect new outbreaks, it is useful to take into consideration people's reaction when they find a specimen of the exotic beetle or when they observe signs of *A. chinensis* presence.

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Dating exit holes of *Anoplophora chinensis* in an outbreak site in Rome, Italy

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Introduction

Anoplophora chinensis is a longhorned beetle native to Eastern Asia which develop on a number of broadleaf plants accidentally introduced in Europe; this species is a serious pest and strong emergence measures applied.

A. chinensis generally colonise healthy trees, feeding into the wooden parts mainly at the base of the trunks and in the roots. Adult beetles emerge from the infested trees producing a circular exit hole in the bark. Using techniques of annual growth rings analysis for dating age of trees, and assuming that the plant at time of felling was alive, it is possible to date the time of *injury* occurrence.

Materials and methods

Wooden material colonised by *A. chinensis* was obtained from the infested site in Rome during the eradication activities performed by the Plant Protection Organisation (PPO) of Lazio Region.

By preparing the cross-sections the stump of infested trees were sectioned progressively, using an all-purpose or sabre saw, producing approximately 3-5 cm broad cross-sections. Surfaces of the cross-sections were sanded using different abrasives of decreasing grain size using belt and orbital sanders. Each exit hole was dated by annual growth ring count using stereomicroscope and Lega dendrochronograph system.

Results

Investigations conducted on the wooden material collected in Rome, showed that the emerging from infested trees of the first specimens of *A. chinensis* is dated to occur in 2002; considering that the species takes 1 or 2 years for juvenile development in the infested site in Rome, we speculate that the first introduction of *A. chinensis* was the year 2000 or 2001, or even previously.

Acknowledgments

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Application of several technologies in the production chain in China to control the citrus longhorn beetle *Anoplophora chinensis*

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Introduction

Citrus longhorn beetle (CLB) *Anoplophora chinensis* and Asian longhorn beetle (ALB) *A. glabripennis* are both wood-boring beetles that can cause serious damage to a wide range of tree species (EPPO, 2004). Both CLB and ALB are quarantine species of Europe. In recent years CLB and ALB were introduced in several occasions with imported woody plants and packing wood materials from primarily China into the Netherlands (EPPO). The Dutch government took quarantine measures and eradicated all host plants within a 100 m range of an infestation, at high economic costs. Meanwhile, the export of CLB host plants from China to Europe declined dramatically during the last several years.

The most effective way to prevent the invasion of CLB, is to improve the pest control technology or strategy at the origin of primary production, during production or post-production process. In order to reduce the risk of CLB and ALB infection in host plant products and wood packing materials in China, the Dutch Ministry of Economy, jointly with the Chinese authority AQSIQ, has initiated a research project to seek for improvement in the prevention and control of CLB and ALB problems.

Materials and methods

In this joint research project, monitoring and control technologies will be evaluated for the feasibility of application in China. For monitoring and control purpose lure-baited traps will be tested for the effectiveness in catching adult CLBs (NVWA, 2010; Haack *et al.,* 2010; Yasui *et al.,* 2011). In an earlier study, CT and X-ray scan has been shown to be able to detect beetle larvae inside stems or branches of woody plants (Mol *et al.,* 2011, unpublished data). This technology will be evaluated for the detection of CLB larvae in host plants or ALB larvae in wood materials. Earlier research had proved a controlled atmosphere temperature treatment (CATT) to be effective in killing mites and nematodes in strawberry plants (Van Kruistum, 2009). The CATT technology will also be tested for the applicability in killing various stages of CLB and ALB in woody plants and wood packing materials.

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Visual inspection and application of new detection method on discovering low *Anoplophora chinensis* (Forster) population density in Croatia

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Abstract

A. chinensis, originally from China, was firstly detected on *Acer palmatum* potted plants by visual inspection in nursery Turanj, near Zadar, Croatia, in 2007. A non-destructive detection method was applied with trained dogs from Austria for the first time in Croatia and one infested *Lagerstroemia indica* was found.

Introduction

The status of *A. chinensis* is regulated in EU by Council Directive 2000/29/EC and Commission Decisions 2008/840/EC and 2010/380/EU. Non-destructive detection methods, acoustic detection, use of x-rays and of trained dogs for detection of infested wood, shrubs and trees were studied and used in different countries.

Presented are results of four years of visual inspection and use of non-destructive method with trained dogs.

Methods

Symptoms of insect presence were searched on host plants from April to September.

Trained dogs searched hedge of *Prunus laurocerasus*, gardens, alleys, parks and forestry as well as host plants in the nursery. Two pair of dogs alternated every 15 minutes early in the morning and late in the afternoon to avoid high temperatures (35°C) during the day in June and July 2011.

Molecular analyses have been performed to confirm the origin of larvae found by dogs.

Results

One hundred and eighteen visual inspections were performed in different areas of Croatia and 128 samples were collected. All of 112 larvae of citrus longhorned beetle (CLB) were found only in the nursery of the first discovery. Infested were 105 plants of *A. palamtum* and seven *Rosa* spp. (Figure 1) in the period 2008 to 2010.

Dogs found one *L. indica* infested with *A. chinensis.* Molecular analysis confirmed insect origin was from the same population discovered in 2007.

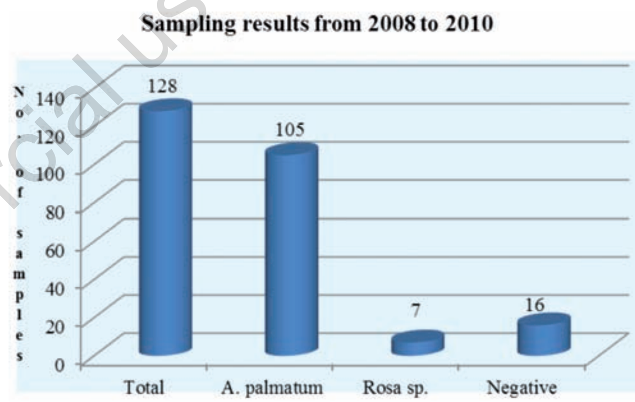

Figure 1. Results of the survey.

Discussion and conclusions

Use of dogs to discover low CLB population density was successful, while dogs searched thousands of plants of which only one was infested. The molecular analysis confirmed that larvae found on *L. indica* belonged to the same population imported from China in 2007.

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