REDIA, XCV, 2012: 89-92

TECHNICAL NOTE

GIUSEPPINO SABBATINI PEVERIERI(*) - GIADA BERTINI (**) - PAOLA FURLAN (*)
GIOVANNI CORTINI (*) - PIO FEDERICO ROVERSI(*)

ANOPLOPHORA CHINENSIS (FORSTER) (COLEOPTERA CERAMBYCIDAE) IN THE OUTBREAK SITE IN ROME (ITALY): EXPERIENCES IN DATING EXIT HOLES

(*) *Consiglio per la ricerca e la sperimentazione in agricoltura, Research Centre for Agrobiology and Pedology, via Lanciola 12/a, 50125 Florence, Italy;* e-mail: giuseppino.sabbatini@entecra.it
(**) *Consiglio per la ricerca e la sperimentazione in agricoltura - Forestry Research Centre, Viale Santa Margherita, 80, 52100 Arezzo, Italy;* e-mail: giada.bertini@entecra.it

Sabbatini Peverieri G., Bertini G., Furlan P., Cortini G., Roversi P.F. – *Anoplophora chinensis* (Forster) (Coleoptera Cerambycidae) in the outbreak site in Rome (Italy): experiences in dating exit holes.

Anoplophora chinensis is a poliphagous woodboring beetle native to Eastern Asia, and is a serious pest accidentally introduced into Europe. Currently two infested areas are present in Italy and other infestations have occurred in the past in other European countries, while interceptions in entry ports are made yearly by National Plant Protection Organizations of the European Community. *A. chinensis* is a quarantine invasive pest, and strong measures are applied worldwide to prevent its introduction, spread and to eradicate its populations. The presence of exit holes of adult beetles on infested trees is one of the most important diagnostic signs to detect an *A. chinensis* infestation. Exit holes are visible on the bark surface of infested trees for several years, but the hole is after that enclosed by the reaction tissue (callus) of the plant; thus, after some years the exit hole is no longer visible. Such injuries produced by beetle activity can be recognised after many years by inspection of the wood after cross-sectioning of the tree. It is possible to date the time of occurrence of the injury using the annual growth ring method for tree age analyses. Surveys conducted on the wooden material collected in the infested site in Rome indicate that the first *A. chinensis* adult emergences from infested trees of the city occurred in 2002. Since the species takes in the infested site 1 or 2 years for juvenile development, we can speculate that the first introduction of *A. chinensis* into the city of Rome took place in the years 2000 or 2001.

KEY WORDS: Citrus longhorned beetle, xylophagous insect, quarantine pest.

INTRODUCTION

Anoplophora chinensis (Forster) is a longhorned beetle native to Eastern Asia (China, Japan, Korea), commonly known as the Citrus Longhorned Beetle (CLB) due to the severe damages recorded in citrus orchards (ADACHI, 1988, 1990; LINGAFELTER and HOEBEKE, 2002). Despite its common name, this species is able to develop on a number of broadleaf plants, including shrubs and trees of forest, urban, fruit and ornamental species. *A. chinensis* attacks plants of almost any size and age, from potted plants and "bonsais" up to monumental specimens; only plants with very small trunk diameter may not be subject to its attacks (LINGAFELTER and HOEBEKE, *l.c.*; HAACK *et al.*, 2010). *A. chinensis* is a serious pest accidentally introduced into Europe, and in Italy currently present in two areas: in the Lombardy Region (Northern Italy) and in the city of Rome (Lazio Region, Central Italy) (EPPO, 2012). Infestations have occurred in other European Countries, while interceptions in entry ports are made several by NPPOs (National Plant Protection Organizations) of the European Community (HAACK *et al.*, *l.c.*; VAN DER GAAG *et al.,* 2010; EPPO, *l.c.*). *A. chinensis* is a quarantine invasive pest, and strong measures are applied worldwide to prevent its introduction and spread, and to eradicate its populations (EPPO, 2010; EU, 2012; HAACK *et al.*, *l.c.*). In the European Community, its eradication is currently carried out by destructive methods including chipping or burning of infested trees; destruction is not aimed only at trunks and branches but also at the root system (EU, *l.c.*).

A. chinensis is a woodboring insect which generally colonizes healthy trees but can repeatedly attack the same tree for many years. After years of repeated attacks, larvae disrupt the tree's vascular tissue and can lead to the tree's death, causing also structural weakness (HAACK *et al.*, *l.c.*). The beetle needs approximately 1 - 2 years for juvenile development (3 years in colder countries) (see VAN DER GAAG *et al.*, *l.c.*), and the females lay their eggs mainly at the base of the trunk of susceptible trees or on exposed roots. The larvae feed at first in the phloem tissue under the bark and later become xylem feeders, boring tunnels more deeply into the woody parts of plants (Fig. I, 1). Adult beetles emerge producing a circular exit hole by chewing a thin portion of bark separating the pupal chamber burrowed in the xylem from the external environment (Figs. I, 2 and 3).

The presence of exit holes on trees is one of the most important diagnostic signs to detect *A. chinensis* infestation and holes are visible on the bark surface of infested trees for several years (Fig. II, 1). The exit hole dug by adult beetles in the wood of the infested plant can be considered an injury like any other event causing a damage to the trunk (accidental damage from machinery or working tools or from natural events). Plants respond to injuries occurring during their life cycle by many chemical and anatomical reactions; these reaction tissues remain in the wood structure (RADEMACHER *et al.*, 1984; SCHWEINGRUBER, 2007). The reaction ability of the plant to the insect injury depends on many factors (*i.e.* plant species, vegetative condition and environmental characteristics)

– Received 30 July 2012 Accepted 24 September 2012 4 December 2012

Fig. I – *A. chinensis*: a well grown larva in a gallery in the xylem of an infested tree (1); adult specimen (female) during the emergence from an infested tree (2); adult specimen (male) just emerged and walking on the stump of a tree (3).

but the exit hole is eventually enclosed by the reaction tissue (callus) and after some years the hole is no longer visible (Figs. II, 2 and 3). However, such injuries can be recognised after many years by inspection of the wood after cross-sectioning of the tree. It is possible to date the time of occurrence of the injury using the annual growth ring method for tree age analyses, i.e. by counting backwards from the year of felling to the margins of the exit hole (if the tree is still alive at the time of felling). Dating damage caused by woodboring insects is a feasible approach in studies of an insect outbreak history (SCHWEINGRUBER, *l.c.*). Previous studies on dating exit holes of *Anoplophora glabripennis* (Matschulsky), a species close to *A. chinensis* in many aspects, suggested the possibility to analyse the dynamics of an infestation through the years and the pattern of infestation in a given area (SAWYER *et al.,* 2004; SAWYER, 2007). The aim of the present study was to date *A. chinensis* exit holes at the infested site in Rome in order to obtain information on the dynamics of the infestation.

MATERIAL AND METHODS

Wooden samples colonized by *A. chinensis* were obtained from the infested site in Rome during the eradication campaign performed by the Regional Plant Protection Organization of the Lazio Region. In the infested site, during the period 2009 - 2012 were felled 48 infested trees, and the strength eradication procedures permit to preserve, for the dendrochronological analysis, the stumps of 11 trees, almost belonging all to *Acer negundo* (other plant species were *Aesculus hippocastanum* and *Ulmus umilis*). Trees were sampled randomly, but three trees which showed old signs of infestation were also intentionally selected. The wooden material were stored under quarantine restrictions in a multi-compartmentalized wire mesh insect cage in the infested area. Sampled trees, with diameters from 25 to 120 cm at the root collar, were growing in public parks or in private gardens. Trees were still alive at the time of felling and in good vegetative condition upon visual inspection.

SAMPLING OF TREE STUMPS COLONIZED BY *A. CHINENSIS*

The collection of wood samples from trees colonized by *A. chinensis* involved several difficulties due to the ethology of the females, which tend to oviposit at the base of the tree trunks near the soil surface or on shallow roots, and the behaviour of the larvae, digging galleries mainly with a downward direction in the tree trunk and in the roots. Therefore, exit holes are mainly present in the basal part of the trees and cutting trees at ground level in order to obtain the most part of the stump is a demanding task; this however can lead to an undesired lost of wooden material interested potentially by *A. chinensis* exit holes.

In the infested site the felled trees were attacked exclusively by *A. chinensis*, since only this species emerged from the wooden material stored in the insect cages. Stumps of trees were cut at a height of 20-50 cm from the ground level with a chainsaw. The root collar was cut close to the ground line so as to collect as much of the wood as possible, whereas, the tree root system was not sampled. Collection of the wood samples required much care to preserve the entire section of the stump or at least most

Fig. II – *A. chinensis* exit holes: current year (1); exit holes fully closed by the plant reaction (2); comparison of two exit holes fully closed by the plant reaction (3), with clear margins of the hole (a) and with margins no longer observable due to the tangential expansion of the bark during tree growth (b).

part of it in order to have a wide cross-section surface on which perform the annual ring analysis. Stumps were put on pallets under a roof in a shaded site, ensuring sufficiently dry conditions to avoid saprotrophic fungal activity that could cause wood deterioration and staining, but also avoiding excessive drying of the wood, with consequent cracks that might compromise the cross-section analysis.

PROCESSING OF THE WOOD MATERIAL

The entire cross-section of each collected stump was carefully inspected and all clearly visible exit holes were marked and recorded. Special attention was given to suspected old exit holes (exit holes that seem completely enclosed by callus material), which provide the best opportunity to date older exit holes and thus to obtain information on the outbreak history and introduction date of the pest into the area. The use of a petrol-powered or electric chainsaw to cross-section the stump was avoided because the chain and its vibration during cutting might cause a significant loss of woody tissue. Cross-sections were cut with an all-purpose/sabre saw and/or a hand saw, and when necessary with the aid of log wedges.

The stump was sectioned progressively step by step from one end to the opposite one, producing approximately 3-5 cm wide cross-sections. Both surfaces of the cross-sections were inspected for signs of fully closed exit holes not recognised from the outside. Identified exit holes were carefully cut in the middle of their sections so as to obtain two cross-sections, each with half of the sign of the exit hole (Figs. III, 1 and 2).

The cross-sections were sanded with abrasives of decreasing grain size (P60, P100, P150, P240, P400), using belt, orbital and delta sanders. After sanding, the samples were cleaned with an air compressor.

ANALYSIS OF THE ANNUAL RINGS FOR EXIT HOLE DATING

Each exit hole was dated by annual growth ring analysis using different magnifications under a stereomicroscope (Nikon SMZ-2B) and a dendrochronograph system (Lega) (Fig. III, 3). The annual rings of the tree were counted from the outer side (the cambium layer under the bark) to the inner side until the margins of the exit hole were reached. Since the date of felling was known and the trees were still alive at the time of felling, the age of the exit holes dug by the beetles could be determined. To minimize the possibility of counting false rings, each ring was checked by following its track on the cross-section; rings that disappeared for no visible reason or rings that did not show a typical early/late wood tissue pattern were not considered annual growth rings. In the present work, it was assumed that the tendency of tree species to present missing rings due to lack of annual growth did not apply to the infested area, given the sub-Mediterranean climate of the city of Rome.

Staining products such as safranine, methyl blue and chalk/graphite powder were also used to try to improve the visibility of the annual rings in dubious cases. At times, a cutter was used to remove a thin woody tissue layer from the cross-section in an attempt to obtain a more clearly observable surface.

RESULTS AND DISCUSSION

The data on dating of *A. chinensis* exit holes of the outbreak site in Rome are shown in Figure IV. In total, 44 exit holes were analyzed; most of them were recognised before the felling of the tree, while 10 exit holes were detected only after cross-sectioning of stumps because they were completely closed by the callus and no external signs were visible on the bark. All 10 of these exit holes correspond to the older holes (years 2002, 2003, 2004 and 2005). Only 4 completely closed exit holes were detected before cross-sectioning, at the time of tree felling, due to the presence of a visible circular dimple on the bark. They were dated 2005, 2007, 2009 and 2011 respectively. The rest of the exit holes were dated to 2007, 2008 and 2010. On the whole, half of the total number of exit holes were dug in 2008, the year the pest was detected in Rome. Few holes were discovered in the years after starting the eradication program and all them were dated to be excavated in the period 2009 - 2011.

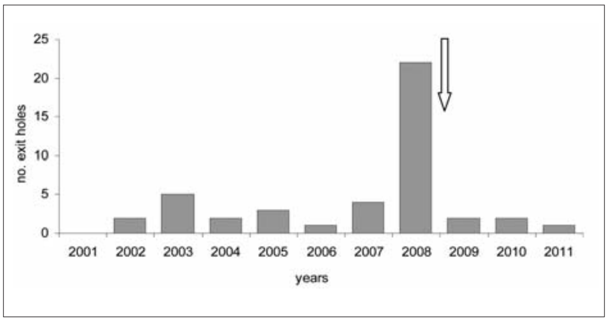

Fig. IV – Number of *A. chinensis* exit holes inspected and dated at the infested site in Rome (data referring to stumps of 11 infested trees felled in the period 2009 - 2012. The arrow indicates the year when the eradication program started.

Fig. III – Cross-sections of *A. negundo* trunks with *A. chinensis* exit holes partially (1) and fully closed (2) by the plant reaction, and a cross-section prepared for annual ring analysis (3).

Surveys conducted on the infested material collected in Rome showed that the first *A. chinensis* emergences from infested trees occurred in 2002. Since the species takes in Rome 1 or 2 years for the juvenile development, we can speculate that the first introduction of *A. chinensis* into the city took place in the years 2000 or 2001.

The data reported in Figure IV show that, six years after the emergence of the first *A. chinensis* specimens in 2002, the number of exit holes increased considerably in 2008, suggesting a clear population growth. However, the area of the infestation site in Rome did not expand considerably, taking into account that the infested site was restricted to a circular area of approximately 700 m of diameter (EPPO, 2012.; VAN DER GAAG *et al.*, 2010). The infested area in Rome, where host trees are numerous and many of them are very large, the *A. chinensis* population appears to have multiplied steadily in a limited area, exploring more or less the available susceptible trees in the area. Therefore, *A. chinensis* has shown infestation dynamics similar to those of *A. glabripennis*, whose infestations do not spread rapidly (SAWYER *et al.*, 2004), at least when hosts are available and no adverse factors influence beetle establishment in the environment. When such conditions no longer exist, adult specimens move in search of more suitable sites.

ACKNOWLEDGMENTS

This work was carried out with a grant from the EUPHRESCO - ANOPLORISK project and with the support of the Regional Plant Protection Organization of the Lazio Region.

REFERENCES

ADACHI I., 1988 – *Reproductive biology of the white-spotted longicorn beetle,* Anoplophora malasiaca *(Thomson) (Coleoptera: Cerambicidae), in citrus trees.* - Applied Entomology and Zoology, 23: 256-264.

ADACHI I., 1990 – *Population studies of* Anoplophora malasiaca *adults (Coleoptera: Cerambycidae) in a citrus grove.* - Researches on Population Ecology, 32: 15–32.

EPPO, 2010 – *EPPO Plant Quarantine Data Retrieval System (PQR) version 5.0* (last update 2012-03-28). - Available at: http://www.eppo.org/DATABASES/pqr/pqr.htm (Accessed on June 8th, 2012).

EPPO, 2012 – *Archives of the EPPO Reporting Service.* - Available at: http://archives.eppo.org/EPPOReporting/Reporting_Archives.htm (Accessed on June 8th, 2012).

EU, 2012 – *Commission implementing decision of 1 March 2012 as regards emergency measures to prevent the introduction into and the spread within the Union of* Anoplophora chinensis *(Forster)* [notified under document C(2012) 1310 (2012/138/EU)]. - Official Journal of the European Union, Legislation Series L64 (55): 38-47.

HAACK R.A., HÉRARD F., SUN J., TURGEON J.J., 2010 – *Managing invasive population of Asian Longhorned Beetle and Citrus Longhorned Beetle: a worldwide perspective.* - Annual Review of Entomology, 55: 521-546.

LINGAFELTER S.W., HOEBEKE E.R., 2002 – *Revision of the genus* Anoplophora *(Coleoptera: Cerambycidae).* - The Entomological Society of Washington, Washington, D.C., USA, 236 pp.

RADEMACHER P., BAUCH J., SHIGO A.L., 1984 – *Characteristics of xylem formed after wounding in* Acer, Betula, *and* Fagus. - IAWA Bulletin, 5 (2): 141-150.

SAWYER A.J., 2007 – *Infestation dynamics of the Asian Longhorned Beetle in the United States.* - In: Proc. XVII USDA Interagency Research Forum on Gypsy Moth and Other Invasive Species 2006, GTR-NRS-P-10, pp. 6-7.

SAWYER A.J., MASTRO V., EMENS B.C., DENHOLM T., 2004 – *Analysis of the Asian Longhorned Beetle infestation in Jersey City, NJ.* - In: Proc. XV USDA Interagency Research Forum on Gypsy Moth and Other Invasive Species, GTR-NE-332, p.70.

SCHWEINGRUBER F.H., 2007 – *Modification of the Tree-Ring structure due to destructive agents in wood and bark.* - In: Wood Structure and Environment, Timmel T.E. and Wimmer R. (eds.), Springer Verlag, Berlin, Heidelberg, pp. 179-227.

VAN DER GAAG D.J., SINATRA G., ROVERSI P.F., LOOMANS A., HÉRARD F., VUKADIN A., 2010 – *Evaluation of eradication measures against Anoplophora chinensis in early stage infestations in Europe.* - EPPO Bulletin, 40 (2): 176-187.