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SCIENTIFIC OPINION

# Scientific Opinion on a technical file submitted by the US Authorities to support a request to list a new option among the EU import requirements for wood of *Agrilus planipennis* host plants[1]

**EFSA Panel on Plant Health (PLH)**[2, 3]

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## ABSTRACT

This document presents the scientific opinion of the Panel on Plant Health on the technical file submitted by the US Authorities to support a request to list a new option among the EU import requirements for wood of *Agrilus planipennis* host plants. The option under consideration is a heat treatment at 60 °C for 60 min to eliminate possible infestations of the wood by the emerald ash borer (EAB). The experiments leading the US Authorities to propose this option are presented in a scientific peer reviewed publication, Myers et al. (2009). The analysis of the aggregated data published by Myers et al. (2009) and based on a Probit regression model showed that the proposed treatment cannot guarantee a control level of 99 % or higher. The analysis of the individual data either from the original measurements or from a corrected dataset, using a Probit regression model, showed that it is likely to observe one live EAB out of an infestation of 100 after the proposed heat treatment of 60 °C/60 min. To ensure a control level of 99 % the temperature of the heat treatment of 60 min should be higher than 70 °C. Results obtained with a Poisson log linear model based on individual data showed that the estimated probability that one insect or more per $m^2$ survive the proposed heat treatment was higher than 0.6 and that there is a 0.1 probability that three insects or more per $m^2$ survive the proposed heat treatment. Based on these results, the Panel concludes that *A. planipennis* is likely to survive the proposed heat treatment of 60 °C/60 min with a low uncertainty, and that the alternative option proposed in the technical file submitted by the US Authorities for wood does not guarantee the wood to be free of *A. planipennis*.



## KEY WORDS

*Agrilus planipennis*, Ash, emerald ash borer (EAB), firewood, *Fraxinus* spp., hard wood, heat treatment

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## SUMMARY

Following a request from the European Commission, the Panel on Plant Health was requested to provide a scientific opinion on a technical file submitted by the US Authorities to support a request to list a new option among the EU import requirements for wood (except in the form of dunnage, spacers, pallets or packing material) of *Agrilus planipennis* host plants. The request was supported by a scientific publication:

Myers SW, Fraser I and Mastro VC, 2009. Evaluation of heat treatment schedules for emerald ash borer (Coleoptera: Buprestidae). Journal of Economic Entomology, 102, 2048-2055.

The Panel analysed the relevant literature pertaining to the biology, host-plants and geographic distribution of *A. planipennis*, common name Emerald Ash Borer (EAB) and gave particular attention to the published articles describing control measures, with a special focus on that of Myers et al. (2009).

During the critical review of the different datasets provided by the US Authorities, the Panel found important inconsistencies. Therefore, in order to reduce uncertainties on the results of the analyses, four datasets were considered to explore the dependence of the model outcome on the possible input datasets.

The analysis of the aggregated data used by Myers et al. (2009) based on a Probit regression model showed that the proposed heat treatment of 60 °C/60 min cannot guarantee a control level of 99 % or higher. The analysis of the individual data using a Probit regression model showed that it is likely to observe one surviving emerald ash borer out of an infestation of 100 after the proposed heat treatment of 60 °C/60 min. To ensure a control level of 99 % the temperature of the heat treatment of 60 min should be higher than 70 °C. Results obtained with the Poisson log linear model showed that the estimated probability that one insect or more per $m^2$ survive the proposed heat treatment was higher than 0.6 and that there is a 0.1 probability that three insects or more per $m^2$ survive the proposed heat treatment.

The rate of survival of EAB prepupae after heat treatment documented in the additional published studies that were examined, suggests that individuals may survive after exposure to 55 ºC for 120 min, to 56 ºC for 60 min and to 60 ºC for 30 min. Therefore none of these treatments are effective in eliminating the EAB from infested wood. These results do not allow any conclusion regarding the effectiveness of the heat treatment under scrutiny (60 °C/60 min).

Based on the results of the analyses it performed, the Panel concludes with a low uncertainty that *A. planipennis* is likely to survive the proposed heat treatment of 60 °C/60 min, and that the alternative option proposed in the technical file submitted by the US Authorities does not guarantee the wood to be free of *A. planipennis.*

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## BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The current European Union plant health regime is established by Council Directive 2000/29/EC on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community (OJ L 169, 10.7.2000, p.1).

The Directive lays down, amongst others, the technical phytosanitary provisions to be met by plants and plant products and the control checks to be carried out at the place of origin on plants and plant products destined for the Union or to be moved within the Union, the list of harmful organisms whose introduction into or spread within the Union is prohibited and the control measures to be carried out at the outer border of the Union on arrival of plants and plant products.

*Agrilus planipennis* Fairmaire, the emerald ash borer, is a serious pest of several woody plant species (*Fraxinus* L., *Juglans mandshurica*, *Ulmus davidiana*, *U. parvifolia* and *Pterocarya rhoifolia*). It is known to be present in Canada, China, Japan, Mongolia, Republic of Korea, Russia, Taiwan and USA, where it causes extensive damage.

*Agrilus planipennis* Fairmaire, is a regulated harmful organism in the European Union, currently listed in Section I, Part A, Annex II of Council Directive 2000/29/EC while present on plants intended for planting, other than plants in tissue culture and seeds, wood and bark of *Fraxinus* L., *Juglans mandshurica*, *Ulmus davidiana*, *Ulmus parvifolia* and *Pterocarya rhoifolia*, originating in Canada, China, Japan, Mongolia, Republic of Korea, Russia, Taiwan and USA. It is currently not known to occur in the EU.

The import requirements for wood (except in the form of dunnage, spacers, pallets or packing material) of host species are listed in Section I, Part A, Annex IV of Council Directive 2000/29/EC and they include the requirement that:

- the wood other than in some specific forms should either originate in an area established by the national plant protection organisation in the country of export as being free from *Agrilus planipennis* in accordance with the relevant International Standards for Phytosanitary Measures or have been squared so as to remove entirely the round surface;
- the wood in the form of chips should either originate in an area established by the national plant protection organisation in the country of export as being free from *Agrilus planipennis* in accordance with the relevant International Standards for Phytosanitary Measures or should have been processed into pieces of not more than 2.5 cm thickness and width.

By the letter of 29th March 2010 the US authorities submitted a request for listing a new option (heat treatment of 60 degrees Celsius for 60 minutes) among the EU import requirements for wood of *Agrilus planipennis* host plants. The request is supported by a scientific article entitled 'Evaluation of Heat Treatment Schedules for Emerald Ash Borer (Coleoptera: Buprestidae)' by S. W. Mayers et al. (J. Econ. Entomol. 102 (6): 2048-2055 (2009).
This request was discussed at the Standing Committee on Plant Health in June 2010 and the Commission decided to seek a scientific opinion from EFSA.

## TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

EFSA is requested, pursuant to Article 29(1) and Article 22(5) of Regulation (EC) No 178/2002, to provide a scientific opinion on a technical file submitted by the US authorities to support a request to list a new option among the EU import requirements for wood (except in the form of dunnage, spacers, pallets or packing material) of *Agrilus planipennis* host plants.

In particular, EFSA is requested to determine whether the alternative option included in the US request for wood of *Agrilus planipennis* host plants provides a comparable level of protection of the Union against the introduction of *Agrilus planipennis* as those currently stipulated in Section I, Part A, Annex IV of Council Directive 2000/29/EC.

## EFSA DISCLAIMER

In application of Article 39(1) of Regulation (EC) No 178/2002, the present opinion keeps confidential , part of the data provided in the technical file submitted by the United States Authorities in relation with their request to list a new option among the EU import requirements for wood of *Agrilus planipennis* host plants (namely raw data used by Myers et al. (2009) in their publication on wood heat treatment schedules for *Agrilus planipennis*).

Please refer to the European Commission letter dated 10 June 2011 which takes into account the indications provided by the US Authorities (ref. Ares(2011)626613 available at http://registerofquestions.efsa.europa.eu/roqFrontend/questionsListLoader?panel=ALL).

## ASSESSMENT

## 1. Introduction

This document presents the scientific opinion of the Panel on Plant Health on the technical file submitted by the US Authorities to support a request to list a new option among the EU import requirements for wood (except in the form of dunnage, spacers, pallets or packing material) of *Agrilus planipennis* host plants. The new option under consideration is a heat treatment at 60 °C for 60 min to eliminate possible infestations of the wood by the emerald ash borer (EAB). The experiments supporting the proposal of the US Authorities are presented in the following scientific peer reviewed publication:

Myers SW, Fraser I and Mastro VC, 2009. Evaluation of heat treatment schedules for Emerald Ash Borer (Coleoptera: Buprestidae). Journal of Economic Entomology. 102(6), 2048-2055.

The Panel in this scientific opinion undertakes the evaluation of the relevant experiments described and analysed by Myers et al. (2009).

### 1.1. Scope of the Opinion

EFSA is requested to determine whether the treatment under scrutiny provides a comparable level of protection of the EU against introduction of the EAB as those stipulated in Council Directive 2000/29/EC. The Panel restricts its assessment to the effectiveness of the new option proposed by the US Authorities. The Panel does not compare the level of protection of this treatment with that of the measures outlined in the Section 1 Part 1 Annex IV of Council Directive 2000/29/EC.

The technical file submitted by the US Authorities relates to heat treatment of firewood of ash. The opinion covers in its scope all hardwood from potential host species of *A. planipennis* including other commodities of these host species (chips, logs, …) except in the form of dunnage, spacers, pallets or packing material.

### 1.2. Current regulations in the EU

*Agrilus planipennis* Fairmaire, is a regulated harmful organism in the European Union, currently listed in Section I, Part A, Annex II of Council Directive 2000/29/EC while present on plants intended for planting, other than plants in tissue culture and seeds, wood and bark of *Fraxinus* L., *Juglans mandshurica*, *Ulmus davidiana*, *Ulmus parvifolia* and *Pterocarya rhoifolia*, originating from Canada, China, Japan, Mongolia, Republic of Korea, Russia, Taiwan and USA. It is currently not known to occur in the EU.

The import requirements for wood (except in the form of dunnage, spacers, pallets or packing material) of host species are listed in Section I, Part A, Annex IV of Council Directive 2000/29/EC and they include the requirement that:

- the wood, other than in some specific forms, should either originate in an area established by the national plant protection organisation in the country of export as being free from *A. planipennis* in accordance with the relevant International Standards for Phytosanitary Measures or have been squared so as to remove entirely the round surface;
- the wood in the form of chips should either originate in an area established by the national plant protection organisation in the country of export as being free from *A. planipennis* in accordance with the relevant International Standards for Phytosanitary Measures or should have been processed into pieces of not more than 2.5 cm thickness and width.

## 1.3. Evaluation methodology

### 1.3.1. Methodology

The Panel followed the EFSA guidance on evaluation of pest risk assessments and risk management options prepared to justify requests for phytosanitary measures under Council Directive 2000/29/EC (EFSA, 2009). The Panel evaluated the heat treatment described and analysed in the Myers et al. (2009) publication and examined the scientific basis of the proposed treatment. The Panel also scrutinised other studies relevant to heat treatment of hard wood to eliminate the EAB.

The Panel evaluated the experimental design described in the publication and focused its evaluation on experiments 1, 2 and 3 which were relevant to the proposed treatment of 60 °C during 60 min. The data and statistical analysis presented by the authors were assessed.

The Panel re-ran the analysis of Myers et al. (2009), following the same modelling approach using a Probit regression model. The Panel also performed some additional computations based on the Poisson regression model.

Based on the results of the different analyses, the Panel provides its overall conclusions.

### 1.3.2. The data requests

In the review process the US Authorities assisted the Panel providing the raw data used by the authors of the publication (hereafter called "the original measurements" - Appendix 1). During the critical review of the data, important inconsistencies were found. Thereafter the US Authorities provided a second set of individual data matching the aggregated data used by Myers et al. (2009) (hereafter called "the corrected dataset" – Appendix 1).
Therefore, to perform a thorough evaluation and re-analysis of the estimated survival rates and temperatures and to explore the dependence of the model outcome on the possible input datasets, the panel used both the original data files first received and their corrected version received subsequently. Data were extracted from the files provided to EFSA to guarantee a clear and well documented dataset. The variables in the datasets were, if possible, neither transformed nor re-calculated. The sources of all values used in the reanalysis are described in the Appendix 1.
To detect inconsistencies in the data files several comparisons and recalculations were performed: comparison of duplicated information in the data files, recalculation of all steps of aggregation from individual to aggregated data, recalculation of the reported values in the publication by Myers et al. (2009).

Therefore the data and additional information provided by US Authorities considered by the Panel for the evaluation consists of four datasets as detailed in Appendix 1:

- Aggregated data as reported in Myers et al. (2009) from the individual data provided in the corrected dataset
- Aggregated data from the original measurements
- Individual data from the corrected dataset
- Individual data from the original measurements

## 2. Biology of *A. planipennis*

### 2.1. Taxonomy

The taxonomy and nomenclature has been retrieved from the Integrated Taxonomic Information System (ITIS, 2011).

| | |
|---|---|
| Kingdom: | Animalia |
| Phylum: | Arthropoda |
| Class: | Insecta |
| Order: | Coleoptera Linnaeus, 1758 |
| Family: | Buprestidae Leach, 1815 |
| Genus: | Agrilus Curtis, 1825 |
| Species: | *Agrilus planipennis* Fairmaire, 1888 |

**Synonyms**
*Agrilus feretrius* Obenberger, 1936
*Agrilus marcopoli* Obenberger, 1930
*Agrilus ulmi* Kurosawa, 1956

**Common names:**
Emerald ash borer, EAB

### 2.2. Distribution and host range

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) is native to China, Korea, Japan, Mongolia, Russia (Far East) and Taiwan (Haak et al., 2002).

*Agrilus planipennis* is an East Asian species. Its current distribution includes northeastern China (Jilin, Liaoning, Heilongjiang, Inner Mongolia, Hebei, and Shandong), Japan (Hokkaido, Honshu, Kyushu, Shikoku), Korea, Mongolia, Russia (Moscow region and Russian Far East), Taiwan (Yu, 1992; Haack et al., 2002; Baranchikov et al., 2008), the United States of America and Canada.

In North America it is currently causing significant damage to ash trees (*Fraxinus* spp.) (Cappaert et al., 2005; Poland and McCullough, 2006). By October 2010, EAB had been found in 15 states of the USA (Hausman, 2010). Furthermore, it is projected that EAB has the ability to expand its range across 25 states in the next 10 years due to the extensive host tree range and a lack of effective control measures (Kovacs et al*.*, 2009).

Depending on its distribution, the pest infests different ranges of susceptible hosts. In its current area of distribution, its host range consists of species belonging to genus *Fraxinus*, with the exception of Japan, where natural hosts of other genera have also been reported. The following major points on EAB – host relationships as regards the distribution of the pest can be outlined:

- In China the larvae develop mainly in the trunks of dying or severely stressed ash trees of the species *Fraxinus chinensis* (Liu et al., 2003, Zhao et al., 2005); in Japan, EAB is also reported from *Juglans ailantifolia*, *Pterocarya rhoifolia* and *Ulmus davidiana* var. *japonica* (Haack et al., 2002). In China, the North American ash species planted as ornamentals (*Fraxinus americana*, *F. pennsylvanica* and *F. velutina*) are more susceptible to EAB attack than the Asian ash species and outbreaks have almost exclusively occurred on those introduced tree species (Liu et al., 2003; Zhao et al., 2007).
- In North America – the USA and Canada – EAB was first identified in 2002 (Cappaert et al., 2005; Timms et al., 2006). The larval development of the pest occurs exclusively in *Fraxinus* spp., although females occasionally lay eggs on other tree genera (Anulewicz et al., 2008). All major eastern North American ash species (*F. pennsylvanica*, *F. americana*, *F. nigra*, *F. quadrangulata*, and *F. profunda*) are susceptible to EAB (Cappaert et al., 2005; Smith, 2006; Anulewicz et al., 2007).
- In the European part of Russia, infestations of EAB were observed on the introduce*d F. pennsylvanica* and on *F. excelsior*, with the former being more widely distributed in Moscow and more severely attacked (Mozolevskaya and Izhevskiy, 2007; Volkovich, 2007).

Appendix 2 contains a comprehensive list of confirmed hosts in nature and experimental hosts under artificial conditions of EAB compiled by the Panel from the literature.
In the EU the pest is not known to occur. However, *Fraxinus* spp which is considered as the major host of EAB is widely distributed in Europe. The most common species of the genus *Fraxinus* are *F. excelsior* and *F. ornus*. The native distribution map of *F. excelsior* in Europe is presented in the map below (figure 1).

Apart from the native species, many other *Fraxinus* species are available in Europe in specialised nurseries and are planted in parks and gardens: *Fraxinus Americana, F. angustifolia, F. berlanderia, F. bungeana, F. caroliniana, F. chinensis, F. dipetala, F. floribunda, F. griffithii, F. holotricha, F. latifolia, F. mandshurica, F. mariesii, F. nigra, F. oregona, F. ornus, F. pallisiae, F. paxiana, F. pennsylvanica, F. platypoda, F. potamophila, F. profunda, F. quadrangulata, F. rotundifolia, F. sieboldiana, F. sogdiana, F. spaethiana, F. syriaca, F. tomentosa, F. velutina, F. xanthoxyloides* (Hillier, 2010).

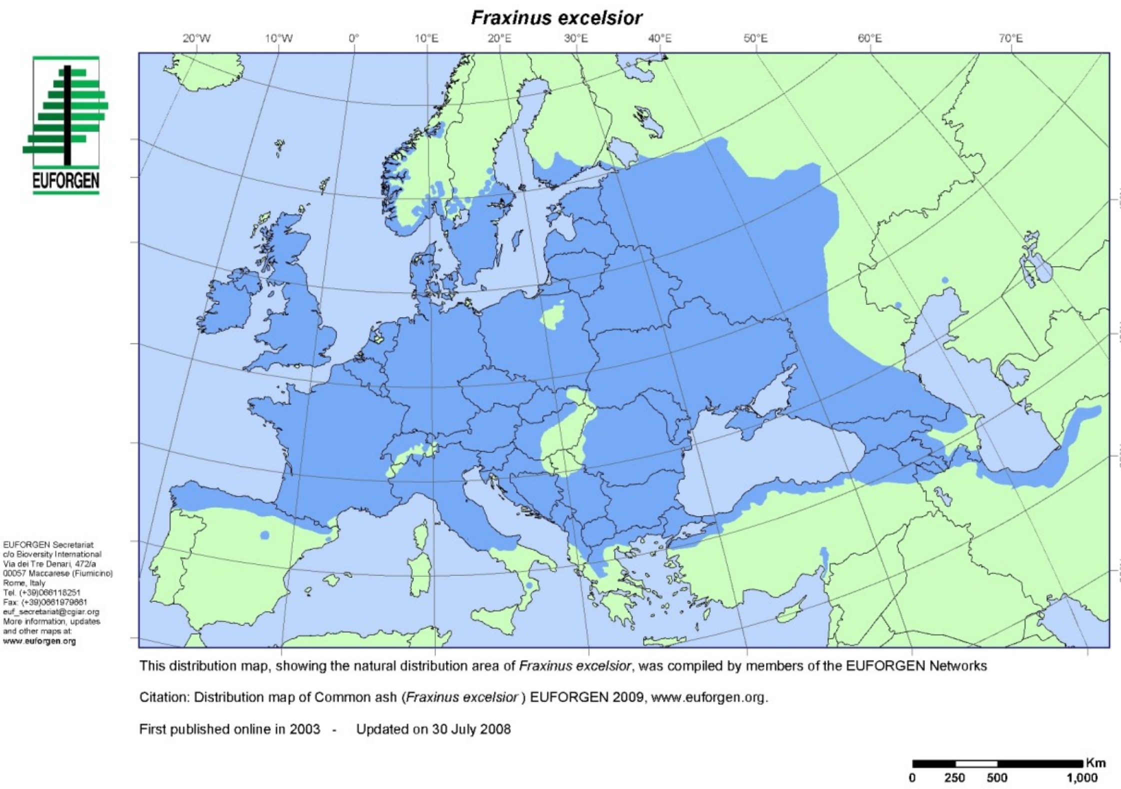


**Figure 1** Distribution map of Common ash (*Fraxinus excelsior*). EUFORGEN, 2009.

## 2.3. Life cycle

EAB has both a one and two year life cycle in China (Wei et al., 2007) and the United States (Cappaert et al., 2005). The duration of the life cycle may depend on various factors such as population density, climate, food quality, oviposition time, defensive response of the host, etc. It has been observed that low density populations on vigorous ash trees tend to support a two year life cycle while stressed trees with higher beetle population densities tend to support a one year life cycle (Cappaert et al., 2005).

Wei et al. (2007) established a relationship between the duration of the frost free period in three provinces of China and the duration of the life cycle. It takes at least 150 frost free days (with minimum temperatures above zero degrees Celsius) for EAB to complete one generation. In areas where the duration of the frost free period does not fulfil this requirement, the pest has a two year life cycle. Conversely, in areas with more than 150 frost free days per year, EAB develops through a one year life cycle.

### 2.3.1. Eggs

Adults lay eggs after a pre-oviposition period of approximately 10 days for mating and egg maturation (Wang et al., 2010). The start date and duration of the oviposition period depend on the local climatic conditions and mainly on temperature. In the province of Tianjin, China, situated next to the Yellow Sea coast, oviposition has been observed from early May to late June or early July (Wei et al., 2007; Wang et al., 2010). In Heilongjiang province, situated in the northern part of the country, oviposition has been observed from early June until early July (Wei et al., 2007). Oviposition usually takes place under bark flaps or in vertical slits on the trunk. Eggs are usually laid individually, but up to 7 eggs have been observed together in one location (Wang et al., 2010).

The eggs have an average length of 1.23 mm and an average width of 0.96 mm. Initially they are ivory white or jade green in colour and become fulvous to brown in 3 – 4 days. Eggs deposited in Tianjin during mid to late May hatched in 17 – 19 days at 18 – 23 °C, while eggs laid in late June hatched after 12 – 13 days at 24 – 26 °C (Wang et al., 2010).

#### 2.3.2. Larvae

According to Wei et al. (2007) the larval stage is the longest one of the life cycle of the insect with a duration of approximately 308 days for a one year cycle and 673 days for a two year cycle. It starts in late May and continues to mid-April the following year or early May the year after, respectively. There are four larval instars, differentiated by the length of the urogomphi, the width of the peristoma, the width of the prothoracic plate (Wang et al., 2010) and the width and height dimensions of the head capsule (Cappaert et al., 2005).

During the full-grown last (IV) instar larva reaches an average length of 13 – 22 mm and width of 3 – 4 mm. This is the overwintering stage of the pest in case of a one year life cycle. It usually builds a pupal cell (overwintering chamber) in the xylem. When a two year life cycle is observed, second and third instar larvae may also overwinter in their galleries between the xylem and the phloem (Timms et al., 2006; Wei et al., 2007). Overwintering prepupae have low supercooling points reaching -30 °C, which are achieved by accumulation of high concentrations of glycerol and synthesis of antifreeze agents, contained in the haemolymph. Also, cuticular waxes reduce inoculation from external ice (Crosthwaite et al., 2011).

#### 2.3.3. Pupae

The pupal stage is observed from early April to mid-June (Wei et al., 2007; Wang et al., 2010). The pupa is exarate and rhombic in shape. It is 11 – 16 mm long and 3 – 5 mm wide. The average duration of the pupal stage at 18 – 20 °C is 20.6 ± 0.7 days (Wang et al., 2010).

#### 2.3.4. Adults

Metamorphosis occurs from the end of April to early July (Wei et al., 2007; Wang et al., 2010). The adults remain in the pupal cells for an average of 8.67 days and emerge from the tree when conditions are favourable. Before emergence, they chew a D-shaped hole in the tree bark through which they exit. After emergence adults crawl upwards or fly to the canopy (Wang et al., 2010).

Adults are 7.5 – 13.5 mm long, with elongate bodies and metallic, emerald green elytra (McCullough and Katovitch, 2004). They feed on leaves in the tree canopy under strong sunlight and high temperature (>25 °C). Mating takes place one week after emergence.

#### 2.3.5. Feeding habits

The newly hatched larvae feed in the outer phloem and gradually bore into the cambial region of the host tree trunk as their development progresses. The larvae produce S- shaped galleries in the cambial region (Wang et al., 2010), interfering with the tree's ability to translocate water and nutrients (Poland and McCullough, 2006; Hausman, 2010). Mature larvae (IV instar) bore overwintering chambers in thick outer bark or from 4 to 16 mm in depth in the outer sapwood of young trees (McCullough et al., 2007; Wang et al., 2010). A low percentage of the larvae may overwinter in their galleries without boring overwintering cells. Once having entered the overwintering chamber, they stop feeding (Wang et al., 2010). In this stage, the mature larvae are also called prepupae.

A study on the patterns in the within-tree distribution of EAB performed by Timms et al. (2006) shows that larval galleries are most likely to be found in trees with bark thickness of 1.5 – 5 mm and stem diameter of 4 – 13 cm, predominantly on the southwestern side of the trunk. The authors suggest that bark thickness is the most important variable affecting the distribution of the galleries.

#### 2.3.6. Vulnerability of life stages to heat treatment

Late larval instars or prepupae which have already entered their overwintering chambers are most resilient to heat treatment. During this stage they are folded into oval cells within the sapwood or thick bark, where they may be protected from desiccation (McCullough et al., 2007).

### 2.4. Conclusion

All life stages of the pest, including adults can be present in wood of *Fraxinus* spp. originating from locations infested with EAB. These life stages can be found in the bark (eggs, larvae and in cases of thick bark – prepupae, pupae and adults), the outer phloem (young larvae), the cambial region (developing larvae) and the outer sapwood (prepupae, pupae, adults). In its native area on the Asian continent (Japan), *A. planipennis* is also reported to develop on hosts of the genera *Juglans*, *Pterocarya* and *Ulmus*. Development on these hosts in North America has not been observed.

Late larval instars are most resistant to heat treatment, as they are protected from dessication by the prepupal chamber and are most deeply located into the tree trunk, at a depth of 4 – 16 mm.

## 3. Review of heat treatments on EAB other than Myers et al. (2009)

Other experimental heat treatments to eliminate EAB from infested wood described in literature are presented in Appendix 3.

In heat treating of ash firewood, one practical concern is to estimate the time required to heat firewood of various forms and sizes to the lethal temperature. The heating time can vary widely depending on a number of factors such as wood piece size, wood density, initial moisture content, initial wood temperature, heating temperature, and heating medium (Wang et al., 2009).

McCullough et al. (2007) carried out an experiment on the effects of chipping, grinding, and heat treatment on survival of *A. planipennis* in the sapwood of *F. americana*. Infested wood and bark chips were treated at 25/40/60 °C for 8/24/48 h. Survival was higher in wood chips at 40 °C, and no insect survived at 60 °C. Finally, prepupae in wood chips were subjected to 40/45/50/55/60 °C for 20 min and 120 min. Some survival was recorded at all temperatures with the 20 min exposure. No survival was recorded at 60 °C/120 min. Their results showed that after exposure of infested chips to 55 ºC for two hours, 16.7 % of the EAB prepupae in the wood survived. The authors remark that heating rates might be important (although not tested explicitly here). Heating rates were between 0.04 and 0.1 °C/min in commercially manufactured kilns for treating wood pallets; heating rates in the experiments of McCullough et al. (2007) ranged from 0.20 to 0.37 °C/min.

Goebel et al. (2010) exposed EAB infested ash firewood to heat treatments at temperatures of 46 ºC and 56 ºC for 30 min and 60 min and investigated the emergence of beetles from the treated wood. Their results showed that the treatment at 56 ºC for 60 min did not result in full control of EAB, as several beetles subsequently emerged from the treated wood. Exposure to heat before reaching 56 °C was very long (~46 h). Heating rate was approximately 0.02 °C/min. The wood remained about 4200 min (70 h) in the kiln.

Nzokou et al. (2008) performed experimental kiln heat treatments on halved logs of infested ash at temperatures of 50, 55, 60 and 65 ºC for 30 min and investigated the subsequent emergence of adults from the treated wood. All treatments produced highly significant reductions in the mean adult insect emergence. However, although temperatures of 50 and 65 ºC resulted in complete control of adult insect emergence, a few adults emerged from some of the 55 ºC and 60 ºC treated logs preventing any claim of full control for all treatment temperatures used. The duration of exposure to heat was not clear from the data. However, it appears that logs took ~2 h to reach 65 °C. Heating rate was approximately 0.53 °C/min (the heating started from 2 °C).

Wang et al. (2009) did not test the insect survival after heat treatments. However, the authors evaluated different heat treatment options for various firewood operations and developed heat treatment schedules and heating time tables. They first conducted laboratory heating experiments using green and air dried ash firewood and obtained heating time data for different heating schemes. Mathematical models were developed to estimate heating times for heating conditions not tested in the experiment. Heating time tables were developed for a series of heating temperatures and initial wood temperatures. Their field heat treatment trial in a commercial dry kiln facility indicated a significant difference in heating times between the laboratory kiln runs and the field kiln run. The laboratory experiments were designed to heat treat green and seasoned ash firewood to meet the heat treatment standard for EAB at that time (a minimum firewood core temperature of 71.1 °C for a minimum of 75 min as prescribed in treatment T-314a of the USDA APHIS PPQ treatment manual before 2011). The research approach was to obtain experimental data that addressed the most important factors that influence heat sterilisation of firewood, i.e., heating medium, heating temperature, wood density, initial moisture content, and initial wood temperature.

Sobek et al. (2011) analysed the physiology of EAB larvae and pupae subjected to the temperature regime as indicated in the ISPM No 15 (FAO, 2009), 56 °C/30 min, at a facility treating pallet wood under protocols that followed the Canadian official guidelines for treatment with particular attention to mechanisms allowing increased resistance to heat (heat shock response and expression of heat shock proteins). The larvae were very tolerant to high temperatures without any heat pre-treatments (some individuals survived exposure up to 53 °C). High temperature survival was increased by either slow warming or pre-exposure to elevated temperatures. The authors suggest that the phenotypic plasticity of EAB may lead to high temperature tolerance very close to conditions described in an ISPM No 15 standard heat treatment.

**Conclusion**

The rate of survival of EAB prepupae after heat treatment, documented in the various studies examined above, suggests that individuals may survive after exposure to 55 ºC for 120 min, to 56 ºC for 60 min and to 60 ºC for 30 min. These results do not allow any conclusion regarding the effectiveness of the heat treatment under scrutiny of 60 °C for 60 min.

The studies reported different heating rates that might influence insect heat tolerance through the formation of heat shock proteins.

Field research on heat treatment (production scale) suggests that laboratory results under similar conditions do not always correspond to the results obtained in real conditions due to a variety of factors, among which is the type of equipment used and the method of heating.

# 4. The evaluation of Myers et al. (2009)

## 4.1. The experimental design

The publication Myers et al. (2009) presents the results of four experiments. Only the first three were directly related to heat treatment of wood. The fourth experiment concerned the survival of EAB larvae removed from wood logs and directly exposed to heat treatment; this experiment was not considered in this opinion.

The design of the first three experiments is summarised below in table 1. Heat treatments with temperatures ranging from 50 °C to 65 °C and a duration equal to 30 min were tested in experiment 1. Heat treatments with temperature equal to 50 °C or 55 °C and duration equal to 30 min or 60 min were considered in experiment 2. Heat treatments with temperatures ranging from 45 °C to 65 °C and duration equal to 30 min or 60 min were tested in experiment 3. The new option proposed by the US Authorities i.e. 60 °C/60 min was tested in experiment 3 only. All the three experiments included a control and 4 to 6 replicates for each temperature-duration treatment. The number of emerging adults was counted in the controls and after each heat treatment by inspecting the barrels containing the wood pieces. The initial numbers of insects in the treated pieces of wood were not counted. Wood temperatures were measured using probes located in the wood at 3.5 cm depth. Measured temperatures were higher than the target temperatures. For example, in experiment 3 and treatment 60 °C/60 min, the average and maximal temperatures were equal to 62.2 °C and 63.8 °C respectively.

**Table 1:** Experimental conditions in experiments 1, 2 and 3 summarised from Myers et al. (2009)

| | Experiment | | |
|---|---|---|---|
| | **No. 1** | **No. 2** | **No. 3** |
| **Material** | Firewood of green ash (*Fraxinus pennsylvanica* Marshall) | | |
| **Harvest** | | Harvested about 30 days before use | |
| **Date** | a: 19th – 22nd Dec. 2006<br>b: 8th – 12th Jan. 2007 | March 2007 | January 2008 |
| **Infestation** | Trees showing symptoms of EAB infestation in Livingston and Washtenaws counties, MI | | |
| **Preparation** | Ash bolts were cut to about 40 cm length and split to half, quarter or sixth section pieces, depending on the diameter, equally sized like commercial firewood | | |
| **Storage** | 10 days after cut and split, 9 days stored in walk-in cooler at 4 °C,<br>24 h in laboratory at 23 °C to standardise the minimal core temperature to 23 °C | | |
| **Treatment** | 2 drying ovens of 0.07 $m^3$ (Precision Econotherm). Wood was stacked on a single steel rack positioned about 6 cm from the floor. Initial temperature of the oven was 80 °C. | 0.14 $m^3$ environmental chamber (Blue-M) "*with vapour pressure humidity control system, which adds moisture to the air by increasing temperature of an internal water reservoir*"[4]. Wood was stacked on a single steel rack positioned about 10 cm from the floor. Initial temperature of the oven was 80 °C. | 0.09 $m^3$ environmental chamber (Espec). Wood was stacked on two racks positioned about 23 cm and 50 cm from the floor. Initial temperature of the oven was 80 °C. |
| **Control of heat** | Measured 3.5 cm below bark surface to the midpoint of the firewood, perpendicular to the grain direction in each piece of firewood by AWG copper-constantan T-Type thermocouples. Maximum temperature experienced by EAB larvae "*was equal or greater than the treatment target temperature*"[4]. | | |
| **Temperatures tested** | Control (untreated)<br>50/55/60/65 °C | Control (untreated)<br>50/55 °C | Control (untreated)<br>45/50/55/60 °C<br>and 65 °C (only 30 min) |
| **Duration tested** | 30 min | 30, 60 min | 30, 60 min |
| **Temperature and Duration** | Temperature was monitored at one minute intervals. Individual pieces were removed, when they reached the desired temperature-time combination. "*To minimize the amount of internal firewood temperature exceeded the target temperature; oven temperature was lowered to about 5 °C above the target temperature once it was reached in all pieces of wood in the oven.*"[4] | | |
| **Moisture** | Ambient humidity | Near 100 % RH (0 °C wet bulb depression) | Ambient humidity |
| **Replications** | 4 | 4 | 6 |
| **No of wood pieces** | 6 per replication | 8 per replication | 4 per replication |
| **Bark area** | Measurement of bark surface area by length and width | | |
| **Detection of EAB** | Daily (Mon-Fri) counting of emerged EAB until 5 consecutive days without new emerged EAB in the whole experiment. Inspection of the barrel for further EAB at the end of the experiment. | | |

[4] Citation from Myers et al. (2009)

### 4.2. Uncertainties on the characteristics of the wood material used by Myers et al. (2009) in the experiments

When listing the uncertainties the Panel considered both the publication Myers et al. (2009) and the data provided by the US Authorities.

- Because the experiments of Myers et al. (2009) used firewood from only one ash species (*F. pensylvaniva*), the results have to be extrapolated to:
  - all forms of imported wood (except in the form of dunnage, spacers, pallets or packing material
  - and wood from all host plants of *A. planipennis*, especially all hardwood.

  Uncertainties may result from different layouts/geometries or different characteristics (e.g. density, initial moisture content) of the wood.

- The US Authorities did not provide information on the possible levels of infestation of the wood exported to the EU. Uncertainties may occur if the experimental infestations are not representative.

- No assumptions on the spatial distribution of the infestation on the trees/logs were mentioned. Thus this influencing factor was not controlled in the experiments. Uncertainties may occur when different infestation levels were assigned to the different treatment groups.

- The description of the experiments does not include information about:
  - the thickness of the bark.
  - the completeness of the bark.
  - the correctness of the assumption of a rectangular form of the bark area.
  - the relation between bark quality and occurrence of EAB.

  This may result in biased estimates of the bark area.

- In each experiment, only one control group was used for all treatments. The infestation of each individual piece of firewood was not assessed in the experiments. Furthermore, no individual control for each experimental lot has been considered. This may result in biased estimates.

- Data analysis in the study of Myers et al. (2009) used only aggregated data. Therefore, the variation within each treatment group (e.g. the variation of infestation between the barrels of firewood in the control group) was not used in the data analysis. This may result in under-estimated standard deviations / too small confidence limits of the estimates.

- The influence of the initial moisture content of the firewood on the effectiveness of the treatment was not regarded in the experiment. As only fresh wood has been used by Myers et al. (2009), the results may not cover all intended applications of the treatment.

- The experiments were only conducted in laboratory ovens. The firewood was individually stacked on steel racks. The control of the temperature regime for each individual piece of firewood is not applicable outside laboratory conditions. The heating rate was significantly higher under experimental conditions (0.20 – 0.37 ºC/min) than in commercially manufactured kilns for treating wood pallets (0.04 – 0.1 ºC/min). The Panel did not find any information on experiments comparing the effect of different heating rates on the effectiveness of the treatment against EAB. Uncertainties may result in the application of these experimental findings to treatments in industrial settings. Faster heating rates may prevent EAB from adapting to adverse environments, e.g. by producing heat shock proteins (Sobek et al., 2011).

- The individual measurements of the temperature for each piece of firewood were not used in the data analysis with aggregated data of Myers et al. (2009). Only the average and maximal temperatures for each treatment are reported in the article. The average mean core temperature was always 3 to 5 °C above the intended temperature, single wood pieces might have reached maximum core temperatures about 5 to 10 °C above the intended temperature. Furthermore Myers et al. (2009) used only the intended temperature as input in their analysis. This may lead into an over-estimation of the effectiveness of the heat treatment.

- The detection level of the experiment is about 1 live larva out of 100 EAB in the wood of one barrel. In the publication, the reasons for stopping the EAB adult collection after 5 days without emergence were not described. This may lead to missing detections or unrecognised infestation levels below the limit of detection.

Taking into account the uncertainties listed above, the Panel considers the uncertainty on the conclusions of the publication Myers et al. (2009) high.

## 4.3. Presentation of the analysis performed by Myers et al. (2009)

### 4.3.1. Effect of heat treatment on the number of EAB adults

The effect of heat treatment on the number of ash borer adults was tested using a single factor mixed model analysis of variance (ANOVA) with random block effects (PROC MIXED)

In experiment 1, results showed that all the heat treatments significantly decreased the number of adults compared to the controls.

No insect was found in experiment 2 in any of the tested heat treatments.

In experiment 3, results show that the heat treatments based on a temperature of 45 °C and 50 °C did not significantly decrease the number of adults. Treatments 55 °C/60 min, 60 °C/30 min, 60 °C/60 min, and 65 °C/30 min significantly decreased the number of adults. No insects were found in the treatments 60 °C/30 min, 60 °C/60 min, 65 °C/30 min.

### 4.3.2. Effect of temperature on mortality rate

Another statistical analysis was carried out by Myers et al. (2009) in order to estimate the mortality rate caused by the heat treatment in function of the temperature and the duration. Mortality rates were first calculated for each heat treatment of experiments 1 and 3 from the counted numbers of insects in both treated wood pieces and controls. As the initial numbers of insects were not counted in the treated pieces of wood, the counted numbers of insects in the controls were used as a proxy for the initial number of insects before treatment. The mortality rates were then related to temperatures using two Probit models, one for the 30 min duration, and one for the 60 min duration. Model parameters were estimated from experiments 1 and 3 by maximum likelihood.

The fitted Probit models were used by the authors to estimate the temperature values leading to the control levels (i.e., mortality rate) of 99 %, 99.9 %, and 99.99683 %. The estimated temperatures were equal to 56.2 °C, 58.5 °C and 61.4 °C respectively, for a heat treatment duration of 60 min. Based on these estimations, the proposed treatment of 60 °C/60 min leads to a control level of 99.9 %, but not of 99.99683 %.

90 % confidence intervals were reported by the authors for the control levels 99 and 99.99683 %, but not for the control level 99.9 %. The Panel considers that confidence intervals should be reported for

all control levels. In addition, the 90 % and 95 % confidence intervals should be computed in order to avoid an under-estimation of the level of uncertainty in the estimations.

Considering the upper bound of the 90 % confidence intervals reported by the authors and the missing data of table 3 of the Myers et al. (2009) publication, the Panel concludes that the uncertainty of the effectiveness of the 60 °C/60 min heat treatment is high.

### 4.4. Additional statistical analysis performed by EFSA

The US Authorities provided EFSA with several datasets of Myers et al. (2009) to allow thorough evaluation and re-analysis of the estimated survival rates and lethal doses (temperatures). To explore the dependence of the model outcome on the possible input datasets, the Panel considered four datasets (numbering by the Panel):

- Dataset 0: Aggregated data as reported in Myers et al. (2009) from the individual data provided in the corrected dataset;
- Dataset 1: Aggregated data from the original measurements;
- Dataset 2: Individual data from the corrected dataset;
- Dataset 3: Individual data from the original measurements;

Poisson models were computed in addition to Probit models. A Poisson model could be fitted to insect counts directly, and does not require the calculation of mortality rates. The Panel thus considers that this type of model may be more suitable to analyse the data obtained in experiments 1 and 3.

Probit and Poisson regression models were used on both the original and the corrected datasets. Both the aggregated data and individual data were used in the Probit regression analyses. The Poisson regression analyses were performed using the individual data.

#### 4.4.1. Re-analysis of the aggregated data using a Probit regression model

The main objective of the re-analysis is to calculate the missing confidence intervals (CI) of table 3 of Myers et al. (2009), and to explore the dependence of the results on the choice of the specific model.

The analysis of the aggregated data follows the same strategy as Myers et al. (2009). The authors describe the method as follows:

*"A generalized linear model was used to perform Probit regression analysis on the pooled data from experiments 1 and 3, for 30 and 60 min treatments separately (data from exp 2 was excluded due to the difference in heating conditions and a lack of differential response across treatments). Percent mortality was estimated using the control emergence from each experiment and the number of adults per meter bark surface was standardized across all experiments. Regression parameters and lethal dose (temperature) estimates were calculated via maximum likelihood estimates using the PoloPlus software package."* (Myers et al. (2009), p. 2050).

The first data files received by EFSA provided additional information to understand the standardisation approach used in the data analysis. The results of each treatment (combination of duration and temperature) were standardised to the number of EAB that survived in firewood with 2 m² bark area by applying a corresponding adjustment factor. The same adjustment factors were used for both parts of experiment 1. The numbers of EABs in firewood with 2 m² bark area of the control groups were rounded to full numbers and used as a reference for the corresponding treatments.

This procedure was followed on the aggregated Dataset 0 to get results as close as possible to the results of Myers et al. (2009).

The Probit regression was defined on the whole dataset with individual parameterisation for each treatment time (30 or 60 min).

$$\frac{k_{ij}}{n_i} \approx \text{Binomial}(n_i, \pi_{ij}) \quad \text{with an additional “over-dispersion” of } \sigma$$

$$\text{Pr}obit(\pi_{ij}) = a_0 + b_0 \cdot T_j + a_1 \cdot 1(d_j = 30\text{min}) + b_1 \cdot 1(d_j = 30\text{min}) \cdot T_j$$

where
- i — is the index of the experiment: 1.1 / 1.2 or 3
- j — is the index of each treatment (combination of duration and temperature) in experiment i
- $T_j$ — is the temperature of treatment j: 45 °C / 50 °C / 55 °C / 60 °C or 65 °C
- $d_j$ — is the duration of treatment j: 30 min or 60 min
- $1(d_j$=30 min) — is the indicator (true=1, false=0) for a duration of 30 min
- $k_{ij}$ — is the number of EAB survived treatment j in experiment i, standardised to 2 m² bark area
- $n_i$ — is the number of EAB survived in the control group in experiment I. standardised to 2 m² bark area

Thus the model for a duration of 60 min is: $\text{Probit}(\pi_{ij}) = a_0 + b_0 \cdot T_j$

and for a duration of 30 min: $\text{Probit}(\pi_{ij}) = (a_0 + a_1) + (b_0 + b_1) \cdot T_j$

The parameter of possible over-dispersion σ was estimated using data obtained for both treatment durations. All computations were done using the GLM procedure of the SAS software package, version 9.1. This software allows non-integer responses ($k_{ij}$) and numbers of objects ($n_i$), as they appeared in the data analysis after the standardisation to firewood with 2 m² bark area.

The data analysis of Dataset 0 results in an estimated survival rate of 0.0004 % (95 % CI: 0.000 – 5.544 %) of the proposed treatment 60 °C/60 min. The lethal dose (temperature) for a control level of 90 % is estimated to 55.8 °C (90 % CI: 53.5 – 61.7 °C), which is very close to the results obtained and published by Myers et al. (2009). The remaining differences might be caused by using unrounded numbers of insects ($k_{ij}$ and $n_i$) and the estimation of an over-dispersion. No details on the rounding procedure and model fit are given in Myers et al. (2009).

The Panel used confidence intervals to a level of 95 % for their evaluation. For a 60 min duration and a control level of 99 % a necessary lethal temperature of 55.8 °C (95 % CI: 53.2 – 64.2 °C) was calculated, and for a control level of 99.9 % a lethal temperature of 57.8 °C (95 % CI: 54.5 – 68.8 °C) was calculated.

As the upper bounds of the confidence intervals were higher than the proposed temperature of 60 °C, it can be concluded that the analysis of the Dataset 0 does not show that the proposed treatment 60 °C/60 min guarantees a control level of 99 % or a control level of 99.9 %.

Several inconsistencies were identified when evaluating the original measurements. As the explanations provided subsequently were not sufficient to clarify the problems identified, the Panel used both data sources to perform the analyses: the original measurements included in the first data files and the later provided corrected data.

In Dataset 1 each experiment (1.1, 1.2 and 3) was standardised separately using the specific bark area for calculating the adjustment factors. The Panel considers this procedure more accurate because of the noticeable differences in the bark areas between the experiments.

Dataset 1 indicates one EAB that survived after the treatment of 60 °C/60 min. As a consequence, the data analysis performed by the Panel indicates higher survival rates for the proposed treatment, namely 0.162 % (95 % CI: 0.001 – 4.407 %) and higher lethal temperatures for a duration of 60 min at a control level of 99 %: 57.5 °C (95 % CI: 54.5 – 64.4 °C) or at a control level of 99.9 %: 60.6 °C (95 % CI: 56.8 – 69.8 °C).

As the upper bounds of the confidence intervals were higher than the proposed temperature of 60 °C, the Panel concludes that the analysis of the Dataset 1 does not prove that the proposed treatment of 60 °C/60 min guarantees a control level of 99 % or a control level of 99.9 %.

The results of the analyses performed by the Panel are presented in tables 2 and 3. Figure 2 shows the survival rate for a 60 min heat treatment for different temperatures and compares the results of the analysis obtained by the Panel using Datasets 0 and 1. The results are highly influenced by the survival of EAB at 60 °C/60 min as indicated in Dataset 1.

**Table 2:** Survival rates and confidence intervals for the proposed heat treatment of 60 °C for 60 min estimated from the four datasets

| *Dataset* | *Estimated survival rate, %* | *90 % confidence interval, %* | | *95 % confidence interval, %* | |
|---|---|---|---|---|---|
| **Dataset 0** | 0.004 | 0.000 | 2.409 | 0.000 | 5.544 |
| **Dataset 1** | 0.162 | 0.003 | 2.842 | 0.001 | 4.407 |
| **Dataset 2** | 0.896 | 0.069 | 6.228 | 0.039 | 8.431 |
| **Dataset 3** | 0.624 | 0.050 | 4.414 | 0.029 | 6.027 |

**Table 3:** Necessary lethal temperatures and confidence intervals for a heat treatment of 60 min to reach a given control level estimated from the four datasets

| *Dataset* | *Control level, %* | *Estimated temperature, °C* | *90 % confidence interval, °C* | | *95 % confidence interval, °C* | |
|---|---|---|---|---|---|---|
| **Results of Myers et al. (2009)** | 99.0 | 56.2 | 54.3 | 59.9 | – | – |
| | 99.9 | 58.5 | – | – | – | – |
| **Dataset 0** | 99.0 | 55.8 | 53.5 | 61.7 | 53.2 | 64.2 |
| | 99.9 | 57.8 | 54.9 | 65.4 | 54.5 | 68.8 |
| **Dataset 1** | 99.0 | 57.5 | 54.8 | 62.7 | 54.5 | 64.4 |
| | 99.9 | 60.6 | 57.2 | 67.5 | 56.8 | 69.8 |
| **Dataset 2** | 99.0 | 59.8 | 56.5 | 66.7 | 56.1 | 69.0 |
| | 99.9 | 63.8 | 59.5 | 73.1 | 59.0 | 76.4 |
| **Dataset 3** | 99.0 | 59.1 | 56.0 | 65.3 | 55.6 | 67.4 |
| | 99.9 | 63.3 | 59.1 | 71.9 | 58.6 | 74.8 |

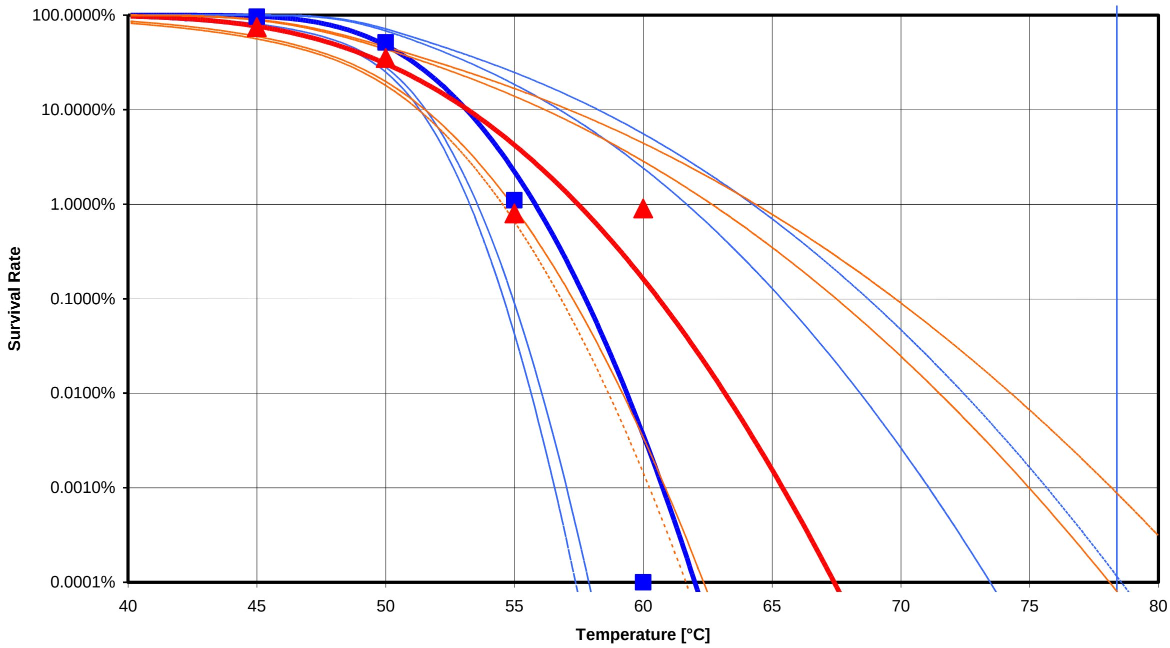


**Figure 2** Comparison of the estimated survival rates after a heat treatment of 60 min for different temperatures estimated from aggregated data of Dataset 0 (blue) and Dataset 1 (red).
(Bold curve = estimator, thin curve = 95 % or 90 % confidence bands)

### 4.4.2. Analysis based on individual data

#### 4.4.2.1. Analysis of survival rates using a Probit regression model

The datasets provided by the US Authorities include individual data of the treated barrels of firewood. High variation between the survival rates of EAB in the barrels of one treatment has been noticed for different time/temperature combinations. The analysis of the aggregated data does not take into account this variation and under-estimates the width of the confidence intervals. The Panel therefore performed a similar analysis for the individual data based on Dataset 2 and Dataset 3.

The average density of EABs in each control group was estimated by the ratio of the total number of detected EABs divided by the total bark area in the control group. The number of EABs in each barrel $n_{ij}$ is calculated as product of the bark area of the barrel and the density of EABs in the corresponding control group. j is here the index of the different barrels in experiment i. No standardisation is applied.

The estimates of survival rates for a heat treatment of 60 min at 60 °C and the lethal temperatures for different control levels are very similar between Dataset 2 and Dataset 3 (see tables 2, 3 and figure 3). The differences do not influence the results on individual data.

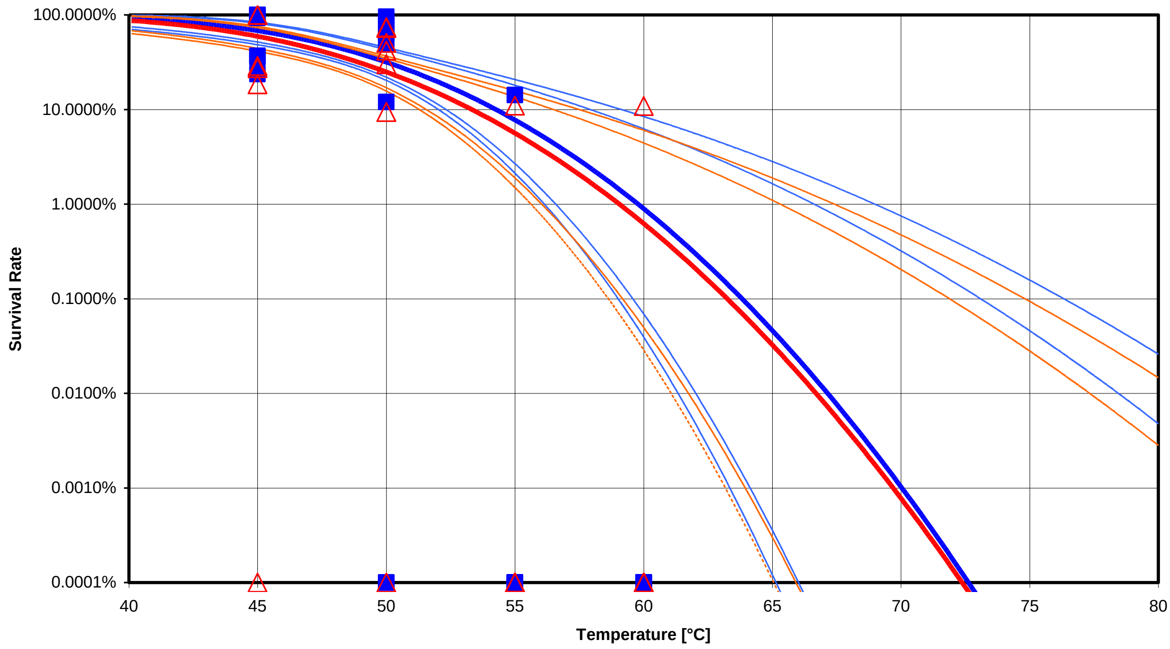


**Figure 3** Comparison of the estimated survival rates after a heat treatment of 60 min for different temperatures estimated from individual data of Dataset 2 (blue) and Dataset 3 (red). (Bold curve = estimator, thin curve = 95 % or 90 % confidence bands)

However, when the additional variation (uncertainty) obtained using the individual data is considered, the results change considerably (figure 4).

The survival rate of the proposed heat treatment of 60 °C for 60 min changed from 0.004 % (95 % CI: 0.000 – 5.544 %) to 0.896 % (95 % CI: 0.039 – 8.431 %) when using the aggregated data (Dataset 0) or individual data (Dataset 2). The necessary lethal temperature for a heat treatment of 60 min changed from 55.8 °C (95 % CI: 53.2 – 64.2 °C) to 59.8 °C (95 % CI: 56.1 – 69.0 °C) for a control level of 99 %; and from 57.8 °C (95 % CI: 54.5 – 68.8 °C) to 63.8 °C (95 % CI: 59.0 – 76.4 °C) for a control level of 99.9 %.

Considering these results from the individual data, the Panel concludes that it is likely to observe one live insect out of an infestation of 100 EAB after the proposed heat treatment of 60 °C for 60 min. To ensure a control level of 99 % the temperature of the 60 min heat treatment should be higher than 70 °C.

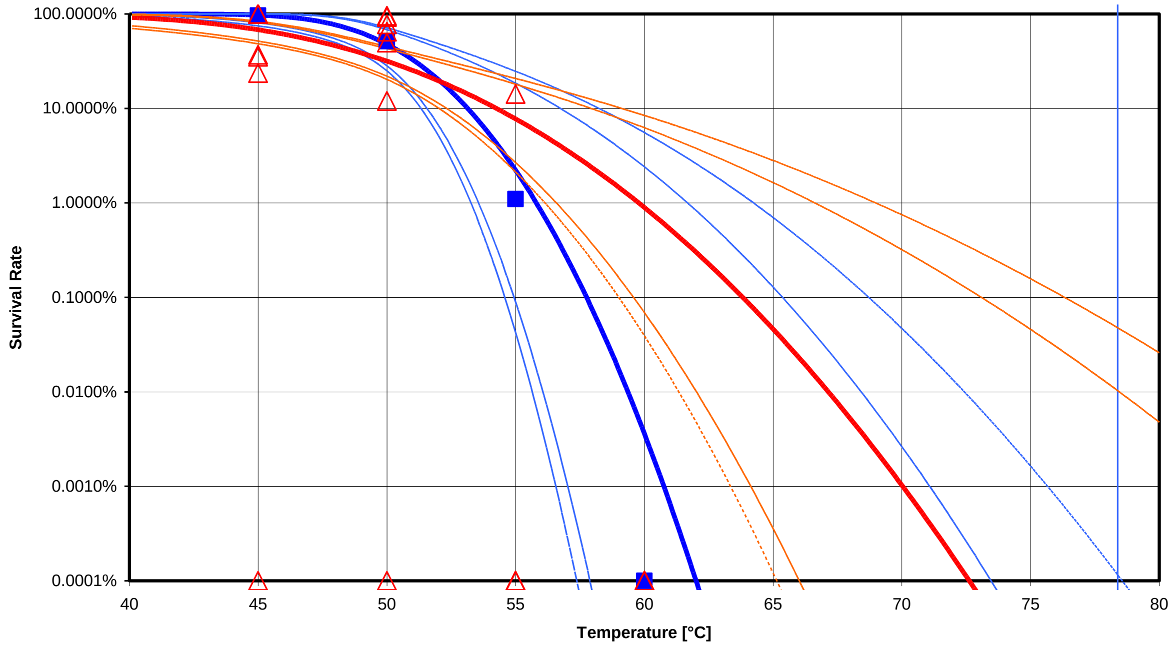


**Figure 4** Comparison of the estimated survival rates after a heat treatment of 60 min for different temperatures estimated from Dataset 0 (aggregated data) (blue) and Dataset 2 (individual data) (red). (Bold curve = estimator, thin curve = 95 % or 90 % confidence bands)

The Panel observed a large over-dispersion in the fits of the Probit regression models indicating problems in the underlying assumption of homogeneous infestations of all barrels of each experiment (1.1, 1.2 or 3). This assumption is crucial for calculating the survival rates.

4.4.2.2. Analysis of the number of survivals using a Poisson regression model

A Poisson model can be fitted to insect counts directly, and does not require the calculation of mortality rates. The Panel thus considers this model more suitable to estimate the probability of an EAB surviving a heat treatment of 60 min.

A Poisson log-linear regression model was fitted to Dataset 2 and Dataset 3. Only the individual data obtained in experiment 3 with a heat treatment duration equal to 60 min were considered.

Another advantage of this model is that it can take into account the difference of the bark areas considered in the different barrels. The number of survivals is expected to be higher in barrels with large bark area than in barrels with small bark areas.

The model is defined by

$$Y_{ij} \approx Poisson\ (\mu_{ij})$$

$$\log \mu$$

where $Y_{ij}$ is the number of insects found alive after the $i^{th}$ heat treatment at temperature $T_i$ in the $j^{th}$ barrels, $s_{ij}$ is the bark area of the wood pieces included in barrel $j$, is the expected value of the number of survivals, $i$=1, …, 4, $j$=1, …, $n_i$, and are two parameters relating the log of the expected number of survivals to the temperature. According to this model, the expected value of the number of survivals is expressed as $\mu$

and were estimated using Dataset 2 and Dataset 3 with the target temperatures (45, 50, 55, or 60 °C). The two resulting models were then used to compute the expected number of survivals in function of the temperature. Confidence intervals of the estimators (95 %) were estimated by taking into account possible over-dispersion of the data. Probabilities of 0, 1, 2, 3, 4 and 5 survivals per $m^2$ after the proposed 60 °C/60 min heat treatment were computed from the fitted models assuming a Poisson probability distribution. All computations were performed using the function GLM of the R statistical software v. 2.11.1 (cran.r-project.org).

Figure 5 shows both the data and the fitted models. The curves indicate the estimated expected numbers of insects that survive the heat treatment in function of the temperature and their corresponding 95 % confidence intervals. At 60 °C, the estimated expected number of survivals was equal to 1.11 survivals per $m^2$ of wood bark (sd=0.85) with Dataset 3, and was equal to 1.02 survivals per $m^2$ of wood bark (sd=0.8) with Dataset 2.

Figure 6 shows the variation of the number of insects that survive the heat treatment at 60 °C during 60 min. The estimated probability that one insect or more per $m^2$ survive the proposed heat treatment is equal to 0.67 and 0.64 with Dataset 3 and Dataset 2 respectively. According to the distributions reported in figure 6, there is a 0.1 probability that three insects or more per $m^2$ survive the proposed heat treatment.

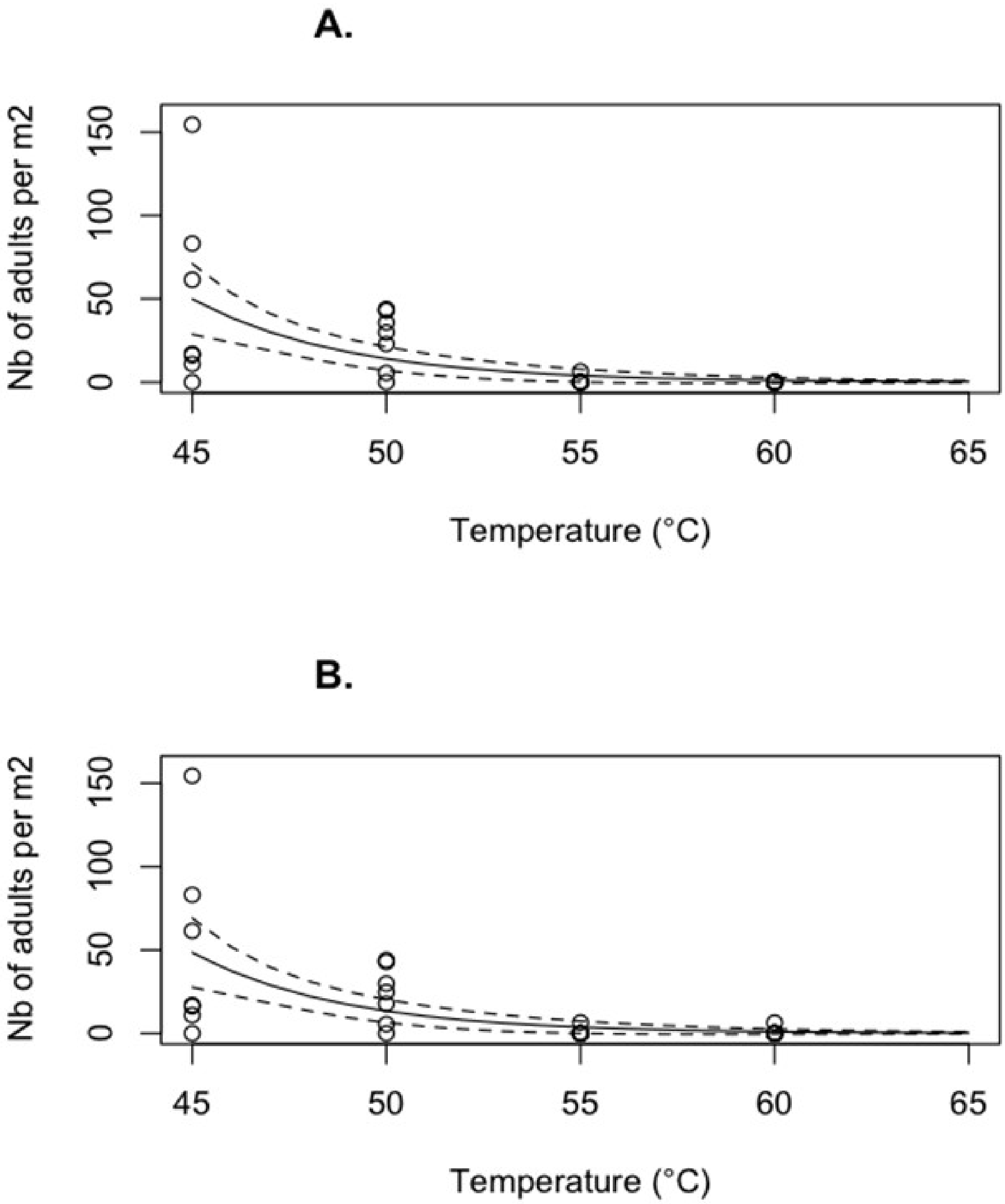


**Figure 5** Number of survivals in function of the temperature of the heat treatment obtained using Dataset 3 (A) and Dataset 2 (B). Points correspond to measurements obtained in barrels of experiment 3 (duration=60 min), the continuous curve indicates the estimated expected numbers of survivals, and the dashed lines indicate 95 % confidence intervals.

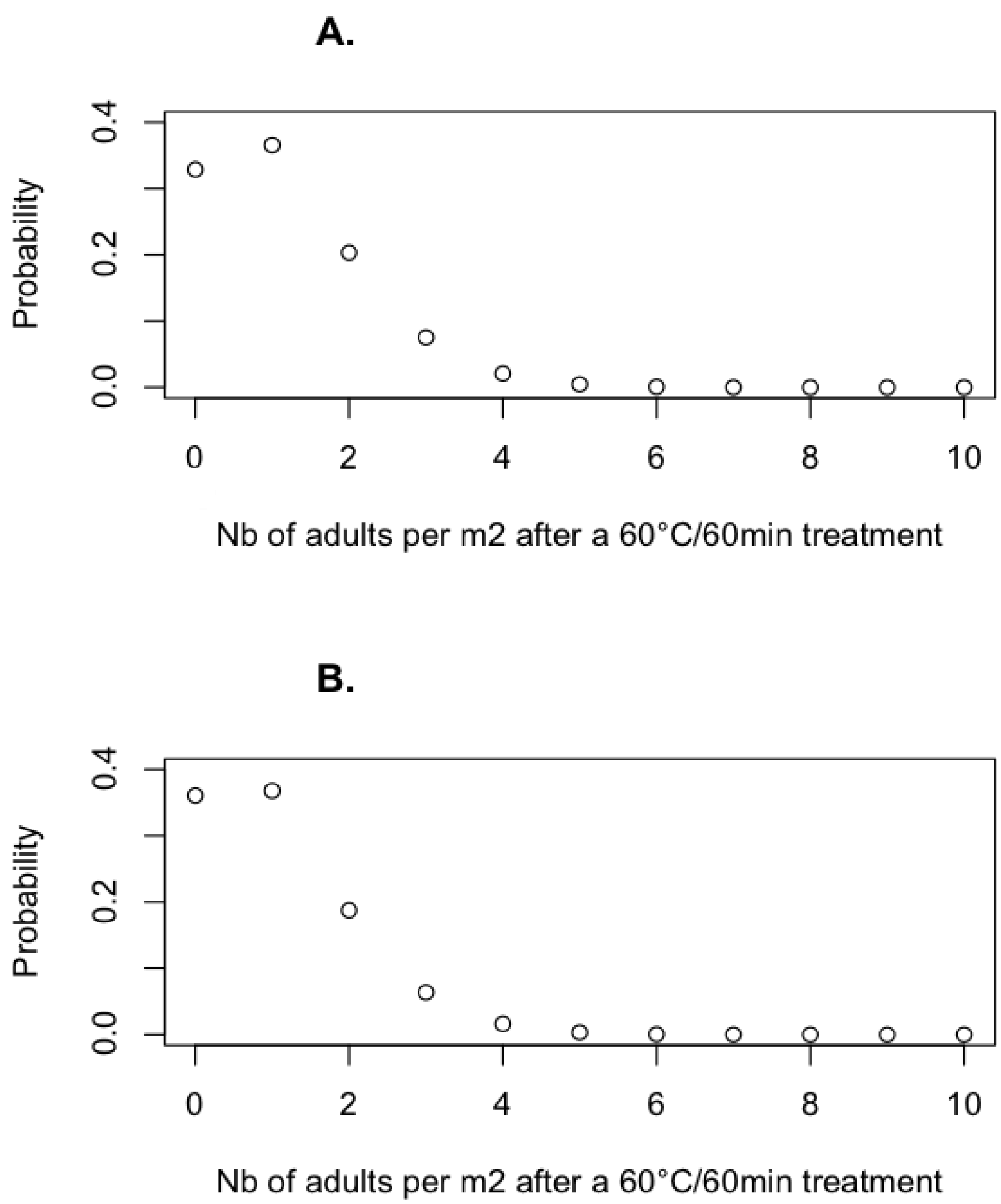


**Figure 6** Probabilities to obtain 0 to 5 survivals after the proposed heat treatment (60 °C during 60 min) assuming a Poisson distribution. Expected values of the Poisson distribution were computed using the fitted models shown in figure 5 using Dataset 3 (A) and Dataset 2 (B).

## 5. Uncertainties

- Table 3 of the publication of Myers et al. (2009) does not include confidence intervals for a control level of 99.9 %. The calculation of the number of objects (n) is not explained in the article. The scrutiny of additional data and information provided by the US Authorities revealed important inconsistencies in the experiments, therefore the Panel considers the results of Myers et al. (2009) analyses uncertain.

- The re-analysis using the original measurements, reveals errors in the standardisation of the survival rate and incorrect calculation of the number of objects (n) in the original analysis. This problem may have a small effect on the conclusion of the Probit analysis, but no effect on the Poisson analysis.

- Myers et al. (2009) do not provide information on the appropriateness of their statistical analysis, e.g. no information on the goodness of fit for the model. Results and conclusions of the additional analysis performed by the Panel using the original dataset are opposite to those presented by Myers et al. (2009).

- The results obtained by the Panel using different statistical models and datasets are very consistent.

The Panel considers the uncertainty about the conclusions of the publication by Myers et al. (2009) high. As the results obtained by using different statistical models and datasets are very consistent, the Panel therefore considers that the uncertainty about its conclusion is low.

## CONCLUSIONS

The Panel on Plant Health was requested to provide a scientific opinion on a technical file submitted by the US Authorities to support a request to list a new option among the EU import requirements for wood of *Agrilus planipennis* host plants.

The Panel restricts its assessment to the effectiveness of the new option proposed by the US Authorities. The Panel does not compare the level of protection of this treatment with that of the measures outlined in the Section 1 Part 1 Annex IV of Council Directive 2000/29/EC.

The technical file submitted by the US Authorities relates to heat treatment of firewood of ash. The opinion covers in its scope all hardwood from potential host species of *A. planipennis* including other commodities of these host species (chips, logs, …) except in the form of dunnage, spacers, pallets or packing material.

The supporting documents of the technical file consist of a peer reviewed publication by Myers et al. (2009) and data files provided by the US Authorities. Only the first three experiments of the publication Myers et al. (2009) were directly related to heat treatment of wood.

During the critical review of the different datasets provided by the US Authorities, the Panel found important inconsistencies. Therefore, in order to reduce uncertainties on the results of the analyses four datasets were considered to explore the dependence of the model outcome on the possible input datasets.

The analysis of the aggregated data used by Myers et al. (2009) based on a Probit regression model show that the proposed heat treatment of 60 °C/60 min cannot guarantee a control level of 99 % or higher. The analysis of the individual data using a Probit regression model show that it is likely to observe one surviving emerald ash borer out of an infestation of 100 after the proposed heat treatment 60 °C/60 min. To ensure a control level of 99 % the temperature of the heat treatment of 60 min should be higher than 70 °C. Results obtained with the Poisson log linear model show that the estimated probability that one insect or more per $m^2$ survive the proposed heat treatment was higher than 0.6 and that there is a 0.1 probability that three insects or more per $m^2$ survive the proposed heat treatment.

The rate of survival of EAB prepupae after heat treatment documented in additional published studies examined by the Panel suggests that individuals may survive after exposure to 55 ºC for 120 min, to

56 ºC for 60 min and to 60 ºC for 30 min. Therefore none of these treatments is considered effective in elimination of EAB from infested wood. These results do not allow any conclusion regarding the effectiveness of the heat treatment under scrutiny (60 °C/60 min).

Based on the results of the analyses presented in this opinion, the Panel concludes that *A. planipennis* is likely to survive the proposed heat treatment 60 °C/60 min with a low uncertainty, and that the alternative option proposed in the technical file submitted by the US Authorities does not guarantee the wood to be free of *A. planipennis.*

## Documentation provided to EFSA

1. Letter, 5 August 2010. Submitted by the European Commission, ref. SANCO E1/DB/svi (2010) 518955.

2. Myers SW, Fraser I and Mastro VC, 2009. Evaluation of heat treatment schedules for Emerald Ash Borer (Coleoptera: Buprestidae). Journal of Economic Entomology. Vol 102(6), 2048-2055.

3. E-mail, 5 January 2011, from European Commission (DG SANCO/E7) to EFSA (PLH unit), including original data in the form of 2 Excel files (CONFIDENTIAL).

4. E-mail, 4 March 2011, from European Commission (DG SANCO/E7) to EFSA (PLH unit), including answers to EFSA's request for clarifications on the original data and an Excel file with corrected data (CONFIDENTIAL).

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# APPENDICES

## APPENDIX 1: DATA AND INFORMATION PROVIDED BY THE US AUTHORITIES (CONFIDENTIAL)

### EFSA DISCLAIMER

In application of Article 39(1) of Regulation (EC) N° 178/2002, the present opinion keeps confidential part of the data provided in the technical file submitted by the United States Authorities in relation with their request to list a new option among the EU import requirements for wood of *Agrilus planipennis* host plants (namely raw data used by Myers et al. (2009) in their publication on wood heat treatment schedules for *Agrilus planipennis*).

Please refer to the European Commission letter dated 10 June 2011 which takes into account the indications provided by the US Authorities (ref. Ares(2011)626613 available at http://registerofquestions.efsa.europa.eu/roqFrontend/questionsListLoader?panel=ALL).

## APPENDIX 2: CONFIRMED HOSTS IN NATURE AND EXPERIMENTAL HOSTS OF *AGRILUS PLANIPENNIS*

### 1. CONFIRMED HOST PLANTS OF *A. PLANIPENNIS* IN NATURE

| Species | Common name | Reference | Presence in the EU |
|---|---|---|---|
| *Fraxinus americana* L. | White ash | Mastro and Reardon (2003)<br>Anulewicz et al. (2008) | Present as an ornamental (Hillier, 2010) |
| *Fraxinus chinensis* Roxb. | Chinese ash | EPPO (2005) | Present as an ornamental (Hillier, 2010) |
| *Fraxinus chinensis* Roxb. subsp. *chinensis* | Bai La Shu | Mastro and Reardon (2003)<br>Haack et al. (2002) | N/A |
| *Fraxinus chinensis* Roxb. subsp. (Hance) A. E. Murray | Hua Qu Liu | Mastro and Reardon (2003)<br>Haack et al. (2002) | N/A |
| *Fraxinus excelsior* L. | European ash<br>Common ash | Volkovich (2007) | Wide spread in EU, planted and natural forests<br>Austria, Belgium, Bosnia-Herzegovina, , Bulgaria, Croatia, Czech republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxemburg, Macedonia, Netherlands, Norway, Poland, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, ex-Yugoslavia., United kingdom (CABI, 2011) |
| *Fraxinus japonica* Blume ex K. Koch | Japanese ash | EPPO (2005) | N/A |
| *Fraxinus lanuginosa* Koidz. | Wollflaumige esche | EPPO (2005) | N/A |
| *Fraxinus mandshurica* Rupr. | Manchurian ash | Mastro and Reardon (2003)<br>Haack et al. (2002)<br>Anulewicz et al. (2008) | Present as an ornamental (Hillier, 2010) |
| *Fraxinus nigra* Marsh. | Black ash | Mastro and Reardon (2003)<br>Anulewicz et al 2008 | Present as an ornamental (Hillier, 2010) |
| *Fraxinus pennsylvanica* Marsh. | Green ash<br>Red ash<br>Downy ash | EPPO (2005)<br>CABI (2011)<br>Anulewicz et al. (2008) | Austria, Bulgaria, Czech republic, Germany, Romania (Flora Europaea, 2011)<br>United Kingdom: present as an ornemental (Hillier, 2010) |
| *Fraxinus profunda* Bush | Pumpkin ash | Lyons (2008) | N/A |
| *Fraxinus rhynchophylla* Hance | Oriental ash | EPPO (2005) | N/A |
| *Juglans ailantifolia* Carr. | Japanese walnut | CABI (2011) | N/A |
| *Juglans mandshurica* Maxim. | Manchurian walnut | EPPO (2005) | Planted in Italy (CABI, 2011) |
| *Juglans mandshurica* | Japanese walnut | Haack et al. (2002) | N/A |

| | | | |
|---|---|---|---|
| Maxim. var. *sachalinensis* Miyabe et Kudo | | | |
| *Juglans mandshurica* var. *sieboldiana* Maxim | Manchurian walnut | Haack et al. (2002) | N/A |
| *Juglans mandshurica var. japonica*. | Manchurian walnut | Haack et al. (2002) | N/A |
| *Juglans sieboldiana* Maxim. | Walnut, Siebold | EcoPort Foundation (2008) | N/A |
| *Pterocarya fraxinifolia* (Lam. ex Poir.) Spach | Caucasian wingnut | EcoPort Foundation (2008) | Widely planted in EU, though never on a large scale (Flora Europaea, 2011) |
| *Pterocarya rhoifolia* Siebold & Zucc. | Japanese wingnut | EPPO (2005)<br>Haack et al. (2002)<br>Mastro and Reardon (2003) | N/A |
| *Ulmus davidiana* Planch. | Japanese elm | EPPO (2005) | N/A |
| *Ulmus davidiana* Planch. var. *japonica* (Sarg. ex Rehder) Nakai. | Japanese elm | Haack et al. (2002) | N/A |
| *Ulmus japonica* (Sarg. ex Rehder) Sarg. | Japanese elm | CABI (2011) | N/A |
| *Ulmus propinqua* Koidz. | Japanese elm | EPPO (2005) | N/A |

Note: N/A indicates data not available

## 2. EXPERIMENTAL HOST PLANTS OF *A. PLANIPENNIS*

| Species | Common name | Reference | Presence in the EU |
|---|---|---|---|
| *Carya glabra* (P. Mill.) Sweet | Pignut hickory | Mastro and Reardon (2003) | Planted for timber in Germany (Flora Europaea, 2011) |
| *Carya ovata* (P. Mill.) Koch | Shagbark hickory | Mastro and Reardon (2003) | Planted for timber in central Europe, Czech republic, Germany and Romania. (Flora Europaea, 2011) |
| *Celtis occidentalis* L. | Hackberry | Mastro and Reardon (2003) | Planted in Croatia and Hungary (CABI, 2011) |
| *Forestiera* spp. Poir. | Swampprivet | Mastro and Reardon (2003) | N/A |
| *Forsythia* spp. (Thunb.) Vahl. | Forsythia | Mastro and Reardon (2003) | N/A |
| *Fraxinus quadrangulata* Michx. | Blue ash | Lyons (2008)<br>Mastro and Reardon (2003)<br>Anulewicz et al. (2008) | Present as an ornamental (Hillier, 2010) |
| *Fraxinus uhdei* (Wenz.) Lingelsh. | Shamel ash | Mastro and Reardon (2003) | N/A |
| *Fraxinus velutina* Torr. | Velvet ash | Mastro and Reardon (2003) | Present as an ornamental (Hillier, 2010) |
| *Juglans cinerea* L. | Butternut | Mastro and Reardon (2003) | Occasionally planted for timber in Denmark and Romania (Flora Europaea, 2011) |
| *Juglans nigra* L. | Black walnut | Mastro and Reardon (2003) | Extensively planted for timber in parts of Central and Eastern Europe: Austria, Belgium, Bulgaria, Croatia, Czech republic, France, Germany, |

| | | | |
|---|---|---|---|
| | | | Hungary, Italy, Netherlands, Romania, Slovakia, Spain, Switzerland, United Kingdom, ex-Yugoslavia (CABI, 2011) |
| *Ligustrum* spp. L. | Privet | Mastro and Reardon (2003) | Austria, Belgium, Britain, Bulgaria, Czech republic, Denmark, France, Germany, Greece, Ireland, Switzerland, Netherlands, Spain, Hungary, Italy, ex Yugoslavia, Portugal, Norway, Poland, Romania, Sweden (Flora Europaea, 2011) |
| *Syringa* spp. L. | Lilac | Mastro and Reardon (2003) | Ornamental tree in Europe |
| *Ulmus americana* L. | American elm | Mastro and Reardon (2003) | N/A |
| *Ulmus parvifolia* Jacq. | Chinese elm | Mastro and Reardon (2003) | N/A |
| *Ulmus pumila* L. | Siberian elm | Mastro and Reardon (2003) | Unconfirmed records of planted in Italy and present in Spain (CABI, 2011) |

Note: N/A indicates data not available

## APPENDIX 3: STUDIES OF HEAT TREATMENTS FOR EMERALD ASH BORER

The following studies are presented: Myers et al. (2009), Goebel et al. (2010), Nzokou et al. (2008) and McCullough et al. (2009)

NB – Only the heat treatments of wood pieces or chips are considered here. Wood chipping or grinding and *in vitro* insect survival are not considered)

| **Myers et al. (2009)**: Evaluation of heat treatment schedules for Emerald Ash Borer (Coleoptera: Buprestidae) | | | | | | |
|---|---|---|---|---|---|---|
| Expt | Date | Temperature tested ( °C) Measurement | Duration | Moisture | Sample size & nature Assessments | Results |
| 1 | December 2006 January 2007 | Control + 50/55/60/65<br><br>Thermocouples inserted each in a hole 3.5 cm deep (3.5 cm exceeds the depth at which EAB larvae are found) | 30 min | Ambient | 4 replicates of 6 pieces of wood per treatment<br><br>41.6*17.5*13.2 cm logs cut shortly before from attacked trees<br><br>Wood collected from heavily infested stands of ash. Assumed that majority of the EAB within were fourth instar and prepupal stage, with a smaller proportion of earlier stage larvae<br><br>**Measurements**:<br>Wood placed after treatment in photoeclectors and emerging beetles counted | **December**<br>60 °C/30 min : 1.4 beetles ± 1.4 / m²<br>(mean: 64.3 °C ±0.5; max: 68.1 °C ± 1.0)<br>65 °C : 0 ± 0 / m²<br>(mean: 67.9 °C ± 0.3; max: 70.7 °C ± 0.8)<br>Control: 43.1 ± 14/ m²<br><br>**January**<br>60 °C : 3.8 ± 1.2 / m²<br>(mean: 63.9 °C ± 0.6; max: 68.1 °C ± 1.0)<br>65 °C: 0 ± 0 / m²<br>(mean: 68.1 °C ± 0.3; max: 71.3 °C ± 0.6)<br>Control: 22.1 ± 3.6 / m² |
| 2 | March 2007 | Control + 50/55 | 30/60 min | 100 % relative humidity | 4 replicates of 8 pieces of wood per treatment<br>Logs: see. expt 1 | No survival at 50 or 55 °C (30 and 60 min)<br>Control: 42.0 beetles ± 11.8 /m² |
| 3 | January 2008 | Control + 45/50/55/60/65 | 30/60 min (only 30 min at 65 °C) | Ambient | 6 replicates of 4 pieces of wood<br><br>Logs: see. expt 1 | 60 °C/30 min : 0 beetles ± 0 / m²<br>(mean: 61.5 °C ± 0.1; max: 63.2 °C ± 0.2)<br>60 °C/ 60 min : 0 ± 0 / m²<br>(mean: 62.2 °C ± 0.2; max: 63.8 °C ± 0.4)<br>65 °C/30 min : 0 ± 0 / m²<br>(mean: 67.2 °C ± 0.4; max: 68.4 °C ± 0.4)<br>Control: 45.9 ± 12.5 / m² |

**Goebel et al. (2010):** Failure to Phytosanitize Ash Firewood Infested With Emerald Ash Borer in a Small Dry Kiln Using ISPM-15 Standards

| Expt | Date | Temperature tested ( °C) | Duration | Moisture | Sample size and nature<br>Assessments | Results |
|---|---|---|---|---|---|---|
| | Completed by mid-March 2008 | 46 °C<br>56 °C<br><br>Recorded within a 2.54 cm layer below the surface on 3 pieces of wood, a split, triangular piece, a large roundwood piece, and a small roundwood piece in the middle of each stack | 30 min<br>60 min | ambient | ~ 100 pieces/expt:<br>70 % split<br>30 % round (among which small (<10 cm diam) and large (>4 cm)<br><br>45-61 cm in length<br><br>Firewood collected from a stand with a heavy infestation<br>Collected in Dec 2007 & Jan 2008 most insects: older larvae or prepupae<br><br>**Measurements:**<br>D-shaped exit holes counted | 46 °C / 30 min : 283 exit holes/96 pieces (2.95/piece)<br>46 °C / 60 min : 119 exit holes/101 pieces (1.18)<br>56 °C / 30 min : 17 / 101 (0.17)<br>56 °C / 60 min : 42 / 100 (0.42)<br>Control 1 : 322 / 100 (3.22)<br>Control 2: 181 / 64 (2.83) |

| **Nzokou et al. (2008):** Kiln and microwave heat treatment of logs infested by the emerald ash borer (*Agrilus planipennis* Fairmaire) (Coleoptera: Buprestidae) | | | | | | |
|---|---|---|---|---|---|---|
| Expt | Date | Temperature tested (°C) | Duration | Moisture | Sample size & nature | Results |
| | unspecified | 50<br>55<br>60<br>65<br><br>Kiln: two thermocouples inserted into the center of the log, 1 cm into the phloem | Logs were removed 30 min after the temperatures of the core thermocouple reached levels of 50/55/60/65 °C. | Ambient | Logs ~0.9 m, further divided into two halves, one half reared indoors, the 2d reared indoors.<br><br>Length 419-464 mm<br>Diametre: 142-159 mm (oven)<br>9-13 mm (µwave)<br><br>Randomized complete blocks with 5 treatments replicated 4 times<br><br>Green logs were cut from infested ash trees in Feb-March (late larvae/prepupae)<br><br>**Measurements:**<br>Logs subsequentlyy reared indoors and outdoors, in photoeclectors | **Indoor (kiln)**<br>Control : 58.7 ± 17.1 beetle/m²<br>50 °C : 0.0 ± 0.0 beetle/m²<br>55 °C : 1.9 ± 1.8 beetle/m²<br>60 °C : 1.0 ± 1.0 beetle/m²<br>65 °C : 0.0 ± 10.0 beetle/m²<br><br>**Indoor (kiln)**<br>Control : 47.2 ± 25.0 beetle/m²<br>50 °C : 0.0 ± 0.0 beetle/m²<br>55 °C : 1.2 ± 1.2 beetle/m²<br>60 °C : 0.0 ± 0.0 beetle/m²<br>65 °C : 0.0 ± 0.0 beetle/m²<br><br>Myers et al. (2009): "*Similarly, Nzokou et al. (2008) observed emerald ash borer emerging from logs heated to 60 °C for 30 min, whereas a 65 °C treatment did not allow adults to emerge*" |

**McCullough et al (2007)**: Effects of chipping, grinding, and heat on survival of emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae), in Chips.

| Expt | Date | Temperature tested (°C) | Duration | Moisture | Sample size & nature | Results |
|---|---|---|---|---|---|---|
| 4 | April-June 2004 | 25 °C<br>40 °C<br>40 °C<br>40 °C<br>60 °C<br>60 °C<br>60 °C | 48 h<br>08 h<br>24 h<br>48 h<br>08 h<br>24 h<br>48 h | ambient | **Effects of heat treatments I**<br>- 56 bark- & 56 wood-sentinel chips chiseled from infested trees, each with a prepupa<br>- bark sent chips: 8.3 * 3.3 * 2.0 cm<br>- wood sent chips: 6.5 * 3.1 * 1.3 cm<br>- 4 bark- or wood-sentchips in 30 * 22 * 12 cm boxes filled with clean chips (28 such boxes)<br>- 2 boxes with bark- & 2 boxes with wood-sent chips assigned to each time regimes/temp combination: (48 h/constant 25 °C; 8 h/40 °C; 24 h/40 °C; 48 h/40 °C; 8 h/60 °C; 24 h/60 °C; 48 h/60 °C)<br>- boxes inspected regularly later for emerging beetles<br>- sent chips finally dissected | - 48 h, constant 25 °C : 75 % survival<br><br>Survival in bark chips sign lower than in wood chips<br><br>- 8-48 h , 40 °C: 37.5 - 50 % (bark); > 75 % (wood)<br>- 8-48 h , 60 °C: 0 % (bark and wood)<br>- 24 h, 60 °C<br>- 48 h , 60 °C |
| 5 | February 2005 | 25 °C<br><br>40 °C<br>45 °C<br>50 °C<br>55 °C<br>60 °C<br><br>40 °C<br>45 °C<br>50 °C<br>55 °C<br>60 °C | 4d<br><br>20 min<br><br><br><br><br><br>120 min | ambient | **Effects of heat treatments II**<br>- 160 sapwood sentchips chiseled from infested trees.<br>- 12 chips assigned to each of 11 heat/time regimes: constant at 25 °C for 4 days; exposure to 40/45/50/55/60 °C for 20 min; exposure to 40/45/50/55/60 °C for 120 min<br>- Chips examined after experiments | - 120 min, constant 25 °C: 75 % survival<br>- 20 min, all Temp: 30-64 % survival<br>- 120 min, 55 °C: 17 % survival – 0 % pupation<br>- 120 min, 60 °C: 0 % survival – 0 % pupation<br><br>*"At 55 °C, just slightly below the regulatory standard of 56 °C, 50% of prepupae survived 20 min of exposure, and 17% survived 2 h of exposure."* (McCullough et al., 2007)<br><br>" *McCullough et al. (2007) reported that emerald ash borer prepupae were able to survive in wood chips at 60 °C for 20 min, but not 120 min*" (Myers et al. (2009)) |