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# DISCUSSION OF AVAILABLE METHODS TO SURVEY AND CONTROL THE PINE WOOD NEMATODE AND ITS INSECT VECTORS, IN COMMERCIAL WOOD

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**ABSTRACT**
In a globalised society the worldwide trade is vital for every country economy and the pallets, crates and dunnage are ever present. These are often made of wood from dead trees that circulate all around the world, and have been associated with the dispersal and introduction of exotic pests and diseases. To rule the commodities circulation the International Standards for Phytosanitary Measures were established, where ISPM nº 15 is specific regulation for wood packaging material in international trade. The recent review of the Annex I with the approved treatments includes the fumigation with methyl bromide (MB) and sulphuryl fluoride (SF), and the heat treatment using conventional steam or dry kiln heat chamber (HT) and using dielectric heating (DH). A critical review is made on the efficacy of the approved methods to eliminate the *Bursaphelenchus xylophilus* and its insect vectors and on the probability of the wood becoming infected again after treatment.

Keywords: Pine wood nematode, *Monochamus*, ISPM, eradication.

**RÉSUMÉ**
**SURVIE DU NEMATODE DU PIN ET DE SES INSECTES VECTEURS DANS LES BOIS COMMERCIALISES - INVENTAIRE ET ANALYSE CRITIQUE DES METHODES DE TRAITEMENT UTILISABLES.**
Dans une société mondialisée, le commerce mondial est vital pour l’économie des pays, où les palettes, caisses et bois d'arrimage sont toujours présents. Ceux-ci sont souvent construits à partir du bois provenant d'arbres morts, qui circulent partout dans le monde, et ont été associés à la dispersion et à l'introduction des ravageurs et des maladies exotiques. Pour écarter la circulation des matériaux d'emballage en bois, ont été créés les Normes Internationales pour les Mesures Phytosanitaires, avec l’ISPM nº 15. Dans la récente révision de l'annexe I, où les traitements sont approuvés, il y a la fumigation au bromure de méthyle (MB) et fluorure de sulfuryle (SF), le traitement thermique utilisant la vapeur conventionnelle ou la chambre de chaleur de four sec (HT) et en utilisant un chauffage diélectrique (DH). Un examen critique est fait sur l'efficacité de ces méthodes pour éliminer le nematode du pin (*Bursaphelenchus xylophilus*) et leurs insectes vecteurs, et sur la possibilité du bois devenir infecté à nouveau, après le traitement.

Mots-clés : Nématode du Pin, *Monochamus*, ISPM, éradication.

## 1 - INTRODUCTION

In general, the control measures that may be used in the management of Pine Wilt Disease (PWD) caused by the Pinewood Nematode (PWN) *Bursaphelenchus xylophilus* Steiner et Buhrer Nickle, are scarce. The methods for the control can be applied directly to the agents (nematode and/or insect-vector) or indirectly to the host, to improve the development and vigor of the plants and, consequently, their resistance to pests and diseases. These methods can be grouped in five categories: 1) cultural – forestry management carried out to improve the vigor and vitality of the trees, starting on the forest planning and ending on the sanitary survey with the removal and destruction of symptomatic plant material with destruction of the agents here harbored; 2) genetic – increasing plant resistance to pests and diseases or to abiotic adverse factors through the genetic improvement of plants or a careful selection of the plant origin. 3) biological – introduction of living organisms with the ability to control pathogens, which can be parasitoids (insects), diseases (fungi, nematodes, virus, bacteria) or predators (insects, birds, mammals); 4) biotechnical –methods, usually very effective, with low costs and without environmental impacts, which affect and manipulate the behaviour of the agent or its interactions with the host; 4) chemical – direct application on the host, according to the chemical group and method of acting (ingestion, contact, fumigation). The efforts to limit the PWD affected areas worldwide are primarily focused on the insect vector population (*Monochamus* spp.) when inside the host (autumn-early spring), with the removal and destruction of the infected woody material, including the small sized branches of the canopy, and later during the flight period of the insect, through capture techniques (trapping with chemical lures) or through the increase of mortality agents (natural enemies).

Wood from dead or dying trees is often used in the making of lower quality wooden products, such as wood packaging (pallets, crates, dunnage), which are vital in today´s globalised society. It's estimated that over 1,5 billion pallets are produced every year, using approximately 60 million cubic meters of timber. Pallets are the most abundant wood packaging, and in the United States they represent nearly 80 percent of all packaging and 90 percent of which are made of solid wood (TIMCON, 2014).

The worldwide trade has been associated with the dispersal and introduction of various exotic insects and pathogens (e.g., Allen & Humble, 2002; Zahid *et al.*, 2008; Leal *et al.*, 2010; Haack *et al.*, 2014), such as bark and wood-boring insects (Haack, 2006; Haack & Petrice, 2009; Wermelinger *et al.*, 2013), but also fungi and nematodes, all detected in wood packaging interceptions (Braasch *et al.*, 2001; Tomiczeck *et al.*, 2003).

All wood from PWN affected countries (either of symptomatic and healthy pine trees) intended for circulation, transfer or export, needs to follow the International Standards for Phytosanitary Measures No. 15 (ISPM 15), developed by the International Plant Protection Convention (IPPC) that directly addresses the need to treat wood materials of thickness over 6 mm, used to ship products between countries. Its main purpose is to prevent the international transport and spread of diseases and insects that could negatively affect plants or ecosystems.

The application of ISPM 15 procedures reduced the pest infestation rates of WPM entering the United States to 0,1% occurrences on the phytosanitary surveys (Haack *et al.*, 2014).

To complement these measures, is also currently under member's consultation the new ISPM 28 that addresses sulphuryl fluoride fumigation to control both nematodes and insects in debarked wood.

## 2 - REGULATION OF WOOD PACKAGING MATERIAL IN INTERNATIONAL TRADE (ISPM 15)

ISPM 15 affects all wood packaging material (pallets, crates, dunnages, etc.) and previously included the Methyl Bromide (MB). Fumigation during 24 h with 40 g/m3 of methyl bromide is very effective since it will reach 100% PWN mortality (Soma *et al.*, 2001). However, this product is harmful to human health and degrades the ozone layer and since 2008 is currently being phased out of use globally and is no longer registered for use in the USA and EU, with recommendation to evaluate alternative treatments, which must be at least 99,99683% effective (PROBIT 9), and supported by strict research requirements (Magnusson & Schröder, 2009; Schröder *et al.*, 2009).

**2.1 - Heat Treatment (HT and DH)**

Until 2013 the only alternative treatment was the Heat Treatment, either with Thermal Shock (HT) or using Dielectric Heating (DH). The thermal shock treatment requires that wood materials should be heated in a schedule that attains a minimum core temperature of 56 °C for at least 30 minutes. The treated wood is stamped or branded, with a mark of compliance, colloquially known as the "wheat stamp". In Portugal there are about 280 companies providing HT, authorised and supervised by the national phytosanitary entities.

The dielectric heating, using microwaves or radio waves needs to achieve a minimum temperature of 60ºC for a continuous minute, throughout the entire profile of the wood (including the surface). For wood exceeding 5 cm thickness, dielectric heating at 2,45 GHz requires bidirectional application or multiple waveguides for delivery of microwave energy to ensure uniformity of heating.

In both heat treatments procedures temperature sensors and data recording equipment are calibrated in accordance with the manufacturer’s instructions specified by the National Plant Protection Organizations (NPPO).

However, in some situations heat treatment apparently allows the survival of a small percentage of quarantine wood borers larvae, such as *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) (Haack *et al.*, 2014).

The heat treatment by thermal shock for the eradication of pests intends to reduce the time needed for kiln-drying the sawn timber that was much higher with commercial standards. With a final temperature of 82 ºC, common in more conventional ovens, all organisms in the wood are eliminated (Dwinell, 1994; USDA, 1995).

**1.2 - Sulphuryl Fluoride fumigation (SF)**

Fumigation of woody material was used worldwide with methyl bromide, reaching 100% mortality in the different insect development stages (Kobayashi, 1988), and a mixture of sulfuryl fluoride (doses of 30 and 50 g/m3) and methyl bromide (doses of 10 and 20 g/m3), during 24 h. But methyl bromide was banned and replaced in Chine by aluminum phosphite and achieves 98% mortality (Xu, 2008) while, in Korea, since 2002, fumigation has been with 1 L/$m^3$ of metham sodium in wood piles constructed of 1-2 $m^3$ of infected trees cut into sections and immediately covered with 0,1 mm thick vinyl, achieving 100% of mortality of insects after 7 days (Shin, 2008).

In May 2015’s revision of Annex 1 (Approved treatments associated with wood packaging material) and Annex 2 to ISPM 15 was included the phytosanitary treatment using fumigant sulphuryl fluoride (SF). The revised Annexes are currently under members’ consultation, until 30 September 2015.

Sulfuryl fluoride (SF) is a broad-spectrum fumigant used to control rodents and insects. Was first registered in 1959, and is known to have better timber penetration qualities than methyl bromide (Scheffrahn *et al.*, 1987). Currently, SF (commercial brands Vikane, ProFume and Zythor) is registered in various countries for the control of stored-product insects and wood-destroying pests. In the EU, SF has been granted Annex 1 listing under EU Directive 98/ 8/ EC (Biocides), for Product Type 8 (Wood Preservative) and Product Type 18 (Insecticides). More recently, Annex 1 listing under EU Directive 91/ 414 (Pesticides) was granted with effect from 1 November 2010, and the pest types include insects and nematodes.

The fumigation of wood packaging material with SF must be in accordance with a schedule that achieves the minimum concentration- time product (CT) in the ambient atmosphere over 24 or 48 hours at the temperature and final residual concentration specified in Table I.

**Tableau I:** Concentration de fluorure de sulfuryle pour la fumigation de l'emballage en bois.

**Table I:** Minimum concentration- time product (CT) over 24 or 48 hours for wood packaging material fumigated with sulphuryl fluoride.

| Temperature (°C) | Minimum CT (g·h/m3) | Minimum final concentration (g/m3) |
|---|---|---|
| 20 and above for 48 h | 3.000 | 29 |
| 30 and above for 24 h | 1.400 | 41 |

One example of a schedule that may be used for achieving the specified requirements is shown in Table II.

**Tableau II:** Exemple de programme de traitement avec fluorure de sulfuryle pour la fumigation de l'emballage en bois.

**Table II:** Example of a treatment schedule that achieves the minimum required CT for wood packaging material treated with sulphuryl fluoride.

| Temperature (°C) | Minimum target CT dosage (g·h/m3) | Dosage (g/m3) | Minimum concentration (g/m3) at: 0,5 h | 2 h | 4 h | 12 h | 24 h | 36 h | 48 h |
|---|---|---|---|---|---|---|---|---|---|
| 20 and above | 3 000 | 120 | 124 | 112 | 104 | 82 | 58 | 41 | 28 |
| 30 and above | 1 400 | 82 | 87 | 78 | 73 | 58 | 41 | *NA | *NA |

*NA, not applicable.

Small increases in the treatment time (e.g. –1-2 hours) may be permitted to achieve the required CT if the minimum final concentration is not reached. The minimum temperature of the wood must be not less than 20°C and the minimum exposure time must be not less than the time stated for each temperature.

NPPOs must ensure that a long list of 13 factors is appropriately addressed by the entities involved in the application of sulphuryl fluoride treatment under this standard.

## 3 - SURVIVAL OF THE PINEWOOD NEMATODE AND ITS INSECT VECTOR

### 3.1 – Nematode survival in *Pinus pinaster* wood

In the initial period on infection, *B. xylophilus* reproduces abundantly and a population peak is detected at 8–12 weeks. Subsequently, the populations declined and became dominated by third-stage resistant larvae (JIII), and in the final sample nematode abundance was very low. Nematode decline was correlated with a decrease in the moisture content (MC) to below wood fibre saturation (Sousa *et al.*, 2011a).

When a tree is infested by *B. xylophilus*, the nematode increases in numbers during the first weeks to occupy the all host, therefore eggs and juveniles dominate in the populations. Wood kept in laboratory at room conditions, the nematode populations gradually declined in the subsequent weeks (from 16 to 40), and were dominated by JIII. Nematode populations reached very low numbers in the final sample, and disappeared completely in 48% of the replicates (Sousa *et al.*, 2011a).

This study has demonstrated that when trees infested by *B. xylophilus* are felled, the nematodes can survive and continue their development both in branches and sawn wood for prolonged periods of time, which in the current experiments was over 40 weeks from being brought to the laboratory. Halik & Bergdahl (1990) found high populations of the nematode in wood chips after 12 weeks and suggested that under favourable conditions nematode abundance could remain high for well beyond that period, while Tomminen *et al.* (1991) detected it in wood chips after 130 days.

This trend is directly correlated with the decrease in the wood moisture content (MC) observed during the first 12–16 weeks, when it reached values below the fibre saturation point of the wood at approximately 28% MC (Table 2). And the final breakdown of nematode populations occurred when the MC was below 15%.

As the ambient conditions were favourable for development, the decrease in the nematode populations probably reflected a decline in food availability in wood over time, as fungi are an essential food source for the mycophagous life stages of *B. xylophilus* once the living cells of the host tree are dead (Mamiya, 1983; 1984; Kishi, 1995; Maehara *et al.*, 2005). This was further enhanced by the decrease of the MC in all wood materials, because wood with low moisture has lower capacity to harbour mycophagous populations of *B. xylophilus* as the blue-stain fungi require MC above fibre saturation to develop (Viitanen, 1997; Schmidt, 2006).

### 3.2 – Insect vector survival in processed wood

The preventive clear cuts in the areas of asymptomatic and healthy pines adjacent to infestation sites followed by physical elimination of logs and branches by burning or chipping wood, seem to decrease significantly the occurrence of trees with PWD in Korea (Kwon *et al.*, 2011).

Burning is the control measure that has been most widely used because it has a lethal effect on the PWN and on the vector (Yamane, 1981). Special protection measures must be taken in order to prevent forest fires or the raising of the soil temperature to levels that can stimulate the germination of root pathogenic fungi such as *Rhizina undulata* (Kamata, 2008).

The production of wood chips is being increasingly recommended in Japan and China as it allows an economic use of the wood (for production of paper pulp or wood pellets) (Xu, 2008), while in Korea fumigation has been used since 2002 (Shin, 2008).

Many times the wood from dead trees is used in lower quality wooden products, such as pallets or boxes. The survival of the insect vector (*Monochamus galloprovincialis* Fab.) was assessed in sawn boards (1200 x 100 x 25 mm) and blocks (160 x 95 x 95 mm), the size wood pieces widely used. The majority of the larvae were killed when sawing the wood, although some adults successfully emerged from the boards (10% survival) and blocks (37%). These results represent a contribution to the quantification of the risks of dispersing pine wilt disease through wood packaging materials, confirming that untreated wood can support healthy and abundant *B. xylophilus* populations for sufficient time for vectors surviving the sawing process to complete their development, to emerge and disperse the nematode (Sousa *et al.*, 2011a).

Our results suggest that if pine wood infested with both the *B. xylophilus* and its insect vector is moved in trade, whether as a wood product or as pallet ⁄ packaging material, there will be a period over 40 weeks (over 10 months) during which it poses a risk of disseminating *B. xylophilus*. This period is sufficient for *M. galloprovincialis* surviving the initial sawing of the wood to emerge and transfer *B. xylophilus* to susceptible hosts in new locations (Sousa *et al.*, 2011a).

In Portugal, a method has been developed that prevents the spread of the insect vector to new locations during the transport of wood by using two types of an insecticide net: 1) Complion™ from

BASF, a polyester fabric impregnated with alpha-cypermethrin, mainly used to protect green wood from attack by scolytids; and 2) Bayer-net, which is impregnated with deltamethrin. The assays carried out revealed that the nets were very effective for newly emerged adults of *M. galloprovincialis*. After exposure to the net, even over short periods (1 min), the insects that were still alive were unable to fly, feed or move normally, and all were dead after 6 h of exposure (Naves *et al*., 2013).

This dispersion pathway can also be prevented by application of ISPM 15 phytosanitary measures, which are effective in killing the *Monochamus* larvae and the pine wood nematode in the wood (Dwinell, 1987; Wang, 2010).

### 3.3 – Nematode survival in ISPM 15 treated wood

In spite of the proved effectiveness of ISPM 15 methods in eradicating over 99,9963% of the nematodes presented in the treated wood, the few survivors can resists many months or even multiply and recolonize the wood within few weeks, as long as wood moisture content is above 20% (Sousa *et al.*, 2011a, 2011b).

Initial survival of the nematode in SF fumigation during 24 h at 20°C was due to nematode survival in egg stage (Bonifácio *et al.*, 2014). It is known that insect eggs are less susceptible to SF fumigation than other life stages because the shell limits the passage of the gas; therefore, to control of eggs required an increased exposure time to 48h. In fact assays in 2010 with SF fumigation for 24 h at 20°C eradicated completely NMP from 77,5% of the 80 boards, but in boards where some egg nematodes survived and hatched allowing the populations rebuild and rapidly increased in subsequent wood samples until 86 times in average the initial survivors within 20 days (Bonifácio *et al.*, 2014)(Table III).

**Tableau III**: Succès de l'éradication du *Bursaphelenchus xylophilus* après l’application du fluorure de sulfuryle sur la bois de *Pinus pinaster* infecté et taux de multiplication des nématodes survivances. (0) nématode absent, (+) nématode présent (basé sur Bonifácio *et al.*, 2014)..

**Table III:** Eradication success of the sulphuryl fluoride application on infected *Pinus pinaster* wood and multiplication rates of *Bursaphelenchus xylophilus* survivals (based on Bonifácio *et al.*, 2014).

| Number of boards | Wood sampling 1 d | 3 d | 21 d |
|---|---|---|---|
| 62 | 0 | 0 | 0 |
| 12 | 0 | + | + (38x) |
| 6 | + | + (4x) | + (86x) |

Later assays in 2012 with SF fumigation at 20°C with similar methodology, eradicated completely NMP from 14,5% of 55 boards, but in the rest some nematode eggs survived and hatched. However when WMC decrease to around 20% by the 21$^{st}$ day , those nematodes died (10 boards), while in other boards that were had more moisture nematodes prevailed and reproduced, even when there were undetected in the 3$^{rd}$ day wood sampling (14 boards), like in 2010 (Table IV).

**Tableau IV:** Succès de l'éradication du nématode du pin (*Bursaphelenchus xylophilus*) par le fluorure de sulfuryle sur bois de pin maritime infecté, et teneur en humidité (WMC en pourcentage). (0) nématode absent, (+) nématode présent (basé sur Bonifácio *et al.*, 2014).

**Table IV:** Eradication success of the Sulphuryl Fluoride application on infected *Pinus pinaster* wood by *Bursaphelenchus xylophilus* (PWN), and average wood moisture content (WMC in percentage) of the treated boards. (0) absence of PWN; (+) presence of PWN (based on Bonifácio *et al.*, 2014).

| **Number of boards** | **Wood sampling** | | | | | |
|---|---|---|---|---|---|---|
| | **Before SF** | | **3 d after SF** | | **21 d after SF** | |
| | **PWN** | **WMC** | **PWN** | **WMC** | **PWN** | **WMC** |
| 8 | + | 37,5 | 0 | | 0 | |
| 10 | + | 31,4 | + | 23,4 | 0 | 17,6 |
| 14 | + | 75,1 | 0 | 55,5 | + | 22,1 |
| 23 | + | 76,4 | + | 46,9 | + | 18,5 |

### 3.4 – Re-infestation of treated wood

The key factors determining nematode transfer is temperature, the nematode load of the donor wood and the MC of the recipient wood, with a 'barrier' of 20% MC below which it becomes unsuitable for nematode transfer. There was successful nematode transfer from donor to recipient wood materials kept at 25°C, as pine wood nematodes were detected in recipient boards, long-blocks and blocks (with natural MC) and in heat-treated boards. There was no transfer to kiln dried or in-service boards, or in the board-to-board treatments at 10°C (Table V) (Sousa *et al.*, 2011b).

**Tableau V:** Succès du transfert du *Bursaphelenchus xylophilus* à partir de bois donneur du *Pinus pinaster* infecté pour le bois des différentes origines ou des traitements préliminaires (basé sur Sousa *et al.*, 2011b).

**Table V:** Pine wood nematode *Bursaphelenchus xylophilus* transfer success from infected donor *Pinus pinaster* boards to recipient boards with different origins or preliminary treatments (based on Sousa *et al.*, 2011b).

| | Recipient nematode free wood | | | |
|---|---|---|---|---|
| | Freshly-cut | | | Used wood |
| | Natural WMC | Heat-Treated | Kiln-dried | |
| PWN infected donor wood | 100% | 89% | 0% | 0% |

When it occurred, nematode transfer took place within the first week in all experiments, being more successful and abundant in recipient wood with high MC, such as the freshly cut wood (Fig. 1).

This may explain why nematodes of the genus *Bursaphelenchus* are sometimes detected in wood despite having phytosanitary certificates indicating that heat-treatment has apparently been carried out (Gu et al., 2006; Zahid et al., 2008).

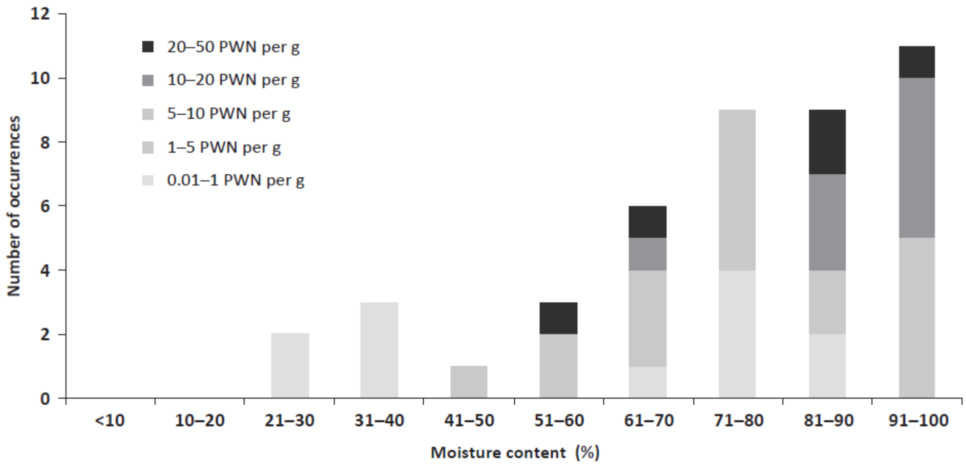


**Figure 1:** Succès du transfert du *Bursaphelenchus xylophilus* à partir de bois donneur du *Pinus pinaster* infecté et relation avec la humidité du bois (Sousa *et al.*, 2011b).

**Figure 1:** Pine wood nematode *Bursaphelenchus xylophilus* transfer success from infected donor *Pinus pinaster* boards to recipient boards in relation to wood moisture content (Sousa *et al.*, 2011b).

Wood unsuitable for nematode transfer was found to have an MC around or below 20%, regardless of having been kiln-dried or dried naturally, as was the case for boards taken from pallets in service. Once wood is sawn, and especially when converted into wood in service (e.g. as constructed pallets), it loses moisture at a rapid rate and, from the current experiments with fresh pine wood from recently felled trees, consistently drops to below fibre-saturation point within approximately 6 weeks. Therefore wood in service (as traded wood or as packaging wood) also has an identical risk period as a potential recipient of *B. xylophilus*. On the other hand, some bark and wood boring insects are able to colonize heat-treated wood, and complete its development, mainly if the wood has still some bark attached (Haack *et al.*, 2014).

## 4 - CONCLUSIONS

If pine wood containing *B. xylophilus* is moved in trade, whether as a wood product or as packaging, it will have a finite period of <40 weeks during which it poses a risk of transfer if it comes into contact with a suitable recipient piece of wood. During this risk period, if *B. xylophilus* infested wood comes into contact with wood with an MC around or above 30%, then nematode transfer can take place rapidly. In such cases, there is a further risk period of up to 40 weeks in the newly infested batch of wood, during which it also presents a risk of transfer to other suitable recipient pieces of wood.

Wood packaging material subjected to ISPM15-compliant heat treatment or fumigation can subsequently become infested with pine wood nematode under favourable ambient conditions, therefore the detection of *B. xylophilus* in ISPM15-marked wood packaging materials cannot always be considered as evidence of incorrect treatment or failed policy.

Heat treatment and SF fumigation are effective in killing the *Monochamus* vectors in the wood; this process diminishes the threat of introducing the nematode to new locations through wood packaging materials, even if the wood is re-infested with the *B. xylophilus* after treatment.

Despite the resilience of the JIII stage, the nematode populations decline and became extinct over time, confirming that once removed from the field there appears that any given piece of wood has finite capacity to support continued breeding and survival of *B. xylophilus*.

New treatments against the Pine wood nematode are continuously being tested. For instance, a new fumigant (Hydrogen Cyanide) (Douda *et al*., 2015 ) or exposing pine wood to high pressure processing (HPP), 30 MPa for 5 min, apparently eliminates the nematode, which could be use at industrial level (Fonseca et al., 2014). However, these are only preliminary assays and its efficiency needs to be proved meeting IPPC requirements (Magnusson & Schröder, 2009; Schröder *et al.*, 2009).

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