

**REPORT OF THE FIFTH MEETING OF THE INTERNATIONAL FORESTRY  
QUARANTINE RESEARCH GROUP MEETING (IFQRG)  
ROME, ITALY  
SEPTEMBER 10-14, 2007**

<b>Definitions and Abbreviations</b>	
IFQRG	International Forestry Quarantine Research Group
IPPC	International Plant Protection Convention
NPPO	National Plant Protection Organization
ISPM	International Standard for Phytosanitary Measures
EAB	Emerald Ash Borer
RPPO	Regional Plant Protection Organization
SOD	Sudden Oak Death
CPM	Commission on Phytosanitary Measures
TPFQ	Technical Panel on Forest Quarantine
TPPT	Technical Panel on Phytosanitary Treatments

<b>1</b>	<b>Opening</b>
	Peter Kenmore, Secretary to the IPPC opened the meeting. He encouraged the technical work done by the IFQRG which supports the science based work that is done by IPPC.
<b>2</b>	<b>IFQRG background</b>
	Eric Allen provided an overview of the role of IFQRG in relation to IPPC and worldwide support of NPPO risk identification, assessment, and management in the form of a presentation.
<b>3</b>	<b>IPPC report</b>
	<p>Brent Larson provided an update on the approval of recent standards by the IPPC. Larson indicated that ISPM No. 28 provides new international processes for the approval of treatments. A call for the submission of new treatments has been forwarded to NPPOs, particularly those that would assist in the management of ISPM No. 15. It is recognized internationally that the removal of the use of methyl bromide, would create some difficulties for a number of countries.</p> <p>Larson also indicated that the IFQRG meeting has minimal representation from developing countries. The group should make stronger efforts to obtain funding support to allow developing countries</p>

	<p>to attend.</p> <p>Larson indicated that the IPPC does not compile information on when countries implement ISPM No. 15. Countries themselves may report on the implementation by entering national information on the IPP. IFQRG requested the IPPC Secretariat to develop a mechanism to allow access to global information on the implementation of ISPM No. 15 at a glance. .</p> <p>Larson reported that FAO legal services have advised the IPPC Secretariat that the use of the ISPM no. 15 mark should follow the mark prescribed in the standard without deviation. Larson also reported that in the near future the registration of the ISPM No. 15 mark in countries would come up for renewal. Tom Searles indicated that legal services acting on behalf of the ALSC would be willing to assist in the renewal of registrations in relation to ISPM No. 15..</p>
<b>4</b>	<b>Report on the activities of the TPFQ</b>
	<p>A presentation on the activities of the panel was provided by Greg Wolff who clarified the roles of, and relationship between, the TPFQ and IFQRG, and communicated highlights of recent TPFQ work. Of most significance to IFQRG was the TPFQ's work on revision of ISPM No. 15. Wolff summarised key issues (a tolerance for bark on WPM, retreatment of repaired and remanufactured WPM, and criteria for considering efficacy of alternative treatments) considered during the revision and explained how these issues led to some of the specific requests that the TPFQ had made of IFQRG.</p>
<b>5</b>	<b>Report on the activities of the TPPT</b>
	<p>A presentation on the activities of the panel was provided by Mike Ormsby. Ormsby indicated that the Technical Panel will be involved in issues relating to phytosanitary treatments including collecting, reviewing and recommending them to be used internationally. The Panel will identify and collect existing treatments which are internationally needed; evaluate treatments and recommend which ones should be included in standards adopted by the CPM; classify the treatments in a logical manner (by pest, groups of pests, commodities, crops, etc.) and review existing phytosanitary treatments included in adopted standards. and recommend updates as needed. The TPPT will also propose drafts to the Standards Committee; develop a procedure for the submission of new proposals for treatments and their evaluation by the TPPT; collect information on regulated pests and treatments needed for those pests so that recommendations can be made to research institutions and when needed, identify experts on treatments.</p>
<b>6</b>	<b>CSIRO Timber Forum; SOD science symposium; Sirex</b>

	<b>symposium</b>
	A number of updates were provided. A number of presentations are included in the documents circulated to participants.
<b>7</b>	<b>Updates on regional plant protection organizations (RPPO)</b>
	Shane Sela, Andrei Orlinski, Mike Ormsby and Eric Allen (on behalf of COSAVE) gave updates on the principle activities of RPPOs. Alice provided an update pertaining to pest situations in Uganda
<b>8</b>	<b>Review of the risks of bark on marked wood packaging</b>
	<p>Eric Allen provided an update on the results of the IPPC bark survey that was undertaken in the spring of 2007. A notation that Australia reported “little or no bark and no pests observed” was reported as erroneous by Australian participants. The reference in the document will be removed.</p> <p>Australian participants requested that Table 6 in the summary report be reviewed, as the numbers reported in the presentation at the meeting appeared to be in conflict with the numbers given in the table. It was unclear if numbers in the table represented single individuals or interceptions of individuals.</p> <p>Bob Haack provided a presentation developed by Lee Humble regarding the importance of moisture content and quality of wood/bark to insect development. They suggested that:</p> <ul style="list-style-type: none"> <li>- The risk posed by infestations present prior to treatment are virtually eliminated by treatment</li> <li>- Once wood is milled, residual bark and wood begin drying</li> <li>- The shortest drying times coincide with the timing of flight and breeding seasons of bark and wood-borers (spring and summer months)</li> <li>- The experimental evidence demonstrates that dried bark patches are a less suitable substrate for oviposition and larval development than is intact bark on logs</li> <li>- Residual bark patches, less than or equal to 50 sq. cm, may have sufficient area for establishment of breeding galleries for small bark beetle species (e.g. <i>Pityogenes</i> sp.) based on form of galleries but drying from edges will rapidly reduce quality of resource and likely preclude successful breeding</li> <li>- The IPPC audit of bark on wood packaging provides no evidence of</li> </ul>

increased moisture content (and hence substrate quality) in treated packaging; 73% of the wood packaging measured was less than or equal to 20% moisture content.

Further Bob Haack indicated that in studies undertaken in an infested forest in Michigan, reproduction of bark beetles and long horned beetle was severely limited by sizes of bark less than 100 sq. cm.

Greg Wolff summarized the proposed wording included in the proposed revision of ISPM No. 15 by the TPFQ. IFQRG reviewed the wording included in Wolff's presentation. IFQRG understood that the proposal by TPFQ is:

*"Pieces of bark should be less than 3cm in width, or if wider than 3cm, the total surface area of each bark piece should be less than 50 sq. cm."*

Australian members reported that while Australia has a nil tolerance for bark, they will be reviewing its technical justification for requiring bark freedom in light of the information presented at the meeting.

While recognizing the value of the TPFQ position on a tolerance for bark, representatives of the Canadian and U.S. industry raised a concern that the proposed changes in requiring reduced bark occurrence could increase the cost in the production of wood packaging.

A small group of IFQRG participants met and the following statement was agreed to by IFQRG:

IFQRG, having considered the data from a number of monitoring exercises and scientific evaluations, supports the TPFQ proposal that pieces of bark should be less than 3cm in width, or if wider than 3cm, the total surface area of each bark piece should be less than 50 sq. cm. It notes that this recommendation will significantly reduce the risk from pests associated with bark on wood packaging material marked in accordance with ISPM No 15 in a way that should not have an unfavourable impact on trade

**9 Plants for planting**

Kerry Britton summarized developments in the NAPPO Plants for Planting standard. She indicated that the standard establishes requirements for systems approaches to control the movement of

	<p>pests on nursery stock based on pest guilds. Britton suggested several ways that the scientific community could assist in this effort by identifying and quantifying the risks associated with plant for planting movement.</p> <p>Brent Larson reported that an IPPC Expert Working Group for Plants for Planting panel has met twice and it is hoped that a draft ISPM will be submitted to the SC by May 2008. The general approach being suggested by the panel is a systems approach</p>
<p><b>10</b></p>	<p><b><i>Phytophthora ramorum</i> and its occurrence in forest products</b></p>
	<p>Shane Sela provided a summary of the discussions held at the NAPPO Forestry Panel Session held at the SOD Science Symposium. In general, conifer wood poses little risk for the movement of the pathogen. Roddie Burgess pointed out that Brown and Brasier, 2007 reported that the pathogen may be moved in xylem tissue of beech, maple and oak as reported in a recent publication.</p>
<p><b>11</b></p>	<p><b>Risks associated with the regulatory control of repaired/remanufactured wood packaging</b></p>
	<p>Greg Wolff summarized the proposed regulatory controls to be applied to repaired and remanufactured wood packaging. The current proposal of TPFQ is that NPPOs have responsibility for confirming that marked repaired and remanufactured wood packaging is constructed only using ISPM No. 15 treated wood. If this process is in question then re-treatment should occur. John McDaniel and Gordon Hughes provided presentations outlining the production of repaired/remanufactured wood packaging in the U.S. and Canada.</p> <p>The members of IFQRG reviewed significant information and were not able to reach any specific conclusions regarding specific phytosanitary risks associated with repair and remanufacture. However, during the discussion the members did recognize that the conditions for inventory controls in repair facilities create potential for the mixing of treated and not treated wood during re-manufacture or repair. Additionally, several members raised concerns such as non-compliant wood packaging material which may enter repair and remanufacture facilities and felt that re-treatment may reduce potential inventory control issues.</p> <p>Several members also felt that certain sectors of the re-manufacturing and repair industry may operate in an area which is difficult to regulate and so standardized requirements of treatment may not have the desired risk reducing effect.</p>

	<p>IFQRG recognized that there may be some greater risk in repairing multiple components of wood packaging as opposed to simple repairs of a single piece, for example.</p>
<b>12</b>	<p><b>Review of the applicability of heat treatment to the control of some pests shown to be somewhat thermo-tolerant</b></p>
	<p>Ron Mack indicated that APHIS CPHST believes that more research is needed to clarify the preliminary findings of EAB thermo-tolerance.. Eric Allen indicated that similar research is underway in Canada.</p> <p>Eric Allen provided a presentation regarding the nature of heat treatment in kilns and provided a model that demonstrated that outer portions of wood pieces are treated to higher temperatures than the requirements of 56°C for 30 minutes. For example insect pests such as EAB, which inhabits the area near the bark surface, will be treated significantly higher than core temperatures, because the surface of the wood is significantly higher.</p> <p>There was generally agreement amongst IFQRG members that organisms present at the surface of the wood are likely exposed to much higher temperatures during kiln heat treatment, but are also more likely to be exposed to fumigants as well.</p> <p>Roddie Burgess raised the issue that should the TPFQ change the specification for heat treatment to read that 56C should be applied throughout the profile of the wood (reflecting the situation with dielectric heating) then there is a potential for wood to be treated to a maximum of 56C at all points throughout the wood, and render the kiln models inappropriate.</p>
<b>13</b>	<p><b>Solar kiln technology</b></p>
	<p>Eric Allen reported that Jonathan Banks (Australia) was demonstrating that solar kiln treatment experiments were showing success in achieving heat treatment requirements. In some cases supplementary heating was required.</p>
<b>14</b>	<p><b>Heat treatment</b></p>
	<p>Adnan Uzunovic reported that in experiments that he conducted, HT treatments were successful in killing most fungal organisms tested. He also pointed to evidence that in treated naturally infested wood the prevalence of aggressive saprophytic molds e.g. <i>Trichoderma</i> is likely to suppress any surviving fungi of concern.</p> <p>Mike Ormsby and Eric Allen showed similar data confirming that heat</p>

	<p>treatment as currently prescribed in ISPM no. 15 is sufficient for most of the fungi tested.</p> <p>TPFQ has recommended that IFQRG produce guidelines on heat treatment. IFQRG members agreed that such a document would assist countries in adopting heat treatment procedures.</p>
<p><b>15</b></p>	<p><b><i>Ips</i> diagnostics</b></p>
	<p>Eric Allen provided a list of quarantine species identified by various countries and interception information.</p> <p>IFQRG concluded that although quarantine lists may identify some of the critical species of concern to countries, there are many other species that potentially pose a quarantine risk which have not been identified by countries (e.g. <i>Agrius planipennis</i> was not originally identified as a problem species).</p> <p>IFQRG identified a number of experts that could serve on a diagnostic protocols expert working group.</p> <p>Eckehard Brockerhoff provided a presentation on bark beetle interceptions and establishment in new areas. It was clear from this information that although <i>Ips</i> could be a significant invasive species, it is not a particularly common genus to become established in new areas although it is commonly intercepted on wood packaging.</p> <p>Brockerhoff provided a demonstration of a LUCID key system which could be used for the identification of a number of bark beetle genera. Brockerhoff indicated that the key will be made available upon request.</p> <div style="border: 2px solid black; padding: 10px; margin-top: 10px;"> <p>IFQRG concluded that numerous diagnostic tools for <i>Ips</i> and other bark beetle genera are already available and that the work of Brockerhoff and others should be considered by TPFQ, as it suggests that there are a number of other bark beetle species of potentially greater quarantine importance. Therefore diagnostic work concerning other bark beetle genera should be considered by the TPDP.</p> </div>
<p><b>16</b></p>	<p><b>ISPM No. 15 treatment development</b></p>
	<p>Mike Ormsby provided an overview of the standards prescribed in ISPM No. 28 and specific details on treatments evaluated under the standard.</p>

Mike Ormsby provided a presentation outlining the proposed groupings of pests suggested for treatment testing and the general requirements suggested by the TPFQ. Bob Haack and others suggested that treatment testing should consider the location (lifestyle) of insects within the wood. Haack also indicated that in some cases, the pests suggested to be tested are rare and difficult to obtain sufficient samples. There was a suggestion by a number of individuals that the specification of *Anoplophora* and *Agrilus* is too confining as some countries may not be able to access these organisms. Andrei Orlinski suggested the use of surrogates.

Adnan Uzunovic and Kelli Hoover provided presentations on efficacy testing for insect and nematode controls done in the past and provided details on the statistical approaches that could be used for future treatment work.

Industry members indicated that the \$5 million needed to test pinewood nematode is likely too significant for industrial treatment developers.

The group formed three sub-groups and reviewed the information proposed by the TPFQ regarding efficacy testing for alternative treatments. The sub-groups were requested to focus on identifying/clarifying the list of pests/processes necessary for approving alternative treatments. The information proposed by the groups was reviewed in plenary and is summarized in an appendix to this report.

Over some time, the group discussed the equivalency of the approved treatments (heat treatment and methyl bromide fumigation) in relation to the proposed list of organisms identified by the sub-groups. Some members suggested that the requirements being proposed is much more extensive than the standards required for the approval of methyl bromide and heat treatment. The group concluded that ISPM No. 28 and the need to ensure appropriate control of pests on wood supports developing an effective science-based standard for evaluating the treatments.

Brent Larson suggested that more work in relation to heat treatment and methyl bromide would be useful and establish a baseline of comparison to be used when considering new ISPM No. 15 treatments. A gap analysis has been proposed in the action items arising from this meeting. Once this analysis is complete, IFQRG members would be encouraged helping fill these gaps.

	<p>Mike Ormsby indicated that the TPPT has not established any specific efficacies for treatments to be submitted under ISPM No. 28.</p> <p>Ormsby also provided a summary of the IPPC process for the adoption of treatments. Members requested a written summary of that process.</p> <p>There was discussion by the group on undertaking independent testing of fungi in relation to treatments to assist companies developing treatments</p> <p>IFQRG members were unclear on the specific definition efficacy and requested clarification of the term from the TPPT.</p>
<b>17</b>	<b>Fumigation Information</b>
	<p>A presentation on sulphuryl fluoride's applicability as a treatment in ISPM No. 15 was provided by Suresh Pabhrakan and Mike Drinkall. A presentation on fumigation, processing and chemical pressure impregnation as means of control of Sirex was provided by Ron Mack. An overview of measuring fumigation concentrations was provided by Dennis Glennon.</p>
<b>18</b>	<b>Vacuum Treatment Information</b>
	<p>Zhangjing Chen provided scientific evidence on testing of low pressure vacuum treatment successfully killing a number of wood pests.</p>
<b>19</b>	<b>Chemical Pressure Impregnation Information</b>
	<p>Christoph Shauwecker provided an overview of chemical pressure impregnation testing.</p>
<b>20</b>	<b>Comparison of the efficacies of sulphuryl fluoride and microwaves with heat treatment and methyl bromide</b>
	<p>Eric Allen reviewed the data available for heat treatment and methyl bromide. He indicated that for the most part the information suggests that the work done on these two treatments is related to dose response. If similar information is available for sulphuryl fluoride and microwaves these can then be compared to determine the equivalency of these treatments with what is currently accepted in ISPM No. 15.</p> <p>Such work could address the concerns raised by Brent Larson regarding the potential differences in approval of heat treatment and methyl bromide and future treatments evaluated under any testing criterion that is being suggested.</p>

<b>21</b>	<b>Dielectric(Microwave and Radio Frequency) Heating Information</b>
	<p>Kelli Hoover provided an overview of current work on microwave testing for pests on wood. Ben Wilson provided additional information and testing of radio frequency heating as a potential treatment mechanism. Both systems appear to be sufficient to meet the heat treatment requirement, but both are looking at determining if the application of frequency is sufficient to disrupt or kill pests within the wood without having to meet the heat treatment requirement of 56°C for 30 minutes.</p> <p>Mike Ormsby raised the issue that in the case of dielectric heating it could be possible to kill pests at existing temperatures but at much lower treatment times. As such, perhaps the standard for heat treatment could be lowered in treatment time (e.g. 65°C for 3 minutes).</p>

<b>19</b>	<b>Next meeting</b>
	September 15 – 19, 2008, Rome, Italy

<b>NEXT STEPS</b>		
<b>Responsible Person</b>	<b>Action Item</b>	<b>Due Date</b>
<b>Chen</b>  <b>Supporting: Uzunovic, Haack, Humble and Demano</b>	Team will produce a summary of the rate of drying of bark.	September 2008
<b>Allen</b>	Provide a list of <i>lps</i> information and relevant experts to Brent Larson, Standards Officer, IPPC	<b>September 16, 2007</b>
<b>Ormsby</b>	To summarize the information developed by the subgroups of IFQRG with regards to pests required to be tested for approval as treatment in ISPM No. 15	<b>October 1, 2007</b>
<b>Allen</b>  <b>Supporting: Barak, Pabhrakan, Drinkall, Letham, Janoviak, Kawakami, Ormsby, Stirling</b>	Finalize a comparison document of heat treatment/methyl bromide with sulphuryl fluoride, microwave and several new treatment submissions (MITC, Phosphine, hydrogen cyanide, methyl iodine)	<b>October 30, 2007</b>
<b>Sela</b>  <b>Evans, Ormsby, Burgess, Searles</b>	Develop guidelines on heat treatment application procedures	September 2008
<b>Mack</b>	Review the FAO fumigation manual and compare it with other manuals and provide a report on the utility of the document	September 2008
<b>Allen</b>	Review opportunities to undertake testing of fungi related to treatment efficacies	September 2008
<b>Ormsby</b>	To obtain a summary of the process used in adopting treatments	September 2008

Wolff	To obtain clarity on the term, efficacy as it is intended under ISPM No. 28	September 2008
Sela	Request that the Secretariat develop a reporting protocol for ISPM No. 15	October 30, 2007

## **Appendix: ISPM 15 Treatment Criteria Working Group Reports**

### **Questions asked of working group:**

- 1a.** What is the shortest list of pests (in your break out-group task) that IFQRG should recommend to be tested in order for a treatment to be approved?
- Recognizing that heat treatment and methyl bromide have been approved. What is the minimal number of Genera / species of pests that need to be tested to convince NPPOs and the CPM that an acceptable global level of protection has been achieved.
  - How do the practical aspects of treatment application affect the list?
- 1b.** What are the reasons for these choices?
- 2a.** What is the prevalence in wood (standing trees, logs, lumber...) of the organisms identified above in Question 1?
- 10       100       1000       10,000,       100,000       > 100,000
- 2b.** Given the prevalence of the pests in wood, how many individuals need to be used to convince NPPOs and the CPM that an acceptable global level of protection has been achieved?
- 3.** What aspects of the organism's occurrence in wood (where it lives/feeds) should be considered to ensure appropriate treatment testing?

### **Insect Group Responses:**

- Focus on where pests are in wood;
- Need to bear in mind where pests are present globally (availability);
- Need to give consideration to any climatic considerations;
- Need a science-based approach to satisfy CPM;
- Identify typical representative target pests, not necessarily named pests
- Preferably tested on naturally infested material, any artificial testing to be proved under natural conditions;
- Efficacy testing must use a dose response approach to demonstrate 100% mortality at a confidence level of 95% ( $p < 0.05$ ). A minimum sample size of 30 units (pieces of wood) at each dose was selected based on the sample size traditionally used in bioassay experiments for pathogens and pesticides.
- Controls should be representative of level of infestation of tested pieces

Group	Life stage	Number <sup>1</sup>
Cerambycids, Buprestids – 3 spp. representing both families, covering both shallow and deep wood examples	Larval	30
Siricids, Cossids – at least 1 from either family	Larval	30
Scolytidae, Curculionidae, Platypodidae – 1 phloem feeding spp + 1 wood feeding spp.	Larval, adult	30
Isoptera – 1 species	adult	30
Lychtids, bostrichids, anobiidae –1 spp. from any family	larval	30

### **Fungal Group Responses**

1. Shortest list of pests that IFQRG should recommend

Tests should include a representative(s) from following fungal groups: decay, canker causing/root pathogens fungi, Vascular Wilts and bluestain fungi (Alternative grouping Basidiomycota, Ascomycota, Oomycota).

Groups	Fungi	Replication*
Decay (in standing trees and/or green wood products)	<i>Phellinus</i> sp.	60
	<i>Heterobasidion annosum</i>	60
	<i>Armillaria</i> sp	60
	<i>Postia sericiomollis</i>	60
	<i>Innonotus rickii</i>	60
Canker causing/ root pathogens	<i>Chrysosporthe cubensis</i>	60
	<i>Ceratocystis fagacearum</i>	60
	<i>Leptographium wagneri</i>	60
	<i>Phytophthora ramorum</i>	60
Wilt fungi	<i>Ophiostoma novo-ulmi</i>	60
	<i>Fusarium circinatum</i>	60
Bluestain (deep penetrating rather than surface stain and an associate of bark beetle)	<i>O. minus</i>	60
	<i>Ceratocystis polonicum</i>	60
	<i>Leptographium</i> sp	
	<i>Ceratocystis</i> sp	

\* Should include at least 3 isolates per species

Chosen isolates should be a type likely to be resistant (e.g. chlamydospore producing)

Isolates to be tested after being grown on wood following the Uzunovic et al protocol on the IFQRG site

<sup>1</sup> Number = number of infested pieces of wood, not number of organisms. One organism per piece of wood is sufficient.

Replication of 60 gives 95% reliability (efficacy) at 95 % confidence for any single treatment dose. This replication data will also allow creating the mortality curves with good fit and from these curves the other efficacy levels can be extrapolated (including probit 9 level (0.99997)).

The group suggested doing the experiments in two stages. First stage to include screening on larger number of fungi (such as a number of isolates for each of the above list, or other examples that may become relevant at a later date) with smaller replication (5-10) to get an idea of which isolates are more tolerant to treatment (the worst case scenario) and then do the second part with full amount of replication (60) with only one or a few chosen test organisms.

Collaborative research is encouraged to cover as many selected test organisms as possible.

The submission should provide documentation (literature review, preliminary test data) to support the selection of organisms for final full- scale testing.

### **Nematode Group Responses**

Nematodes to be included in tests for new treatments for submission to ISPM 15 (Answers and remarks according to “questionnaire”)

**1 a)** *Bursaphelenchus xylophilus* (substitute *B. mucronatus*)

**1 b)**

- *B. xylophilus* is the only known (scientific proven) wood nematode to be pathogenic on trees and which is occurring in wood.
- Laboratory tests with *B. xylophilus* can be done anywhere in the world as in the small pieces no vector beetles occur. To work with artificially *B. xylophilus* infested wood pieces, no complicated quarantine measures have to be followed in the lab.
- *B. mucronatus* has the same host trees, has the same vector and is from taxonomical point of view the closest related *Bursaphelenchus* species but is believed not to pathogenic.
- General request on substitute species: same host tree, same vector

**2 a)** More than 100,000 nematodes.

- Examples: inoculation tests with saplings > 1000 Nemas/g dry weight; up to 40,000 Nemas per vector beetle.

**2 b)** As there is an existing protocol which was basis for 56/30 in the current version of ISPM 15, the group felt this is applicable to current discussions. This protocol was basis of negotiations between NA and EU and the result

is accepted world wide (though most people do not know that this was basis for 56/30).

- In general laboratory testing (artificially infested) should be confirmed by operational conditions (natural infested material in operational sizes). But,
- If efficacy proof can be made under operational conditions using natural infested material, this should be acceptable.
  - **Laboratory test**
    - To be able to compare results with the one from 56/30 protocol samples should be inoculated with 1000 nemas each; (? Should the method be described in more detail, like sample size etc.)
    - Number of samples according to protocol presented by Forintek. The group felt to mention the efficacy rate and the confidential interval rather than mentioning a number (which was 60 using the Gomperts distribution as statistical basis) → 95% confidence that is 95% reliable.
    - Make sure that untreated control has 100% survivors.
  - **Field test**
    - No recommendation on specific number of nematodes in the wood samples
    - Number of samples as in laboratory test
    - Confirmation is necessary that the range of B.x. life stages is there, because there is no evidence that there is a most resistant life stage up to now. (L<sub>1-4</sub>, L<sub>III</sub> and adults; L<sub>IV</sub> only occurs under the presence of beetles shortly before the emerge)

3)

- Distribution of nematodes in tree (and as result in wood) is unequal.
- Number of each life stage varies according to stage of disease development, time of year, temperature, nutrition (food source)