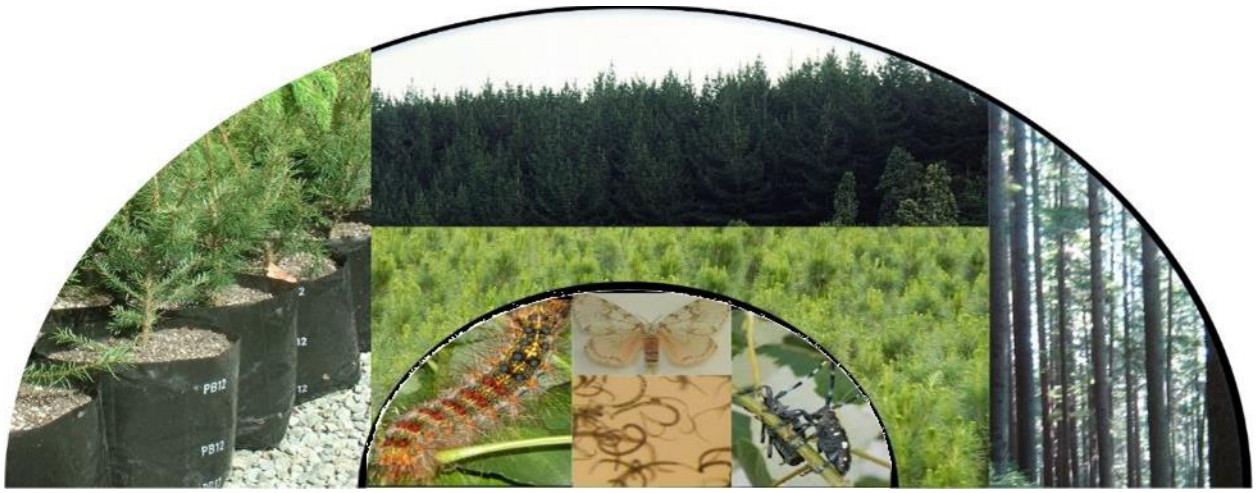

International Forestry Quarantine Research Group

International Meeting # 19

2 September – 30 Sept 2022

Proceedings of the Virtual Symposium

MARCH 20, 2023
INTERNATIONAL FOREST QUARANTINE RESEARCH GROUP
SCIENCE STEERING COMMITTEE

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While every effort has been made to ensure the information in this report is accurate, the International Forestry Quarantine Research Group does not accept any responsibility or liability for error of fact, omission, interpretation or opinion that may be present, nor for the consequences of any decisions based on this information.

The Mission of IFQRG

The mission of the International Forestry Quarantine Research Group (IFQRG) is to support and address critical forestry quarantine issues for the global plant health community through scientific analysis, discussion and collaborative research.

IFQRG is an independent, open international body providing scientific analysis and review of global forestry-related phytosanitary issues. The IFQRG serves as a forum for the discussion and clarification of key issues related to the phytosanitary implications of global trade with forest plants and products.

IFQRG's goal is for membership to include global representation from scientific, industrial and phytosanitary organizations from both developed and developing nations. Membership is open to suitably qualified individuals who have demonstrated expertise in disciplines relevant to plant health. IFQRG endeavors to recruit members from all FAO regions.

To become a member of IFQRG, the individual submits a short biography or curriculum vitae to the Science Steering Committee (SSC) outlining research or other relevant experience. Membership applications will be accepted by the SSC if information on the applicant indicates they would be a suitable member of IFQRG. There is no membership fee.

Symposium Proceedings

These proceedings communicate the discussions and conclusions from the 2022 International symposium number 19 of the International Forestry Quarantine Research Group. The symposium was held on-line (virtually) on the 2nd, 9th, 16th and 30th September, 2022.

List of Abbreviations

ALSC	American Lumber Standards Committee
APPPC	Asia Pacific Plant Protection Commission
CFIA	Canadian Food Inspection Agency
CLSAB	Canadian Lumber Standards Accreditation Board
CPM	IPPC Commission on Phytosanitary Measures
CRADA	Cooperative research and development agreement
CWPCA	Canadian Wood Pallet and Container Association
DH	Dielectric Heating
EAB	Emerald Ash Borer (*Agrilus planipennis*)
EDN™	Ethanedinitrile (C_2N_2)
EPPO	European Plant Protection Organisation
FPSA	Forest Products System Approach
HACCP	Hazard Analysis and Critical Control Points
HT	Heat Treatment
IFC	IPPC Implementation and Facilitation Committee
IFQRG	International Forestry Quarantine Research Group
IFU	Implementation and Facilitation Unit
IPPC	International Plant Protection Convention
IPRRG	International Pest Risk Research Group
IRSS	Implementation Review and Support System
ISPM	International Standards for Phytosanitary Measures
ISPM15	ISPM No. 15 *Regulation of wood packaging material in international trade*
ISPM28	ISPM No. 28 *Phytosanitary treatments for regulated pests*
ISPM42	ISPM No. 42 *Requirements for the use of Temperature Treatment as Phytosanitary Measures*
IUFRO	International Union of Forestry Research Organizations

IYPH	International Year of Plant Health
MBr	Methyl bromide
MW	Microwave
NAPPO	North American Plant Protection Organization
NEPPO	Near East Plant Protection Organization
NGS	Next Generation Sequencing
NPPO	National Plant Protection Organisation
OECD	Organisation for Economic Co-operation and Development
OTUs	Operational taxonomic units
PCE	Phytosanitary Capacity Evaluation
PMRG	Phytosanitary Measures Research Group
PWN	Pine Wood Nematode (*Bursaphelenchus xylophilus*)
RoP	Rules of Procedure
RPPO	Regional Plant Protection Organisation
SC	IPPC Standards Committee
SSC	IFQRG Science Steering Committee
STDF	Standards and Trade Development Facility
ToR	Terms of Reference
TPFQ	IPPC Technical Panel for Forest Quarantine
TPPT	IPPC Technical Panel for Phytosanitary Treatments
USDA-APHIS	United States Department of Agriculture- Animal and Plant Health Inspection

MEETING REPORT

1. Welcome Address

The meeting of IFQRG 19 was hosted online by the Pennsylvania State University, USA. Forests and their sustainable management all over the world have presented researchers with many challenges during the past decades. Responses with solutions were and are being only possible because of scientific cooperation among institutions and their dedicated scientists.

2. Opening of the meeting

IFQRG Chair, Dr. Michael Ormsby, opened the meeting and welcomed all participants.

3. Introductions

A list of the recorded participants is provided in Appendix 1.

4. Symposium Agenda

Day 1 - Friday, Sept 2nd (2.5 hours)

Topic - Molecular Tools	Speaker
Meeting Logistics	Kelli Hoover, Penn State University, USA
Greetings & Introduction to IFQRG	Mike Ormsby, MPI New Zealand / Eric Allen, Canada
Improved assessment and mitigation of phytosanitary risks associated with seed trade	Iva Franić SLU, Sweden
Specificity and sensitivity of LAMP assays for early detection of two *Agrilus* pests: Emerald ash borer (*A. plannipennis*) and bronze birch borer (*A. anxius*)	Donnie Peterson SLU, USA
Using environmental RNA for the detection of live pinewood nematodes and to assess the efficacy of phytosanitary measures	Caren Helbing, University of Victoria, Canada
Detection of insect pests in shipping containers in Australia using Environmental DNA	Alejandro Trujillo-Gonzalez, University of Canberra, Australia
Summary, additional questions and discussion	Adnan Uzunovic FPInnovations Canada/ Meghan Noseworthy, Canadian Forest Service

Day 2 - Friday, Sept 9th (2.5 hours)	
Topic - Forestry Phytosanitary Programs and Treatments	**Speaker**
Meeting Logistics	Kelli Hoover Penn State University, USA
Daily update	Mike Ormsby MPI, New Zealand
FAO Forestry Update	Shiroma Sathyapala FAO Forestry, Rome
Wood Commodity Systems Approach Guidance - Draft Annex to ISPM 39	Meghan Noseworthy Canadian Forest Service, NRCan
Heat treatment based on vacuum/steam technology to eliminate pinewood nematodes in the naturally infested pine logs	Chen Zhangjing Virginia Tech, USA
Ethane dinitrile treatment of the oak wilt fungus in oak logs	Anna Yang USDA
Summary, additional questions and discussion	Thomas Schröeder Plant Health Unit of the Federal Ministry of Food and Agriculture, Germany

Day 3 - Friday, Sept 16th (2.5 hours)	
Topic - Phytosanitary Treatments	**Speaker**
Meeting Logistics	Kelli Hoover Penn State University, USA
Daily update	Eric Allen, Canada
Regulatory aspects of log export	Tyrone Jones USDA APHIS, USA
Update – global research, registration, and commercialisation activities for EDN™	Matt Hall Draslovka, Australia
Carbon and the Wood Packaging Industry: Finding a path forward	Brad Gething National Wooden Pallet & Container Association, USA
Radio Frequency (RF) Phytosanitation of Wood Packaging Material (WPM); Update on Development of Certification Protocols and Advancements for RF Technology	Karolina Szymona Penn State University, USA
Mortality of bark- and wood boring Coleoptera exposed to different chamber and wood core temperatures	Toby Petrice/ Bob Haack USDA Forest Service, USA
Summary, additional questions and discussion	Ron Mack USDA/ Kelli Hoover Penn State University, USA

Day 4 - Friday Sept 30th (2.5 hours)	
Topic - Surveillance Tools - New Tools and Technology	**Speaker**
Meeting Logistics	Kelli Hoover Penn State University, USA
Daily update	Mike Ormsby MPI, NZ
Enhancing implementation of ISPM 15: Regulation of wood packaging material in international trade	Barbara Peterson & Janka Kiss IPPC
Efforts to improve surveillance efficacy for detection of exotic jewel beetles	Jon Sweeney Canadian Forest Service, NRCan
Comparison of Intercept Trap Fluids and Aerial Spore Collectors to Survey Fungal Spores	Jean Bérubé Canadian Forest Service, NRCan
Challenges in semiochemical lure development for spotted lanternfly (*Lycorma delicatula*)	Mariam Cooperband / Ron Mack USDA APHIS, USA
Summary, additional questions and discussion	Kelli Hoover Penn State University, USA/ Eric Allen, Canada

5. Presentation Abstracts

Day 1 – September 2nd: Molecular Tools

1.0 Greetings ORMSBY

Bio: Dr. Mike Ormsby manages a team of scientists in the New Zealand Ministry of Primary Industries that assesses pest risks to New Zealand and oversees research programmes to support pest management. Dr Ormsby has worked in the phytosanitary area for over 24 years and has been a member of IFQRG and the IPPC technical panels for treatments and forest quarantine since 2005.

1.1 IFQRG Introductory Remarks ALLEN

Author: Eric Allen

Presenter Bio: Dr. Eric Allen, now retired, was head of the Forest Invasive Alien Team with the Canadian Forest Service at the Pacific Forestry Centre in Victoria, Canada for more than 20 years. He worked extensively on non-indigenous species that impact forest ecosystems; their biologies, their movement with international trade, and the assessment of mitigation measures. During his career, he was the founder and chair of the International Forestry Quarantine Research Group (IFQRG), member of the North American Plant Protection Organization (NAPPO) Forestry Panel and expert groups on Forestry Systems Approaches and Contaminating Organisms and the International Plant Protection Convention (IPPC) Technical Panel on Forest Quarantine. Through these fora he has supported the creation of a number of regional and international standards for phytosanitary measures and guidance documents for both and has recognized the importance of teamwork and collaboration through bringing global experts from science, industry and regulatory communities together to solve phytosanitary issues in forestry.

Eric gave a summary on the history of IFQRG since its formation in 2003. Eric described IFQRG as a policy-focused science research organization with participants from over 40 countries with wide backgrounds that include scientists, regulators and the industry. IFQRG is not an advocacy group. IFQRG provides independent science/research and analysis in support of international forest phytosanitary policy. IFQRG encourages a culture of discussion and open dialog and problem solving. Much work is done in association with IPPC and their technical panels and working groups. Historically IFQRG gave significant contributions that included refinements of ISPM 15, new perspectives on treatment efficacy and others. This year (third year of virtual meeting) we have had 176 participants registered from 22 countries and the first session on molecular tools was attended by 80 participants.

Day 1 Session Moderator: Adnan Uzunovic

Bio: Adnan Uzunovic was a senior research scientist for over 20 years at FPInnovations (Canadian Wood research institute), working on wood protection from pests that cause deterioration or market issues, developing various test methodologies, conducting research on the management of wood pests, and participating in different national and international forums. Adnan has been a member of IFQRG since its establishment and other relevant groups serving various functions in support of IPPC phytosanitary standards development and their implementation, and a member of the International Group of Wood preservation. Currently Adnan provides scientific advice and representation to Canada Wood to support regulation and biosecurity of wood products exports and imports.

Adnan gave a short introduction to the session. He pointed out that rapid development of molecular technology, the advent of next generation sequencing and metagenomics have allowed analysis of DNA and RNA of many organisms (at the same time) directly from the environment/commodities (eNA). A lot of research has already been done, and the methodology has been used routinely around the world, revolutionizing assessments and surveys of environments and commodities. Many other applications are being explored, including the use in biosecurity to inform management decisions. Analysis of such complex data sets are done through bioinformatics and are based on the use of reference collections and other types of data. A number of issues are under discussion to improve uniformity and consistency for the technology to be used uniformly and consistently. Examples of problem areas include: inconsistent practices, poor quality eNA, detection tools, high false negatives/ positives, cross contamination and inhibition. In addition, it is known that current DNA barcoding is not universal and sometimes it is impossible to distinguish beyond family and genus level. Many species cannot be identified depending on the existing reference databases. Genetic databases need to be standardized and verified; they are continually improving. It may be challenging to determine if an invasive species is present and a threat using molecular tools since there is a need to address if it is alive, if it can establish; these are the key questions relevant to quarantine agencies. These tools are in use by some NPPOs to aid surveillance and management. Molecular technology has potential to complement existing tools to manage plant health. The use of molecular tools should be standardized and should not impact trade unnecessarily and unjustifiably.

1.2 Improved assessment and mitigation of phytosanitary risks associated with seed trade FRANIĆ

Authors: Iva Franić, Patrick Sherwood, Oskar Skogström, Rene Eschen and Michelle Cleary

Presenter bio: For her Ph.D. project Dr. Iva Franić studied insect and fungal communities of tree seeds and twigs on a large scale to determine the main drivers of the observed diversity patterns and to assess the phytosanitary risk associated with the movement of plant material. She obtained her PhD from University of Bern and has been working on her project at CABI Delémont and WSL in Switzerland. She is currently doing her postdoc at Southern Swedish Forest Research Centre, SLU Alnarp where she looks more closely into vertical transmission of fungal endophytes of tree seeds, phytosanitary treatments of seeds, and utilization of various molecular methods for the assessment of fungal communities in plant tissues.

Abstract: High-throughput sequencing reveals high diversity of seed-borne fungi, including potential plant pathogens that may be introduced with seeds and cause damage in the new areas. Risk assessment of seed trade as a pathway for harmful fungi requires additional information about the viability of those fungi, and about the transmission of seed-borne fungi into seedlings. Furthermore, the development of environmentally friendly treatments for the reduction of fungal inoculum in seeds is needed for mitigating the risks of seed trade. We present the results of the study that characterized the fraction of seed-borne fungi of *Pinus sylvestris* and *Fagus sylvatica* that are vertically transmitted. Around 70% of fungal taxa associated with seeds were also found in seedling. Total of 13 out of 110 (12%) and 12 out of 70 (17%) potentially pathogenic fungal genera were identified from seeds and seedlings of *P. sylvestris* and *F. sylvatica*, respectively. We also present the on-going studies that look into (1) the efficacy of DNA vs RNA high-throughput sequencing for the assessment of viable fungi in seeds and (2) the efficacy of UV-C light and heat treatments in eliminating viable fungi from seeds. The results of those studies will facilitate improved assessment and mitigation of phytosanitary risks associated with seed trade.

Iva commented that they could not ID many of the encountered species depending on the reference databases that are incomplete. In their research they used multiple approaches: Traditional plating and identification using PCR based on ITS and Sanger Sequencing, rDNA metabarcoding from seed tissue looking at whole community (amplifying ITS region using PCR) and rRNA metabarcoding ensuring with the latter to target those actively synthesizing RNA, thus pointing that they are alive so the research reveals total and viable seed-borne fungi. Iva's group will continue comparing these methods in the next year. DNA metabarcoding reveals 10x higher diversity than traditional plating. It was found that 70% of the fungi found with seeds was also found in seedlings that indicate potential of transfer of pathogens via seeds (vertical transmission) and potential of establishment that is high. Iva suggested stricter regulation of seed trade and the use of treatments (UVC light and/or heat 55 °C for 8 hours or higher temperatures for shorter time) that will affect pathogens but not the vitality of seeds.

Questions and comments:

Q: Is it important if the detected organisms are viable or not? If they are invasive that is enough to raise alarm. Perhaps not enough for quarantine action, but enough for serious detection program to begin! **A:** It is important they are viable for the concern. If we come up with the procedure to kill those organisms, then detection of DNA of these organisms is not alarming enough to stop trade in the commodity.

Comment: Additional commenter pointed out there is a clear need for a risk assessment.

Comment: It is interesting that in the EU they did not have regulations for seeds of other forest tree species apart from *Pseudotsuga menziesii* and *Pinus*. However, from 2019 it is required that all forest seeds need a phytosanitary certificate for import, so we are a bit safer. Iva's work clearly shows there is a potential problem. Commenter liked Iva's suggestion on the future control of pathogens in seeds. The question is: what do these export countries test for before providing a phytosanitary certificate as it is not standardized and rather is often based on visual inspection, and traditional plating method may miss pathogens.

Q: Another participant was very intrigued by metagenomics results and observations for the differences in performance between the DNA and RNA results. They wondered if the research group used the same metabarcoding primer sets for both DNA and RNA? **A:** Yes, we will extract RNA and synthesize complementary DNA, and then will use the same parts of ITS, the same primers and the same bioinformatics pipeline.

The participant commented further that one thing her group is noticing is that universal primers for metabarcoding are not necessarily that great in terms of reproducibility. One possibility that may account for the difference in performance is that the primer sets you are using don't work well with the DNA because of the interaction with non-target sequences whereas the RNA will be a different kind of mix. They were also struck by the large percentage of unknowns. This suggests a need for more genomic resources to whittle down. **Q:** Also, does the barcode that is chosen (25% mix) mean you can't distinguish? **A:** No, the bar chart I showed just shows those identified to genus and which ones could be parsed to the trophic guild. That was not how many were identified. There is lot of variability depending on primers we used and tissues we worked with and also dependent on geographic scale (like twigs study on global scale from around the world, we could only ID 15% species) in Seed study we got from Europe and North America, we identified 30% to species, all with the same primers.

Iva also asked the commenter if the differences they see in RNA, not reflected in DNA are caused by the same issues. The commenter pointed that it is possible, the mixture is different so potential interactions can reduce efficiency of replication. We try to figure out ways that primers sets we use compare different things.

Q: On possible seed treatments - there was a question regarding UV-C penetration: is this only a surface sterilization? **A:** UV-C would control just what is on the surface. Treatment with heat would likely penetrate.

Q: Does 55 °C for 8 h damage seeds in any way and is the temperature efficacious for the pathogens of concern? **A:** If the seeds are dried, then longer exposure should not do anything. We are conducting germination tests for all time/ temperature combinations. 55 °C was efficacious for pathogens of concern such as *Diplodia* and *Fusarium* and some published papers suggested 55 °C/ 8 hours is efficient.

Q: Have you identified core seed microbiomes for both species? What is the average number of OTUs/ASVs you find per seed sample? Presumably heat treatment selects for thermotolerant species e.g., some Dothideomycetes and Eurotiales - do you see a pattern? **A:** We didn't do this for this study, but we did it earlier. So, the number of OTUs per seed lot is 10-15 and can go lower or higher. Yes,

we identified core seed microbiomes for both, but we did not compare yet between micobiomes before and after the heat treatments and we are planning to do that.

Q: What is the size of seed? **A:** Depends, they can be different. We looked to see if the size of seeds influenced the number of fungi in the seed and we did not find any correlation. Maybe the shape of the seed or outside structure plays a role.

Comment: Another method to consider is using DNA-binding dyes like propidium monoazide to selectively amplify viable cells. This method is used for bacteria and to some extent fungi in agricultural products.

1.3 Specificity and sensitivity of LAMP assays for early detection of two *Agrilus* pests: Emerald ash borer (*A. plannipennis*) and bronze birch borer (*A. anxius*) PETERSON

Authors: D. L. Peterson[1*], K. Kyle[2], Aurélien Sallé[3], F. Pecori[4], Duccio Migliorini[4], A. Santini[4], N. Luchi[4], and M. Cleary[1]
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Presenter bio: I am a forest entomologist that is interested in woodborers, their ecology, and their potential to be invasive species. I have studied how emerald ash borer (EAB, *Agrilus planipennis*) performs on and impacts evolutionary naive hosts from North American. I recently worked on implementation and improving a novel, terrestrial eDNA method to survey for forest and agricultural pests. I have begun a Marie Curie Postdoctoral Fellowship in Sweden to study EAB on European naive hosts and developing novel early detection tools.

Abstract: Among forest pests, Buprestids are an emerging threat to broadleaf forests across the world. Species like emerald ash borer (*Agrilus planipennis*) and bronze birch borer (*A. anxius*) pose serious threats to ash (*Fraxinus* spp.) and birch (*Betula* spp.) stands, respectively, where they are currently established. If these beetles were introduced to Europe, native populations of their host tree species would suffer dramatic losses. Due to their cryptic lifestyle, feeding on vascular tissue of their host plants, Buprestids and other woodborers can be difficult to observe or detect. Early detection tools are vital to swiftly implement eradication measures and prevent the establishment of introduced species. Detection methods using PCR or qPCR assays to target specific taxa can take hours to prep and run the results. However, loop-mediated isothermal amplification (LAMP) eDNA assays are highly specific and sensitive and can provide results within 30 min after extraction. In this study, we designed a novel LAMP assay for *A. anxius* and investigated the specificity and sensitivity of both this assay and a previously developed LAMP assay for *A. planipennis* for its use as an early detection tool in European forests. We found that both assays were specific to the targeted species when tested against 12 other European *Agrilus* species, five other Buprestids, two Scolytinae, and five Cerambycid woodborers. The sensitivity of the two assays varied with the *A. planipennis* assay amplifying at a concentration as low as 0.02 pg/µl, whereas the *A. anxius* assay amplified at 3.2 pg/µl. These results demonstrate that both assays make for a highly specific and sensitive tool that can be used to detect and monitor for the spread of *A. anxius* and *A. planipennis*, if or when, respectively, they are introduced to European forests.

Keywords: early detection, emerald ash borer, bronze birch borer

Donnie Peterson pointed that the next steps need to address if plant chemistry and different tissues influence DNA detection; compare qPCR vs LAMP, if with lower DNA conc. there is more inhibitory influence and testing best best methods for recovering wood borer eDNA from host material?

Q: A participant was excited to see the use of molecular tools for such challenging needs. They were not surprised that the Lamp method is less sensitive than PCR. They wondered if there are any actions towards standardizing the sensitivities of the assays that they are working with. One of the things we're trying to do in North America is to express assay performance in a standardizable way using Gblocks etc. So, you showed pictograms/microliter, that is from an extract? That is hard to standardize as each sample is different. USDA suggested using Gblocks – synthetic DNA to standardize so it is accessible to everyone and see how reproducible it is. **A:** No, I just used what I was familiar with. Rutgers group was starting to work on this and talking about standardizing that.

Q: What is being sampled in the field: rainwater? Plant material? **A:** Rainwater is popular, but the downside is that it has to rain when you sample. For EAB they sampled tree cores, DNA may degrade in phloem. The longest stage is larvae. Also, it may be possible to use leaf clippings for adults. Exploring these methods is important.

Q: Are you testing the sampling method in areas already infested, such as North America for EAB and BBB? **A:** Yes, the intention is to look this summer for proof of concept in the field for plant inhibition situation.

Q: A participant pointed that there is a recent study where they immersed hemlock branches in water, shook them up, and used that for the sample- and it worked well. **A:** Yes, it is another method, Judy Lockwood at Buckers University used spray aggregation by using backpack sprayer then spray foliage of plant and collect rinse water and then filter it. It worked for target species.

Comment: The RT LAMP method was mentioned not to be sensitive enough, but it was species specific when used on *Bursaphelenchus xylophilus* (PWN) and *B. mucronatus.*

1.4 Using environmental RNA for the detection of live pinewood nematodes and to assess the efficacy of phytosanitary measures HELBING

Authors:
Caren C. Helbing[1], Gwylim Blackburn[2], Isabel Leal[2], Stacey Kus[3], Vanessa C. Thompson[1], Esme John[2], Adnan Uzunovic[4], Jacob J. Imbery[1], Luís Fonseca[5], and Joana Cardoso[5]
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[5] Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Coimbra, Portugal

Presenter bio: Dr. Caren Helbing is a Professor in the Department of Biochemistry & Microbiology at the University of Victoria in British Columbia, Canada. She directs an internationally recognized

research group that investigates biomolecules to understand and promote animal, environmental, and ecosystem health. She is shared co-lead of the iTrackDNA Genome Canada/BC/Québec large-scale applied research project that introduces innovations in non-destructive precision genomics for environmental impact tracking in a global climate change era.

Abstract: Conventional pinewood nematode, *Bursaphelenchus xylophilus*, detection in wood products involves morphological analysis of species-specific characteristics after nematode extraction from wood. This analysis is time-consuming and requires specialized expertise in nematode morphology and taxonomy. Moreover, morphological distinction of eggs and juvenile stages from other related species is problematic, prompting the need for gDNA-based discrimination methods. However, these methods can only detect nematode presence, not viability. Because only living organisms are regulated as quarantine concerns, molecular tools that can distinguish between live and dead pinewood nematodes, such as those based upon short lived RNA molecules, will provide increased confidence in confirming the effectiveness of phytosanitary measures, including new phytosanitary treatments. This presentation discusses the progress made on developing environmental RNA-based assays as a means for viable pinewood nematode detection in wood products. In her talk Caren pointed that the eDNA technology can pick more easily cryptic species, you can sample more frequently, different windows, more sites in less time, test single sample for multiple species at same time or different times with different assays. Sampling is less invasive, no pathogen transfer risk.

Comments:

Caren pointed out that there are barriers to uptake of this revolutionized methodology. Inconsistent data quality is eroding confidence for their use and standardization. The iTrack project is trying to address these issues. It is a large project in Canada planning to deliver user driven eDNA and eRNA taxa-specific assays, targeting 100eDNA and 10eRNA kits. The project aims to help decision making and to support standards. An extensive number of controls, for sample integrity and assay performance are built in. The keystone of eNA workflow success is the assay. Poor assay will compromise the entire study and decision making. The work on PWN live-dead detection assay includes establishing appropriate eRNA targets for key nematode stages. Caren pointed out a need for work to be done to create enough transcriptomics (RNA) information that can be used for assays. Also there is a need to verify that the target sequence we selected comes from RNA and not from contaminated genomic DNA. Also there is a need to confirm that RNA is found in different life stages.

Another aspect of this project is to support national eDNA standards. Last year Canada published one standard on eDNA requirements terminology. iTrack is supporting a second standards-performance criteria for targeted qPCR-based eDNA analysis in the coming year. The standard is to ensure consistent predictable outcomes through minimum compliance requirements. The group is happy to assist in integrating technology into international standards, working with IFQRG and IPPC. The iTrack group hopes to be part of the solution to increase confidence and set the foundations for reliable eNA applications in phytosanitation, forestry and ecology.

Q: A participant wondered about integrating transcriptomics into barcoding. In terms of the RNA stability there are differences between the molecules RNA vs normal mRNA. Which molecules are constitutive versus transitive? How big are the detection barcodes? Degradation among or between animal types can ensure it is representative to viability **A:** This must be empirically evaluated for the application it was intended for and there is no way around it. There are no one-size fits all. **A:**

Alluding to micro RNAs etc. Caren commented on micro RNAs in environmental samples: one thing to be aware of is that they are very highly conserved among many species thus we must be aware of this. So, if nervous grad students are trying to assess micro RNAs that are contaminants it will be an issue.

Q: Are you looking at any *Phytophthora* spp. (e.g., *P. ramorum*) or other oomycetes/fungal pathogens in iTrackDNA? **A:** Currently we are not. We are limited to 100 animal species and haven't tackled the fungal world yet.

Q: TaqMan can only see what you look for. Are you not seeing a bulk of what may be introduced? What about a meta barcoding as a first screen then TaqMan for what seems to be present? **A:** When you use TaqMan approach you are very specific so you can get high sensitivity, specificity and reproducibility in the assay being used. The challenge with using metabarcoding as the first screen on a routine basis is reproducibility. It is difficult to come up with standardised methodologies for it. We tackled that in Canada in standards for eDNA use.

1.6 Detection of insect pests in shipping containers in Australia using Environmental DNA TRUJILLO-GONZÁLEZ

Author: Shams, F.[1], Soroka, J.[1], Gleeson, D.[1] & Trujillo-González, A.[1]

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Presenter bio: Alejandro Trujillo-González; Senior Research Fellow; Institute for Applied Ecology; University of Canberra\W www.ecodna.org.au; T @AlejandroT_G; Canberra ACT, 2601 Australia

Alejandro Trujillo-Gonzalez is a Senior Research Fellow at the University of Canberra and the Principal Scientist of Australia's National eDNA Reference Centre. He has over 12 years of research experience in parasitology, molecular methods, environmental DNA and biomonitoring and surveillance. Alejandro explores the utility of eDNA-based screening methods to detect pests, parasites, and pathogens of importance to Australia. His projects involve the development of standardised eDNA assays and guidelines for the operational use of eDNA screening and testing novel eDNA technologies for their suitability as reliable screening tools in Australian biosecurity.

Abstract: Exotic insects pose important biosecurity risks to Australia, where widespread incursions could cost the country billions of dollars to manage and minimize. Environmental DNA (eDNA) and RNA (eRNA)-based methods could offer non-invasive, sensitive detection tools to inform biosecurity officers on the presence of high-risk pests across the biosecurity continuum. The shipping trade is a known pathway for multiple insect pests globally, wherein species such as Khapra beetle and brown marmorated sting bug have been detected as a contaminant pest on multiple imported goods. The National eDNA Reference Centre (NRC) at the University of Canberra is validating the use of eDNA/eRNA to screen shipping containers for pest risks through the Australian Government Department of Agriculture, Fisheries and Forestry (DAFF) funded project. The project aims to optimise Real Time PCR assays to detect trace DNA and RNA from samples collected inside shipping containers and examine the approach rate of high priority insect pests of biosecurity concern to Australia. A total of 2119 dust samples were collected from 2000 shipping containers arriving at an empty shipping container park in Brisbane during May-August of 2021. These containers were not

subject to biosecurity conditions and were selected randomly for sampling on a daily basis by staff at the site. Dust samples were then shipped to the NRC to be processed and tested for insect eDNA/RNA. Herein, we present results obtained for *Trogoderma granarium*, *Halyomorpha halys*, *Wasmannia auropunctata* and *Lymantria dispar asiatica*. A total of 2112 dust samples were tested for eDNA/RNA of the aforementioned species using either published or novel species-specific probe assays. A total of 229 samples (10.08 %) showed positive detection for *T. granarium* eDNA, of which 16 (0.75 %) tested positive for RNA. Similarly, a total of 17 samples (0.8 %) showed positive detection for *H. halys* eDNA, of which 8 (0.4 %) tested positive for RNA. A total of 40 samples (1.89 %) showed positive detection for *W. auropunctata* eDNA, of which 4 (0.2 %) tested positive for RNA and 31 samples (1.46 %) showed positive detection for *L. dispar asiatica* eDNA, of which 3 (0.14 %) tested positive for RNA. Of importance, two shipping containers with positive eDNA/RNA results were recommended to undergo further examination and were confirmed to contain either live khapra beetle specimens or khapra beetle larvae skins. This data is being analysed by DAFF to study pest approach rates and risk profiling of container arrivals. We discuss the benefits and limitations of using eDNA/RNA-based detection and how it could improve surveillance of priority pests in future Australian biosecurity responses.

Comments: The presenter's talk was pre-recorded therefore he could not take direct questions after the talk.

1.7 Summary, additional questions and discussion UZUNOVIC/ NEHME

Q: A participant commented that molecular tools are very promising but still hard to use as a regulatory tool. We regulators are still relying on pest detection or confirmed infested individuals to trigger any regulatory response. Do you expect metabarcoding to become useful for regulatory responses in the near future? **A:** Targeted assays yes, they are the wedge, they will allow for this type of technology to be used. Metabarcoding will be more challenging. Many researchers are working on it to figure out how to standardize it.

Comment: Alejandro's presentation highlights the need, the importance of justification of import conditions and emergency measures for regulators. We could have a hunch as regulator that there may be a problem on the pathway, but the SPS agreement that most countries are signatories to, indicates a need to justify measures taken. Alejandro's presentation and work provides that hunch – that shipping containers may be infested without having goods in them and perhaps that is how the pests are potentially getting around. That could be an issue. In this particular case, the work justified that Khapra beetle is a real threat and led to the weight of the response, supporting that emergency measures were technically justified. However, we have to be aware of the fact that you cannot slap measures on things and not have them questioned. These tools can be used in this way initially to justify, from a regulatory point of view, what we are doing. This IFQRG group consists of a good mix of regulators and researchers who want answers, but the key is whether these can be applied in real life situations.

Q: A participant would like to ask Alejandro what else he would do with that information. If you find live RNA in a container, but you do not know where that container comes from, are you going to take the product that is in the container? Is he working with the Sea Container Task Force on this for future planning? It is good information to foresee what may be coming and for looking at pathways, but it is

difficult to regulate containers. **A**: It all fits into that wider picture. We need to know if this is a ubiquitous pest that may be associated with containers that go around the world or is it possible to regulate specific countries for this pest? Is there a standardized measure that we need to apply to commodities that may be put in these containers?

Comment: A participant commented that for 15-20 years APHIS had a test where they put LED light in containers and it was attached to sticky surfaces and they caught all kinds of things: snails, insects and other critters inside those containers to analyze. They dropped this monitoring idea as shippers didn't want this kind of surveillance in their containers. Participant commented that this method holds a promise and it should be used.

Comment: It is difficult to trace things back through the containers supply chain. The Sea Containers Task Force under the IPPC is working on this. Some of this technology will be looked at the Workshop on Sea Containers in Sept 19-20, 2022 in London. Things have changed in terms of what the shipping industry is willing to do with the sea container supply chain. Having information on what may or not may be traveling in the containers is of interest to them as well as us.

Q: Is there a potential problem related to the large amount of information gathered through eNA methods and subsequent triggering of regulatory action? **A:** There is a fear that when one has large amounts of data one can always find something that could potentially trigger a regulatory action. It is very important to start as soon as possible talking about what do we do with this type of data. Are there examples of how one can utilize such data? Similarly, genomics-based data sets are being grappled with in the human health area, and this could potentially provide a way on how to use the data. We need to be cognizant and careful of having a high rate of false positives and false negatives.

Comment: This technique is a very sensitive tool, but false positives are an issue. In the IPPC realm we can only regulate live pests. So, we cannot regulate when for example, a khapra beetle has left a bit of eNA. If the sensitive techniques captured bits of eRNA in absence of a live pest what action can be taken? Researchers are encouraged to think about this and to work closely with regulators to ensure that the questions they are answering are working towards refinement of how eNA is used as a regulatory tool. For example, Alejandro's talk showed that even the smoking gun that he provided with his research indicating he found live *Khapra* beetles could be a deluge that regulatory bodies must go to all these things, and they may not have the manpower to look at every incident raised by these sensitive techniques. There are many logistics issues so when we all put our heads together, we may come up with ways to use these tools most effectively without them becoming burdensome.

Comment: The aquatic world of invasive species has been tackling this issue very extensively for example, on incidental versus live finds for zebra mussels entering northwestern part of states. False positives are hugely problematic, for example dead/decaying matter is causing huge potential for false positives. The North American community is tackling these issues on false positives and negatives, eDNA versus eRNA, and viability measures and could join this group in helping solve the issues.

Q: Is anyone working on an international standard or guide on how to interpret eDNA or eRNA results in a regulatory arena? **A:** Canada is working on this, and I believe has a national standard on eDNA. There are also guidelines that are being developed in Europe and Australia. There is growing interest to coordinate. The challenge is to get the coordination going to have people tackle the various aspects of eDNA eRNA and their challenges. In Canada the aim is to have everybody using the same technical language first, with defined terminology, discussion on minimum reporting requirements and then build on that in in a stepwise manner. We would like to get together with others. The iTrack

project is to an extent facilitating that but we need more interaction with international partners. Adnan pointed out that there are several activities and documentation on this topic: Australian "Environmental DNA development guide for biomonitoring:, and "Environmental DNA test validation guidelines" that 150 scientists put together; Canadian national standard " Environmental DNA (eDNA) reporting requirements and terminology" and Canadian working concept document, *Performance criteria for targeted qPCR-Based eDNa analysis*; there is a Quads working group (Australia, New Zealand, United States and Canada) entitled: *Managing regulatory issues from new diagnostic technology*. IPPC also has 2019 CPM recommendation: (R-8) *Preparing to use high-throughput sequencing (HTS) technologies as a diagnostic tool for phytosanitary purposes*; https://www.ippc.int/en/publications/87199/.

The moderator commented at the end of the session, that when IFQRG works normally (not virtually) we would have a full four days face-to-face. After presentations and discussions, there would be plenty of time to discuss and identify possible ways forward. A smaller working group may be formed and tasked to work on particular aspects and to report to larger groups to then make conclusions and produce final meeting proceedings. A collaboration may be formed to continue after the meeting to address tasks and prepare for the next meeting. Next year IFQRG will hopefully be back in-person providing an opportunity to continue this approach to tackling solutions to current issues. Perhaps IFQRG could be a hub to initiate needed coordination among researchers on the various aspects of eDNA eRNA and their challenges with international partners/stakeholders and with important input from regulators.

Day 2 – Sept 9: Forestry Phytosanitary Programs and Treatments

2.0 Greetings & Introduction HOOVER

Kelly Hoover on behalf of the chair Mike Ormsby welcomed the participants and gave some technical information concerning the online version of IFQRG 19.

2.1 IFQRG Introductory Remarks ALLEN

Author: Eric Allen

Presenter Bio: Dr. Eric Allen, now retired, was head of the Forest Invasive Alien Team with the Canadian Forest Service at the Pacific Forestry Centre in Victoria, Canada for more than 20 years. He worked extensively on non-indigenous species that impact forest ecosystems; their biologies, their movement with international trade, and the assessment of mitigation measures. During his career, he was the founder and chair of the International Forestry Quarantine Research Group (IFQRG), member of the North American Plant Protection Organization (NAPPO) Forestry Panel and expert groups on Forestry Systems Approaches and Contaminating Organisms and the International Plant Protection Convention (IPPC) Technical Panel on Forest Quarantine. Through these fora he has supported the creation of a number of regional and international standards for phytosanitary measures and guidance documents for both and has recognized the importance of teamwork and collaboration through bringing global experts together.

Eric Allen gave a summary on the history of IFQRG since it formed in 2003. Eric described IFQRG as a policy-focused, science research organization with participants from over 40 countries with a wide background, including scientists, regulators and the industry. IFQRG is not an advocacy group. IFQRG provides independent science/research and analysis in support of international forest phytosanitary policy. IFQRG encourages a culture of discussion, open dialog, and problem solving. Much work is done in association with IPPC and their technical panels and working groups. Historically IFQRG gave significant contributions that includes refinements of ISPM 15, new perspectives on treatment efficacy and others. This year (the third year of virtual meeting) we have had 176 participants registered from 22 countries and the first session on molecular tools was attended by 80 participants.

Day 2 Session Moderator: Dr. Thomas Schröder

Bio: Dr. Thomas Schröder is a forest scientist. He has over 20 years of research experience in forest quarantine, including as head of the forest quarantine laboratory of the Institute for National and International Affairs of the Federal Research Institute JKI in Braunschweig/Germany. Thomas has been working in the IFQRG since its foundation. In relation to ISPM 15, he served on the IPPC Technical Panel on Forestry Quarantine (TPFQ) from its inception until 2016. Among other things, the TPFQ was active in the revision of ISPM 15 and drafting other forestry related ISPMs. He also authored the Guidance Document on the Implementation of ISPM 15 in Germany and is co-author of the IPPC Guidance Document on ISPM 15. Currently, Thomas is a Senior Officer in the Plant Health Unit of the Federal Ministry of Food and Agriculture in Germany.

Thomas Schröder gave a short introduction on the session and remarked that the discussion on phytosanitary treatments has been one of the core activities of IFQRG over the past years. ISPM 15 and ISPM 15 treatments were the starting point of IFQRG as mentioned by Eric A. already. Therefore, the presentation and discussion of phytosanitary wood treatments has a long history in IFQRG. For example, dielectric heating was on the agenda of the first IFQRG meeting in Rome. At every meeting up to now, phytosanitary treatments played a key role. Some of them, such as EDN, where a presentation is in the current section are on the agenda for many years now and both industry and regulators are looking forward to alternatives to MBr and SF fumigations.

2.2 Wood Commodity Systems Approach Guidance – Draft Annex to ISPM 39 NOSEWORTHY

Author: Meghan Noseworthy

Presenter bio: Meghan Noseworthy works for the Canadian Forest Service of Natural Resources Canada as the research manager of the forest phytosanitary research group. She is the Secretary of IFQRG and the new NAPPO Forest Quarantine Research Group, a member of the International Plant Protection Convention (IPPC) Technical Panel on Phytosanitary Treatments and Chair of the expert working group for the draft annex on systems approaches for wood commodities to ISPM 39, *The international movement of wood*.

Abstract: The draft annex to ISPM 39 (*The international movement of wood*) on systems approaches for wood products was drafted in June 2022 to provide technical guidance on the types of phytosanitary measures available to reduce pest risk associated with wood commodities. A description of the components and next steps for the annex are provided.

Discussion:
Comment: It takes years to develop an ISPM. It is often forgotten in the discussion how time-consuming the process is to make the result robust. Simple, short-term changes are not possible.

Comment: The question arose whether a new phytosanitary treatment of wood packaging after use is always necessary. A more environmentally friendly standard is desired. A participant has formed a team to look at different aspects of ISPM 15. However, there is a current lack of young people who can devote themselves to scientific issues.

Comment: A participant supported this aspect. Forestry issues themselves need to be brought to the attention of the younger generation. Only in this way can good forestry practice be developed in such a way that it fits in with current issues.

Comment: A participant noted that the concept of the systems approach fits very well with this demand, both on the scientific and on the administrative side. The individual components of a systems approach could be examined individually in small projects and then brought together to form a whole. Such projects should be interesting for students.

2.3 FAO Forest Health Programme Update SATHYAPALA

Author: Shiroma Sathyapala, FAO

Presenter Bio: Dr. Shiroma Sathyapala, Forestry Officer FAO has been leading the Forest Health and Protection Program in FAO, Rome, Italy since 2014. Through this programme, FAO assists, advises and supports countries and regions to safeguard the health and vitality of forests, forest ecosystems and trees outside forests, with special reference to insect pests, diseases and other harmful biotic and abiotic agents.

Abstract: FAO forest protection and health programme supports countries to safeguard the health and vitality of forests, forest ecosystems and trees outside forests, with special reference to insect pests, diseases and other harmful biotic and abiotic agents.

In 2022, technical assistance to countries addressed issues on forest decline, chestnut blight, enhancing forest resilience towards native bark beetle outbreaks, invasive plant and supported improving institutional capacities. FAO continues to facilitate the Forest Invasive Species Network activities in Africa, Asia and Pacific, Europe and Central Asia and Near East. As part of regional activities, FAO completed a review of early warning and early action systems for major forestry pests in Europe and central Asia. As global activities, FAO is in the process of revising the Guide to implementation of phytosanitary standards in Forestry and creating a new guide to support the development of national forest Biosecurity strategies.

Discussion:

Comment: A participant raised to the problem of the lack of young professionals. How can it be ensured that the work presented is secured in the long term? Sathyapala explains that FAO is working to attract more young people. The aim is to make work in our field more attractive, not only at FAO level. Sathyapala sends information material on recruiting young people to the participant.

Comment: A participant suggested that Sathyapala establishes contacts with interested countries for research cooperation due to her good expertise, which Sathyapala is in favour of. Interest needs to be generated with the relevant governments.

Q: In pandemic times, IFQRG has experienced significantly more participants due to the online format. However, it has also been observed that countries where it is nighttime at event times are significantly underrepresented. Can FAO provide travel funds for participants from developing countries to enable their participation in physical meetings? **A:** Sathyapala explained that face-to-face meetings are important. There are options for cooperative meetings or small meetings especially if a country offers training. Then there could be travel support.

Comment: A participant suggested introducing unknown pests and promoting the concept of "unknown pests" as part of the International Day of Plant Health. another participant suggested that we need more than rules and regulations, but also the way of thinking have to be changed. Education is also needed.

Q: A scientist from a developing country asked for research support in the chat and another participant questioned whether STDF project funding is possible. **A:** Yes, but they have to be very specific.

2.3 Heat treatment based on vacuum/steam technology to eliminate pinewood nematodes in the naturally infested pine logs ZHANGJING

Author: Chen Zhangjing

Presenter Bio: Dr. Chen Zhangjing is a research scientist at Virginia Tech University. His work involves vacuum steam sanitation of logs, pallets, chips and recently fruit. That includes the treatment of *ohia Ceratocystis huliohia* and *C. lukuohia*, Thousand Cankers Disease, oak wilt disease and pine wood nematode.

Abstract: The pinewood nematode, *Bursaphelenchus xylophilus*, is lethal to pine trees. The objective of this study was to evaluate the efficiency and efficacy of heat treatment based on the use of saturated steam at 85 °C and vacuum to eliminate pinewood nematodes in the naturally infested pine logs. Forty, 2.5 m long pine logs with small end diameter ranging from 26 cm to 33 cm, were heat-treated using three schedules of 48 °C/15min, 56 °C/30min and 60 °C/60min to log center at the initial vacuum of 100 mmHg. Survivors were found at 48 °C /15 and 56 °C /30. The pinewood nematodes were completely killed at the schedule of 60 °C/60min. The average treatment time to reach the lethal level is less 668 minutes.

Discussion

Comment: A participant pointed out that in many trials the ISPM 15 parameters 56/30 were confirmed as effective. It is therefore necessary to examine in detail why there were surviving nematodes at 56/30 and even 60/60 in the present trials. Zhangjing confirmed that further trials are

needed. The temperature distribution in the wood could be one reason. The borehole for the probe also needs to be considered in the trials.

Comment: A participant noted that it is important for future trials to understand how the heat distribution occurs in the wood and why there are survivors even though the 56/30 was reached at all measuring points. Zhangjing also considers "cold spots" possible in the wood. It is too early to call for a revision of the ISPM 15 parameters (56/30), more trials are needed.

Q: Since there have been survivors at 60/60: Which treatment parameter needs to be increased? **A:** Both.

Comment: 56/30 is effective for sawn timber. It is not enough for stronger dimensions like logs. The trials made it clear that there were surviving nematodes that could reproduce.

Comment: A participant pointed out that WPM is not always thin dimension.

Comment: It is important to distinguish between the lethal dose and the transmission of that dose. In case of doubt, it is not the dose that is wrong, but a problem of transferring this dose (temperature) correctly to the target object (nematode).

Comment: There are many other influencing factors for an effective heat treatment. Therefore, great caution is required when interpreting the results. Actually, there should be no difference in the effectiveness of 56/30, whether boards or logs are treated.

Response: Zhangjing explains that sampling took place on the day of treatment, there was no incubation of the wood samples.

Comment: supported the comments of the two previous participant commenters. The trials presented were a first investigation with pine logs. There had been good success with other types of wood and other harmful organisms. Logs are probably not the target of phytosanitary treatment as they are not commercially efficient. Possibly the application is more with wood chips.

2.4 Survival of the oak wilt fungus, *Bretziella fagacearum*, in red oak logs fumigated with ethanedinitrile (EDN) YANG

Author: Anna Yang

Presenter Bio: Anna Yang is a Pathways Intern with the Northern Research Station, U.S. Forest Service and a Ph.D. student at the University of Minnesota in St. Paul, MN. She has conducted research related to the detection and management of oak wilt since 2011. Since 2016, she has worked on several phytosanitary trails evaluating alternatives to methyl bromide treatment of oak and walnut logs. Anna has a B.S. in Plant Biology and M.S. in Plant Pathology (University of Minnesota).

Abstract: Following the ban of methyl bromide (MB) fumigation of oak logs with intact bark in 2020, the European Food Safety Authority panel of Plant Health now recommends a systems approach in tandem with sulfuryl fluoride (SF) treatment, yet SF fumigation is unable to fully eliminate *Bretziella fagacearum* in oak logs. Fumigation with ethanedinitrile (EDN) is considered another promising alternative phytosanitary fumigant to MB. We evaluated the efficacy of EDN in a series of experiments conducted in 2020 and 2021 on red oak logs from oak wilt-affected trees by

comparing the rate of *B. fagacearum* isolation before and after fumigation. Logs (mean 15.2 to 91.4 m long; mean 12.2 to 34.7 cm diameter) were obtained from red oak *(Quercus rubra* or *Q. ellipsoidalis*) trees that were naturally infested (NI) or artificially inoculated (AI) with the pathogen. The logs were fumigated for 24, 48, and 72 hours with 120 g/m^3 EDN. Frequencies of pathogen isolation from sapwood chips before treatment were higher for AI logs than for NI logs. EDN treatments greatly reduced the frequency of viable pathogen; however full eradication only occurred in experiments using the smallest log diameters (9 to 14 cm). Our results suggest that, similar to fumigants such as MB and SF, EDN may have limited penetration in green logs.

Discussion:

Comment: The results fit exactly with the previous discussion: dose and dose transfer. The existing laboratory results still need to be transferred to use dimensions, as effective fumigation depends on many factors. Seabright as co-author confirms this.

Comment: A participant expressed concern that wood infested with harmful organisms is circulating worldwide and asks the question what leads to this? Wrong dose, fraud...? What can IFQRG do in this context? A new guideline for ISPM 15 is needed so that phytosanitary treatments are better implemented.

Q: Are EDN and SF as effective as MBr? **A:** Seabright confirms fairly similar efficacy data, however 100% kill rate has not been seen with any of the three fumigants.

Comment: A participant pointed out that ISPM 15 does not speak of 100% kill rate. The treatments include demobilisation in addition to killing. Future research on sublethal effects is needed. Saprophytes may also have an influence on treatment success. If one limits the evaluation of a phytosanitary treatment only to the aspect of killing, one may leave out other important aspects.

Comment: A participant asked for data on complaints and research on the phytosanitary risk of wood packaging. There should also be different analyses, e.g. pallets versus dunnage.

Comment: A participant referred to a publication by Haack that is currently in progress. It is problematic that after 20 years of implementation of ISPM 15 we are still having discussions about effectiveness.

2.5 Summary, additional questions and discussion SCHRÖDER/ NEHME

Schröder thanked all the speakers and participants for their contributions and discussion as well as Kelly and Eric for facilitating the presentations and monitoring the chat. He referred to the first international Plant Health Conference in London from 21 to 23 September, hoping to meet one or the other IFQRG symposium participants there in person. This week will also be historically significant, albeit sadly, as the Queen's funeral was announced on Monday 19th.

Day 3 – September 16: Phytosanitary Treatments

3.0 Greetings & Introduction ALLEN

Day 3 Session Moderator: Ron Mack

Ron Mack is a Commodity Treatment Specialist with USDA-APHIS-PPQ S&T, where he has broad responsibility for treatment development with particular focus on wood. Research interests include new technology development and industrial processes as they relate to commercialization. Ron has been a long-term member and contributor to the International Forest Quarantine Research Group (IFQRG), and has more recently been involved with projects to support NAPPO. He received a Bachelor's degree in Wildlife Management and a Master's degree in Entomology, both from the University of Maine.

3.1 Regulatory aspects of log export JONES

Author: Tyrone Jones

Presenter Bio: Tyrone began working for USDA, APHIS, Plant Protection and Quarantine (PPQ) in October of 1996. Since that time, he has served as a PPQ technician; PPQ officer; a PPQ supervisor; a Customs and Border Protection supervisor for bioterrorism control; a PPQ Export Specialist for Canada and Mexico, including wood packaging material issues worldwide. For the past 15 years he has served as the Trade Director for Forestry Products, managing the import, export, and trade of international forestry products. He also served as a member of the TPFQ; IPPC working groups; working groups for NAPPO; and IFQRG.

Abstract: The presentation will review the regulatory steps involved in the export of logs to a trading partner. It will include the initial request for access and the basic steps followed for adaption of the request by the trading partner; the inspection and auditing processes used to maintain the efficacy of the program; the corrective actions taken through ISPM 13 when issues occur with a shipment; and the final actions which can occur when the trading partner loses confidence in the safeguarding issues taken.

Tyrone began presentation with the industry request to APHIS that typically kick starts the bilateral negotiation involved in the export regulatory process. APHIS requires this request to be written for record keeping purposes. APHIS will then reach out to state (Dep. of Ag), or if concerns a larger region, then US Forestry will be contacted for background information on such things as genus, species, processes proposed, etc. S&T will then review this information, then the completed packet will be sent to the importing NPPO for basic review. Importing NPPO can ask questions on information, then they publish a PRA that will either approve or deny the request. If approved, it moves on to mitigation step, where a procedure is developed by NPPO for impact. Additional review precedes final publication, which can take up to 2 years.

Once approval for access is given by importing country, a program maintenance plan is developed. This involves things like random sampling, log staging that includes sufficient spacing for inspection prior to shipping. All products have some form of treatment (e.g., debarking, KD, fumigation), and these practices are regulated through inspection of required paperwork. Lab testing for some products may be required. No export is allowed until required reporting is completed to the satisfaction of importing NPPO.

Auditing of workplace by both industry and USDA inspectors was discussed. This can be accomplished on either an annual or biannual schedule. HT and fumigations are audited to make sure numbers (HT charts) and quantities (fumigation) match up appropriately for adequate treatment. Regarding conformance, industry and ACO's are both involved. Major conformance issues are subject to suspension until corrections are approved. If there's a problem, importing country sends

back a notice of non-compliance with ISPM-13. Corrective action would then be taken, with records sent to NPPO. If non-compliance continues, procedures may be added to ensure that proper remedy is accomplished. Importing NPPO's may schedule visits to conduct their own audits. They may want to see that a pilot program as an example is performing as it should. An outright ban could happen aimed at a specific level (county, state, region, country) under a worst-case scenario. A plan would then have to be resubmitted and the entire process would start over again.

Q: Does the US have a similar process in place for import of logs to the US? **A:** Information is sent to APHIS, then to S&T for further evaluation. It then goes out for a 30–60-day comment period, where entire world gets to see it. All comments are responded to. There would be internal review after that by a number of departments.

Q: What role would adoption of a new process by exporter such as US have on adoption of process by our trading partner (importer)? **A:** It shows that the country has confidence in the treatment. The import country would still want to do their own evaluation.

Q: Do you ever restrict cutting of logs to certain seasons, such as winter, when pests would not be flying and laying eggs? **A:** If it's in a protocol, then yes, but nothing like that is in there currently. There was some time ago when ash was being moved.

3.2 Update – Global research, registration, and commercialisation activities for EDN™ HALL

Authors: Dr Matt Hall, Phytosanitary & Market Access Manager, Draslovka Services/ PO Box 973, North Melbourne, VIC, 3051, Australia/ Email: matt.hall@draslovka.com / Phone: +61 426 957 165

Presenter bio: Dr Matt Hall is the Phytosanitary and Market Access Manager for Draslovka Agricultural Solutions, dedicated to securing the global registration and approval for the company's environmentally sustainable solutions for the treatment of goods in international trade. Matt has over fifteen years of experience working with phytosanitary products and processes, with a career which spans the public, private and non-profit sectors. Matt has worked as a researcher, consultant, lecturer, and manager. He completed his bachelor's degree in Agricultural Science and PhD in Horticulture at the University of Sydney and his Master of Business Administration at Griffith University. Matt is also an Adjunct Associate Professor at Central Queensland University, where he contributes to the Institute for Future Farming Systems.

Abstract: Ethanedinitrile (EDN™) is being commercialised worldwide as an alternative phytosanitary treatment to methyl bromide (MB) and sulfuryl fluoride (SF) for forest products. EDN is registered in Australia, New Zealand, Malaysia, South Korea, Russia and the Czech Republic (under critical use permit), with approval progressing for several countries. Current investments in R&D are focused on market access and registration activities in Australia, Canada, the USA, and Uruguay. In Australia, the focus is on local efficacy data for *Sirex noctilio* to support bilateral discussions between Australia-India, New Zealand-India and other countries. In Canada and Uruguay, local efficacy studies are required to support the registration of EDN in these countries. In the USA, research is focused on efficacy studies of *Bursaphelenchus xylophilus* in logs and wood chip. In mid-2017, a submission to the IPPC to include EDN as a chemical treatment of wood pests under ISPM 28 was lodged. At that time, it was deemed that there was insufficient data for the committee to make a decision. We are currently working with the New Zealand Ministry for Primary Industries (MPI) as

the NPPO to prepare a new application for the inclusion of EDN as a chemical treatment under ISPM 28. Since the first application, significant global research has been undertaken in New Zealand, South Korea, Australia, the Czech Republic, Canada, the USA, and Russia. In total, over 50 studies on forest pests of logs, sawn timber, and wood chip have been completed under a wide range of abiotic conditions. A new desktop project commenced in early 2022 which aims to consolidate existing raw data for the concentrations of EDN in the treated space over time under different conditions. Collaborators include research organisations in New Zealand, the Czech Republic, Canada, and the USA. This project will improve decision-making processes by creating greater value from existing data that is not publicly available. This information will be used to propose dose by time concentration tables so that commercial fumigators can determine whether a fumigation is successful and whether a top-up dose is possible to avoid a failed fumigation.

Keywords: forestry products, fumigation, ISPM 28, market access

Matt began with an overview of the presentation that described EDN as a broad-spectrum fumigant that is highly effective against timber insects, nematodes and pathogens. The main focus of the company is to place EDN for forestry products and soil, essentially a general replacement for MB and SF.

In comparing EDN and MB, there are a number of advantages for EDN. Low boiling point means no need for a vaporizer. Vapor pressure is roughly double that of MB, so more effective at pushing into voids. EDN is less dense than MB, so this will assist in quicker post treatment ventilation. Specific volume is greater for EDN than MB, so more gas/kg is created. EDN molecular weight is less than MB, so fumigation equilibrium concentration can be achieved more quickly. Lastly, endpoint concentration of EDN is very low, which is advantageous for ventilation and buffer zone creation.

Draslovka is promoting three major uses for EDN in its worldwide quest for registration by individual countries. The first is post-harvest phytosanitary treatment of import and export timber and logs for control of insects, nematodes and pathogens. Second is pre-plant soil treatment for control of nematodes, pathogens, and weeds in turf grass, sports turf, and golf courses. Third is pre-plant treatment for control of nematodes, pathogens, and weeds in some horticultural crops such as strawberries, melons, and cut flowers.

Matt described the evolution of registration in individual countries, beginning with Australia in 2013. He makes the point that registration is different than market access approval. A number of countries have active registrations for timber and logs, including Australia, South Korea, Malaysia, Russia, and New Zealand. A number of additional countries are working on registrations for timber and logs, and these include the United States, Uruguay, EU, India, South Africa and Canada, which he hopes will be complete sometime in 2023-24.

Matt worked through an example of Australian R&D to highlight operational aspects of EDN fumigation of Eucalyptus logs in ship's hold to be exported to Malaysia. 100 g/ m^3 for 24 hours in a very large space (8,153 m^3) with 56% load factor and 14-18 °C temperature. Matt touted the value of additional application lines for this kind of fumigation as it assists gas diffusion.

Another example study on NZ R&D using EDN on bark beetles in lab through scaled-up work under tarp. Large insect replicates in excess of 30,000 beetle life stages total (larvae, pupa, adults). In all cases, mortality was 100% at dose and time specified.

Canadian R&D example highlighted work on PWN in blocks and logs. These EDN fumigations were conducted at lower temperatures (10-14.2 °C) and at 50 and 100 g/ m^3 across four exposure times (1,3.6, 12, 18, and 24 hours). No PWN survivors were found after 6 days in all treated material. For comparison, commercial application would be 80-120 g/ m^3 for 24 hours.

USA R&D example with EDN on PWN in white pine blocks and woodchips. The wood block experiments were conducted at low temperatures (5, 10 °C) over a range of doses (0-150 g/ m^3) at 24 hours and were completely effective at killing all PWN at concentrations of 30 g/ m^3 and above. The work with woodchips resulted in complete mortality of PWN at 75 g/ m^3.

Preliminary work on oak wilt and thousand canker disease in USA R&D trials was accomplished at 120 g/ m^3 for 24 hours. Treatment volumes were in excess of 52 m^3 under tarpaulin, and 68 m^3 in container. Pathogen growth was monitored for 21-27 days post EDN fumigation, and no viable pathogen growth was observed after this period.

Draslovka submitted an application in 2017 for ISPM 28 consideration, but it was judged that there was insufficient data for a decision. Given the number of promising EDN treatment studies conducted since that time, a resubmission is planned for the end of 2022. Matt also discussed the compilation of 26 separate reports on EDN that are to be reviewed against the requirements of ISPM 28.

Going forward, Draslovska is working with NPPO's on commercialization aspects, to include decisions on what is considered a good EDN fumigation, and what would be considered a failure. It's important to identify CTs from successful fumigations as part of this exercise. Matt brought up an example of the kind of information that can be gleaned through publications (e.g., effective dose for control of nematodes/pathogens at different times and temperatures, initial and final concentrations), and also highlighted information generally not provided in publications that would be useful for discussion on commercialization, like CT values and concentrations in the treated space during fumigation.

In an overview of the project concept, operational monitoring requirements are being considered and proposed. Sampling tube placement, monitoring table development (similar to MB), and confidence around CT calculations were discussed.

Q: EDN does disassociate into components. What is actually measured? **A:** In 2018, we put out a publication on this matter. EDN is highly soluble and sorbed. HCN is a breakdown component. HCN in empty trailer space and in treated space is essentially the same.

Q: Is EDN easily available and costly? **A:** In a recent Europe meeting, EDN was discussed at $16/kilo in AU dollars. This is comparable to MB, and less than SF. The challenge for EDN is the commercial dose rate of 80 - 120 g/ m^3, and MB concentrations are typically less, so MB may be less expensive. But on a per kilo basis, EDN and MB are about the same. Regarding SF, as we move to carbon neutrality, offsets will potentially add costs to use of SF.

Q: Can you talk about penetration depth into softwoods and hardwoods? And is it easy to recover EDN when released after a cycle so that it can be used again? **A:** EDN penetrates well and is more consistent than MB. In wet timber, everything is difficult. We have good comparison data between hardwoods and softwoods. Much better on sawn timber, and insect galleries help. As a broad summary, EDN is as good or better than MB at penetrating bark. As far as recovery, there is no EDN

left essentially. 700 ppm. This keeps aeration times down and is better for bystanders and buffer zones.

3.3 Carbon and the Wood Packaging Industry: Finding a path forward GETHING

Authors: Brad Gething

Presenter bio: Brad Gething, PhD, is the Vice President of Science & Technology for the National Wooden Pallet & Container Association. Brad works with wood pallet standards, initiates and oversees industry research projects, and serves as lead point of contact for PDS™ pallet design support and education. He has been a member of IFQRG for over 10 years, dating back to his work as a graduate assistant helping to develop DH heat treatment schedules for ISPM 15. Brad earned a MS and PhD degree in Materials from Penn State University, and a BS degree in chemical engineering from Bucknell University.

Abstract: This presentation will lay the groundwork toward understanding the challenges and opportunities that are emerging for the wood packaging industry as the world becomes more carbon conscious. Several facets of carbon accounting and accounting will be explored, with he intended purpose of creating a dialogue within IFQRG to identify research and project priorities.

Brad begins by discussing the overall purpose of the presentation, beginning with conversation starter, then determining importance of this topic to IFQRG. What does IFQRG want to do on this topic, if in fact they consider it important enough to work on? Looking for a path forward as an interested group.

Focus on substance, not rhetoric. This begins with an environmental product declaration, or EPD. It's essentially a communication tool of environmental impacts. It gives users, consumer an idea on environmental impact.EPD's help purchasers better understand a product's sustainable qualities and environmental repercussions. Main objectives of an EPD are development of credible and transparent eco-labeling, provision of Life Cycle Assessment (LCA) information on wood products (similar to nutritional labels), and maintenance of open markets. What is the value of an EPD? NWPCA EPD is 3rd party certified, UL in this case. NWPCA worked with experts in the Forest Service - life cycle assessment done by them independently. This EDP was the first of its kind in the packaging, distribution space. Until now, EPD's mostly in the building industry. NWPCA tried to raise the bar in terms of transparency, reliability of data.

Looking at a table of life cycle assessment results. Focus on GWP, which is standard approach to CO^2 equivalency, for 100,000 lbs of pallet loads of product delivered with wooden pallets. GWP calculations across many segments of industry (e.g., tree growth and harvest, transportation, manufacturing, use and recycling, and end of life) was performed. Certain practices in industry led to carbon reduction. These offsets exist because of valuable industry practices such as coproducts-thermal recovery in wood boiler, end-of-life-thermal recovery in wood boiler, reuse of boards, and steel recycle. The reason these offsets exist is due to the unique nature of wood. Sawdust can be processed as boiler fuel. Reuse of board components can help provide new pallets. This all leads to carbon reduction. Valuable industry practices have been around awhile but have been mostly thought of as value added in the past.

A discussion on sustainable forestry carbon cycle emphasizes that carbon in trees comes from the atmosphere. This idea of borrowing carbon from the atmosphere makes it unique. Let's look at the components of the carbon cycle for better understanding of carbon storage and where it's liberated.

Beginning with the atmosphere, companies and governments are now responding to climate change. Carbon reporting and carbon credits are important pieces from an industry perspective. CO^2 concentrations over time are measured and inform policy. Forest dynamics affect industry, and there are a number of categories to consider (carbon credits for timber preservation, sustainable forest management, more carbon sequestration the better, and forest decay rates).

With regard to WPM manufacturing, companies want to know the carbon footprint. Managing this footprint, and also determining the "built environment" with respect to carbon storage are critical to businesses getting a handle on this. From new WPM introduction, through recycling to end of service, companies are following this closely. WPM reuse/repair maximizes service life, and also reduces timber harvest. WPM recycling/end of service promotes extended service life.

WPM landfilling offers the opportunity for sequestering carbon under anaerobic conditions. NWPCA research has found that pallets in general have a 95% recycle rate. We may not like the idea of landfilling pallets, but there are advantages from a carbon sequestration standpoint.

So how does this all relate to IFQRG? When we consider heat treatments, we found from a 2016 US industry survey on pallets that 97 million kg of CO^2 per year was produced to heat treat pallets in the US. There is then serious carbon implication to heat treating, and these implications as well for any adjustment made in heat treatment schedules. This is something to consider by the group.

As forests become more susceptible to decay, there is a carbon implication here as well. Brad referenced a paper by Leigh Greenwood that focuses on this issue of forest decay. Healthy forests mean less susceptibility to decay, with a benefit of more ability to sequester carbon. The wood products industry clearly has a role to play here.

Where to go from here? There's a great opportunity to crunch the numbers and better understand the true implications of carbon. Let's think about how we as a group (IFQRG) might work on this together.

Q: Has anyone compared carbon impact of plastic vs wood packaging? **A:** Chuck Ray, while at Penn State, did an LCA on this, and it's available in the literature. Take home message is that it's more favorable for wood than plastic.

Q: Is there anything in the latest [US] Climate Bill that industry could advantage from a carbon perspective? **A:** Our Public Affairs person handles this for NWPCA. Let me just say how do we increase our cogeneration opportunity? It's a pretty big capital expense, and there's clear carbon savings on this. How do we make this easier? Electric forklifts a huge opportunity. They are quieter, offer ergonomic advantages. There's a clear carbon advantage there.

Comment: One of the roles IFQRG has is that we are concerned with carbon outputs, and minimizing these as best we can. We recommend treatments, so we should start to calculate the costs of these, along with the costs of any retreatments. We should balance retreatment in ISPM-15 as an example against carbon costs and come up with the math so that we can make good decisions.

Q: Can you estimate the CO^2 impact for a 1 °C increase in 56/30? **A:** Mark Gagnon at Penn State has done work has done work as an example on efficiency of RF versus conventional heat treatment. Maybe we could get Mark more involved in this by crunching numbers. Kelli mentioned that RF has proven to be more efficient, and Karolina will discuss this in the next talk.

Comment: Just to follow up on what a previous participant said, with recycled pallets, we should look at effects of desiccation of pallets, whether kiln dried or organically dried, in the supply chain. Are we having a positive impact on phytosanitary safety? Efficacy of treatment versus excess. At what point does benefit of one outweigh the other?

3.4 Radio Frequency (RF) Phytosanitation of Wood Packaging Material (WPM); Update on Development of Certification Protocols and Advancements for RF Technology SZYMONA

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Presenter Bio: Dr. Karolina Szymona obtained her Ph.D. at Warsaw University of Life Sciences in Forest Sciences. She works as a researcher at Penn State University in the project related to the development of the radio frequency phytosanitation for wood packaging materials in compliance with ISPM-15 international standard. She conducts experiments and assists with data and economic analyses and extension activities related to the project.

Abstract: Our Penn State University research team, with the support of USDA NIFA funding and in collaboration with USDA APHIS, is developing a radio frequency (RF) sanitizing method for wood packaging material (WPM). We are in the final stages of development, focusing on optimizing the process to provide reliable and cost-effective methods of monitoring the temperature of the workload to verify that it reaches 60 °C for 1 min per the ISPM 15 treatment standard. Methods of controlling and verifying the temperature are crucial in developing the certification protocol for RF treating units. Representatives from the Canadian Lumber Standards Accreditation Bureau (CLSAB) and American Lumber Standards Committee (ALSC) are assisting and providing feedback as we develop protocols to certify RF treatment units in compliance with ISPM-15.

Karolina begins with an overview of the dielectric standard in ISPM-15, which calls for 60 °C/1 minute hold throughout the profile of the wood. The current RF testing unit was built for the research group in 2017 as a semi-commercial unit, capable of treating 1200 bd ft or 3 m^3 of dead stacked material. This would equate to 82 standard GMA pallets.

The RF unit was recently upgraded at the end of 2022 with a solid-state power system (50 KW) replacing the older oscillator-based system. The original 5 electrode system (top and bottom, with 3 intermediate electrodes) was replaced by a single intermediate, winged electrode with the original top and bottom electrodes retained. This new 3 electrode plate configuration improved on overall heating efficiency from 62% for the older system to 95% for the finished upgrade. The single intermediate electrode also allows for ergonomic advantage as it makes loading easier.

A review of past work includes a number of replications on recycled pallet material that included fasteners, to be sure that metal embedded in the wood didn't create arcing within the chamber. Additional studies included work on salt-soaked wood to see if improved conductivity would lessen treatment times, an important consideration for dunnage material at ports that could be soaked in salt water prior to treatment. Grain direction was also assessed in the form of pallet blocking, which is one of the most difficult pallet components to heat in a timely manner.

The focus on testing with the upgraded power source and winged electrode has been on common pallet species and dimensions. Replicated testing on Eastern white pine dunnage, along with red oak and tulip poplar stringers. Spruce, pine and fir (SPF) will be tested in the near future.

Temperature monitoring has been accomplished through a number of methods to date. These include fiber optics, FLIR thermal images, pop-up thermometers, and dedicated probes at end of stack that monitor air temperature. The hope is that air temperature can be correlated with the in-wood probe temperatures to allow for a simplified commercial monitoring that just involves air temperature alone.

Karolina showed an animated video of the chamber with loading and unloading wood sequence.

A bit more on the pop-up sensors that are placed in all boards within a stack to ensure that every board is meeting minimum temperature requirement of 60 °C for 1 minute. These pop-up sensors actually are calibrated for 62.8 °C, so there's a level of conservatism that ensures that all boards reach 60 °C. These sensors retail for approximately 10 cents per piece commercially, but bulk commercial purchase could bring cost down further. They are uniformly 1 and ⅞" in length. The company would make custom sizes for an additional cost.

A sample chart of red oak and poplar stringers and their heating times is displayed with corresponding air temperatures. Heating time for wood is just over 60 minutes, but the tests were extended so that air temperature data could be gathered for analysis.

A slide of thermal images post treatment shows a dramatic difference in heating uniformity between the older 5 plate system and the upgraded 3 electrode plate system with winged electrode. The winged electrode helps to redirect the electromagnetic field over the outer edge of the stack, which is typically the coolest location.

A bit on industry engagement and outreach. Prior work has involved 11 pallet manufacturers total, with detailed analysis on 4 of these businesses. Presentations have been accepted by the Forest Product Society.

Anticipated work includes engagement and facilitation of RF commercial unit adoption with North American pallet manufacturers, to include selection of the right operator, SBIR and/or grant relief for tech adoption, and joint assistance tech support and data sharing. The RF research group will also be involved with technology dissemination, assist with IPPC dielectric guidance document, and make port visits on behalf of RF evaluation and placement for dunnage treatment.

For takeaways then on this presentation, here are the following:

1. Upgraded RF unit provided enhanced treatment time and uniformity
2. Pop-up thermometers and monitoring of the chamber air temperature might provide an easy and inexpensive way to confirm treatment completion

3. Additional replicated tests are needed to inform certification protocol

Q: Is there a measurable correlation between the air temperature in the kiln and the actual surface or subsurface (not at the core) temperature of the wood itself? I assume the air temperature is directly related to/influenced by the wood temperature as the air is not actually being heated by the RF waves? **A:** We didn't look at surface temperature of wood correlated with air temperature. There is some heat radiating from the workload. Placement of probes for air temperature matters. They should be placed in the same position for a consistent result. Air temperature probes placed at the end of the stack at the door end. Kelli added that surface temperature is always cooler than the core. Getting heat through the profile of the wood is not difficult. Waves penetrate the whole stack. Ron discussed the edge of stack being the coolest location, and how this offers advantage from a monitoring perspective. Addition of a pressure envelope actually hold escaping steam/heat to the stack and improves heating uniformity along the edge.

Q: In other treatments, we've seen failure due to delivery of dose as we've discussed. It seems that in this case that we can deliver the RF treatment consistently throughout the load? Is there a way to measure all spots so that we are absolutely sure that there is no treatment failure? **A:** Pop-up thermometers ensure that temperatures are received by all boards in the stack. The 63 °C calibrated pop-up is actually a conservative measure to ensure that all boards have reached 60 °C minimum.

Q: Because of the very good penetration of RF (vs MW) into the wood, do you think it is worth it to continue research on MW in the frame of ISPM-15? **A:** Our research team chose bulk treatment with RF, as this appealed most to industry. We're also comfortable with our findings on economics of RF bulk treatment that this approach will be desired and placed in industry. MW system is one piece of wood at a time through a conveyed system due to limited penetration. We don't work with MW, and haven't worked through economics of its use for WPM, so can't speak to whether or not it has a place in industry.

Q: Could you introduce which part is the coldest point? And what is penetration depth? **A:** The outer edge of the stack is the coldest area, and penetration is meters using RF.

Q: Have you tested mixed woods in a load, as this is commonly done in industry? **A:** Yes, we have run spruce- pine- fir and plan to run more stringers with mixed softwoods. We also plan to run mixed hardwoods. Our independent trials of red oak and poplar gave similar treatment times, and we're encouraged by this due to disparity in density between these two species. This bodes well for a positive mixed hardwood result.

3.5 Mortality of bark- and wood boring Coleoptera exposed to different chamber and wood core temperatures PETRICE

Authors: Toby R. Petrice and Bob A. Haack, USDA Forest Service, Northern Research Station, Lansing, MI

Presenter Bio: Since 1997, I have worked for the USDA Forest Service, Northern Research Station, focusing on the biology, ecology and management of invasive wood boring insects including pine shoot beetle, Asian longhorned beetle, European oak borer, and emerald ash borer. My current position is a Research Entomologist with a major focus on the biological control of emerald ash borer.

Undergraduate: Fairmont State University, Fairmont, West Virginia, 1994.
Masters: West Virginian University, Morgantown, West Virginian, 1997
PhD: Michigan State University, East Lansing, MI, 2020.

Abstract: We conducted studies to determine the survival of the bark- and wood borers in the coleopteran families Buprestidae, Curculionidae, and Cerambycidae when exposed to different chamber and wood core temperatures, with a focus on the current ISPM15 heat treatment standard of 56 °C wood core temperature for 30 minutes. Treated bolts were cut from naturally infested trees and exposed to chamber temperatures ranging from 60 to 75 °C and wood core temperatures ranging from 50 to 60 °C. Results demonstrated that most borers were killed at core temperature of 56 °C and mortality increased with increasing chamber temperature, while no borers survived wood core temperatures of 60 °C. Study results will be published in the Journal of Economic Entomology in late 2022.

Toby introduced the topic and mentioned that the study was actually accomplished in 2010 and 2011, and they are just now publishing the results. A rundown on the common bark and wood infesting borers. There are thousands of species worldwide in the major groups that we considered here for the study. For example, Scolytinae has over 6,000 species worldwide, with more than 600 found in North America and approximately 70 species considered exotics here in the US. Similarly, Buprestidae (> 15,000 WW, >700 NA and 20 exotics in US) and Cerambycidae (>36,000 WW, 1,200 NA and 20 or so exotics in US) groups present challenges as exotics here in the US.

The feeding strategies of these groups vary, with a general rule that insect development is faster if feeding on the outer portion of the tree (inner bark, sapwood) versus the heartwood or near center. Wood moisture also has effect on development rate, with drier wood generally coupled with slower development.

Some examples of high profile borers were discussed, with Asian longhorn beetle, Emerald ash borer and Redbay ambrosia mentioned as exotic introductions, whereas walnut twig beetle and goldspotted oak borer are endemics that have moved geographically within North America to create problems.

An overview of papers that address heat treatments for EAB was discussed, and the differing methods were highlighted to offer insight as to why the results differed. McCullough et al. (2007) looked at survival in wood chips. Test 1: 40 °C and 60 °C for 8, 24, 48 hours. All died at 60 °C. Test 2: 40, 45, 50, 55 and 60 °C for 20 and 120 minutes. All died at 60 °C for 120 minutes, 17% survival at 55 °C /120 minutes. Myers et al (2009) conducted heat treatment in split firewood. Test 1: 50, 55, 60, 65 °C for 30 minutes at depth of 3.5 cm. All died at 65 °C, a few survived at 60 °C and below. For this study, probe was inserted 3.5 cm, but firewood averaged 14 cm in thickness. Goebel et al (2009) also tested EAB in split and whole firewood. Test 1: 46 and 56 °C for 30 and 60 minutes at a depth of 2.5 cm. Some EAB survived all treatments, but over 85% mortality at 56 °C. This study probed to 2.5 cm depth, but firewood size varied. Sobek et al. (2011) looked at heat shock protein response of EAB in vitro, no survival at 56 °C. Lastly, McQuarrie et al. (2020) conducted the most realistic approach that evaluated milling process as well as the heat treatment of EAB. No survival was observed at 56 °C, and milling process killed most insects. Maximum wood thickness in this study was 6.25 cm.

Toby and Bob wanted to design and conduct a realistic evaluation of the ISPM 15 standard (56 °C for 30 minutes) by comparing survival across different chamber and core temperatures for a range of borer species. They settled on 4 major *Agrilus* species (EAB, twolined chestnut borer, European oak borer, and bronze birch borer), *Ips* species, Cerambycids *Monochamus* and *Acanthocinus*, and the

Curculionid *Pissodes.* In ash, the EAB larvae were mature J stage in high density numbers. For the pine borers, trees were cut in summer, then allowed to become infested.

All wood material for this study were cut bolts with bark intact. Dimensions weren't discussed, but it appears that bolts were in the range of 15 cm diameter and 75 cm in length. Check with paper on this. To prepare for heat treatment, each bolt was drilled in two locations. One hole was to center at the midpoint of total length, the other hole was drilled at a depth of 1 cm at the midpoint of length. T-ype thermocouples were then inserted, and hole was backfilled with a combination of sawdust and toothpick, then sealed with plumbers' putty. Probed bolts were treated in a Lunaire steady state chamber at a ramping of 1 °C/minute. Each log was removed after 30 minutes at target core temperature. Core temperature target temperatures included 50, 53, 56, 60 °C groups. Chamber target temperatures included 60, 65, 70, and 75 °C groups. Treated and control bolts were stored in tubes on a rack, then dissected at a later date. For EAB, percent survival was positive up to 65 °C chamber temperature for 50 °C core temperature. At 53 °C core temperature, there was some survival at 60 °C chamber temperature only. For 56 and 60 °C core temperatures, there was no survival at 60 °C and 65 °C and above chamber temperatures, respectively. For bronze birch borer, there was no survival at 56 °C and 60 °C core temperatures for any of the measured chamber temperatures. For twolined chestnut borer, there was some survival at both the 53 and 56 °C core temperatures, but not at the 60 °C core temperature. European oak borer had some survival at 53 °C core temperature up to 65 °C chamber temperature. It also had some survival at 56 °C core temperature at 65-75 °C chamber temperature, and interesting finding. No European oak borer survived the 60 °C core treatment. Pine borers at 56 °C to core had Ips surviving up to 70 °C chamber temperature, Cermbycidae surviving up to 65 °C chamber temperature, and *Pissodes* weevil completely killed across all chamber temperatures. *Chrysobothris* also had a few survivors at 56 °C core temperature. At 60 °C core temperature, *Ips*, Cerambycidae and *Pissodes* were all killed.

To summarize the study, the ISPM 15 standard significantly reduces, but does not eliminate borer survival in round bolts with bark on. No borers were able to survive the 60 °C core treatments. Generally speaking, increasing chamber temperatures increased mortality. This was even true of the tests at 53 °C core temperature. Survival varied among borer species and/or tree species. This could be due to some level of interspecific heat tolerance, or some wood related factor like wood moisture, density, species, etc. All these considerations may have impact. Lastly, we should be considering a systems approach for wood sanitization. The milling process greatly decreases the number of insects present. MacQuarrie study discusses this. Also, important to remember that WPM is different from roundwood or firewood with bark in place. This last sentence is important to remember. The material used in this study would not qualify as WPM, so the results here should not be taken as a charge to replace the existing standard.

Q: Why choose a ramping temperature of 1 °C? **A:** I don't actually remember; it's been so long since the study was initiated. The idea was to select a rate that approximated smaller industrial kilns. A participant suggested too that this is close to the ramping schedule that currently exists in industry. Haack mentioned that at the time the study was designed, other papers suggested this rate was appropriate and seemed reasonable.

Q: Did you verify if surviving insects would have been able to complete their life cycle or reproduce? Were they neutralized even if not dead? **A:** We didn't do that. It could be that many insects had shorter lifespans or were not able to reproduce. If beetles were less healthy, they could reproduce

less. For *Agrilus* as an example, you need fresh foliage to continue the development. It would have been nice to do this.

Q: Some people may look at this result and feel that 56 °C /30 is not adequate, and a change to 60 °C is warranted. Should we focus on temperature, or maybe modify guidance and how we deliver dose and run our kilns? **A:** No need to raise temperature, for most life stages are reduced in milling process. The 60 °C schedule is designed for sawlogs and firewood.

Q: How long did you track survival? **A:** We didn't do this.

Q: Wood boring insect pests are serious pests worldwide. In Ethiopia, there's a wide research gap in identification and management of longhorn beetles. Young Forest Entomology researchers want to collaborate with any senior entomologist. Is there any chance for research collaboration? **A:** I don't know. I'm sure there's someone in US who would be interested in collaborating.

Q: Did the wood temperature probe get inserted into the geometric center of the logs? **A:** Yes, the hole drilled was at geometric center of length, and also of diameter of the bolt.

Q: Except for dunnage, wood packaging is made from milled lumber. Given that ISPM 15 is based on a systems approach, debarking, sawing and HT 56 °C/30 mins. Thus, should the science community differentiate dunnage risk vs other WPM, e.g., pallets, skids, and crates? **A:** Good point. Survival will vary with amount of processing. Dunnage still required to be free of bark per ISPM 15. Ron mentioned that NAPPO group intends a special focus on dunnage here shortly once project receives the go ahead.

EXTRA QUESTION SESSION

We started with more thoughts on the carbon presentation, as we had some outstanding questions remaining there.

Comment: In the spirit of IFQRG, anyone on the call want to get in touch with Brad Gething and begin this process of crunching numbers, please feel free. At the Pacific Forestry Centre, there's a carbon accounting team that could conceivably help with this.

Comment: The paper that Brad alluded to on quantification of carbon…non-native forest pests are variable, some very low, some very high, so it's difficult. It's important to quantify things like increase in treatment temperature vs potential losses. Science is tricky, so this is a firm justification for getting it done.

Q: Brad, you have expressed in the past that your group may have money available for projects like this, true? **A:** Yes, a clean focus on outcome needed though. We'd need to communicate this back to them for consideration. But yes, there are many complications to this whole thing that I didn't even touch on. One being this: currently there's perspective on how you do carbon accounting on consumption of a standing tree. Idea is as soon as you cut a tree down, it can no longer sequester carbon if you limit production and allow tree to stand, you sequester carbon and therefore there's a benefit to that. Increase recycle loop of pallets leads to carbon savings because you're reducing production and sequestration potential of a standing tree. In my mind, you're planting a tree right behind a tree you just cut. From a pure carbon standpoint, not sure whether to keep tree standing, or

plant behind it. There's a lot to unpack here. I'll continue to work on it anyway. If we need to fund to substantiate participation, we may be able to do this.

Comment: Look at all steps in the supply chain, then look at sequestration and put a number to that. May be a good first step.

Comment: Start with the carbon cycle. It's a big ecological question. What happens after the tree is down.

Comment: Just a comment on the idea for supply chain analysis regarding carbon. We're putting out a paper that will provide structure for carbon sequestration, storage, release…similar to pests in and out that has been suggested.

A shift in the general discussion now away from carbon, and back to Matt Hall presentation on EDN.

Q: How far away is the world from using EDN? What are the next steps? What happens to all member countries after ISPM 28 resubmission? Matt, any questions or comments from you on next steps? **A:** No, not specific questions. It's clear to me that global QPS consumption of MB stayed the same at 10,000 tons/yr. It has stayed at that level for the last 25 years. It doesn't seem to me that there's a strong desire at the individual country level to change usage pattern. It's important to economies. Non QPS use around MB has been effective. At the other side, the amount of time it takes to register is long. EDN is toxic and needs to be taken seriously. It just takes a long time to get through registration. Market access needs to be worked through, there are certainly challenges there. Regulatory support to get chemicals registered is not there. EU as an example is difficult. Not a criticism, just an observation.

Q: Just to the wider comment earlier on EDN. Every IFQRG meeting we've had presentations. The global community doesn't seem to be making much progress on adoption. There's convincing argument, but something seems to trip up. Are we getting closer to a viable alternative fumigant to MB? **A:** Mike indicated that EDN is on a good trajectory. There's a serious plan now in place to get there. Going through the process of checking all the boxes. The first 10-15 years with CSIRO, not so serious. Draslovska is approaching this the right way now. Meghan mentioned that Matt's presentation of new information should help.

Day 4 – Sept 30: Surveillance Tools – New Tools and Technology

4.0 Greetings and Introduction ORMSBY/ ALLEN

Session Moderator: Dr. Kelli Hoover

Bio: Professor Kelli Hoover from Penn State University is internationally recognized for her research on invasive species biology and ecology; she has been working on a team for many years on development of radio frequency for treatment of wood packaging materials in compliance with ISPM15 and has been a member of IFQRG since 2006.

4.1 Enhancing implementation of ISPM 15: Regulation of wood packaging material in international trade PETERSON/ KISS

Authors: Barbara Peterson and Janka Kiss

Author bios: Barbara Peterson is a Canadian Food Inspection Agency Official who is on loan to the International Plant Protection Convention (IPPC) Secretariat. She has been working with the Implementation and Facilitation Unit since April 2019. Barbara's main areas of responsibility include coordinating the IPPC's work on e-Commerce and managing the development of IPPC Guides and training materials. She is also responsible for facilitating the development of an IPPC Guide to support the implementation of ISPM 15.

Janka Kiss is a Standard Setting Associate at the IPPC Secretariat. She is mostly working with the Technical Panel on Phytosanitary Treatments since 2016 and holds a degree in plant protection engineering and horticulture. She is working with the Standard Setting Unit since 2016 supporting the Standards Committee's work and coordinating expert input to drafting ISPMs and phytosanitary treatments.

Abstract: The new IPPC guide and its associated treatment manuals will provide practical guidance and case studies to assist national plant protection organizations (NPPOs) with implementing ISPM 15: *Regulation of wood packaging material in international trade.* The guide includes guidance to help NPPOs authorize treatment providers, wood packaging producers and repairers and the treatment manuals provide specific information on applying ISPM 15 approved treatments to wood packaging material. The guide also describes import procedures, the importance of controlling the use of the ISPM 15 mark, the process for identifying non-compliant wood packaging material, and encourages collaboration and communication with other IPPC contracting parties and stakeholders.

Janka Kiss reported on the structure of IPPC governance (see also Noseworthy IFQRG presentation), the functions of the Secretariat and the standard setting process, pointing out that it takes about 5 years from the initiation to the adoption of a standard. She noted that the CPM-16 agreed to retain the topic *Criteria for treatments for wood packaging material in international trade* following the publication of Mike Ormsby's paper, *Elucidating the efficacy of phytosanitary measures for invasive alien species moving in wood packaging material,* in January 2022.

Barbara Peterson reported that the IPPC Guides and training materials for ISPM 15 (HT and fumigation) are in progress. The Treatment Manual has been drafted, peer reviewed and the WG and sub-groups will be reviewing comments. A call for topics for new ISPMs will open next year from May-Sept., submissions go through contracting parties or regional plant protection organizations (RPPOs).
https://www.ippc.int/en/core-activities/standards-setting/list-topics-ippc-standards/list

Questions
Q: What is the status of the Dielectric Heating Implementation Guide? **A:** (Janka) The publication of the implementation guide for DH treatment is pending due to an objection by one of the CPM contracting parties. Barb added that there are existing treatment manuals which have some information on applying DH. There are also some licensing and intellectual property issues.

4.3 Efforts to improve surveillance efficacy for detection of exotic jewel beetles SWEENEY

Authors:

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Presenter Bio: Jon Sweeney is a Research Scientist with Natural Resources Canada, Canadian Forest Service, Atlantic Forestry Centre in Fredericton, New Brunswick. Jon studies ecology and management of forest insect pests with a focus on invasive species. The goal of much of his research is to develop effective tools and strategies for surveillance and control of invasive species inadvertently introduced to North America and other continents via global trade. He is Adjunct Professor at the University of New Brunswick and Acadia University and Subject Editor for the Canadian Entomologist and Journal of Pest Science.

Abstract: There are more than 3000 *Agrilus* species worldwide, many of which breed in tree genera common to forests in North America, Europe and Asia. Phytosanitary treatment of wood packaging has reduced but not eliminated the risk of live insects in shipments and the sheer volume of global trade ensures continued arrival of live woodborers in new habitats. Some of these arrivals may become damaging pests, e.g., emerald ash borer, *Agrilus planipennis*, (EAB) in North America and Eastern Europe. I will present results of field trapping experiments conducted in Canada, China, England, Italy, Poland, and the USA designed to determine ways of improving surveillance of jewel beetles.

Jon reported that EAB prefer green and purple to black traps and that unbaited traps are more effective for Agrilus spp., particularly when place in the sunny part of the tree canopy. Their work also showed that ketols-diols work best for trapping cerambycids, add ethanol for Scolytinae.

Q: Were traps placed in healthy or stressed trees? **A:** No attempt to target stressed trees.

Q: Does it work to put out different types of traps in the same location (some with ethanol some with Keto diol etc.? **A:** Unless the traps are 10 m apart they won't interact, good to have a variety of traps. Combined treatments increase the species caught. For surveillance variety is what you want to go for.

Q: When NZ uses trapping and surveillance for new incursions, we want to know how sensitive they are so we use mark recapture are you thinking of doing that? **A:** We tried that with *Tetropium fuscum*, but this doesn't need to be answered, what does one beetle in a trap mean? We did this with BSLB for range of densities and girdled trees and concluded that one BSLB in a trap was related to density in the host. *T. fuscum* likes girdled trees better. Trapping experiments tell us what works better for

capturing but little idea of what this means for density. It tells you that a beetle is present, not how many. If you don't find it doesn't mean it isn't there.

4.4 Comparison of Intercept Trap Fluids and Aerial Spore Collectors to Survey Fungal Spores BÉRUBÉ

Authors: Jean A. Bérubé, Jeremy D. Allison, Kate Van Rooyen, Cory Hughes, Patrick N. Gagné, Isabelle Ochoa, Jon Sweeney

Presenter Bio: Jean Bérubé is a forest pathologist with Canadian Forest Service in Québec city. He works on early detection of forest invasive pests.

Abstract: Surveillance for early detection of non-native, invasive pathogens requires simple, sturdy, and easy to use collecting devices. In this study, we compared the fungal species detected in wet collection cups of Lindgren traps versus those detected on slides with oiled cheesecloth as aerial spore collectors. DNA was extracted and amplified from both using the primers ITS1F - gITS7G, and Illumina sequencing was used for metabarcoding of fungi present in samples. In 90 samples there were 1277 fungal operational taxonomic units (OTUs). For fungal OTUs only detected by one collection method, insect traps had three times the number of fungal OTUs compared to slides, and this pattern persisted when analyses were restricted to pathogens and forest pathogens. Annually, thousands of insect traps are deployed in North America and the associated trap fluids have added value in forest disease research and monitoring. Jean also reported that greater species richness was found in trap fluids than aerial spore collectors with no significant difference between urban and forest sites. Similarly the abundance of fungal DNA was greater in traps (for forest pathogens) and greater in forests than urban environments. He added that one of the limitations is that we don't know if detection is of a living organism, but can follow up with qRT PCR if organism is of concern to see if it's alive and presents a risk.

Comment - From regulators point of view these studies make us nervous because we require species presence; this how is we regulate products between trading partners. What you mentioned in the latter. Publishing with parameters is important, if taxa are present or if further work needs to be done. These studies can put a halt to trade.

Q: –Did you find species you didn't think you had? Indicated presence you didn't know about? **A:***Diplodia corticola* on oak was suspected, found in the USA. CFIA said to be careful; it may show up and yes, we found it. It was found in Quebec City but there are no sick or dead trees. We are certain it is present and established but hasn't shown as a disease. Also preservatives don't just have fungal spores but Insect DNA. A grad student is looking at preservative to see what insects are present.

Q: Follow up on concern about the presence of pest DNA versus its establishment. RNA needs to be there [indicating a living pest], how would you move to an RNA system, what guidance would you give for preserving RNA? **A:** Anyone who works with RNA knows that it is fragile. Point of view is that metabarcoding is a radar image of what is coming. Then target organisms of interest to do qPCR to see if alive. So, it is a two-tier system.

Q: Could you use the same trapping methodology or change the sampling? **A:** Haven't worked with RNA so will leave that to people who do that work. Temperatures in the forest of 30-40 °C would degrade RNA. Not sure about the types of fluids either.

Q: Looking at effect of insects carrying fungi, have you looked at trap without insects vs with insects to see what the insects are carrying versus not. **A:** This is an experiment they are thinking about doing.

4.5 Challenges in semiochemical lure development for spotted lanternfly (*Lycorma delicatula*) COOPERBAND

Authors: Miriam Cooperband

Presenter Bio: Miriam Cooperband is an Entomologist for the United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), specializing in research on insect behavior and chemical ecology. She received a Bachelor's in Entomology at University of California, Riverside, a Master's degree in Entomology studying the behavior and chemical ecology of a parasitic wasp at Texas A&M University, and a Ph.D. in Entomology on mosquito chemical ecology and behavior at University of California, Riverside. After a postdoc at USDA Agricultural Research Service in Gainesville, FL, in 2008 she started working for APHIS in her current position where she has conducted research on chemical ecology and behavior, and methods development, of numerous forest and agricultural pests and their natural enemies. She has been studying spotted lanternfly (SLF) since 2015, identifying many SLF semiochemicals, developing improved trapping capabilities, and describing its phenology, behavior, biology, and host plant associations.

Abstract: Electroantennograms have revealed that spotted lanternfly (SLF) antennae are able to detect numerous compounds of both plant and SLF origin. Laboratory olfactometer bioassays revealed positive locomotor responses by SLF to many of those compounds, resulting in positive dose responses, blends with improved attraction over single compounds, and revealing differences between the responses of males and females. However, these findings in the laboratory did not translate well to the field. Under field conditions, tests of these compounds formulated into lures have not produced significant results in most cases, demonstrating the challenges of developing semiochemicals into attractant lures for SLF trapping and detection. Negative results are discussed, and possible reasons for differences between laboratory and field results are explored.

Miriam reported on how SLF aggregate, results to date suggest they use vision, host plant kairomones, honeydew volatiles, body volatiles, and substrate vibration

Q: Why do they fly into buildings, hit buildings and stay on the ground? Is the building in the way? **A:** We don't know a lot about what drives their behaviour. When they are in flight and see an object (weak flyers) use the tall objects to launch themselves, crawl up to launch themselves again. Why they sit for awhile? Maybe gravid can't move easily? Tired? Often flying because they've depleted their food resources.

Q: Are the SLF tests in test plots (i.e., controlled) or out on the landscape (many covariates possible). So many moving parts to whittle out noise to get to the signal. Are you looking in lab and landscape. Are non-significant results the result of too much noise? **A:** Non-significant results are all field

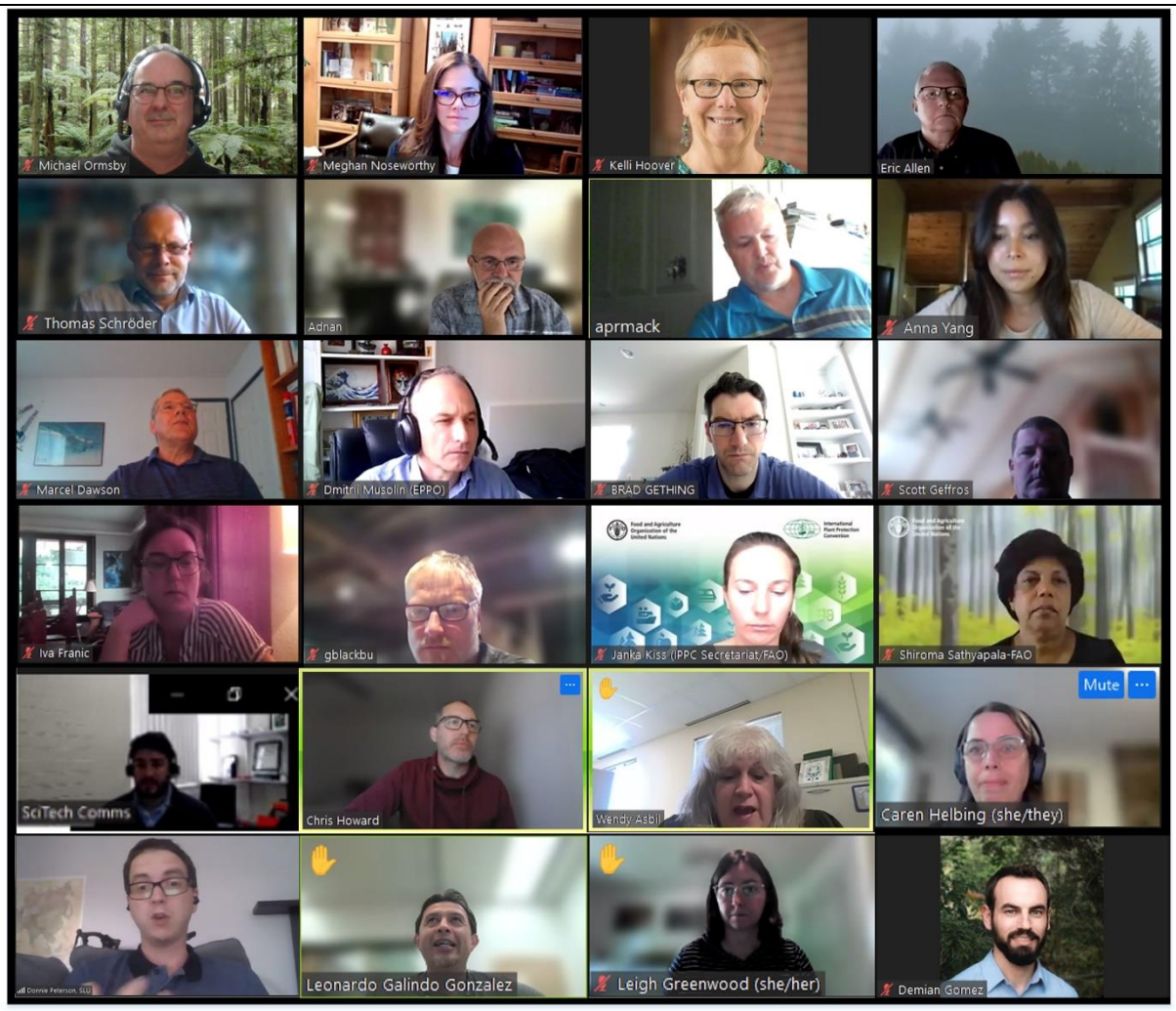
studies. Researchers are very careful how they select trees in a block to avoid noise: same size, same health, trying hard to get rid of as much noise of possible.

Q: Regarding host plant kairomones, is this done in a new country usually? For example, with ALB in the US, it was found on hosts we didn't anticipate. This broadened host range. **A:** We could do work on host profile in country of origin. Some hosts they are attracted to aren't good hosts to feed on.

Comment: Non-significant results are impactful.

Q: Surveillance grid won't work at high densities, but this isn't when we use it. So, would an attractant work at low densities? **A:** The only attractant which shows promise in low density monitoring sites will enhance trap capture chances. If tree of heaven around helps. SLF are initially generalists. Black walnut, wild grape, grape Virginia creeper, and maples after mating because maples senesce later than tree of heaven.

Registrant List



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