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Eastern Region State, Private, and Tribal Forestry | R9–PR–017–24 | February 2025 (admin. rev.)

BOOK OF ABSTRACTS

BEECH LEAF DISEASE WORKSHOP 2021

THURSDAY, APRIL 15, 2021 | VIRTUAL

Cover image: Beech leaf exhibiting symptoms of beech leaf disease. Courtesy photo by Tom Macy, Ohio Department of Natural Resources Division of Forestry.

ACKNOWLEDGMENTS

The workshop organizers greatly appreciate the support of the various collaborators and contributions. We are grateful for the hard work and support of Sherri Wormstead and Autumn Jauck for their behind the scenes efforts that helped this workshop function smoothly.

The abstracts were submitted in an electronic format and were edited only to achieve a uniform format. Each contributor is responsible for the accuracy and content of their own paper. Statements of contributors from outside the U. S. Department of Agriculture may not reflect the policy of the Department.

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INTRODUCTION

Beech leaf disease (BLD) is an emerging disease impacting American beech (*Fagus grandifolia*) and European beech (*F. sylvatica*) in North America. To address this emerging forest health issue, the U.S. Department of Agriculture (USDA) Forest Service Eastern Region State, Private, and Tribal Forestry Forest Health Protection staff convened subject matter experts from various fields and partner agencies to serve as an executive team aggregating current knowledge and guiding future inquiry. This document briefly summarizes our collective understanding of BLD to date as presented at the workshop and creates a foundation that can inform the identification, prioritization, and implementation of survey, research, and outreach needs. The themes and specific suggestions within this report should be viewed as flexible, considering the current limited understanding of the problem, as well as undefined management objectives and yet to be identified stakeholder input.

PARTNERSHIP

In a relatively short time, BLD has become a concern for resource managers across the Eastern United States and Canada. The following organizations have been involved with efforts to understand the disease:

- USDA Forest Service Eastern Region State, Private, and Tribal Forestry Forest Health Protection
- USDA Forest Service Northern Research Station
- USDA Agricultural Research Service
- Connecticut Agricultural Experiment Station
- Connecticut Department of Energy and Environmental Protection Forestry Division
- Indiana Department of Natural Resources
- Maryland Department of Agriculture
- Michigan Department of Natural Resources
- New Jersey Department of Environmental Protection
- Cornell University Insect Diagnostic Laboratory (NY)
- Cornell Cooperative Extension of Suffolk County (NY)
- New York State Department of Agriculture and Markets
- New York State Department of Environmental Conservation
- New York State Office of Parks, Recreation and Historic Preservation
- Ohio Department of Agriculture
- Ohio Department of Natural Resources
- The Ohio State University
- Cleveland Metroparks (OH)
- The Holden Arboretum (OH)
- Lake Metroparks (OH)
- Pennsylvania Department of Conservation and Natural Resources
- Lake Erie Allegheny Partnership (NY, OH, PA)
- West Virginia Department of Agriculture
- Bartlett Tree Research Laboratories
- Davey Tree Institute
- Ontario Ministry of Natural Resources: Forestry (Canada)
- Ontario Forest Research Institute (Canada)
- The Forestry and Forest Products Research Institute (Tsukuba, Japan)

AGENDA: BEECH LEAF DISEASE WORKSHOP 2021

Thursday, April 15, 2021	
8:40 a.m.	**OPENING REMARKS** Danielle Martin, Plant Pathologist USDA Forest Service, Morgantown Field Office, WV, U.S.A. Cameron McIntire, Plant Pathologist USDA Forest Service, Durham Field Office, NH, U.S.A.
	SESSION 1: BEECH LEAF DISEASE BACKGROUND Moderator: Danielle Martin, Plant Pathologist, USDA Forest Service
9 a.m.	**History and Background of Beech Leaf Disease** Connie Hausman, Senior Conservation Science Manager Cleveland Metroparks, Cleveland, OH, U.S.A.
9:25 a.m.	**Beech Leaf Disease: United States Survey and Monitoring Efforts** Dan Volk, Forest Health Project Coordinator Cleveland Metroparks, Cleveland, OH, U.S.A.
9:50 a.m.	**Ontario's Search for *Litylenchus crenatae* ssp. *mccannii* and Beech Leaf Disease** Sharon Reed, Forest Health Research Scientist Ontario Ministry of Natural Resources and Forestry, Sault Ste. Marie, ON, Canada
10:15–10:30	**BREAK**
10:30 a.m.	**Present Status of Beech Leaf Disease Research in Japan** Natsumi Kanzaki, Nematologist Forestry and Forest Products Research Institute, Kyoto, Japan
10:50 a.m.	**Are Birds Vectors of the Nematode *Litylenchus crenatae mccannii*, the Causal Agent of Beech Leaf Disease?** Chris Lituma, Certified Wildlife Biologist & Assistant Professor of Wildlife & Fisheries Resources West Virginia University, Morgantown, WV, U.S.A.
	SESSION 2: STATE REPORTS Moderator: Cameron McIntire, Plant Pathologist, USDA Forest Service
11:15 a.m.	**Increasing Detection, Monitoring, and Sampling for Beech Leaf Disease: Ohio DNR Division of Forestry 2021 Plans** Tom Macy, Forest Health Program Administrator Ohio Department of Natural Resources, Division of Forestry, Columbus, OH, U.S.A.
11:35 a.m.	**Survey and Long-term Plots in Connecticut with Research Update** Bob Marra, Associate Scientist and Forest Pathologist The Connecticut Agricultural Experiment Station, New Haven, CT, U.S.A.
12 p.m.	**Beech Leaf Disease Updates from New York** Maria MoskaLee, Forest Health Specialist Jessica Cancelliere, Research Scientist New York State Department of Environmental Conservation, NY, U.S.A.
12:20–1:20	**BREAK FOR LUNCH**

	SESSION 3: GENETICS, DETECTION, AND TREATMENT Moderator: Enrico Bonello, Department of Plant Pathology, The Ohio State University
1:20 p.m.	**Morphological and Molecular Observations of *Litylenchus crenatae mccannii* and Beech Leaf Disease** Lynn Carta, Plant Pathologist USDA Agricultural Research Service, Mycology and Nematology Genetic Diversity and Biology Laboratory, Beltsville, MD, U.S.A.
1:45 p.m.	**Optimizing Rapid Detection Methods for the Nematode *Litylenchus crenatae*, the Associative Agent of Beech Leaf Disease** David J Burke, Vice President for Science and Conservation The Holden Arboretum, Kirtland, OH, U.S.A.
2:10 p.m.	**Genetic Diversity of *Litylenchus crenatae mccannii* in BLD-affected Trees in Ontario, Canada, and Potential Pathways of Introduction** Katrin N.E. Fitza, Post-Doctoral Fellow University of Pretoria Department of Genetics, Pretoria, South Africa
2:35–2:50 p.m.	**BREAK**
2:50 p.m.	**Diagnosis and Detection of Beech Leaf Disease: A Combined Approach Using High Throughput Sequencing and Infrared Spectroscopy** Carrie Fearer, Ph.D. Candidate The Ohio State University Department of Plant Pathology, Columbus, OH, U.S.A.
3:15 p.m.	**Can Beech Leaf Disease Caused by the Foliar Feeding Nematode *Litylenchus crenatae mccannii* be Managed with Pesticides?** Andrew L. Loyd, Plant Pathologist Bartlett Tree Research Laboratories, Charlotte, NC, U.S.A.
3:40 p.m.	**Experimental Treatment of Beech: Effect of Phosphite 30 Drench Applications** Connie Hausman, Senior Conservation Science Manager Cleveland Metroparks, Cleveland, OH, U.S.A.
4:05–4:20 p.m.	**BREAK**
	SESSION 4: BRAINSTORMING & OPEN DISCUSSION Moderator: Enrico Bonello, Department of Plant Pathology, The Ohio State University
4:20 p.m.	This is an informal session with the purpose of encouraging discussion on future research, collaboration, and funding opportunities.
5 p.m.	**END MEETING**

SESSION 1: BEECH LEAF DISEASE BACKGROUND

Moderator: **Danielle Martin**, Plant Pathologist, U.S. Department of Agriculture Forest Service

Beech Leaf Disease Survey Results

To date, surveys to determine range extent of symptomatic trees have been prioritized in states considered "high risk" based on proximity to early infestations. Survey work includes two components:

1) Examination of existing long-term vegetation plots, as well as establishment of new plots
2) Widespread assessment of forests looking for initial infection.

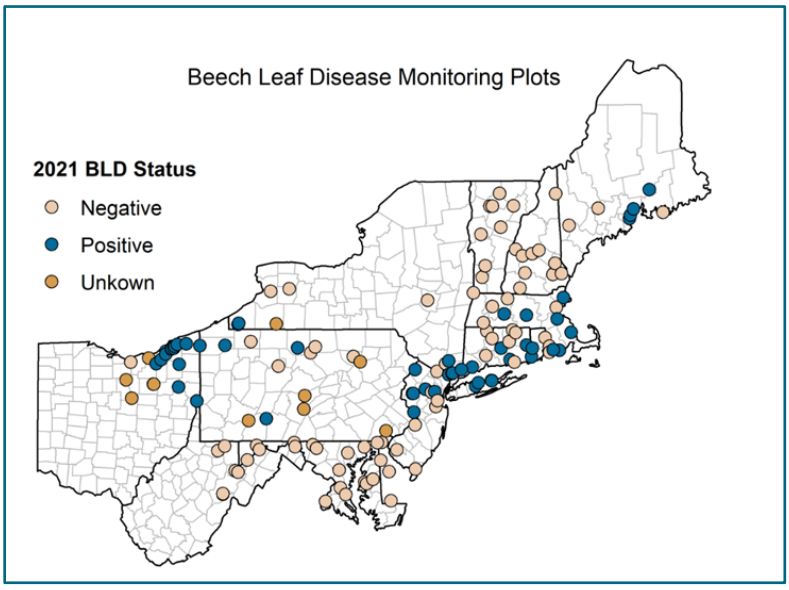
County-level data are based on observed presence or absence of BLD symptoms. The mechanism for dispersal for BLD is an active area of research; when dispersal is better understood, the survey method could be adjusted.

As of 2021, BLD has been detected in 109 U.S. counties (in 10 States) and 11 Canadian counties. First observed in northeast Ohio in 2012, symptoms of BLD have since been detected in Pennsylvania, New York, Connecticut, New Jersey, Virginia, West Virginia, Massachusetts, Rhode Island, Maine and the Canadian Province of Ontario.

Long-term Monitoring Plots

Long term monitoring plot protocols developed by the BLD Survey and Monitoring Team are currently employed in several states. The first BLD monitoring plots were established in 2019 in Ohio, Pennsylvania, and New York. As of 2021, the Forest Service Eastern Region has 151 plots across 12 States. At each plot, data is collected on standardized data sheets to ensure data consistency and facilitate data analyses.

In states with known BLD occurrences, plots are placed within range of BLD symptoms, and in areas with and without Beech Bark Disease. Plots are distributed throughout the various forest types and ecoregions where beech occur. Consideration is given to place plots at varying distances from water sources (rivers, streams, lakes), as nematode spread may be associated with moisture levels. Leaf samples from each plot are submitted to National Plant Network Diagnostic or state clinics for diagnoses and nematode detection. Establishing additional long-term plots in unaffected stands allows observation of disease progression to make comparisons Region-wide.



History and Background of Beech Leaf Disease

Presenter: **Connie Hausman**, Senior Conservation Science Manager
Cleveland Metroparks, Cleveland, Ohio, U.S.A.

ABSTRACT

Understanding the investigation timeline of an emerging disease is critical for recognizing research limitations and current knowledge gaps. Since the initial discovery of BLD in northeast Ohio in 2012, we have made significant strides in documenting leaf, tree, and stand-level impacts of BLD as well as basic biology. This presentation will provide an overview of beech leaf disease to give audience members a strong knowledge base to prepare for remaining presentations.

Keywords

Phenology; monitoring; survey; Beech Leaf Disease.

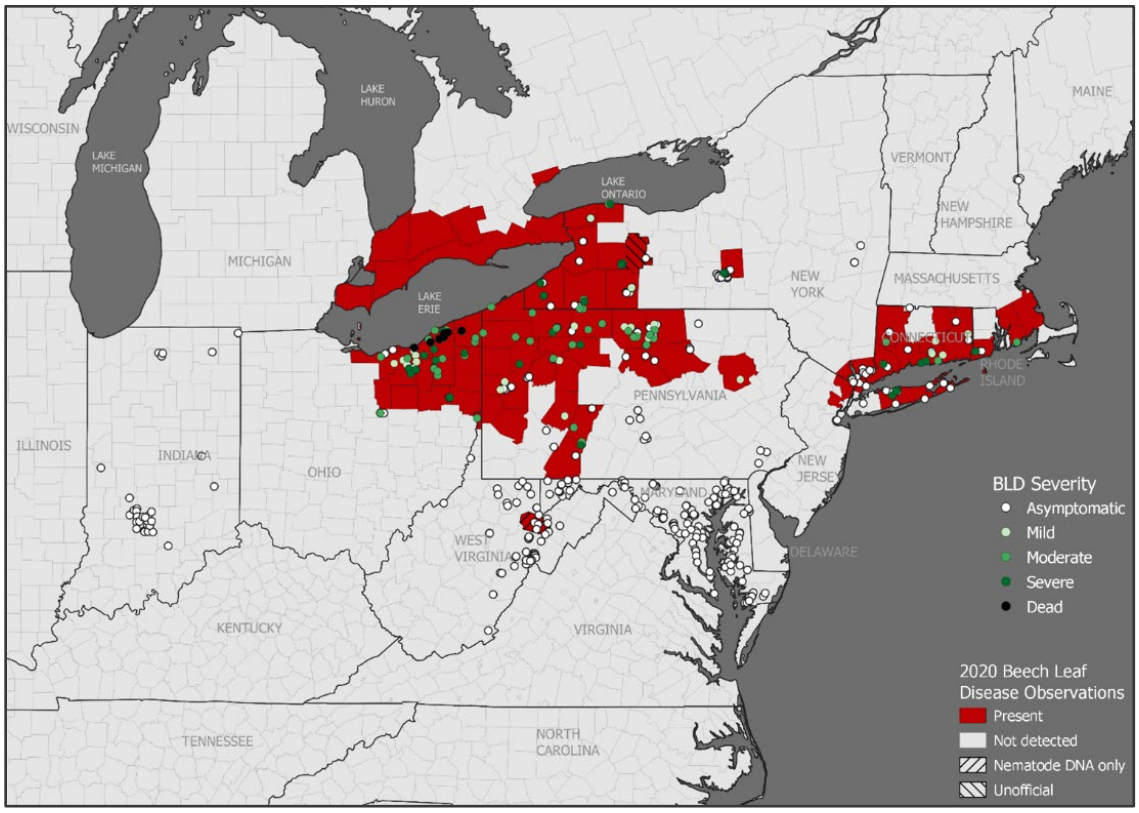
Beech Leaf Disease: United States Survey and Monitoring Efforts

Presenter: **Dan Volk**, Forest Health Project Coordinator
Cleveland Metroparks, Cleveland, Ohio, U.S.A.

ABSTRACT

We will review national beech leaf disease survey efforts through 2020 with guidance on how and where to perform surveys. To assist in collecting uniform BLD data, we developed Tree Health Survey, a smartphone app which provides training on symptom identification and data collection. Tree Health Survey is available to professionals and citizen-scientists, who have both been successful in reporting new occurrences of BLD. We will also highlight our newly established, long-term monitoring project with initial results from 2019 and 2020. This project involves collaboration across multiple states and a significant collaboration with Canada to expand the study across the range of BLD. This project assesses multiple pests including beech leaf disease, *Neonectria* spp., and *Cryptococcus fagisuga*.

Visit http://clevelandmetroparks.com/parks/education/publications for up-to-date BLD information.



Distribution map of BLD survey locations and positive county detections in 2020. Survey locations are colored by BLD severity based on the percentage of symptoms present and canopy remaining. Survey data was captured with a standardized protocol despite using variety of data collection tools (e.g., Tree Health Survey app, Survey123, and others).

Keywords

Phenology; monitoring; survey; Tree Health Survey app.

Ontario's Search for *Litylenchus crenatae* ssp. *mccannii* and Beech Leaf Disease

Presenter: **Sharon Reed**, Forest Health Research Scientist
Ontario Ministry of Natural Resources and Forestry, Sault Ste. Marie, Ontario, Canada

Authors: **Sharon Reed**, Ontario Ministry of Natural Resources and Forestry, Ontario, Canada

Sylvia Greifenhagen, Ontario Ministry of Natural Resources and Forestry, Ontario, Canada

Qing Yu, Agriculture and Agri-Food, Ottawa, Canada

ABSTRACT

From 2018 to 2020, the Ministry of Natural Resources and Forestry led two surveys in Ontario. The first survey described changes in *Litylenchus crenatae* spp. *mccannii* populations in beech tissues over time in areas with and without beech leaf disease. At beech leaf disease (BLD) sites, populations in symptomatic leaves steadily increased over the growing season while asymptomatic leaves had none or small numbers of nematodes. The nematodes overwintered in detached leaves on the forest floor and in buds. The second survey examined the distribution of BLD, scale, and BBD in southwestern Ontario forests. Beech leaf disease is rapidly becoming the most important pest despite the presence of scale since the 1960's and BBD since the early 2000s. Beech leaf disease was the primary pest of saplings while overstory trees either had scale or scale and beech leaf disease. Guidelines for beech management in BLD areas will need to be modified to reflect changing conditions.

Keywords

Beech leaf disease; Canada; Ontario; survey.

Present Status of Beech Leaf Disease Research in Japan

Presenter: **Natsumi Kanzaki**, Nematologist
Forestry and Forest Products Research Institute, Kyoto, Japan

Authors: **Natsumi Kanzaki**, Kansai Research Center, Forestry and Forest Products Research Institute), Kyoto, Japan

Yu Ichihara, Kansai Research Center, Forestry and Forest Products Research Institute, Kyoto, Japan

Takuya Aikawa, Tohoku Research Center, Forestry and Forest Products Research Institute, Morioka, Iwate, Japan

Taisuke Ekino, School of Agriculture, Meiji University, Kawasaki, Kanagawa, Japan

Hayato Masuya, Department of Mushroom Science and Forest Microbiology, Forestry and Forest Products Research Institute, Tsukuba, Ibaraki, Japan

ABSTRACT

Beech leaf gall nematode, *Litylenchus crenatae* was first recognized by Akimoto (2004) causing gall symptom on several broad-leaved tree species including European beech, *Fagus sylvatica*, planted in Hokkaido, Japan. However, the detailed species identification was not conducted at the time. Thereafter, the species was taxonomically described based on the materials from a Japanese beech, *F. crenata*, collected from Iwate, Japan. Preliminary field observation suggested that the life history of *L. c. crenatae* in Japan is basically same as that of *L. c. mccannii* in U.S. and Canada. However, the nematode does not cause visible mortality or severe damage on its host beech trees. So far, our ongoing field collection survey revealed that the nematode is distributed throughout Japan, at least three main islands, Hokkaido, Honshu, and Kyusyu. The other island, Shikoku, has not been surveyed yet. This distribution pattern, i.e., widely distributed without causing severe damage, suggests that *L. c. crenatae* is native to Japan. For further understanding of the nematode, we have to:

1) confirm the phylogenetic relationship between two subspecies, i.e., two subspecies are sufficiently close to each other in ITS sequence, but other more variable genetic loci should be compared;
2) confirm the species status of other leaf galls, i.e., although similar symptom had been found in several broad-leaved trees, the species status has been confirmed for the materials from two beech species, *F. crenata* and *F. japonica*, so far; and
3) conduct wider field survey followed by molecular phylogeographic analysis.

References

Akimoto, M. 2004. Leaf gall symptom on several broad-leaved tree species induced by nematode. In: Proceedings of the 115th Annual Meeting of the Japanese Forest Society. P4009. http://dx.doi.org/10.11519/jfs.115.0.P4009.0. In Japanese.

Keywords

Beech leaf disease; Japan; phylogeny; *Litylenchus crenatae*.

Are Birds Vectors of the Nematode *Litylenchus crenatae mccannii*, the Causal Agent of Beech Leaf Disease?

Presenter: **Chris Lituma**, Certified Wildlife Biologist and Assistant Professor of Wildlife and Fisheries Resources
West Virginia University, Morgantown, West Virginia, U.S.A.

Authors: **Christopher M. Lituma**, Division of Forestry and Natural Resources, West Virginia University, Morgantown, West Virginia, U.S.A.

Danielle K. Martin, U.S. Department of Agriculture Forest Service, Forest Health Protection, Morgantown Field Office, Morgantown, West Virginia U.S.A.

Matthew Day, U.S. Department of Agriculture Forest Service, Forest Health Protection, Morgantown Field Office, Morgantown, West Virginia U.S.A.

ABSTRACT

Since its discovery in 2012 in Ohio, beech leaf disease (BLD) has emerged as a lethal disease to American beech *(Fagus grandifolia*) and has rapidly spread across parts of the mid-Atlantic. American beech is an important tree in eastern forests because it provides critical mast for wildlife. Vectors that spread *Litylenchus crenatae mccannii* (LCM), the nematode associated with BLD, remain unknown, though limited evidence implicates an avian vector. Avian species may contribute to the rapid spread of BLD through consumption of LCM-infested beech buds and defecating LCM throughout the landscape. Mites may also be playing a role in the distribution of LCM, either independently or via associations with avian hosts. Wildlife are known vectors of many plant pathogens, including many nematode species. My goal is to explore the existing information about birds as vectors of BLD, and present hypotheses related to research opportunities.

Keywords

Beech leaf disease; avian vectors; mites; *Litylenchus crenatae; Fagus grandifolia*.

SESSION 2: STATE REPORTS

Moderator: **Cameron McIntire**, Plant Pathologist, U.S. Department of Agriculture Forest Service

State forest health partners have developed robust beech leaf disease (BLD) survey, monitoring, and research programs aimed towards enhancing BLD detection and informing mitigation strategies. Each state is unique in its approach to addressing forest diseases, and the presence and perceived value of American beech on the landscape can also differ. Sharing these diverse perspectives is valuable for both researchers and land managers. These critical updates also strengthen our understanding of BLD across state lines to allow for a better coordination of our efforts with respect to this emerging disease. In this session, we gather information from the Ohio Department of Natural Resources, the Connecticut Agricultural Experiment Station, and the New York State Department of Environmental Conservation.

Increasing Detection, Monitoring, and Sampling for Beech Leaf Disease: Ohio DNR Division of Forestry 2021 Plans

Presenter: **Tom Macy**, Forest Health Program Administrator
Ohio Department of Natural Resources Division of Forestry, Columbus, Ohio, U.S.A.

ABSTRACT

Since its initial discovery in northeastern Ohio in 2012, beech leaf disease (BLD) has been documented in an expanding area both within Ohio and the Northeastern U.S. and eastern Canada. This update will provide a summary of BLD projects planned for 2021 by the Ohio DNR Division of Forestry. Projects will include expanding BLD detection survey, particularly in those areas at the periphery of known "infested" areas, establishment of more long-term monitoring plots to document the impacts of BLD on American beech and wider impacts to forest ecosystems, and sampling of beech buds and leaves across Ohio to document the presence, and assess populations of, the foliar nematode *Litylenchus crenatae* to examine its relationship with BLD symptoms and damage.

Keywords

Beech leaf disease; American beech; *Fagus grandifolia*; *Litylenchus crenatae*; forest health evaluation and monitoring.

Survey and Long-term Plots in Connecticut with Research Update

Presenter: **Bob Marra**, Associate Scientist and Forest Pathologist
The Connecticut Agricultural Experiment Station, New Haven, Connecticut, U.S.A.

ABSTRACT

Thirty-one State forests, State parks, city parks, and private preserves, in all eight Connecticut counties, were investigated for presence of BLD and for beech stands potentially suitable for long-term monitoring plots. BLD was confirmed at 14 of these sites in Fairfield, Litchfield, Middlesex, New Haven, and Windham Counties, but not in Hartford County. BLD was considered present based on visual observation of characteristic interveinal banding, and subsequently confirmed in the laboratory either directly through observation of the associated nematode, *Litylenchus crenatae mccannii* (*Lcm*) or indirectly by PCR amplification of the *Lcm* mitochondrial cytochrome oxidase gene, CO-I, from DNA extracted from symptomatic leaf material *Lcm*. Eleven long-term monitoring plots were installed in all eight counties. DNA extracted from ~10,000 *Lcm* nematodes was used to generate a whole-genome sequence based on PacBio reads. The resulting sequence is being used to identify microsatellite loci, which will be used in population-genetic analyses to track the origins and movement of the nematode within and among sites.

Keywords

Beech leaf disease; *Litylenchus crenatae; Fagus grandifolia*.

Beech Leaf Disease Updates from New York

Presenters: **Maria MoskaLee**, Forest Health Specialist
New York State Department of Environmental Conservation, New York City, New York, U.S.A.

Jessica Cancelliere, Research Scientist
New York State Department of Environmental Conservation, Delmar, New York, U.S.A.

ABSTRACT

BLD has been detected in 12 counties across New York, with most infestations located in western New York, the lower Hudson Valley, and Long Island. 328 detection surveys were completed from 2018 to 2020, to identify new infestations and delineate existing infestations. Foliar samples were collected from survey sites and sent to Cornell Plant Disease Diagnostic Clinic for nematode confirmation. From 2019 to 2020, 16 long-term vegetation plots were established at 10 sites to monitor stand-level progression of the disease. In 2020, omni-directional intercept traps were deployed in symptomatic and asymptomatic beech stands to survey for leaf-feeding insects that may vector the nematode. Mitigation was conducted to study whether cutting infested trees would stop the spread of a small BLD infestation. A BLD webpage was created on the DEC website to provide up-to-date information and enhance public reporting.

Keywords

Beech leaf disease; *Litylenchus crenatae*; *Fagus grandifolia*.

SESSION 3: GENETICS, DETECTION, AND TREATMENT

Moderator: **Enrico Bonello**, Department of Plant Pathology, The Ohio State University

Scientists from universities, state and Federal agencies, and private companies have made significant recent gains in the molecular detection of *Litylenchus crenatae mccannii* (LCM). LCM specific primers have been developed for both molecular and real-time PCR detection. Treatment methods, while still underway, have been investigated with promising preliminary results. This research is imperative in helping forest managers and landowners control beech leaf disease in forested environments.

Morphological and Molecular Observations of *Litylenchus crenatae mccannii* and Beech Leaf Disease

Presenter: **Lynn Carta**, Plant Pathologist
U.S. Department of Agriculture, Agricultural Research Service, Mycology and Nematology Genetic Diversity and Biology Laboratory, Beltsville, Maryland, U.S.A.

ABSTRACT

In this presentation freeze-fracture low-temperature scanning electron microscopy images of the nematodes from symptomatic leaf and bud tissue illustrate extensive damage to leaves and buds. Nematodes were also imaged entwined around the bodies of mites. Mites also implicated birds through a microbiome marker as possible means of nematode distribution. ITS rDNA and COI DNA markers established that the nematode is closely related to a nematode population on beech trees in Japan where the disease associated with the nematode is not lethal.

Keywords

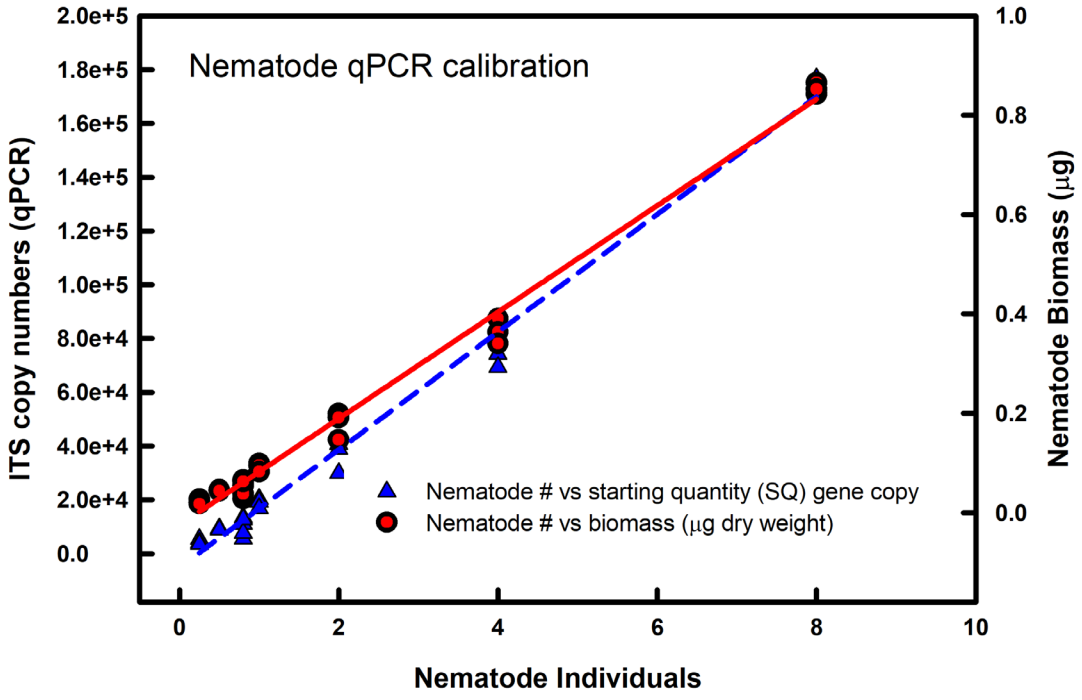
Beech leaf disease; *Litylenchus crenatae*; *Fagus grandifolia*; molecular.

Optimizing Rapid Detection Methods for the Nematode *Litylenchus crenatae*, the Associative Agent of Beech Leaf Disease

Presenter: **David J. Burke**, Vice President for Science and Conservation
The Holden Arboretum, Kirtland, Ohio, U.S.A.

ABSTRACT

Beech leaf disease (BLD) is an emerging threat to beech dominated forests in the Northern U.S. and Canada. The cause of BLD was recently confirmed to be a nematode, *Litylenchus crenatae*, previously undescribed in North America (Carta and others 2020). Although the mode of infestation is uncertain, affected leaves display characteristic interveinal darkening, and thickening of leaf tissue. However, lack of visible BLD symptoms may not reliably reveal the presence of *L. crenatae,* which may persist in low levels in asymptomatic leaves. We have developed DNA-based diagnostic methods for *L. crenatae,* including the use of quantitative PCR (qPCR) that may allow estimates of population size and biomass of *L. crenatae* in beech leaves and buds. General nematode primers TW81 and 5.8SM5 have been previously used for detection of *L. crenatae* with success. Good correlation between the number of nematode adults and copy numbers of the internal transcribed spacer (ITS) region of the rRNA gene have been observed. This indicates that qPCR targeting the nematode ITS region can be useful for determining the level of leaf or bud infestation by *L. crenatae*. However, in samples with low infestation, TW81 and 5.8SM5 can amplify non-target organisms, including other nematode species in low abundance on leaf and bud tissue, as well as DNA of insects such as mites. We have developed a new primer set 33F and 234R that specifically targets *L. crenatae* and eliminates non-specific amplification. Testing of this new primer set is still underway but early results are promising. These methods may be helpful tools for detecting the presence of *L. crenatae* at low populations levels before severe symptoms of BLD appear.



References

Carta, L.K.; Handoo, Z.A.; Li, S. [and others]. 2020. Beech leaf disease symptoms caused by newly recognized nematode subspecies *Litylenchus crenatae mccannii* (Anguinata) described from *Fagus grandifolia* in North America. Forest Pathology. 50(2): e12580. https://doi.org/10.1111/efp.12580.

Keywords

Litylenchus crenatae; quantitative PCR; detection methods; rRNA gene.

Genetic Diversity of Litylenchus crenatae mccannii in BLD-affected Trees in Ontario, Canada, and Potential Pathways of Introduction

Presenter: **Katrin N.E. Fitza**, Post-Doctoral Fellow
University of Pretoria Department of Biochemistry, Genetics and Microbiology, Pretoria, South Africa

Authors: **Katrin N.E. Fitza**, Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002, South Africa

Jeremy Allison, Natural Resources Canada, Canadian Forest Service, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, Canada

Bernard Slippers, Department of Zoology and Entomology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002, South Africa

Sharon E. Reed, Ontario Forest Research Institute, Ministry of Natural Resources and Forestry, Sault Ste. Marie, Ontario, Canada

ABSTRACT

The nematode *Litylenchus crenatae mccannii* is the causal agent of beech leaf disease (BLD). A new *Litylenchus* species has been described as *Litylenchus crenatae* in Japan and a subspecies, *L. crenatae mccannii*, in the United States of America. In North America native and non-native beech are known to be infested with *L. crenatae mccannii*. There are limited studies that have characterized the distribution and genetic diversity of the nematode in Ontario, Canada. In this study samples of beech foliage were collected from 11 locations in southern Ontario. The genetic diversity of *L. crenatae mccannii* from these samples was determined using sequence data from the mitochondrial cytochrome c oxidase subunit 1 (mtCO1) region, the large subunit region (LSU rDNA) and internal transcribed spacer region (ITS rDNA). None of these loci contained any variation amongst the samples. The genotype of *L. crenatae mccannii* found in Ontario is the same as that recently described from the U.S.A. Beech import data show that 92% of beech is imported into Canada from the U.S.A. The results suggest that the U.S.A. could be the origin of *L. crenatae mccannii* nematodes in Ontario, and/or that both countries have imported beech trees from the same source country. To clarify the origin of *L. crenatae mccannii* further sampling and more diverse molecular markers would be needed.

Keywords

Beech leaf disease; *Litylenchus crenatae*; *Fagus grandifolia*; Ontario, Canada.

Diagnosis and Detection of Beech Leaf Disease – A Combined Approach Using High Throughput Sequencing and Infrared Spectroscopy

Presenter: **Carrie Fearer**, Ph.D. Candidate
The Ohio State University Department of Plant Pathology, Columbus, Ohio, U.S.A.

Authors: **Carrie Fearer**, Department of Plant Pathology, The Ohio State University, Columbus, Ohio, U.S.A.

Jason Slot, Department of Plant Pathology, The Ohio State University, Columbus, Ohio, U.S.A.

María-Soledad Benítez, Department of Plant Pathology, The Ohio State University, Columbus, Ohio, U.S.A.

Cristina Rosa, Department of Plant Pathology and Environmental Microbiology, The Pennsylvania State University, State College, Pennsylvania, U.S.A.

Antonino Malacrinò, Department of Evolution, Ecology, and Organismal Biology, The Ohio State University, Columbus, Ohio, U.S.A.

Alison Bennett, Department of Evolution, Ecology, and Organismal Biology, The Ohio State University, Columbus, Ohio, U.S.A.

Caroline Georskey, Masters of Plant Health Management Program, The Ohio State University, Columbus, Ohio, U.S.A.

Anna Conrad, U.S. Department of Agriculture Forest Service, Northern Research Station, Hardwood Tree Improvement and Regeneration Center, West Lafayette, Indiana, U.S.A.

Enrico Bonello, Department of Plant Pathology, The Ohio State University, Columbus, Ohio, U.S.A.

ABSTRACT

When our native forests come under threat from lethal, alien or endemic, invasive pathogens and pests, it is essential to respond quickly and efficiently to try and prevent mass destruction. Given that beech leaf disease (BLD) is a newly emerged disease, we are in the critical time period for rapid response before the disease becomes unmanageable. Our research focuses on diagnosing BLD using comparative metabarcoding of the foliage microbiome and developing an early detection method using near-infrared (NIR) spectroscopy to rapidly respond and manage BLD in the forest. Our results from the metabarcoding analysis determined that the amplicon sequence variant (ASV) corresponding to the suspected nematode causal agent, *Litylenchus crenatae* ssp. *mccannii,* was present in both symptomatic and asymptomatic foliage, yet the ASVs for four bacterial genera, *Wolbachia, Pseudomonas, Erwinia,* and *Paenibacillus,* and one fungal genus, *Paraphaeosphaeria,* were present in only symptomatic tissues. These results suggest that *L. crenatae* ssp. *mccannii* may not be fully responsible for BLD, but rather that other microbe(s) may be contributing to the syndrome. In regard to our second objective, we were able to preliminarily discriminate between leaves from disease-free trees and asymptomatic leaves from diseased trees using NIR spectroscopy coupled with machine learning. The most accurate support vector machine (SVM) model, a form of machine learning used to build and evaluate the accuracy of supervised classification-based disease predictive models, had an overall testing accuracy of 84.3% (N = 181). These results suggest that changes in the leaf chemical composition occur before symptoms appear, so this

approach may serve as an early detection tool that would allow for rapid and targeted disease management.

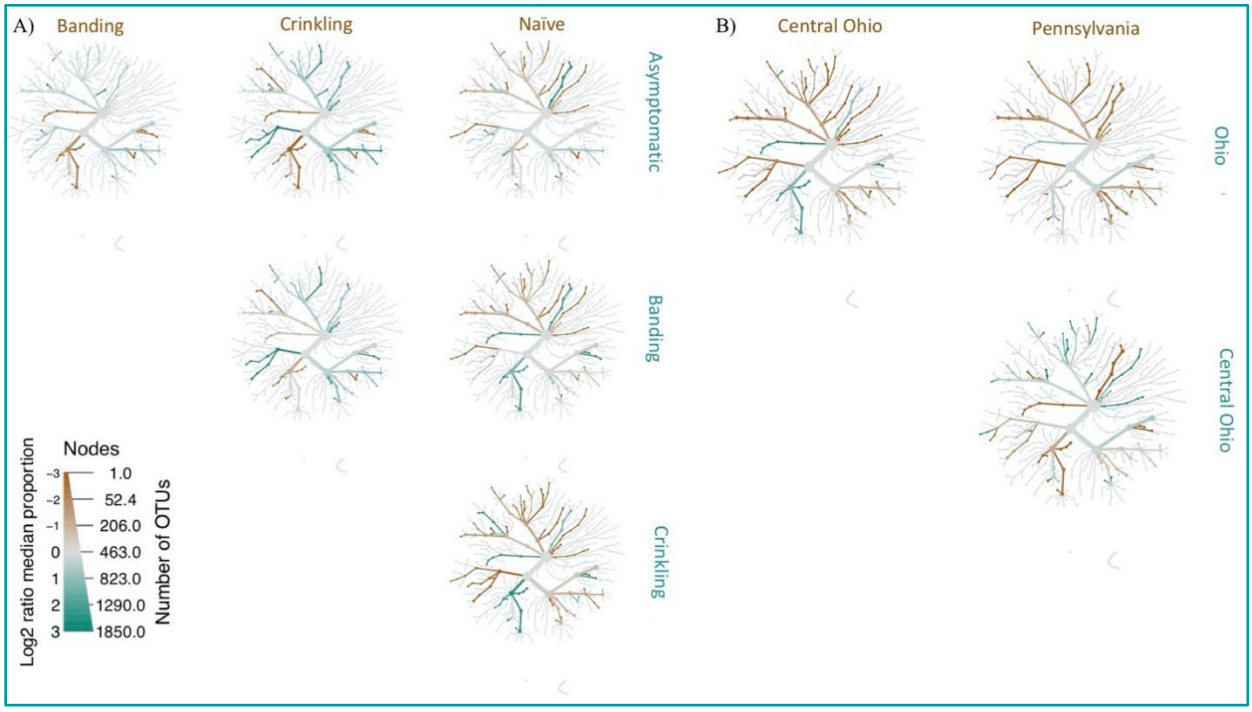


Figure 1.—Differential heat trees showing the differences in the pairwise comparisons of bacterial amplicon sequence variants (ASVs) in *Fagus grandifolia* leaves based on A, symptom type and B, location. Branches and nodes highlighted in blue represent more ASVs of that genus detected in the samples in the categories on the right of the chart, and branches and nodes highlighted in brown represent more ASVs detected in the samples in the categories above the charts (Ewing and others 2021).

References

Ewing, C.J.; Slot, J.; Benítez, M. [and others]. 2021. The foliar microbiome suggests that fungal and bacterial agents may be involved in the beech leaf disease pathosystem. Phytobiomes Journal [e-ISSN: 2471-2906]. 5(3): 335-349. https://doi.org/10.1094/PBIOMES-12-20-0088-R.

Keywords

Metabarcoding; NIR spectroscopy; machine learning; early detection; high-throughput sequencing.

Can Beech Leaf Disease Caused by the Foliar Feeding Nematode *Litylenchus crenatae mccannii* be Managed with Pesticides?

Presenter: **Andrew L. Loyd**, Plant Pathologist
Bartlett Tree Research Laboratories, Charlotte, North Carolina, U.S.A.

ABSTRACT

Beech leaf disease is a concerning and emerging disease in the Eastern United States and Canada. The disease has been observed affecting *Fagus grandifolia, F. sylvaticus,* and *F. orientalis* where it causes symptoms including interveinal leaf galls, epinasty, malformation of buds, and eventual death. The disease is caused by the foliar feeding nematode *Litylenchus crenatae mccannii,* which is a subspecies of a foliar feeding nematode originally described from *Fagus crenatae* in Japan in 2018. There are very few devastating foliar diseases of trees caused by nematodes found around the world. Furthermore, management of nematodes in many pathosystems can be challenging due to a lack of chemistries that are effective. Pine wilt disease caused by the bole infesting pine wilt nematode *Bursaphelenchus xylophilus* is a devastating disease to *Pinus* species native to Asia and Europe. Emamectin benzoate, which can be found in commercially available products such as Mectinite and Tree-äge G4, is an active ingredient that when injected into the tree can prevent establishment of the pine wilt nematode providing 2–3 years of protection of susceptible pines.

Injection efficacy trials were conducted in northeastern Ohio with trees that were already infested with the beech leaf nematode. Products with the active ingredients emamectin benzoate and abamectin were injected into young open grown beech in fall of 2018 or spring of 2019 with the labeled rates for pine wilt nematode prevention. Although symptoms were not significantly reduced in injected trees relative to the untreated trees over two growing seasons, a reduction in nematode numbers were observed in 2019. In a laboratory study different concentrations of emamectin benzoate (43,000, 4,300, 430, and 43 ppm) were used to treat extracted, active beech leaf nematodes and all concentrations resulted with at least 95% mortality, where few died in water treated nematodes over a period of 24 hours. In another study, one hundred, 15–21 cm cuttings of symptomatic beech were collected in northeastern Ohio, wrapped in a moist paper towel and aluminum foil, and processed 24 hrs after collecting. beech leaf nematodes were extracted from cuttings by cutting 6 symptomatic leaf pieces out of the leaves with a standard hole puncher. Cuttings were then placed immediately in tubes with different concentrations of emamectin benzoate, fluopyram, spirotetramat, or distilled water and placed under a fluorescent light for 48 hours to allow uptake of product. After the incubation period, leaves were harvested and processed similarly to the pretreatment extractions. Both emamectin benzoate and fluopyram reduced the number of live, active nematodes compared to the number of active nematodes in the pretreatment extraction. While these data are hopeful, timing of injection, concentration of products, and other management nuances need to be the topic of further investigation.

Keywords

Beech leaf disease; *Litylenchus crenatae*; *Fagus grandifolia*; pesticides.

Experimental Treatment of Beech: Effect of Phosphite 30 Drench Applications

Presenter: **Connie Hausman**, Senior Conservation Science Manager
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Dan Herms, The Davey Tree Expert Company, Kent, Ohio, U.S.A.

Dan Volk, Cleveland Metroparks, Cleveland, Ohio, U.S.A.

Anand Persad, ACRT Services, Stow, Ohio, U.S.A.

ABSTRACT

A pilot project was initiated in 2017 in partnership with the Davey Tree Expert Company to evaluate the efficacy of potassium phosphite (PolyPhosphite 30™) soil injections for management of beech leaf disease. At the time, no knowledge of causal agent was available and exploratory treatment was based on Davey's experience with the product for suppressing symptoms of disease. The project consists of 20 untreated control and 20 treatment trees, all mildly symptomatic initially. Trees are small saplings within a beech maple forest and assumed to be a unique stem (non-clonal) based on distance from each other and distance from any large mature tree. As a precaution, however, a ~4'diameter ring with a min. depth of 10" was created around all trees (control and treated) using a nursery spade to sever any root grafts. Treatment application occurred twice a year for 4 years. Individual tree condition assessments were also conducted every year. After four years, treated trees on average have 3x the canopy cover as control trees. Treated trees also had 37% dead fine twigs/branches, while control trees had 75% dead fine twigs and branches.

Keywords

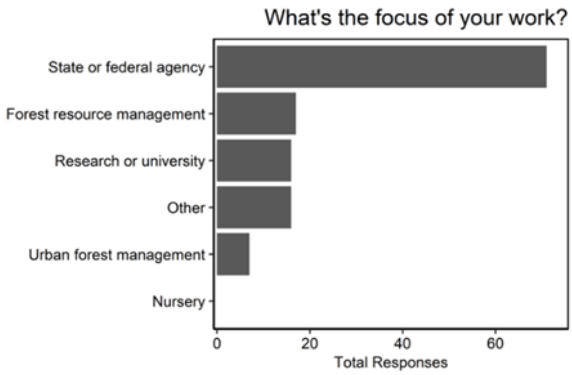
Beech leaf disease; *Litylenchus crenatae*; *Fagus grandifolia*; pesticides.

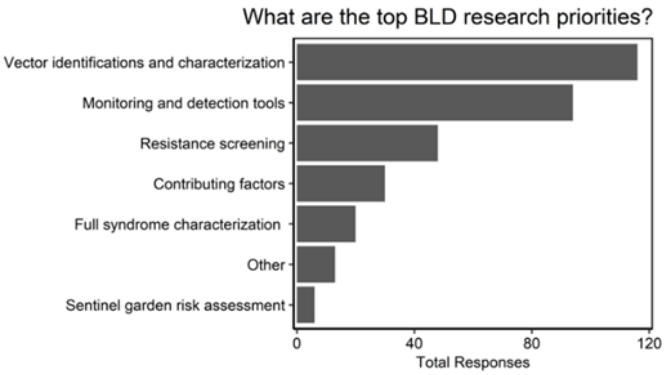
SESSION 4: BRAINSTORMING AND OPEN DISCUSSION

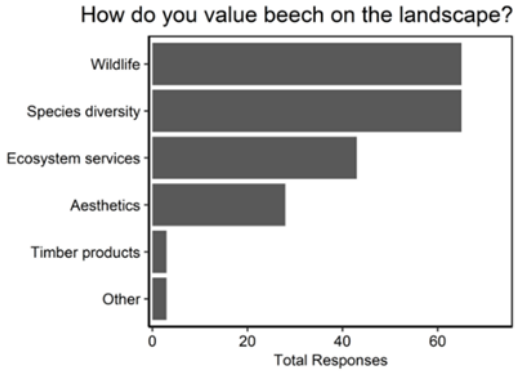
Moderator: **Enrico Bonello**, Department of Plant Pathology, The Ohio State University

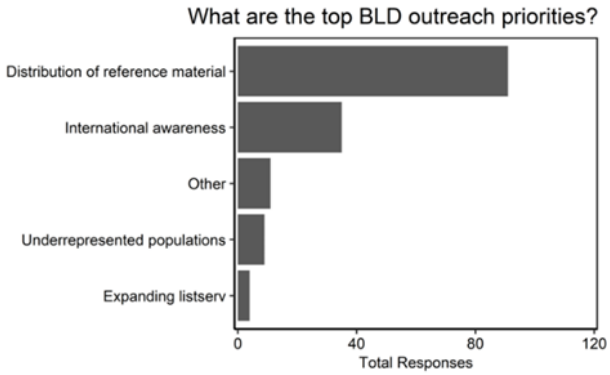
Results of Interactive Poll Questions

At four periods throughout the workshop, we asked all attendees to participate in a poll question regarding their background and their opinions of issues relating to beech leaf disease (BLD). The results of those polls are below. Note that participants were allowed to cast a vote for up to two options.









- Most workshop participants (56%) represented state and Federal agencies. Interestingly, no participants were from a plant nursery background, an area of concern regarding the spread of BLD via ornamental beech cultivars.
- Participants stressed the importance of beech on the landscape for wildlife, as it is a hard-masting tree that many bird and mammal species utilize for its nut crop. Though American beech can produce a fine timber product and was historically an important lumber species, it has fallen out of favor with other hardwood species such as oak and maple. The aftermath of beech bark disease throughout the northeastern region of North America has significantly reduced the wood quality of affected trees.
- Our question concerning research priorities received the most votes of any poll question. Identification and characterization of the BLD vectors was ranked highest, as the uninhibited spread of this disease is likely to continue until the mechanism of transmission can be

determined. Given that many workshop participants are forestry practitioners and land managers, further research on monitoring and detection tools was also deemed as critically important.

- Where BLD outreach is concerned, the distribution of reference material was considered the top priority. Given that the understanding of epidemiology is continuing to evolve, it is important to provide up-to-date information to forestry professionals and the public at large.

Countries Represented

The 2021 Beech Leaf Disease Workshop was attended by 305 people, representing 13 countries. Most attendees were from the United States (85%) and Canada (10%). Within the United States, attendees were from 30 states, primarily in the Eastern Region.