



Annual Report

USDA Award No.: 2020-51181-32135

**Improving boxwood blight mitigation through innovation,
economic analysis and education**

Together we save boxwood crops and plantings!



August 31, 2023

Boxwood Blight Insight Group (BBIG) is a global research and extension consortium consisting of scientists working on a multi-state, transdisciplinary project sponsored by the USDA National Institute of Food and Agriculture – Specialty Crop Research Initiative (SCRI), along with international collaborators and stakeholder partners. BBIG aims to safeguard boxwood—the nation’s #1 evergreen ornamental shrub crop—from the blight disease, thus saving an iconic plant featured in American landscapes since 1653.

BBIG was the recipient of the 2022 USDA NIFA Partnership Award – Program Improvement Through Global Engagement



Table of Contents

PROJECT PARTICIPANTS	2
Project Leadership Team.....	2
Project Co-Directors.....	2
Postdoctoral Associates	2
Graduate Students.....	2
Summer Interns and Other Assistants	2
Advisory Panel and Liaison to AmericanHort.....	3
Key Stakeholder Collaborators	3
Major International Collaborators	4
Project Organization	4
TARGET AUDIENCE	5
Research	6
<i>PhD Dissertations.....</i>	<i>6</i>
<i>Refereed Journal Articles</i>	<i>6</i>
<i>Abstracts (Presented at Professional Meetings).....</i>	<i>7</i>
<i>Invited Presentations at Professional Conferences WITHOUT Abstract</i>	<i>8</i>
<i>Thesis Defense Seminars</i>	<i>8</i>
Global and Stakeholder Partnerships	8
<i>International Boxwood Blight Epidemiology Working Group Meetings Hosted</i>	<i>8</i>
<i>Working with German Scientists on Field Epidemiology while Analyzing and Publishing Multi-year Fungicide Trial Data.....</i>	<i>8</i>
<i>Connecting and Working with Scientists in Southern Europe Investigating whether the Blight Pathogen Populations in That Region are Dominated by Che</i>	<i>9</i>
<i>Collaborating with North Carolina Department of Agriculture and Consumer Services (NCDA&CS), North Carolina Cooperative Extension (NCCE), and Local Farmers in Field Trials Evaluating Selected Antidesiccants and Biological Control Agents</i>	<i>9</i>
<i>International Boxwood Seminar Series</i>	<i>10</i>
<i>Outreach Website – Boxwood Blight Insight Group</i>	<i>10</i>
<i>Launch of the BBIG Boxwood Seminar Series</i>	<i>11</i>
Other Major Extension Products.....	11
<i>Twitter.com/boxwoodhealth</i>	<i>11</i>
<i>BBIG Monthly Digests.....</i>	<i>11</i>
<i>BBIG Newsletters - Quarterly</i>	<i>12</i>
<i>Newsletter and Trade Journal Articles (Excluding Those Published in the BBIG Newsletters).....</i>	<i>13</i>
<i>Workshops</i>	<i>13</i>

<i>Extension Presentations</i>	13
<i>Interactive Virtual Workshops for both English and Spanish Speakers</i>	15
<i>BBIG Showcased in Major National and International Events and Media</i>	15
<i>Consultations</i>	15
<i>Citizen Science Projects</i>	15
<i>Reaching Out to the BBIG Community via BBIG-g@vt.edu and Other Google Groups</i>	15
ACCOMPLISHMENTS	15
What was accomplished under the proposed objectives?	15
Objective 1: To prevent blighted plant materials from entering the nursery trade	16
1.1 <i>Developing on-site commercial strip kits for pathogen detection (Luster)</i>	16
1.2 <i>Oregon nursery survey (Weiland)</i>	16
1.3 <i>Bridging the blight epidemiology knowledge gaps - latent infection under Oregon climatic conditions (Weiland)</i>	17
1.4 <i>Bridging the blight epidemiology knowledge gaps - pathogen colonization, latency and phenotypic diversity (Crouch)</i>	17
1.5 <i>Bridging the blight epidemiology knowledge gaps - cultivar and relative humidity impacts on pathogen sporulation (Shishkoff)</i>	17
1.6 <i>Bridging the blight knowledge gaps - field epidemiology (Hong)</i>	17
1.7 <i>Identifying critical control points at production nurseries (Baysal-Gurel)</i>	18
Objective 2: To better manage the disease at sites of contamination	18
2.1 <i>Evaluating antidesiccants for blight mitigation (Hong)</i>	19
2.2 <i>Further developing biocontrol agents (BCAs) into final products (Hong)</i>	19
2.3 <i>Enhancing boxwood self-defense through systemic acquired resistance (SAR) inducers and selective fertilization (Daughtrey)</i>	20
2.4 <i>Enhancing boxwood self-defense through silicon (Si) treatment and UV radiation (Gouker)</i>	20
2.5 <i>Integrating three key existing technologies for better blight management at sites of contamination (LaMondia)</i>	21
2.6 <i>Preventing less susceptible boxwood cultivars from becoming ‘Trojan horses’ while encouraging their adoption (Shishkoff)</i>	22
Objective 3: To build resilience into boxwood production and plantings	22
3.1 <i>Developing more resistant boxwood cultivars (Gouker)</i>	22
3.2 <i>Cataloging and manipulating boxwood microbiomes against Cps (Kong)</i>	22
3.3 <i>Facilitating adoption of more resistant boxwood cultivars (Hall)</i>	23
Objective 4: To assess the economic benefits of boxwood blight mitigation (Hall)	23
4.1 <i>Developing baseline production model</i>	23
4.2 <i>Analyzing cost/benefit of changes in blight mitigation practice</i>	23
4.3 <i>Partial budgeting: An economic engineering approach</i>	23

4.4 Performing sensitivity tests	24
4.5 Determining the economic return of long-term investment in blight mitigation	24
4.6 Assessing the overall project impact	24
Objective 5: To put research into practice, helping our stakeholders and also the next generation of scientists and educators to achieve sustainable boxwood production and gardening	24
5.1 Translating research into products, protocols, and recommendations	24
5.2 Delivering to end users	25
<i>New initiative – launch of the BBIG Boxwood Seminar Series</i>	<i>25</i>
<i>Meetings with the Advisory Panel (AP)</i>	<i>25</i>
<i>BBIG outreach website updates</i>	<i>25</i>
<i>International Boxwood Seminar Series</i>	<i>26</i>
<i>BBIG Newsletter</i>	<i>27</i>
<i>BBIG Monthly.....</i>	<i>27</i>
<i>Tweets at Twitter.com/boxwoodhealth</i>	<i>28</i>
<i>Conventional means of technology transfer</i>	<i>28</i>
<i>Other nonconventional means of technology transfer</i>	<i>28</i>
5.3 Documenting project impacts	28
<i>Changes in knowledge</i>	<i>29</i>
<i>Changes in practice and condition</i>	<i>29</i>
<i>Other impacts</i>	<i>30</i>
5.4 To prepare a new generation of scientists and educators for tackling increasingly complex issues facing the nation's specialty crop industry in a global economy	31
What opportunities for training and professional development has the project provided?	33
How have the results been disseminated to communities of interest?	33
What do you plan to do during the next reporting period to accomplish the goals?	33
CHANGES/PROBLEMS.....	33
ACKNOWLEDGEMENTS	33

PROJECT PARTICIPANTS

Project Leadership Team

Chuanxue Hong, Virginia Tech

Margery Daughtrey, Cornell University (Extension Leader)

Douglas Luster, USDA ARS Foreign Disease-Weed Science Research Unit (Research Leader)

Jill Calabro, Valent USA Corporation (Advisory Panel Chair)

Olanike Omolehin, Virginia Tech (Project Manager, September 2022)

Project Co-Directors

Charles Hall, Texas A&M University

Jerry Weiland, USDA ARS Horticultural Crops Disease and Pest Management Research Unit

Fulya Baysal-Gurel, Tennessee State University

Fred Gouker, USDA ARS Floral & Nursery Plants Research Unit

Ping Kong, Virginia Tech

Jo Anne Crouch, USDA ARS Foreign Disease-Weed Science Research Unit

James LaMondia, Connecticut Agricultural Experiment Station

Jay Pscheidt, Oregon State University

Luisa Santamaria, Oregon State University

Nina Shishkoff, USDA ARS Foreign Disease-Weed Science Research Unit

Karen Snover-Clift, Cornell University

Postdoctoral Associates

(*landed a new job)

Urmila Adhikari*, Virginia Tech

Vanina Castroagudín*, USDA ARS Mycology & Nematology Genetic Diversity & Biology Lab

Ihsan Khaliq, Virginia Tech

Srikanth Kodati*, Connecticut Agricultural Experiment Station

Xiaoping Li, Virginia Tech

Prabha Liyanapathiranaga, Tennessee State University

Mana Ohkura*, USDA ARS Horticultural Crops Disease and Pest Management Research Unit

Olanike Omolehin*, Virginia Tech

Xiao Yang*, USDA ARS Foreign Disease-Weed Science Research Unit

Graduate Students

(* landed a new job)

Bhawana Ghimire, Tennessee State University

Gabriel Sacher*, Oregon State University

Ravi Bika*, Tennessee State University

Taylor Phelps, Virginia Tech

Summer Interns and Other Assistants

Ava Borden, Virginia Tech

Gloria Chen, Virginia Tech

Kimberly Clark, Virginia Tech

Robert Holtz, Virginia Tech

Cole Jackson, Virginia Tech

Briana Pecora, Virginia Tech
Paulina Rychlik, Cornell University
Thomas Wilchynski, Virginia Tech

Advisory Panel and Liaison to AmericanHort

Jill Calabro (Chair), Valent USA Corporation
Lynn Batdorf, International Society for Horticultural Science
Frank Collier, Pleasant Cove Nursery
Michael Gaines, CW Arborists, Ltd.
Laura Gladwin, Everde Growers
John Keller, Monrovia – Grow Beautifully
Bennett Saunders, Saunders Genetics, LLC
Richard Schnall, Rosedale Nurseries, Inc
Casey Sclar, The Arboretum at Pennsylvania State University
Mark Sellew, Prides Corner Farms
Jennifer Gray, Horticultural Research Institute, AmericanHort

Key Stakeholder Collaborators

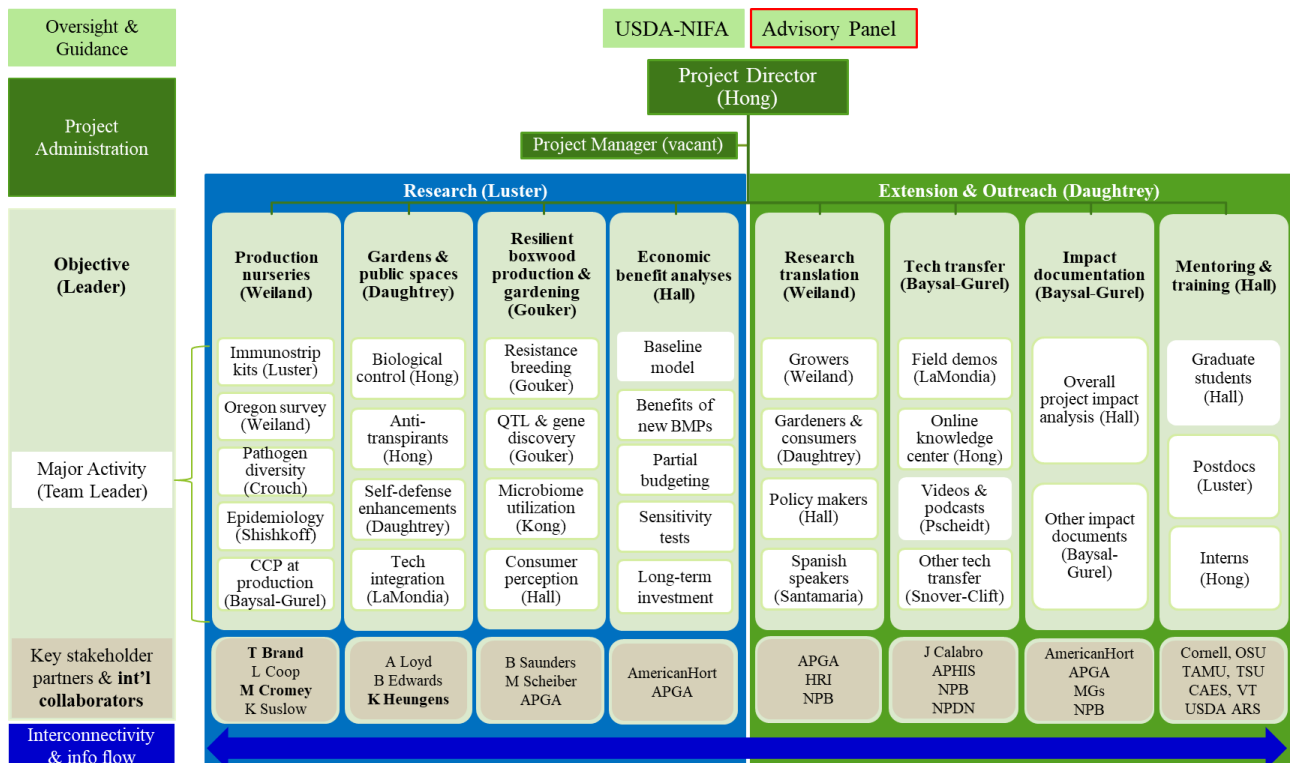
Nilwala Abeysekara, National Ornamental Research Site at Dominican University of California
Urmila Adhikari, AgBiome
Brittany Barker, Oregon State University
Devin Bily, Virginia Department of Agriculture and Consumer Services
Adria Bordas, Virginia Cooperative Extension, Fairfax County
Vanina Castroagudín, USDA Animal and Plant Health Inspection Service
Len Coop, Oregon State University
Marc Cubeta, North Carolina State University
Heather Faubert, University of Rhode Island Cooperative Extension
Andrea Filippone, American Boxwood Society and F² Environmental Design
Dan Gilrein, Cornell Cooperative Extension, New York
Ginger Hemmings, North Carolina Department of Agriculture and Consumer Services
Amanda Holton, AmericanHort
Matthew Howle, Clemson University
Srikanth Kodati, University of Connecticut
Nicolas Leas, AmericanHort
Michael Likins, Virginia Cooperative Extension, Chesterfield County
Andrew Loyd, Bartlett Tree Experts
Mana Ohkura, Oregon State University
Olanike Omolehin, IBM at Baton Rouge
Beth Pantuliano, Longwood Gardens
Karen Rane, University of Maryland Plant Disease Diagnostic Lab
Michele Scheiber, Star Roses and Plants
Rachel Schnaitman, Longwood Gardens
Wolfgang Schweigkofler, National Ornamental Research Site at Dominican University of California
Holly Scoggins, Saunders Genetics, LLC
Melissa Sharifi, Colonial Williamsburg Foundation
Karen Suslow, National Ornamental Research Site at Dominican University of California
Amanda Taylor, North Carolina Cooperative Extension

Chad Taylor, North Carolina Department of Agriculture and Consumer Services
Hsien-Tzer (HT) Tseng, North Carolina Department of Agriculture and Consumer Services
Mina Vescera, Cornell Cooperative Extension, New York
Margaret Williamson, Clemson University
Jean Williams-Woodward, University of Georgia
Xiao Yang, Clemson University
Keith Yoder, Saunders Genetics, LLC

Major International Collaborators

Heinrich Beltz, Research and Teaching Institute for Horticulture, Germany
Thomas Brand, Landwirtschaftskammer Niedersachsen, Germany
Matthew Cromey, Royal Horticultural Society, United Kingdom
Björn Ehsen, Research and Teaching Institute for Horticulture, Germany
Marie Froyen, Flanders Research Institute for Agriculture, Fisheries and Food, Belgium
Kurt Heungens, Flanders Research Institute for Agriculture, Fisheries and Food, Belgium
Thomas Hsiang, School of Environmental Sciences, University of Guelph, Ontario, Canada
Marc Kenis, Centre for Agriculture and Biosciences International, Switzerland
Iryna Matsiakh, Swedish University of Agricultural Sciences, Sweden
Katrijn Van Laere, Flanders Research Institute for Agriculture, Fisheries and Food, Belgium

Project Organization



APGA, American Public Gardens Association; APHIS, USDA Animal and Plant Health Inspection Service; CAES, Connecticut Agricultural Experiment Station; CCP, critical control point; HRI, Horticultural Research Institute; MGs, master gardeners; NPB, National Plant Board; NPND, National Plant Diagnostic Network; OSU, Oregon State University; TAMU, Texas A&M University; TSU, Tennessee State University; VT, Virginia Tech; and USDA ARS, USDA Agricultural Research Service

TARGET AUDIENCE

Growers
Greenery producers
Retailers
Landscapers and ground maintenance personnel
Public garden managers and horticulturists
Trade organizations - local, regional, and national
Consumers
Master gardeners
Boxwood enthusiasts
Arborists
Diagnosticians
Extension specialists and agents
Regulatory personnel
Conservation biologists
Researchers
Policy makers

PRODUCTS

Research

PhD Dissertations

1. Daughtrey, M. L. 2023. Invasive exotic diseases on dogwood, boxwood and impatiens in the United States. Cornell University. May 2023.
2. Sacher, G. O. 2023. Diseases of rhododendron and boxwood: survey and virulence of *Phytophthora* root rot and management of boxwood blight with systemic fungicides. Oregon State University. May 2023.

Refereed Journal Articles

1. Brand, T., Beltz, H., Adhikari, U., Daughtrey, M., Luster, D. G., Kong, P., and Hong, C. X. 2022. Evaluation of fungicides for management of boxwood blight caused by *Calonectria* spp. under field conditions in Northern Germany. *Journal of Plant Diseases and Protection* ([open access](#)).
2. Ghimire, B., Liyanage, K. H. E., Hall, C., and Baysal-Gurel, F. 2023. A modified Delphi study on boxwood blight disease management in the US nursery industry. *HortScience* 58(8):898-906 ([open access](#)).
3. Ghimire, B., Parajuli, M., Liyanapathirana, P., Simmons, T., and Baysal-Gurel, F. 2023. Evaluation of fungicides and antitranspirant for the control of boxwood blight, 2022. *Plant Disease Management Report* No. 17:OT018. <https://www.plantmanagementnetwork.org/pub/trial/pdmr/reports/2023/OT018.pdf>.
4. Hong, C. X. 2023. Building health into new boxwood crops and plantings by making informed cultivar selection. *Plant Health Progress*, Abstract at <https://apsjournals.apsnet.org/doi/10.1094/PHP-01-23-0002-RV>.
5. Kong, P., Daughtrey, M. L., and Hong, C. X. 2023. Differential adaptation has resulted in aggressiveness variation of *Calonectria pseudonaviculata* on hosts *Buxus*, *Pachysandra* and *Sarcococca*. *Journal of Fungi* 9(2), 181 ([open access](#)).
6. Kong, P., Li, X. P., Sharifi, M., Bordas, A., and Hong, C. X. 2023. Leaf endophyte community composition and network structures differ between tolerant and susceptible English boxwood. *Phytobiomes Journal* ([open access](#)).
7. Li, X. P., Omolehin, O., Hemmings, G., Tseng, H. T., Taylor, A., Taylor, C., Kong, P., Daughtrey, M., Luster, D., Gouker, F., and Hong, C. X. 2023. Boxwood phyllosphere fungal and bacterial communities and their differential responses to film-forming antidesiccants. *BMC Microbiology* 23, 219 ([open access](#)).
8. Li, X. P., Tseng, H. T., Omolehin, O., Hemmings, G., Taylor, C., Taylor, A., Kong, P., Daughtrey, M. L., Gouker, F., and Hong, C. X. 2023. Characterization of boxwood shoot bacterial communities and potential impact from fungicide treatments. *Microbiology Spectrum* ([open access](#)).
9. Ohkura, M., Scagel, C. F., and Weiland, J. E. 2023. Rapid and scalable DNA extraction and real-time PCR assay from boxwood tissue for the detection of *Calonectria pseudonaviculata*, causal agent of boxwood blight. *Plant Disease*. 107:1279-1283. <https://doi.org/10.1094/PDIS-06-22-1453-SR>.
10. Omolehin, O., Keller, J., Gouker, F., Daughtrey, M., Luster, D. G., Pscheidt, J., and Hong, C. X. 2022. Combating an invasive boxwood pathogen – *Calonectria pseudonaviculata* –

in the United States by shifting production to less susceptible cultivars. Plant Disease. Abstract at <https://doi.org/10.1094/PDIS-09-22-2124-RE>.

11. Yoder, K. S., Dunn, R. A., Saunders, J. B., Mays, T. E., Yanny, M. D., Hong, C. X., and Scoggins, H. L. 2022. Field performance of *Buxus* cultivars and selections against boxwood leafminer and boxwood blight. Journal of Environmental Horticulture 40(4): 129-142 ([open access](#)).

Abstracts (Presented at Professional Meetings)

1. Daughtrey, M., Luster, D., Calabro, J., Gray, J., Hall, C., Weiland, J., Baysal-Gurel, F., Gouker, F. E., Kong, P., Crouch, J. A., LaMondia, J. A., Pscheidt, J. W., Santamaria, L., Shishkoff, N., Snover-Clift, K., and Hong, C. X. 2023. Fighting a good fight against an invasive pathogen through an international consortium: the Boxwood Blight Insight Group (BBIG). Plant Health 2023 – Annual Meeting of the American Phytopathological Society (APS). Denver, CO, August 12 to 16.
2. Ghimire, B., Liyanage, K., Hall, C., and Baysal-Gurel, F. 2023. Investigating the growers' perceptions on boxwood blight disease management in the U.S. nursery industry. 2023 Annual Meeting of the APS Southern Division. Durham, NC. February 13 to 16.
3. Ghimire, B., Liyanage, K., Hall, C., Pendyala, B., Patras, A., and Baysal-Gurel, F. 2023. An investigation on nursery growers' perception of boxwood blight disease and assessment of efficacy of its management using chemical and innovative alternative approaches. 45th Tennessee State University (TSU)-wide Annual Research Symposium. March 27 to 31.
4. Ghimire, B., Liyanage, K., Pendyala, B., Patras, A., Hall, C., and Baysal-Gurel, F. 2023. Understanding nursery grower's perspectives on managing boxwood blight disease and developing innovative control methods (Oral talk). Annual Meeting of American Society of Horticultural Science. Orlando, FL. July 31 to August 4.
5. Ghimire, B., Liyanage, K., Pendyala, B., Patras, A., Hall, C., and Baysal-Gurel, F. 2023. Understanding nursery grower's perspectives on managing boxwood blight disease and developing innovative control methods (Scholar's Ignite Session). Annual Meeting of American Society of Horticultural Science. Orlando, FL. July 31 to August 4.
6. Ghimire, B., Parajuli, M., Simmons, T., Liyanapathiranage, and Baysal-Gurel, F. 2023. Comparative performance of fungicides, host plant defense inducers and antitranspirant in management of boxwood blight. Plant Health 2023 – APS Annual Meeting. Denver, CO. August 12 to 16, 2023.
7. Kodati, S. and LaMondia, J. 2023. Duration of protection offered by fungicides against boxwood blight. Plant Health 2023 – APS Annual Meeting. Denver, CO. August 12 to 16, 2023.
8. Kong, P., and Hong, C. X. 2023. *Burkholderia* sp. SSG – a powerful new management tool for crop health and production. 12th International Congress of Plant Pathology. Lyon, France. August 20 to 25.
9. Li, X. P., Weiland, J., Ohkura, M., Kong, P., and Hong, C. X. 2023. Comparative analysis of boxwood shoot endophytic microbiota between two major boxwood production regions in the United States. Plant Health 2023 – APS Annual Meeting. Denver, CO. August 12 to 16.
10. Liyanage, K., Ghimire, B., Hall, C., and Baysal-Gurel, F. 2023. Economic impact and management of boxwood blight disease in the U.S. Nursery Industry. 45th TSU University-wide Annual Research Symposium. March 27 to 31.

11. Ohkura, M., Scagel, C. F., and Weiland, J. E. 2023. The effect of host volatiles on spore germination of *Calonectria pseudonaviculata*, the causal agent of boxwood blight. 2023 Annual Meeting of the APS Pacific Division. Tucson, AZ. March 13 to 16.
12. Ohkura, M., Scagel, C., and Weiland, J. 2023. The effect of host volatiles on the sporulation and germination of *Calonectria pseudonaviculata*, the causal agent of boxwood blight. Plant Health 2023 – APS Annual Meeting. Denver, CO. August 12 to 16, 2023.
13. Olsson, A., Shishkoff, N., and Smallwood, E. 2023. Effect of relative humidity on disease severity and sporulation of fungal pathogens *Calonectria pseudonaviculata* and *C. henricotiae*. The 79th Annual Meeting of APS Potomac Division. Fairfax, VA. March 22 to 24.
14. Omolehin, O., Kong, P., Hemmings, G., Taylor, A., Tseng, H. T., Taylor, C., Li, X. P., Khaliq, I., Daughtrey, M., Luster, D., and Hong, C. X. 2023. Field performance of selected biological control agents for protecting Vardar Valley boxwood from infection by *Calonectria pseudonaviculata*. Plant Health 2023 – APS Annual Meeting. Denver, CO. August 12 to 16.

Invited Presentations at Professional Conferences WITHOUT Abstract

1. Ghimire, B., Pendyala, B., Patras, A., and Baysal-Gurel, F. 2022. Ultraviolet inactivation of conidia of boxwood blight pathogen, *Calonectria pseudonaviculata*. Ornamental Workshop on Pests and Diseases. JC Raulston Arboretum, Raleigh, NC. October 18 to 20 (Poster presentation).
2. Hong, C. X. 2023. Antidesiccants for boxwood blight management in gardens and public spaces. The 79th Annual Meeting of APS Potomac Division. Fairfax, VA, March 22 to 24.
3. Shishkoff, N., and Daughtrey, M. 2022. Something wicked this way comes: boxwood blights. Ornamental Workshop on Pests and Diseases. JC Raulston Arboretum, Raleigh, NC. October 19.

Thesis Defense Seminars

1. Daughtrey, M. L. 2023. Invasive exotic diseases on dogwood, boxwood, and impatiens in the United States. Cornell University, by Zoom, April 26.
2. Sacher, G. O. 2023. Diseases of rhododendron and boxwood: survey and virulence of *Phytophthora* root rot and management of boxwood blight with systemic fungicides. Oregon State University, In a hybrid format, May 23.

Global and Stakeholder Partnerships

International Boxwood Blight Epidemiology Working Group Meetings Hosted

- The fifth IBBE meeting on October 17, 2022 with one invited presentation
 - Models for boxwood blight infection and establishment risk, by Drs. Len Coop and Brittany Barker, Oregon State University.

This international group meeting provided a platform to share the latest research and brainstorm future directions in boxwood blight epidemiology.

Working with German Scientists on Field Epidemiology while Analyzing and Publishing Multi-year Fungicide Trial Data

Boxwood blight is caused by two fungal pathogens: *Calonectria pseudonaviculata* (*Cps*) and *C. henricotiae* (*Che*). Currently, *Cps* is widespread in Europe, Western Asia, North America and New Zealand, while *Che*, first identified in 2016, is currently limited only to a few European countries including Germany. Little was known about *Che*, and all published studies have been done under lab conditions. *Che* is known to be more tolerant of high temperatures and less sensitive to some fungicides than *Cps*, thus, potentially more adapted to the U.S. weather conditions and more difficult to control should it be accidentally introduced into this country.

This collaboration produced one open access publication reporting the multi-year field studies evaluating fungicide efficacy in northern Germany and made them available to American stakeholders and international communities. One of the most significant aspects of these studies was the research site with both blight pathogens, *Cps* and *Che*, thus filling major knowledge gaps in our understanding about how fungicides perform against *Che* under field conditions.

While analyzing and publishing the fungicide efficacy data was an add-on after the project was approved, it helped delve into the field data sets and developed a better understanding of boxwood blight field epidemiology.

- Khaliq, I., Brand, T., Daughtrey, M. L., Kong, P., and Hong, C. X. 2023. Investigating weather variables driving boxwood blight epidemics: insights from the field trials with *Buxus sempervirens* ‘Suffruticosa’ in northern Germany between 2006 and 2020. Submitted to Plant Pathology.

Connecting and Working with Scientists in Southern Europe Investigating whether the Blight Pathogen Populations in That Region are Dominated by *Che*

Che could be much more damaging to U.S. boxwood crops and plantings should it be accidentally introduced into this country. We learned from the above collaboration with German scientists that this pathogen was able to quickly outcompete *Cps* in northern Germany. However, it is not known whether *Che*, which is tolerant of high temperature, is present in any of the southern European countries that are warmer than northern Germany. This newly identified knowledge gap is critical to safeguarding boxwood crops and plantings in the U.S. We hypothesized that *Che* is present or even dominating the blight pathogen populations in these countries. To test this hypothesis, we made several efforts:

- Attempted to connect with scientists in France, Italy, Spain and Switzerland who reported the first cases of boxwood blight in these countries.
- Discussed with those in France and Switzerland about the status of this disease and pathogen population research, fostering joint effort to look into this important question.
- Used the opportunity of attending the 12th International Congress of Plant Pathology in Lyon, France to visit some botanic gardens in Spain and France, and to see boxwood plantings and blight disease on-site and collect diseased boxwood shoot samples for isolation and analyses.
- Transferred three isolates of the boxwood blight pathogen from L’Agence Nationale de Sécurité Sanitaire de l’alimentation, de l’environnement et du travail to Virginia Tech through a material transfer agreement for molecularly typing.

Collaborating with North Carolina Department of Agriculture and Consumer Services (NCDA&CS), North Carolina Cooperative Extension (NCCE), and Local Farmers in Field Trials Evaluating Selected Antidesiccants and Biological Control Agents

Working with NCDA&CS (H. T. Tseng, Chad Taylor, Ginger Hemmings), NCCE (Amanda Taylor), and local farmers (names not shown per the research protocol approved by the Virginia Tech Institutional Review Board) was another add-on that has enhanced the project quality, productivity and efficiency for the proposed field trials evaluating selected antidesiccants and biological control agents for their blight mitigation potential. These trials were done in western North Carolina, a hotspot for the disease since its first observation in the U.S. (2011). The NCDA&CS and NCCE collaborators helped to identify the naturally-infested fields for these trials, set them up then worked with the team to apply planned treatments and assess treatment efficacy monthly. One contribution of critical importance was their application of fungicide standard treatments every 3 weeks – this made the trial manageable, as the research fields were more than 5 hours of driving distance from Virginia Beach where the Virginia Tech Team was based.

Looking into how the boxwood foliar microbial community may be affected by antidesiccant products and three fungicide standards was also added to the project after the first treatment in the spring of 2021. This add-on made it possible to integrate antidesiccants and fungicides into a systems approach to boxwood crop health and production, utilizing their full potential as horticultural products now, and looking toward their future use as blight mitigation tools.

These collaborations have resulted in a third submission, in addition to two open access publications in Microbiology Spectrum and BMC Microbiology:

1. Li, X. P., Hemmings, G., Omolehin, O., Tseng, H. T., Taylor, A., Taylor, C., Kong, P., Gouker, F., and Hong, C. X. 2023. Unravelling boxwood phyllosphere epi- and endophytic fungal communities and their responses to three fungicides. Submitted to ISME Communications.

[International Boxwood Seminar Series](#)

Two webinars were conducted during this project year with speakers from USA and Sweden.

1. How do weather and climate impact boxwood blight? A modeling approach to aiding in boxwood disease management. By Dr. Len Coop of Oregon State University, Corvallis, Oregon, September 7, 2022.
2. Calamities of Georgia's boxwood forests. By Dr. Iryna Matsiakh, the Swedish University of Agricultural Sciences, Sweden, December 20, 2022.

These webinars were conducted in a close partnership with the AmericanHort foundation, the Horticultural Research Institute (HRI), and integrated into their tHRive Webinar series. The project team identified the topics of practical importance and recruited world leading scientists of the subject areas to address each topic, while HRI did a lot hard work behind the scene - coordinating, publicizing, hosting via Zoom.us, recording and then posting the recordings at the project outreach website, and providing attendance metrics.

[Outreach Website – Boxwood Blight Insight Group](#)

This website is hosted at the HRI and updated regularly by HRI staff as requested by Project Directors to keep stakeholders and the public informed of the project progress. Major updates included:

- Added a new section – Box Tree Moth – to the [Knowledge Center](#) tab to provide the latest news and research about this invasive pest in the U.S. and meet the emerging needs of our stakeholders. This section currently consists of three subsections:
 - APHIS – white paper and quarantine actions.
 - Webinars – educational programs hosted by BBIG and HRI

- Control strategies – cultivar susceptibility and insecticide recommendations.
- Expanded the Diagnostic Aids section under the [Knowledge Center](#) tab to Diagnostic Aids and Management Strategies to accommodate new factsheets, recommendations, and best management practices that the Team is developing as we have learned from our project. This expanded section now includes following subsections:
 - Blight symptom library
 - Boxwood blight forecasting app and Push notification
 - Scouting, diagnostic guides and tools
 - Boxwood blight resistance
 - Boxwood blight maps
 - Boxwood blight cleanliness programs
 - Sanitizers and sanitation practices
 - Cultural control
 - Fungicide options and chemical protection
 - Best management practices
- Added a new section – Capacity-Building Seminars – to the [About BBIG](#) tab

New materials have been posted under each of these new or modified (sub)sections, in addition to those under the Research Updates tab.

Launch of the BBIG Boxwood Seminar Series

This new Seminar Series was launched in the summer of 2023 to expedite the technology transfer from lab to field. It kicked off with the first webinar – Battling boxwood blight – presented by James LaMondia on August 23.

This is a new product of our partnership with HRI. As with the International Boxwood Seminars, HRI is doing all the legwork – organizing, publicizing, hosting and recording and collecting registrants and attendance data. Likewise, they posted the recording at the BBIG outreach website to continue serving the industry and the public.

Other Major Extension Products

Twitter.com/boxwoodhealth

As of August 28, 2023, 41 tweets were made to keep stakeholders and the public informed of the latest research, educational opportunities, and boxwood health related news including box tree moth.

BBIG Monthly Digests

The BBIG Monthly was published on the first business day of each month. These monthly digests highlighted the latest research, new publications, educational events and programming, keeping stakeholders informed in a more timely fashion.

1. Omolehin, O., Daughtrey, M., Luster, D., Hall, C., Weiland, J., Baysal-Gurel, F., Gouker, F. E., Kong, P., Crouch, J. A., LaMondia, J. A., Pscheidt, J. W., Santamaria, L., Shishkoff, N., and Snover-Clift, K, and Hong, C.X. 2022. BBIG Monthly – September 2022, released September 1.
2. Hong, C. X., Daughtrey, M., Luster, D., Hall, C., Weiland, J., Baysal-Gurel, F., Gouker, F. E., Kong, P., Crouch, J. A., LaMondia, J. A., Pscheidt, J. W., Santamaria, L., Shishkoff, N., and Snover-Clift, K. 2022. BBIG Monthly – October 2022, released October 3.

3. Hong, C. X., Daughtrey, M., Luster, D., Hall, C., Weiland, J., Baysal-Gurel, F., Gouker, F. E., Kong, P., Crouch, J. A., LaMondia, J. A., Pscheidt, J. W., Santamaria, L., Shishkoff, N., and Snover-Clift, K. 2022. BBIG Monthly – November 2023, released November 1.
4. Hong, C. X., Daughtrey, M., Luster, D., Hall, C., Weiland, J., Baysal-Gurel, F., Gouker, F. E., Kong, P., Crouch, J. A., LaMondia, J. A., Pscheidt, J. W., Santamaria, L., Shishkoff, N., and Snover-Clift, K. 2022. BBIG Monthly – December 2023, released December 1.
5. Hong, C. X., Daughtrey, M., Luster, D., Hall, C., Weiland, J., Baysal-Gurel, F., Gouker, F. E., Kong, P., Crouch, J. A., LaMondia, J. A., Pscheidt, J. W., Santamaria, L., Shishkoff, N., and Snover-Clift, K. 2023. BBIG Monthly – January 2023, released January 3.
6. Hong, C. X., Daughtrey, M., Luster, D., Hall, C., Weiland, J., Baysal-Gurel, F., Gouker, F. E., Kong, P., Crouch, J. A., LaMondia, J. A., Pscheidt, J. W., Santamaria, L., Shishkoff, N., and Snover-Clift, K. 2023. BBIG Monthly – February 2023, released February 1.
7. Hong, C. X., Daughtrey, M., Luster, D., Hall, C., Weiland, J., Baysal-Gurel, F., Gouker, F. E., Kong, P., Crouch, J. A., LaMondia, J. A., Pscheidt, J. W., Santamaria, L., Shishkoff, N., and Snover-Clift, K. 2023. BBIG Monthly – March 2023, released March 1.
8. Hong, C. X., Daughtrey, M., Luster, D., Hall, C., Weiland, J., Baysal-Gurel, F., Gouker, F. E., Kong, P., Crouch, J. A., LaMondia, J. A., Pscheidt, J. W., Santamaria, L., Shishkoff, N., and Snover-Clift, K. 2023. BBIG Monthly – April 2023, released April 3.
9. Hong, C. X., Daughtrey, M., Luster, D., Hall, C., Weiland, J., Baysal-Gurel, F., Gouker, F. E., Kong, P., Crouch, J. A., LaMondia, J. A., Pscheidt, J. W., Santamaria, L., Shishkoff, N., and Snover-Clift, K. 2023. BBIG Monthly – May 2023, released May 1.
10. Hong, C. X., Daughtrey, M., Luster, D., Hall, C., Weiland, J., Baysal-Gurel, F., Gouker, F. E., Kong, P., Crouch, J. A., LaMondia, J. A., Pscheidt, J. W., Santamaria, L., Shishkoff, N., and Snover-Clift, K. 2023. BBIG Monthly – June 2023, released June 1.
11. Hong, C. X., Daughtrey, M., Luster, D., Hall, C., Weiland, J., Baysal-Gurel, F., Gouker, F. E., Kong, P., Crouch, J. A., LaMondia, J. A., Pscheidt, J. W., Santamaria, L., Shishkoff, N., and Snover-Clift, K. 2023. BBIG Monthly – July 2023, released July 3.
12. Hong, C. X., Daughtrey, M., Luster, D., Hall, C., Weiland, J., Baysal-Gurel, F., Gouker, F. E., Kong, P., Crouch, J. A., LaMondia, J. A., Pscheidt, J. W., Santamaria, L., Shishkoff, N., and Snover-Clift, K. 2023. BBIG Monthly – August 2023, released August 1.

[BBIG Newsletters - Quarterly](#)

1. Hong C. X., Daughtrey, M. L., Luster, D. G., Hall, C., Weiland, J., Baysal-Gurel, F., Gouker, F. E., Kong, P., Crouch, J. A., LaMondia, J. A., Pscheidt, J. W., Santamaria, L., Shishkoff, N., and Snover-Clift, K. 2022. BBIG Newsletter Volume 3, Issue 4, released November 21.
2. Hong C. X., Daughtrey, M. L., Luster, D. G., Hall, C., Weiland, J., Baysal-Gurel, F., Gouker, F. E., Kong, P., Crouch, J. A., LaMondia, J. A., Pscheidt, J. W., Santamaria, L., Shishkoff, N., and Snover-Clift, K. 2023. BBIG Newsletter Volume 4, Issue 1, released February 24.

3. Hong C. X., Daughtrey, M. L., Luster, D. G., Hall, C., Weiland, J., Baysal-Gurel, F., Gouker, F. E., Kong, P., Crouch, J. A., LaMondia, J. A., Pscheidt, J. W., Santamaria, L., Shishkoff, N., and Snover-Clift, K. 2023. BBIG Newsletter Volume 3, Issue 2, released May 26.
4. Hong C. X., Daughtrey, M. L., Luster, D. G., Hall, C., Weiland, J., Baysal-Gurel, F., Gouker, F. E., Kong, P., Crouch, J. A., LaMondia, J. A., Pscheidt, J. W., Santamaria, L., Shishkoff, N., and Snover-Clift, K. 2023. BBIG Newsletter Volume 3, Issue 3, released August 27.

Newsletter and Trade Journal Articles (Excluding Those Published in the BBIG Newsletters)

1. Batdorf, L. R., and Daughtrey, M. L. 2023. Boxwood blight nomenclature. The Boxwood Bulletin. The Journal of the American Boxwood Society 62:40-42.
2. Baysal-Gurel, F. 2022. Box blight control trial. Chase Digest December 2022 Issue Volume 10(12):5.
3. Bily, D., and Hong, C. X. 2023. [Best management practices for boxwood blight in Virginia nurseries](#). Online publication by Virginia Cooperative Extension – SPES-516NP
4. Hong, C. X. 2023. Boxwood blight – an emerging disease that keeps landscapers and gardeners awake at night. [Virginia Turfgrass Journal \(March/April\):24-26](#).
5. Hong, C. X. 2023. BBIG Team met in the boxwood country – Oregon. Phytopathology News 57(5):11-12.
6. Hong, C. X. 2023. Evaluating antidesiccants as a physical barrier for boxwood blight mitigation under controlled environments. VNLA News – The Virginia Nursery and Landscape Association Magazine. (Summer issue):10-13.
7. Pscheidt, J. W. 2023. Boxwood (*Buxus* spp.) – boxwood blight. [Pacific Northwest Plant Disease Management Handbook](#).

Workshops

1. Weiland, J., Santamaria, L., Pscheidt, J. 2022. Oregon Boxwood Health Workshop, Oregon State University's North Willamette Research and Extension Center in Aurora, OR, October 20.
 - a. Santamaria, L. Scouting tips and educational resources for boxwood diseases and pests.
 - b. Weiland, J. and Ohkura, M. Influence of nursery production practices and environment on boxwood blight.
 - c. Coop, L. and Barker, B. Infection risk app and climate suitability model.
 - d. Pscheidt, J. Fungicide selection and coverage.
 - e. Hos, A. ODA boxwood blight cleanliness program.
 - f. Weiland, J. Field tour to onsite boxwood blight research on plant spacing and irrigation frequency.
2. Pscheidt, J. 2022. Pathogens, life cycles and management principles of boxwood blight and other important plant diseases. Master Gardener Hour Training, Roseburg, Douglas Co., OR. October 18.

Extension Presentations

1. Baysal-Gurel, F. 2022. Vascular streak dieback of redbud and other important woody ornamental disease updates. Tennessee Nursery and Landscape Association Field day. McMinnville, TN. August 25.
2. Baysal-Gurel, F. 2022. Boxwood blight case study. Tennessee Urban Forestry Council State Conference. Burns, TN. November 9 to 10.
3. Baysal-Gurel, F. 2023. Boxwood blight disease management - woody ornamental disease update. UT- 2023 Landscape Short Course/Green Team Landscape Meeting. Franklin, TN. January 12.
4. Baysal-Gurel, F. 2023. Sanitation for boxwood blight management. The Kentucky Green Industry Winter Conferences. January 25.
5. Baysal-Gurel, F. 2023. Boxwood blight disease management. Advanced Turf Solutions Meeting. Lebanon, TN. January 31.
6. Baysal-Gurel, F. 2023. Boxwood blight disease management. Nashville Lawn & Garden Show. in Nashville, TN. March 2.
7. Baysal-Gurel, F. 2023. Dealing with diseases in the landscape. TEMG Intern Training. Virtual presentation. March 28.
8. Baysal-Gurel, F. 2023. Dealing with boxwood diseases in the landscape. Warren County Master Gardener's Class. McMinnville, TN. April 6.
9. Baysal-Gurel, F. 2023. Sanitation practices in nursery production. Cultivate'23, Columbus, OH. July 17.
10. Daughtrey, M. 2022. Research and observations on woody plant diseases. Cornell Long Island Horticultural Research and Extension Center Plant Science Day. Riverhead, NY. September 15.
11. Daughtrey, M. 2022. Are we propagating plant diseases? Eastern Branch of the International Plant Propagators Society Meeting. Hauppauge, NY. September 28.
12. Daughtrey, M. 2022. Something wicked this way comes. GardenComm. Webinar, October 12.
13. Daughtrey, M. 2022. IPM for diseases in the landscape. Farmingdale State College SUNY. November 8.
14. Daughtrey, M. 2023. When it didn't rain, it was droughty. Professional Certified Applicators of Long Island. Melville, NY. January 19.
15. Daughtrey, M. 2023. Diseases to defend your plants against. Back 2 Basics. AmericanHort. Virtual. February 7.
16. Daughtrey, M. 2023. Scouting skills for diseases on broadleaved woody plants. Sharpen Your Scouting Skills Workshop. Branching Out, Cornell Cooperative Extension, Ithaca, NY. Virtual, February 9.
17. Daughtrey, M. 2023. Understanding plant diseases. Master Gardeners of Fairfax County, Virginia. Virtual. February 15.
18. Daughtrey, M. 2023. Diseases of ornamental plants. Certified Nursery and Landscape Professionals Training. Virtual. February 21.
19. Daughtrey, M. 2023. Diseases update. New York State Nursery and Landscape Association. Virtual. March 9.
20. Daughtrey, M. 2023. Diseases after drought and diseases after rain. Long Island Horticulture Conference. Brookhaven National Laboratory, Upton, NY. March 9.

21. Daughtrey, M. 2023. Tree and shrub diseases coming in 2023. Urban Forestry Today. University of Massachusetts. Virtual. March 16.
22. Daughtrey, M. L. 2023. Identifying and coping with diseases of ornamental plants. Tree Committee Meeting, Ladies Village Improvement Society, East Hampton, NY. June 24.
23. Daughtrey, M. L. 2023. Solving disease problems of ornamental plants. Intern Field Day. Cornell University Long Island Horticultural Research & Extension Center, Riverhead, NY. July 11.
24. Daughtrey, M. L. 2023. Research and diagnosis for diseases of woody plants. Nursery Section. Plant Science Day. Cornell University Long Island Horticultural Research & Extension Center, Riverhead, NY. July 13.
25. Pscheidt, J. W. 2023. Fungicide selection and coverage. Pesticide Recertification. Southern Oregon Research and Extension Center. Central Point, OR, January 24.

Interactive Virtual Workshops for both English and Spanish Speakers

Santamaria, L., Baysal-Gurel, F., and Fare, D. 2022. Scouting pest and diseases. TNLA Field Day. McMinnville, TN. August 25.

BBIG Showcased in Major National and International Events and Media

Two posters were presented at the Annual Meeting of the American Society of Horticultural Sciences in Orlando, FL from July 31 to August 4, 2023, and six at the Plant Health 2023 – APS Annual Meeting in Denver, CO from August 12 to 16, 2023, while one poster was presented at the 12th International Congress of Plant Pathology in Lyon, France from August 20 to 26, 2023.

Consultations

Weiland, J.E. 2023. Consultations with two nurseries to assess boxwood samples for boxwood blight. January-June.

Citizen Science Projects

Working with NEWGEN Boxwood Team has resulted in one research publication in the Journal of Environmental Horticulture. The project extended the knowledge about boxwood cultivar resistance not only for boxwood blight but also leafminer.

Reaching Out to the BBIG Community via BBIG-g@vt.edu and Other Google Groups

BBIG-G@vt.edu is a group of twelve Google groups created for different stakeholder groups, and it was used along with local groups such as vce-anr-g@vt.edu for disseminating the latest research and learning opportunities about boxwood blight, reaching 24,604 as of August 25, 2023

ACCOMPLISHMENTS

What was accomplished under the proposed objectives?

This project aimed to better contain and manage an emerging invasive pathogen/disease by taking a systems approach, collaborating with scientists overseas, and partnering with stakeholders. To better serve the horticulture industry and other stakeholders, we added to the project the analyses and documentation of the nation's boxwood production shift to blight-free/less affected regions as well as the inventory shift to less susceptible cultivars. We also

continued the International Boxwood Seminars, with speakers from Sweden and the Pacific Northwest.

Our research and extension programs supported the continual growth (c. 25%) of demand for and sales of boxwood plants in the U.S. as shown in a recent survey of seventeen nurseries in seven major boxwood producing states over a 5-year period from 2017 to 2021. More importantly, they helped to move the nation onto a fast track to sustainable boxwood production and gardening.

Objective 1: To prevent blighted plant materials from entering the nursery trade

1.1 Developing on-site commercial strip kits for pathogen detection (Luster)

We previously identified a protein target by proteomic analysis for generation of antibodies for serological detection of *Calonectria* in infected boxwood leaves. This abundant protein target is unique to the boxwood blight fungi, *Cps* and *Che*. A contract was initiated in May 2022 to generate mouse monoclonal antibodies (mAbs) against two peptides with amino acid sequences derived from the *Calonectria* target protein that we demonstrated in previous studies to be expressed in infected leaves. Four mouse mAbs were received from the contractor in late 2022 and are currently being evaluated for specificity and sensitivity. Current results indicate that two of the mouse mAbs are similar in sensitivity to previously tested rabbit polyclonal antibodies (pAbs, see data in 2022 Annual Report, Objective 1, Subobjective 1.1). Mouse mAbs are harvested from immortal cell cultures, are usually more specific than pAbs, and thus are useful in final development of diagnostic assays. Once fully evaluated, we anticipate that a combination of pAbs and mAbs may be suitable for development of a lateral flow device for detection of *Cps* in the field.

1.2 Oregon nursery survey (Weiland)

The nursery survey was concluded and results are being compiled and analyzed to prepare a research paper. In total, over 8.6 million plants at 20 nurseries were assessed. Preliminary results show that 16 nurseries had boxwood blight and that the disease was more common and severe on plants that were more tightly spaced (Figure 1) and watered more frequently.

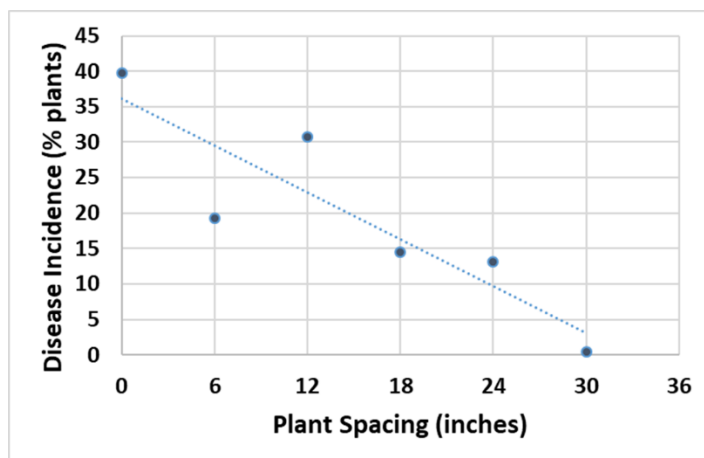


FIGURE 1 BOXWOOD BLIGHT INCIDENCE DECREASED WITH INCREASING PLANT SPACING

A real-time PCR method was developed to detect *Cps* from boxwood samples collected at nurseries. This method was used to confirm results from samples assessed by moist chambers

and to check for false negatives. The real-time PCR method was published in a peer-reviewed journal.

1.3 Bridging the blight epidemiology knowledge gaps - latent infection under Oregon climatic conditions (Weiland)

Experiments to evaluate latent infection are in progress. Plants were inoculated and disease was allowed to develop for eight months or longer. Real-time PCR was then used to evaluate symptomatic and asymptomatic plant tissues collected from infected plants. Preliminary results suggest that latent infection over the long-term is unlikely. Rather, mild symptoms are likely to be overlooked during the inspection process and result in the unintentional movement of infected boxwood plants via nursery trade.

1.4 Bridging the blight epidemiology knowledge gaps - pathogen colonization, latency and phenotypic diversity (Crouch)

Research was interrupted by the shutdown of our lab during COVID19 (lab reopening April 2022) and due to the move of the lab from the USDA Beltsville, MD to USDA-ARS Frederick, MD. Sequencing equipment that was available at the previous facility was not available at the new lab. A new sequencer was purchased and an existing protocol was modified and optimized to be performed on the new MiniSeq DNA sequencer.

1.5 Bridging the blight epidemiology knowledge gaps - cultivar and relative humidity impacts on pathogen sporulation (Shishkoff)

Research was interrupted by the shutdown of our lab during COVID, but this year we were able to continue our work on the effect of different relative humidities (100%, 80%, 60% 40%) on sporulation for different cultivars of boxwood. At different relative humidities, we measured disease severity and sporulation in three replicated trials each on *Buxus sempervirens* ‘Suffruticosa’ and ‘Justin Brouwers’, *B. microphylla* ‘Little Missy’, and *B. ×* ‘Green Mountain’.

1.6 Bridging the blight knowledge gaps - field epidemiology (Hong)

We investigated the field epidemiology of boxwood blight through two case studies. In collaboration with German scientists, we analyzed the data from field trials conducted with *Buxus sempervirens* ‘Suffruticosa’ in northern Germany between 2006 and 2020. These analyses revealed that disease severity significantly increased with increasing mean rainfall per rainy day, daily mean minimum temperature, and daily mean minimum relative humidity. These discoveries provide new leads to calibrate the boxwood blight infection risk model which currently focuses on the temperature during wet hours to predict boxwood blight infection. In the second case study, we analyzed 4-year field data of weekly monitoring boxwood blight dynamics with detector plants in Lambsburg, VA (2014) and Lowgap, North Carolina (2015-2017) – hotspots of boxwood blight where this disease was first discovered and confirmed in the U.S. in 2011. These analyses uncovered that boxwood blight infection increased with increasing rainfall, relative humidity outside of the rainy periods (Figure 2), temperature and leaf wetness. These results accord with those of the first case study in northern Germany, highlighting the importance of incorporating rainfall and relative humidity into the boxwood blight infection risk model.

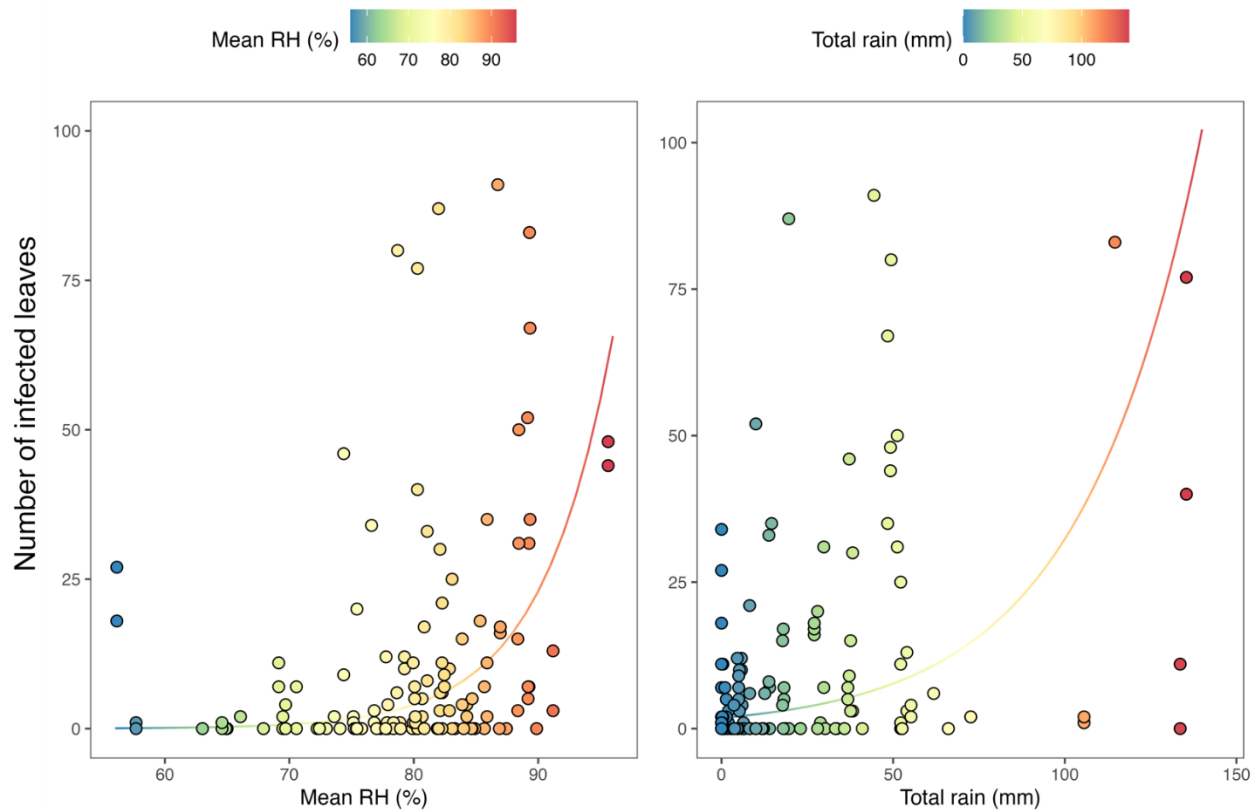


FIGURE 2 GENERALIZED LINEAR MIXED MODEL FIT SHOWING THE EFFECT OF TOTAL RAINFALL AND MEAN RELATIVE HUMIDITY OUTSIDE OF RAINY PERIODS ON THE NUMBER OF INFECTED LEAVES OF DETECTOR BOXWOOD PLANTS IN LAMSBURG, VA (2014) AND LOWGAP (2015-2017)

1.7 Identifying critical control points at production nurseries (Baysal-Gurel)

An online modified Delphi survey was conducted to identify grower perceptions on processes, programs, and practices to limit or prevent the entry and spread of boxwood blight disease. The expert panel consisted of 29 nursery producers who represented a significant portion of boxwood production nationally. The panel members rated boxwood blight as the third most problematic disease with a potential to be number one in the future. Infected boxwood transplants were perceived as the main source of boxwood blight outbreaks, followed by contaminated cutting tools, nursery equipment, containers, plant debris, irrigation water, worker personal belongings, and other crops. According to the panel responses, cultural control methods, inspection, and quarantine of incoming plant material, scouting, and sanitization were the most important practices that can limit or prevent plant diseases during boxwood production. The panel members did not agree that the composted manure could influence the spread of plant disease in boxwood production, although this has been verified by the findings of various previous research experiments. Panel members were very familiar with scouting and employee training, best management practices, and the boxwood blight cleanliness program. This study documented the key components, practices, and procedures in boxwood production that could influence the spread of boxwood blight in nurseries and could be further verified by sampling and laboratory assays to specify the critical control points in the production process. The sampling plan has been finalized according to growers' boxwood production flow diagram.

Objective 2: To better manage the disease at sites of contamination

2.1 Evaluating antidesiccants for blight mitigation (Hong)

The second phase of antidesiccant evaluation with field-grown Vardar Valley and American boxwood at two sites in western North Carolina was continued with the last treatment performed in September 2022 and last data collection late October. As with the first phase study, these trials were conducted in collaboration with NCDA&CS and NCCE, and two collaborating growers. At both sites, selected products – TransFilm, Vapor Gard and Wilt-Pruf – were applied monthly, every 2 or 3 months, and compared with a nontreated control and a fungicide standard – Concert II which was applied every 3 weeks. Phytotoxicity was observed on boxwood treated with Vapor Gard monthly at both sites, thus this product was excluded from further analyses on efficacy. Wilt-Pruf applied monthly consistently reduced boxwood blight on both cultivars by 37 to 39%, which were lower than those by Concert II (80 to 93%). TransFilm applied monthly reduced boxwood blight on American boxwood but not on Vardar Valley. Both products applied at 2-month intervals did not result in any blight control.

Metagenomic analyses indicated that Vapor Gard and Wilt-Pruf had more impacts on boxwood shoot microbial communities than TransFilm. Comparatively, more marked impacts were observed on fungi than bacteria in both community composition and structure. Specifically, they mostly suppressed fungi while promoting bacteria.

2.2 Further developing biocontrol agents (BCAs) into final products (Hong)

The second phase of selected BCA evaluation with field-grown Vardar Valley and American boxwood at two sites – A and B in western North Carolina was continued with the last treatment in September 2022 and the last data collection late October. All five selected BCAs applied at monthly intervals consistently reduced boxwood blight on both cultivars. Specifically, the best performer was *Burkholderia* sp. SSG with control efficacy at 58% on Vardar Valley and 66% on American boxwood at site A and B, respectively (Figure 3). These control efficacies were slightly lower than those by the fungicide standard – Concert II.

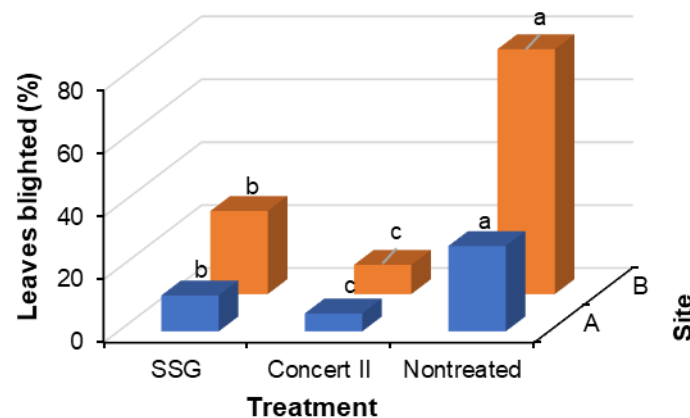


FIGURE 3 BOXWOOD BLIGHT DEVELOPMENT ON VARDAR VALLEY AND AMERICAN BOXWOOD PLANTS TREATED MONTHLY WITH THE BEST PERFORMING BIOLOGICAL CONTROL AGENT - BURKHOLDERIA SP. SSG AND THOSE TREATED WITH CONCERT II AND NONTREATED CONTROL PLANTS AT SITES A AND B

In addition to those evaluated in the field trails, we identified and evaluated a new bacterial biocontrol agent - *Pseudomonas eucalypticola* 1021Bp – from boxwood leaf endophytes. This new strain displayed great potential as a safe and environmentally friendly biofungicide and biofertilizer (Figure 4).

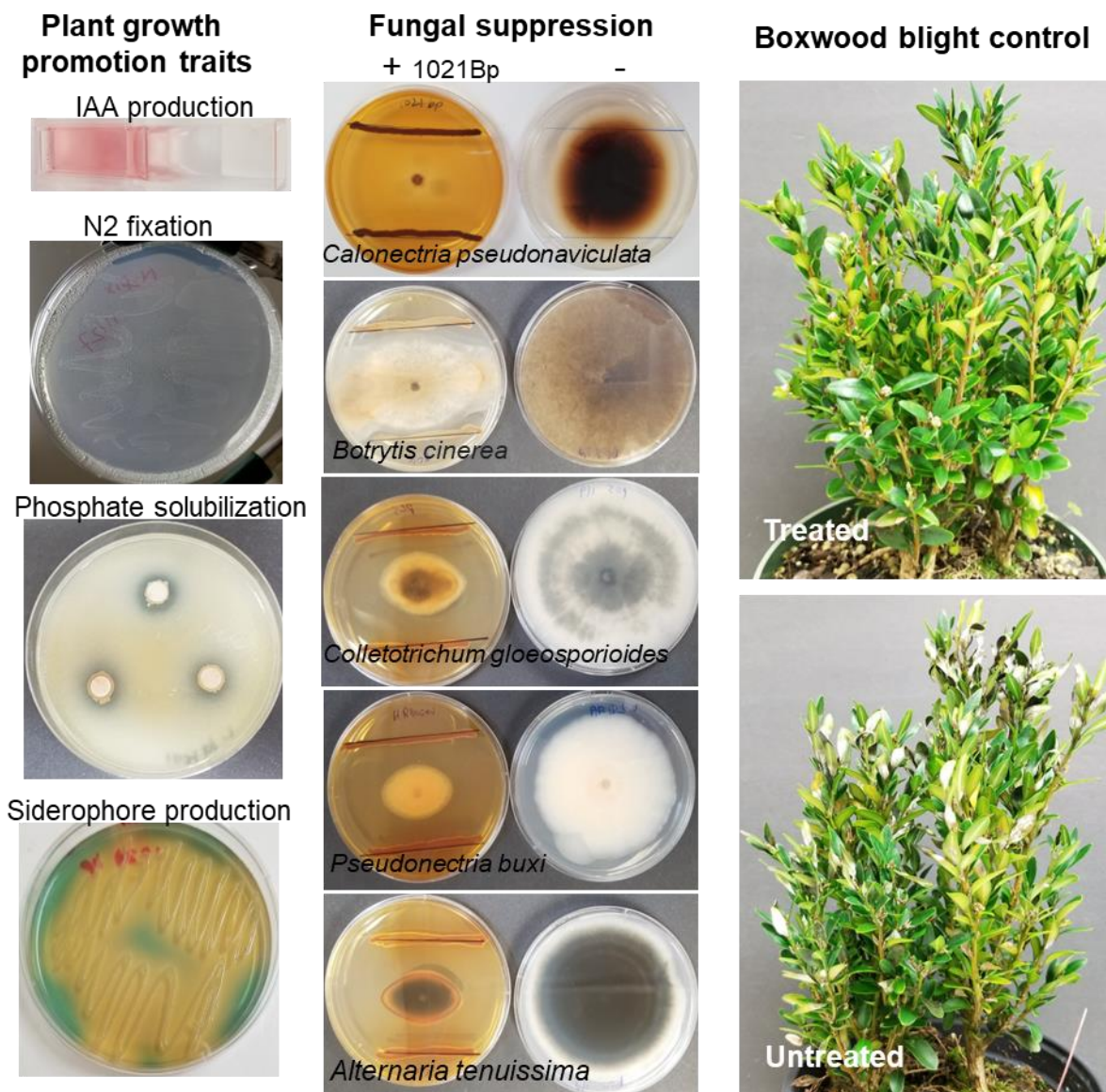


FIGURE 4 PSEUDOMONAS EUCALYPTICOLA 1021BP FROM BOXWOOD SHOWS TRAITS OF PLANT GROWTH PROMOTION, FUNGAL SUPPRESSION AND BOXWOOD BLIGHT CONTROL

2.3 Enhancing boxwood self-defense through systemic acquired resistance (SAR) inducers and selective fertilization (Daughtrey)

Buxus sempervirens ‘Green Mountain’ and ‘Suffruticosa’ have been propagated and grown into small plants suitable for systemic acquired resistance and selective fertilization treatments to be made in early fall 2023.

2.4 Enhancing boxwood self-defense through silicon (Si) treatment and UV radiation (Gouker)

Two independent experiments of 10-week treatments were performed to evaluate the effects of Si treatment on boxwood blight incidence. Two-year old plants of two cultivars, *B. microphylla* ‘Little Missy’ and *B. sempervirens* ‘Suffruticosa’, were treated and evaluated on the effect of continuous Si soil treatment. Treatments were applied during active root growth, at concentrations of 0, 2.5, 5, and 10 mg L⁻¹ with YarVita Actisil (Bio Mineral N.V., Belgium),

whose active compound is choline-stabilized orthosilicic acid. Results show improved growth for both *B. sempervirens* and *B. microphylla*; and initial assay results continue to show reduced severity of blight symptoms for the *B. sempervirens* but not for the *B. microphylla* cultivar. Results also indicated a phytotoxic effect for the highest concentration of treatment at 100 mg L⁻¹ for both cultivars. These experiments are demonstrating a significant effect of Si reducing incidence of boxwood blight on more susceptible cultivars in addition to providing a benefit to enhance growth. Additional observations show increased growth during the second year after Si application compared to nontreated control plants

Two rounds of experiments were conducted to assess the effect of UV-C treatment on *B. sempervirens* ‘Suffruticosa’ using detached leaf assay. One plate each with 12 replicate leaves with treatments of untreated control, 30-sec, 1-min, 2-min, 5-min exposure. Marginal but significant differences between all treatments except control vs 30 sec, control vs 60 sec, and 1 min vs 2 min treatments. Results consistently indicate positive correlation of reduced symptom development with increased UV-C exposure time.

Additional in-vitro studies were done to evaluate plant extracts for inhibiting growth of the boxwood blight pathogen. Specifically, we screened extracts from 22 evergreen plant taxa collected at the US National Arboretum for their ability to inhibit growth of *Cps*. Antifungal activity was determined by measuring the inhibition of mycelial growth of fungal cultures on petri plates. Among the 22 plant taxa tested, eight accessions resulted in over 50% mycelial growth inhibition. These included *Tsuga canadensis*, -79%; *Mahonia aquifolium*, -65%; *Nandina domestica*, -100%; *Pinus echinata*, -53%; *Picea pungens*, -100%; *Magnolia* sp., -63%; *Sanguisorba officinalis*, -50%; and *Cedrus deodara* ‘Devinely Blue’, -91%. Extracts from 10 other taxa resulted in some mycelial growth inhibition, including *Chamaecyparis* ‘Nana gracilis’, -12%; *Ilex crenata* ‘Buxifolia’, -7%; *Kalmia latifolia*, -16%; *Ilex vomitoria* ‘Yellow Fruited’, -43%; *Rosmarinus officinalis*, -5%; *Persea borbonia*, -13%; *Illicium parviflorum*, -4%; *Quercus virginiana*, -36%; *Ilex opaca*, -5%; and *Hedera helix* -1%.

2.5 Integrating three key existing technologies for better blight management at sites of contamination (LaMondia)

The goal for this study was to estimate the duration of protection offered by combination of fungicides with different modes of action on cultivars with low and high susceptibility as well as the interaction of management tactics such as fungicide application, systemic acquired resistance, and cultivar susceptibility. *Buxus microphylla* ‘Tide Hill’ (less susceptible) and *Buxus sempervirens* ‘Justin Brouwers’ (highly susceptible) plants were left nontreated or sprayed with TopBuxus, Actigard, ProConZ and mixtures of the products in all possible combinations. Leaves were collected at 5, 12, 19, 26 days post treatment (DPT). These leaves were inoculated with a drop (50 µL) containing 200 *Cps* conidia. Infection and sporulation data were collected after 7 days of incubation at 25°C and RH >95%. There were no significant differences between any of the treatment combinations for pathogen infection and lesion development except when the SAR was applied alone, which had reduced efficacy. There were no significant differences observed between any treatments for sporulation. The factors cultivar, treatment, and DPT were highly significant ($P < 0.001$), and interaction of cultivar and DPT were significant for both infection and sporulation ($P = 0.01$). We observed that cultivar susceptibility was very important as the fungicide treatments provided a shorter duration of protection for the highly susceptible cultivar, a maximum of 12 days, whereas they provided significantly longer protection on the

less susceptible cultivar, up to 26 days. The Actigard SAR treatment was not different from the nontreated control for disease incidence and sporulation and did not increase the efficacy of the ProconZ or TopBuxus fungicide treatments.

2.6 Preventing less susceptible boxwood cultivars from becoming ‘Trojan horses’ while encouraging their adoption (Shishkoff)

Photographs of symptoms of the cultivars infected during research are being collected to allow better recognition of symptoms.

Objective 3: To build resilience into boxwood production and plantings

3.1 Developing more resistant boxwood cultivars (Gouker)

An RNA-sequencing experiment was initiated and is in progress, using 6-inch liners of *B. sempervirens* ‘Suffruticosa’ and *B. sinica* var. *insularis* ‘Wintergreen’. Three biological replicates as a positive control and 3 biological replicates inoculated with NCB1 were used to isolate RNA and submitted to GeneWiz for Illumina 2×150 bp RNA-sequencing.

RNA leaf-assay was completed and submitted for sequencing using the same aforementioned cultivars using 3 biological replicates, and harvesting tissue at 6 different timepoints – 2, 4, 6, 8, 10, 12 hours post inoculation. RNA extractions and library preps have been submitted for sequencing.

A total of 1.5 TB whole genome sequence data (PacBio and Omni-C sequencing) for *B. sempervirens* ‘Suffruticosa’ and transcriptome data from RNA-seq experiment is now archived on the USDA-ARS HPC Ceres cluster. A draft genome assembly using HiRise assembler has generated genome size of ~1.01 Gbp. An *ab initio* genome annotation was generated using repeat masking, *de novo* gene prediction, and a BUSCO analysis of predicted genes.

Additional RNA libraries are being prepared for sequencing for boxwood (*B. sempervirens*) transcriptome and annotation.

Submitted NCBI BioProject # and SRA #'s for genome sequences and annotation sequences are being summarized and prepared for manuscript submission of boxwood reference genome.

Phenotyping tools are currently in development by developing a pipeline for detection and quantification of boxwood blight from images using machine learning models such as the convolutional neural network (CNN). The first step was data acquisition of boxwood images of both healthy and blight-infected plants. Single-leaf images were taken on controlled white backgrounds for easy repeatability and to minimize the role of the background in the machine-learning process. Boxwood varieties in this study include Wintergreen, Baby Gem, and Winterstar. For the distribution of images, a 70:20:10 split is used for training, validation, and testing images. Data was augmented and preprocessed using the image data generator function in TensorFlow. We then used a VGG16 (transfer learning) and Custom Model for blight predictions. Heat maps were generated for select images to aid in identifying the features of the image that the CNN was able to elucidate. Model performance for VGG and the custom model averaged 96% and 93%, respectively.

3.2 Cataloging and manipulating boxwood microbiomes against *Cps* (Kong)

Our boxwood microbiome studies during the third year resulted in several major discoveries. First, diverse bacteria and fungi live on the surface and internal tissue of boxwood shoots. These included a variety of the bacterial genera with species or strains having beneficial properties to plant growth and fitness. These results help understand the low maintenance nature

of this iconic landscape plant. For example, the composition and network of the endophytic bacterial community is associated with the varied resistance to blight of different English boxwood bushes. Commonly-used fungicides and antidesiccants both impacted boxwood phyllosphere microbiome. Three selected fungicide with chlorothalonil and/or propiconazole as active ingredient(s) impacted the microbial communities more on boxwood surface than internal tissue. Among the most affected were fungal community structure, composition, and co-occurrence networks. Specifically, *Pseudonectria*, a genus including species that causes boxwood Volutella blight, became more abundant after repeated fungicide applications. This discovery helps understand increasing Volutella blight problems being observed on boxwood crops and plantings. Similarly, film-forming antidesiccants with inert polymer compounds as active ingredients had stronger effect on the fungal than bacterial communities. These studies are foundational for fully utilizing the potential of these agricultural chemicals and microbiome in a systems approach to boxwood health and production.

3.3 Facilitating adoption of more resistant boxwood cultivars (Hall)

This objective was delayed due to COVID restrictions regarding in-person research methods, therefore no progress is being reported on this objective to date. This research will involve a mixture of experimental methods that will measure what attributes consumers value most about boxwood blight-resistant boxwood cultivars. The economic returns of long-term R&D investment in boxwood blight resistant plants will be determined through net present value analysis.

Objective 4: To assess the economic benefits of boxwood blight mitigation (Hall)

4.1 Developing baseline production model

To promote the adoption of advanced boxwood blight best management practices (BMPs) within the industry, we analyzed the economic dimensions of all recommended changes relative to current standard disease control practices in the industry. This served as a baseline to determine the socio-economic impacts of our strategies to reduce production- and post-production shrink.

4.2 Analyzing cost/benefit of changes in blight mitigation practice

Specifically, during the early stages of the project, key data collection points were identified, and data management protocols were established to ensure that the appropriate economic-related data were captured during each phase of the project. Meetings were held with team members to identify the appropriate economic cost parameters to assess and develop data collection protocols to ensure consistent and compatible data is acquired during the activities associated with each objective.

4.3 Partial budgeting: An economic engineering approach

An economic engineering approach was used to estimate the initial capital investment, production costs, and product prices for the baseline and alternative nursery production models. The models were simulated with representative best management practices and proper disease control protocols for boxwood. Partial budgeting modeling procedures were then used to measure the costs and potential benefits of short-run changes in BMPs in the production systems analyzed. This is a proven technique widely cited in the literature and is used when comparing

two or more similar production systems. Usually the comparison is between a benchmark system and one or more alternatives, as is the case in this project.

The partial budgeting technique compared the negative effects (costs added) of applying a new treatment relative to a base or standard treatment to the positive effects (cost savings) associated with the new treatment relative to the base or standard treatment. Therefore, in this project, it required the consideration of the returns associated with treatments and changes in the structure of the disease control costs. Aspects of costs and returns that do not change with the treatment relative to the base were not considered in this portion of the analysis. Thus, the technique of partial budgeting examined only the effect of the proposed change in practice, assuming all other aspects of the value chain remain unchanged.

4.4 Performing sensitivity tests

Once the changes in the models were incorporated and analyzed for their individual effects, the sensitivity of the results to various production inputs such as prices, wage rates, and operational conditions were investigated by altering values of the selected variables, one at a time, from the baseline values. The projected variables included in the sensitivity analysis included total labor and plant protection product costs.

4.5 Determining the economic return of long-term investment in blight mitigation

Because the production phase for #3 boxwood extends over multiple years, the effect of delayed returns on the longer-term nature of any investment in labor, materials, and equipment that are necessary to implement some of the proposed changes in production or disease control practices was taken into account.

4.6 Assessing the overall project impact

This part of the project was also delayed because of the COVID pandemic, but will be evaluated by measuring four separate effects including: 1) added costs incurred by the use of alternative materials, cultural practices, and/or disease control treatments; 2) added income resulting from increased yields and/or price premiums associated with higher quality crops; 3) costs savings realized through more efficient disease management practices or reduced inputs; and 4) income that may be lost when substituting one crop for another in the production system. These results will then be analyzed as to their implications for policymakers and stakeholders.

Objective 5: To put research into practice, helping our stakeholders and also the next generation of scientists and educators to achieve sustainable boxwood production and gardening

Translating and delivering research and innovations to the end user has been an integral part of this project as detailed below.

5.1 Translating research into products, protocols, and recommendations

As discussed during the second annual meeting held in Oregon in February, 2023, we made it a priority to translate research results into products, protocols and recommendations and move from lab to field. For example, we developed a list of cultivars consistently resistant to both boxwood blight and leafminer to promote their adoption. We also featured some of the most resistant cultivars – NewGen® Boxwood ‘Independence’ and ‘Freedom’, and ‘Little Missy’ and ‘Littler Mister’ – in the BBIG Newsletters. Likewise, we developed a new map of the boxwood blight pathogen *Che* in Europe to help prevent this potentially more destructive blight pathogen

from entering this country, while updating the U.S. map of the boxwood blight pathogen *Cps* by adding the time of its first confirmed invasion, and shared these new developments in the [Knowledge Center](#) of the BBIG website.

We also used two quarterly meetings to address the stakeholders' emerging needs. First, we met with state pathologists and discussed the pros and cons of conventional methods and molecular diagnostic assays and how to improve and standardize the diagnostic protocol to enable first responders to diagnose boxwood blight with confidence. Second, in response to the emerging interest in fungicide drench study, we brainstormed to move this forward and hopefully develop and materialize it into an operational tool for blight mitigation.

5.2 Delivering to end users

New initiative – launch of the BBIG Boxwood Seminar Series

To facilitate the technology transfer from this project to the end users, we launched this new Seminar Series in partnership with HRI with the first session on August 23, 2023.

Meetings with the Advisory Panel (AP)

- Quarterly meetings with AP members were held via Zoom on the first Friday of September, December, March, and June.
- The second annual meeting with AP and stakeholders was held at the Oregon State University's Willamette Research and Extension Center in Aurora, OR on February 15 and 16, 2023. This meeting was coupled with a tour of several major boxwood producers, learning their management practices and discussing ways to further improve them (Figure 5).



FIGURE 5 OREGON NURSERY TOUR GROUP DURING THE SECOND ANNUAL MEETING

BBIG outreach website updates

As in the past 2 years, HRI continued to host and update the contents as needed. Specifically, the Knowledge Center tab underwent two major updates: 1) expanded the section of 'Diagnostic Aids' to 'Diagnostic Aids and Management Strategies' to accommodate new materials developed by the Team, and 2) added a new section – Box Tree Moth – to meet the emerging need for information on this new invasive insect (Figure 6). Also, a new section – Team Capacity-Building Seminar Series – was added to the About BBIG tab.

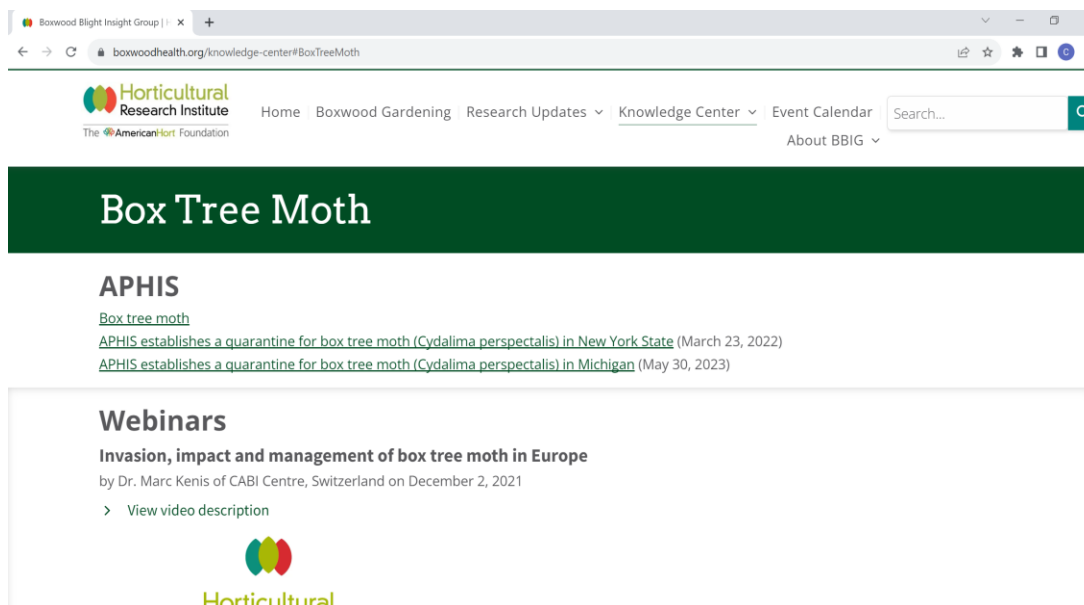


FIGURE 6 BOX TREE MOTH - A NEW SECTION UNDER THE KNOWLEDGE CENTER TAB OF THE BBIG OUTREACH WEBSITE TO MEET STAKEHOLDERS' EMERGING NEEDS ABOUT THIS NEW INVASIVE PEST

International Boxwood Seminar Series

We continued the success of the quarterly Seminar Series this past year with two new webinars – How do weather and climate impact boxwood blight – a modelling approach to aiding in boxwood disease management by Dr. Len Coop of Oregon State University on September 7, 2022, and Calamities of Georgia's boxwood forests by Iryna Matsiakh of Swedish University of Agricultural Sciences on December 20, 2022.

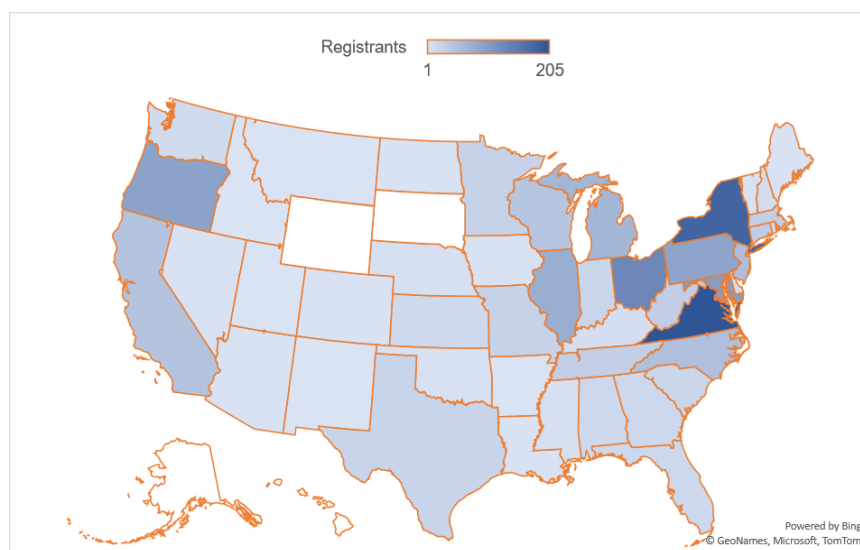


FIGURE 7 NATIONAL REACH TO FORTY-SIX STATES, WASHINGTON DC OF THE INTERNATIONAL BOXWOOD SEMINARS

Overall, this Seminar series reached 46 states in the U.S., plus the District of Columbia (figure 7) with 46% attendees being 'Multipliers' – educators, diagnosticians, regulatory personnel, and master gardeners, and 27% being industry leaders – owners, presidents, CEOs,

managers and head growers. This Seminar series also attracted attendees from 24 other countries on five continents, accounting for 6% of the total. Thus, the U.S. stakeholders and international communities both were well-served by the webinars. This Series was conducted in partnership with HRI; they helped with planning, organizing, publicizing, running and recording the webinars, then posting the recording at the BBIG outreach website. This Seminar Series set a new example of global and stakeholder partnership in addressing new and emerging plant biosecurity issues like boxwood blight.

BBIG Newsletter

As done in the second year, we published four BBIG Newsletters in November 2022, February, May and August 2023, respectively. Each issue began with Featured News, followed by Featured Research, Extension Highlights, and Project Meeting Brief, Recent Publications, etc. as exemplified by Volume 4, Issue 2 (Figure 8). Featured News had two articles introducing NewGen® Boxwood – ‘Independence’ and ‘Freedom’, and other less susceptible cultivars – ‘Little Missy’ and its new companion ‘Little Mister’ – to promote the adoption of these most resistant cultivars. The Featured Research reported the latest major advancements in our research which had been presented first to the quarterly project meeting with all Project Directors and their associates, then to the quarterly meeting with Advisory Panel members. The Extension Highlights covered several topics – major extension activities and accomplishments, plus diagnostic tips. Each issue also included a quick summary of our quarterly deliverables.

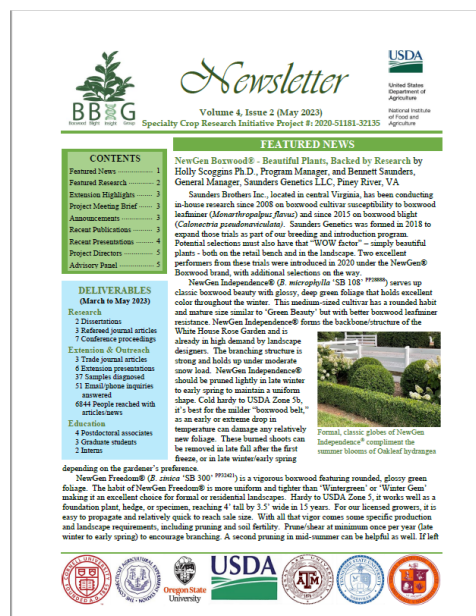


FIGURE 8 A SNAPSHOT OF THE FRONT PAGE OF BBIG NEWSLETTER VOLUME 4 ISSUE 2 (MAY 2023)

BBIG Monthly

This monthly digest provided quick updates on the latest research, extension and outreach programming, and boxwood related news as well as major online resources including [Boxwood Blight Knowledge Center](#), [Boxwood Blight Infection Risk Model](#), [Box Tree Moth](#), and [Boxwood Leafminer Risk Model](#) (Figure 9).

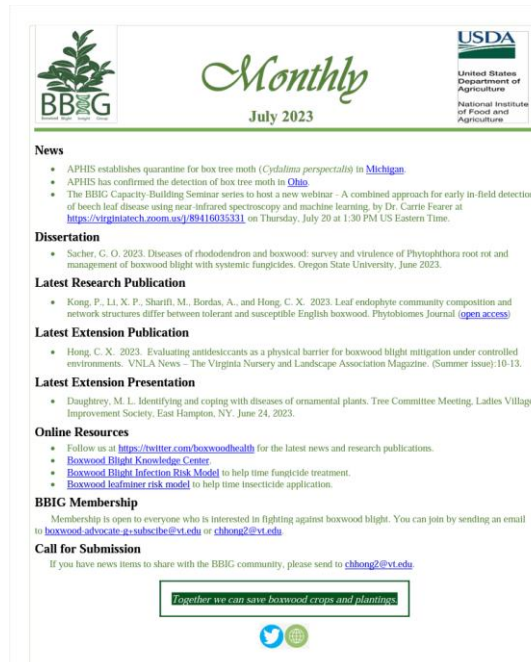


FIGURE 9 EXAMPLE OF BBIG MONTHLY - JULY 2023

Tweets at Twitter.com/boxwoodhealth

Dozens of tweets were used to instantaneously share the latest research, innovations and best management practices as well as boxwood related news with the per tweet views up to 1,239.

Conventional means of technology transfer

- Hosted one virtual meeting of International Boxwood Blight Epidemiology Working Group via Zoom.us on October 17, 2022.
- Reached out to the science and educational communities by publishing eleven refereed journal articles, and giving seventeen presentations at annual meetings of the American Phytopathological Society and other professional conferences.
- Reached out to the environmental horticulture industry and the public through seven newsletter and trade journal articles, two workshops, and 27 additional educational presentations.

Other nonconventional means of technology transfer

- Increasingly used open access publications in *Plant Health Progress* and other journals for improved access to our research data by extension communities and large growers and landscapers.
- Continued to use BBIG Google Group (bbig-g@vt.edu), along with other local and national networks, for dissemination of the project monthly digests – BBIG Monthly and quarterly BBIG Newsletters, as well as the latest research and extension publications, and boxwood health related news. A total of 21,652 boxwood enthusiasts were reached via the BBIG group alone as of August 27, 2023.

5.3 Documenting project impacts

This project has significantly improved boxwood blight mitigation in many ways during the third project year from September 1, 2022 to August 31, 2023.

Changes in knowledge

- Enabled growers, retailers, landscapers, arborists, garden managers and other horticulturists, first responders, and extension specialists and agents to better understand the blight disease, its diagnostic characters and current/latest mitigation technologies. Stakeholders are better able to prevent accidental blight introduction to their properties, identify and scout for blight hot spots, make informed decisions, and act in a timely manner to better manage and contain the disease at sites of contamination should it occur.
- Advanced the science of boxwood biology and disease epidemiology. Specifically, there were several major advancements in the area of boxwood microbiome. First, boxwood phyllosphere bacterial and fungal communities are extremely diverse with many beneficial microbes, helping understand the low maintenance nature of this iconic plant in American gardens and green spaces. Second, both bacterial and fungal communities are affected by selected antidesiccants and fungicides, laying the foundation for fully utilizing the potential of these agricultural chemicals and microbiome in a systems approach to boxwood health and production. Third, leaf endophyte community composition and network differ between English boxwood shrubs of varying susceptibility to the boxwood blight pathogen, providing a new horizon for developing strategies to protect this landmark plant in the historic gardens and other estates. Likewise, we also demonstrated that differential adaptation resulted in aggressiveness variations of the blight pathogen *Cps* on three hosts – boxwood, pachysandra, and sweet box.
- Produced several important innovations and advancements. These included: 1) a rapid and scalable DNA extraction and real-time PCR assay from boxwood tissues for detection of the blight pathogen *Cps*, 2) identification of boxwood cultivars, including many previously not evaluated cultivars and accessions, with resistance to both boxwood blight and leafminer, in collaboration with Saunders Genetics, LLC, and 3) filling a major knowledge gap about the performance of commonly-used boxwood cultivars against *Che* and improving the nation's preparedness for this potentially more damaging pathogen should it be accidentally introduced into this country, in collaboration with German scientists, and 4) new chemistries – both fungicides and antidesiccants, and new application – fungicide drench for blight mitigation.
- Identified critical control points for boxwood blight mitigation. Infected boxwood transplants were perceived as the main source of boxwood blight outbreaks, followed by contaminated cutting tools, nursery equipment, containers, plant debris, irrigation water, worker hygiene, and other crops. Specifically, cultural control methods, inspection, and quarantine of incoming plant material, scouting, and sanitization were found to be the most important practices that can limit or prevent plant diseases during boxwood production. These discoveries will be verified by sampling and laboratory assays to specify the critical control points in the production process in the coming years.

Changes in practice and condition

- The nation's boxwood production continued to shift from severely affected states to less/not affected states or regulated states as demonstrated by the Economic Team.

- Many growers have shifted their boxwood inventory to less susceptible cultivars according to [a recent survey of major boxwood producing states](#) and [the Oregon Nursery Tour](#). It is anticipated that our new publication – [Building health into boxwood crops and plantings by making informed cultivar selection](#) – along with other materials produced from our collaborative research and posted in the [Diagnostic Aids and Management Strategies/Boxwood Blight Resistance](#) section under the Knowledge Center tab of the BBIG outreach website, will further expedite this shift, moving the nation onto a fast track to sustainable boxwood production and gardening.
- Our blight control innovations have been widely adopted as exemplified by the ‘herbal distancing’ to improve air movement and mitigate the risk of water splash dispersal in event of accidental pathogen introduction as shown by all nurseries visited during the [Oregon Nursery Tour](#). Also observed during the same tour were wide adoption of: 1) a closed-loop system by not allowing outside boxwood materials to enter production facility, 2) strict sanitation practices, in addition to growing only less susceptible cultivars.
- Some growers have moved to a systems approach to blight mitigation and boxwood crop health management. A similar approach is also being taken by public garden managers - focusing on blight prevention, trying to detect accidental introduction as early as possible and containing to eradicate the disease where such introduction occurs, as exemplified by the public gardens we have worked with.

Other impacts

The BBIG Team was recognized with [the 2022 USDA National Institute of Food and Agriculture Partnership Award – Program Improvement through Global Engagement](#), setting a new example for fighting together against invasive species and destructive diseases.

- International Boxwood Seminar Series consisted of eight webinars with invited speakers who were world-leading scientists from seven countries. These webinars reached stakeholders in forty-six U.S. states, plus 24 other countries on five continents. The U.S. participants accounted for 94% of the total attendance, with the vast majority being the multipliers – educators, first responders, and researchers (42%) – and industry leaders: business owners, presidents, CEOs and head growers and managers (27%).
- In addition to sharing their expertise through the International Boxwood Seminar series, researchers in Belgium, Germany, and United Kingdom have been collaborating on several important project components including biocontrol and field epidemiology. These collaborations accelerated understanding of the blight biology and fungicide and cultivar performance against the boxwood blight especially in northern Germany where *Che* is dominant.
- AmericanHort and HRI continued to host the project outreach website with a knowledge center and update as needed. This invaluable partnership is projected to provide continual maintenance and service to the horticulture industry and consumers, even after this SCRI project ends. In addition to hosting our International Boxwood Seminar series and newly launched BBIG Boxwood Blight Seminar series, HRI played key roles in planning, organization, publicizing, running, recording, posting recordings, and documenting attendance metrics.
- North Carolina Department of Agriculture and Consumer Services, North Carolina Cooperative Extension and local farmers all played key roles in every important step of

the field trials, including their hands-on efforts to identify naturally infested fields, evaluate antidesiccant and biocontrol agents, make scheduled applications of fungicide standards, and perform monthly blight assessments.

- Again, we had extraordinarily fine support from our Advisory Panel members. In addition to providing feedback at our quarterly meetings and annual meeting, some individuals have shared additional time and talents. For example, **Jill Calabro** has been an integral part of the project leadership team, filling in a number of important perspectives as an industry leader at Valent USA Corporation, former HRI director, and as a plant pathologist during the project planning. **John Keller** contributed to a study analyzing and promoting the boxwood inventory shift to more resistant cultivars in the U.S. to get the boxwood production and plantings on a fast track to sustainability. **Lynn Batdorf** read several manuscripts for the team and provided detailed suggestions for improvement on boxwood nomenclature. Both **John Keller** and **Laura Gladwin** shared their boxwood production practices in addition to what we saw during the Oregon Nursery Tour, helping the Team better understand the system and more effectively address the blight disease.

5.4 To prepare a new generation of scientists and educators for tackling increasingly complex issues facing the nation's specialty crop industry in a global economy

One new graduate student – Taylor Phelps, and two interns – Gloria Chen and Briana Pecora – were added to the BBIG Team at Virginia Tech, while graduate student Gabriel Sacher successfully defended his PhD dissertation, and four postdoc associates – Olanike Omolehin, Srikanth Kodati, Mana Ohkura, and Vanina Castroagudín – all landed dream jobs.



FIGURE 10 TWO NEWEST MEMBERS - BRIANA PECORA (LEFT) AND TAYLOR PHELPS (RIGHT) PRESENT BBIG AT THE PLANT HEALTH 2023 - ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY IN DENVER, CO FROM AUGUST 12 TO 16, 2023

In addition to planning and executing the proposed research activities, analyzing data, and writing for publications, all postdocs, students and interns actively interacted regularly with the rest of the BBIG team including the Advisory Panel via Zoom meetings and email exchanges, gaining an appreciation of how to work with collaborators and industry partners and developing leadership skills. This project also provided them with a variety of other professional training opportunities including attending professional meetings.

- Xiaoping Li, Postdoctoral Microbiome Scientist, continued to coordinate the boxwood microbiome studies with the USDA ARS Horticultural Crops Disease and Pest Management Research Unit in Corvallis, OR, North Carolina Department of Agriculture and Consumer Services, North Carolina Cooperative Extension, and collaborating growers in both North Carolina and Virginia. These collaborations have resulted in publications in two high-impact journals – Microbiology Spectrum and BMC Microbiology.
- Prabha Liyanapathirana, Post-Doctoral Research Associate, continued to assist with diagnosing the boxwood samples received at the Otis L. Floyd Nursery Research Center in McMinnville TN. During the last few years, she has conducted research work on identifying effective fungicide treatments for boxwood blight management. She also delivered an oral presentation to the Warren County, TN Master Gardener Association on boxwood diseases.
- Ihsan Khaliq, Postdoctoral Epidemiologist, coordinated the investigations into field epidemiology of boxwood blight through analyses of data that had been collected by colleagues in northern Germany since 2006 and by those at Virginia Tech between 2014 and 2017.
- Bhawana Ghimire, a graduate student at Tennessee State University, conducted a study to discern the critical control points for boxwood blight disease in nurseries. She also screened for effective chemical management using fungicides, a host plant defense inducer and an antitranspirant. She has also been trained to conduct experiments and characterize the UV-C induced inactivation kinetics of the pathogen.
- Gabriel Sacher, graduate student at Oregon State University, worked on boxwood and rhododendron for his thesis but focused on fungicide management of boxwood blight for the last few years. He discovered that drenching the fungicide flutriafol onto roots was effective at delivering disease-controlling concentrations to boxwood canopies, more effectively than spraying.
- Vanina Castroagudin worked as an ORISE Visiting Research Scientist at the USDA ARS Mycology & Nematology Genetic Diversity & Biology Lab in Beltsville, MD for 4 years and was with BBIG from the beginning. She has now been hired as a Molecular Biologist at the APHIS-Plant Pathogen Confirmatory Diagnostics Lab, also at Beltsville.
- Srikanth Kodati, Postdoctoral Scientist, has been productive on the BBIG Team at the Connecticut Agricultural Experiment Station (CAES) and joined the University of Connecticut as Crop Protection Extension Educator in the College of Agriculture, Health and Natural Resources in early 2023. He will continue to collaborate with Dr. LaMondia examining management of boxwood blight at the CAES.
- Mana Ohkura, Postdoctoral Scholar, assisted with the Oregon nursery survey. In addition, she led a research project to evaluate the effect of irrigation and plant spacing on boxwood blight, experiments to evaluate factors that affect spore germination of the pathogen, and experiments to evaluate latent infection. She wrote a research publication

on the real-time PCR method in the peer-reviewed journal, Plant Disease. Dr. Ohkura will be finishing her BBIG research in September to take on the director's position at the Oregon State University Plant Clinic.

- Olanike Omolehin, Postdoctoral Applied Plant Pathologist and Project Manager, with extra responsibilities for handling communications via Teams, Zoom and email, and assembling reports. Coaching was provided by the Project Leaders as needed. This leadership experience helped Olanike secure the position of Business Analyst at IBM in Baton Rouge, LA in September 2022.

What opportunities for training and professional development has the project provided?

In addition to preparing the next generation of scientists and educators as outlined under Objective 5.4, we hosted and added one webinar – A combined approach for early in-field detection of beech leaf disease using near-infrared spectroscopy and machine learning – to our Team Capacity-Building Seminar Series. This webinar was presented by Dr. Carrie Fearer of University of New Hampshire on July 20, 2023. Among the attendees were our collaborators, in addition to Project Directors and their associates – Postdoctoral Associates, Graduate Students and Interns, and Advisory Panel members. This webinar, along with other training and professional development activities better prepared the Team for tackling increasingly complex issues facing the nation's specialty crop industry in a global economy.

How have the results been disseminated to communities of interest?

We consider research result dissemination an integral part of this project. Please see Objectives 5.1 to 5.3 for details.

What do you plan to do during the next reporting period to accomplish the goals?

We will continue to make efforts to catch up on research components that were delayed due to the COVID-19 pandemic, while striving to do even better for the other research and extension programming—where we have in some cases exceeded what we originally proposed.

CHANGES/PROBLEMS

Although the team has had many achievements in research and outreach over the third year of the grant, the pandemic of COVID-19 caused unprecedented challenges to all the collaborating institutions. All USDA PI's research labs were under a federally-mandated gating phase, with only mission-essential employees permitted to be onsite at 25% facility capacity limit until March 28 2022. This has hampered laboratory-based research objectives. We plan to request a 1-year no cost extension to achieve all proposed project objectives while exceeding in many.

ACKNOWLEDGEMENTS

While a few Advisory Panel members and key stakeholders were recognized above for their significant contributions to this project this past year, many more were not. Among the most significant were: 1) two farmers who provided boxwood crops or on-site assistance for the ongoing field trials; and 2) NCCE Collaborator Amanda Taylor, who purchased American boxwood plants from a local nursery for both antidesiccant and biocontrol study at one research site. These and other much-appreciated demonstrations of support added quality, productivity and efficiency to this project.