



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, 2022

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.

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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)

Xiao Yang, USDA ARS Foreign Disease-Weed Science Research Unit (Project Manager, April to December 2021)

Olanike Omolehin, Virginia Tech (Project Manager, December 2021 to Present)

Co-Project Directors

Charles Hall, Texas A&M University

Jerry Weiland, USDA ARS Horticultural Crops Research Lab

Fulya Baysal-Gurel, Tennessee State University

Fred Gouker, USDA ARS Floral & Nursery Plants Research Unit

Ping Kong, Virginia Tech

Jo Anne Crouch, USDA ARS Mycology & Nematology Genetic Diversity & Biology Lab

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

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 Liyanapathirana, Tennessee State University

Mana Ohkura, Oregon State University

Olanike Omolehin, Virginia Tech

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

Graduate Students

Bhawana Ghimire, Tennessee State University

Gabriel Sacher, Oregon State University

Summer Interns and Other Assistants

Cole Jackson, Virginia Tech

Kimberly Clark, Virginia Tech

Robert Holtz, Virginia Tech

Paulina Rychlik, Cornell University

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.
John Keller, Monrovia – Grow Beautifully
Laura Gladwin, Everde Growers
Bennett Saunders, Saunders Genetics, LLC
Richard Schnall, Rosedale Nurseries, Inc
Casey Sclar, The Arboretum at Pennsylvania State University
Mark Sellew, Prides Corner Farms
Martha Sleeper, Everde Growers
Jennifer Gray, Horticultural Research Institute, AmericanHort

Key Stakeholder Collaborators

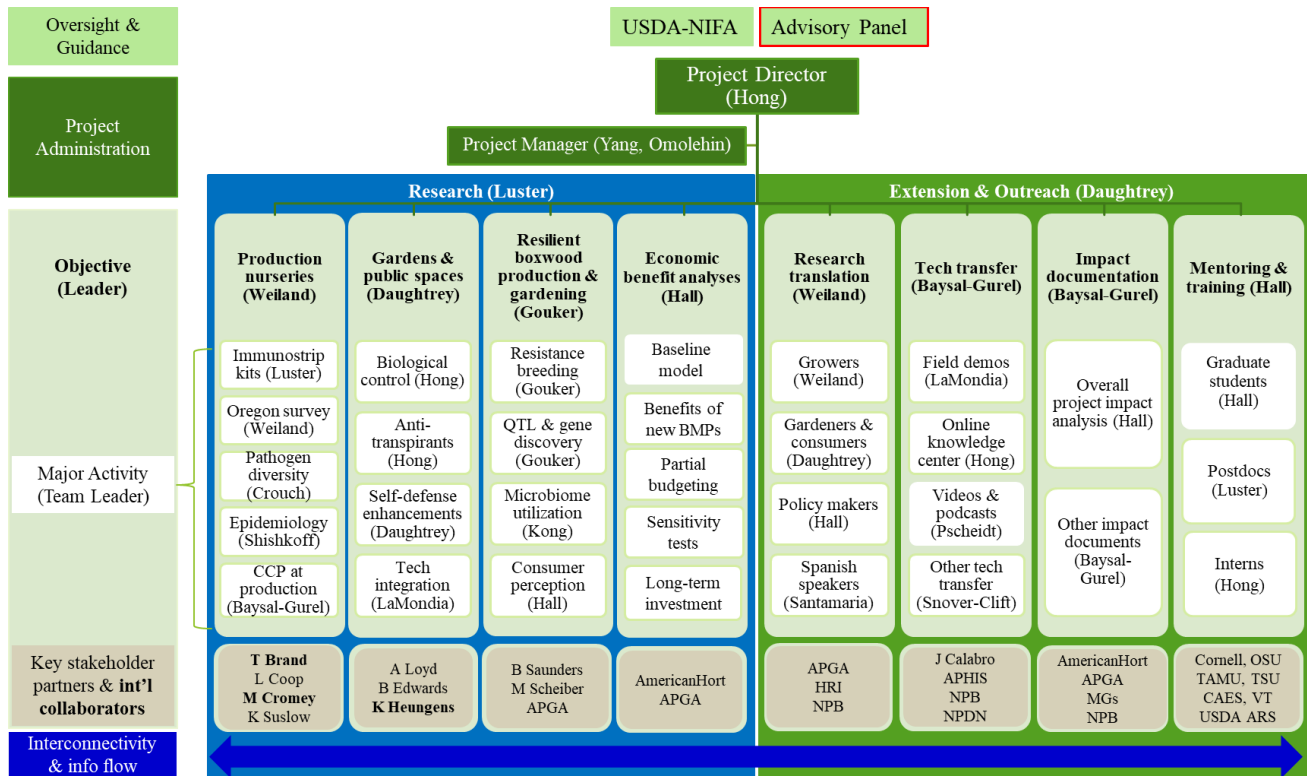
Nilwala Abeysekara, National Ornamental Research Site at Dominican University of California
Brittany Barker, Oregon State University
Adria Bordas, Virginia Cooperative Extension, Fairfax County
Len Coop, Oregon State University
Marc Cubeta, North Carolina State University
Heather Faubert, University 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
Nicolas Leas, AmericanHort
Michael Likins, Virginia Cooperative Extension, Chesterfield County
Andrew Loyd, Bartlett Tree Experts
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
 Katrijn Van Laere, Flanders Research Institute for Agriculture, Fisheries and Food, Belgium

Project Organization



APGA, American Public Gardens Association; APHIS, USDA Animal and Plant Inspection Service; CAES, Connecticut Agricultural Experiment Station; CCP, critical control point; HRI, Horticultural Research Institute; MGs, master gardeners; NPB, National Plant Board; NPDN, 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

(Note: BBIG team members highlighted in bold; BBIG students and postdoctoral associates indicated with an asterisk)

Research

Symposia proposed and moderated

- Ornamental Production Challenges and Perspectives: A Case Study of Boxwood Blight, at the American Phytopathological Society (APS) – Southern Division Annual Meeting in Chattanooga, TN, March 9, 2022. This symposium was organized and moderated by **Fulya Baysal-Gurel** with six invited speakers - **Chuan Hong**, **Jo Anne Crouch**, **Charles Hall**, **Douglas Luster**, **Fred Gouker**, and **Margery Daughtrey**.
- Epidemiology, Diagnosis and Management of an Emerging Disease – Boxwood Blight, at Plant Health 2022 – APS Annual Meeting in Pittsburgh, PA, August 8, 2022. This symposium was organized by **Margery Daughtrey**, **Douglas Luster**, Xiao Yang and **Chuan Hong** and moderated by **Fulya Baysal-Gurel** with five invited speakers – **Nina Shishkoff**, **Jerry Weiland**, Len Coop, Xiao Yang, and **James LaMondia**.

Journal Publications

1. Aliello, D., Guarnaccia, V., Vitale, A., LeBlanc, N., **Shishkoff, N.**, and Polizzi, G. 2022. Impact of *Calonectria* diseases on ornamental horticulture: diagnosis and control strategies. *Plant Disease* 106:1773-1787.
2. Avenot, H. F., Baudoin, A. B., and **Hong, C. X.** 2022. Conidial production and viability of *Calonectria pseudonaviculata* on infected boxwood leaves and twigs as affected by temperature, wetness and dryness periods. *Plant Pathology* 71:696-701 (<http://doi.org/10.1111/ppa.13500>).
3. Barker, B. S., Coop, L., and **Hong, C. X.** 2022. Potential distribution of invasive boxwood blight pathogen (*Calonectria pseudonaviculata*) as predicted by process-based and correlative models. *Biology* 11, 849 (open access at <https://www.mdpi.com/2079-7737/11/6/849/pdf>).
4. **Baysal-Gurel, F.**, Simmons, T., and Jennings, C. 2022. Evaluation of fungicides for the control of boxwood blight, 2021. *Plant Disease Management Report No. 16: OT010*. Online publication.
5. **Baysal-Gurel, F.**, Simmons, T., and Jennings, C. 2022. Evaluation of fungicides for the control of boxwood blight, 2021. *Plant Disease Management Report No. 16: OT021*.
6. Behe, B. K., Huddleston, P. T., and **Hall, C.** 2022. Gardening motivations of U.S. plant purchasers during COVID-19 pandemic. *Journal of Environmental Horticulture* (2022) 40 (1): 10-17 (Open access at <https://doi.org/10.24266/0738-2898-40.1.10>).
7. Brand, T., Beltz, H., Ehsen, B., Adhikari, U.*, **Daughtrey, M. L.**, **Luster, D. G.**, **Kong, P.**, and **Hong, C. X.** 2022. Multi-year field plantings evaluating boxwood cultivars for susceptibility to the blight pathogens (*Calonectria* spp.) in Northern Germany. *Plant Disease* published online as First Look at <https://doi.org/10.1094/PDIS-05-22-1102-RE>.
8. Dhakal, K., Bika, R.*, Ghimire, B.*, Parajuli, M., Neupane, S., Neupane, K., Adesso, K. M., and **Baysal-Gurel, F.** 2022. Arthropod and disease management in boxwood production. *Journal of Integrated Pest Management* 13(1): 18 (open access at <https://doi.org/10.1093/jipm/pmac013>).

9. **Gouker, F.**, Guo, Y., and Pooler, M. 2022. HRM analysis enables efficient detection and differentiation of two boxwood blight pathogens by qPCR assays. *PhytoFrontiers*, published online at <https://doi.org/10.1094/PHYTOFR-09-21-0066-SC>.
10. **Hong, C. X., Daughtrey, M.**, Howle, M., Schirmer, S., Kosta, K., **Kong, P.**, Likins, M., and Suslow, K. 2022. Rapid decline of *Calonectria pseudonaviculata* soil population in selected gardens across the United States. *Plant Disease*, published online as First Look at doi: [10.1094/PDIS-02-22-0443-RE](https://doi.org/10.1094/PDIS-02-22-0443-RE).
11. Kodati, S.*, Allan-Perkins, E., Cowles, R.S., and **LaMondia, J.** 2022. The effect of temperature, leaf wetness period, and cultivar susceptibility on boxwood blight disease development and sporulation. *Plant Disease*, published online as First Look at doi: [10.1094/PDIS-05-22-1022-RE](https://doi.org/10.1094/PDIS-05-22-1022-RE).
12. Kodati S.*, Cowles R., and **LaMondia J.** 2022. Survival of conidia of the boxwood blight pathogen *Calonectria pseudonaviculata* under different relative humidity condition. *Plant Health Progress*, published online as First Look at <https://doi.org/10.1094/PHP-12-21-0142-RS>.
13. **Kong, P.** 2021. Survival of SSG, an endophytic Burkholderia biocontrol agent, on boxwood leaf surface. *Journal of Environmental Horticulture* 39 (4):138-142 (open access at <https://doi.org/10.24266/0738-2898-39.4.138>).
14. **Kong, P.**, Li, X. P.*, **Gouker, F.**, and **Hong, C. X.** 2022. cDNA transcriptome of *Arabidopsis* reveals various defense primings induced by a broad-spectrum biocontrol agent *Burkholderia* sp. SSG. *International Journal of Molecular Sciences* 23(6):3151 (open access at doi: [10.3390/ijms23063151](https://doi.org/10.3390/ijms23063151)).
15. **Kong, P.**, Sharifi, M., Bordas, A., and **Hong, C. X.** 2021. Differential tolerance to *Calonectria pseudonaviculata* of English boxwood plants associated with the complexity of culturable fungal and bacterial endophyte communities. *Plants*. 10, 2244 (open access at <https://doi.org/10.3390/plants10112244>).
16. **LaMondia, J. A.**, Allan-Perkins, E., Kodati, S.* 2021. Factors affecting boxwood blight spread under landscape conditions. *Journal of Environmental Horticulture* 39(3):100-107 (open access at <https://doi.org/10.24266/0738-2898-39.3.100>).
17. Li, X. P.*, **Kong, P.**, **Daughtrey, M. L.**, Kosta, K., Schirmer, S., Howle, M., Likins, M., and **Hong, C. X.** 2022. Characterization of the soil bacterial community in selected boxwood gardens across the United States. *Microorganisms* 10, 1514 at <https://doi.org/10.3390/microorganisms10081514>.
18. Rogers, L. W., Koehler, A. M., **Crouch, J. A.**, Cubeta, M. A. and LeBlanc, N. R. 2022. Comparative genomic analysis reveals contraction of gene families with putative roles in pathogenesis in the fungal boxwood pathogens *Calonectria henricotiae* and *C. pseudonaviculata*. *BMC Ecology and Evolution* 22:79 (open access at <https://doi.org/10.1186/s12862-022-02035-4>).
19. **Weiland, J. E.**, Ohkura, M.*, Scagel, C. F., Davis, E. A., and Beck, B. R. 2022. Cool temperatures favor growth of Oregon isolates of *Calonectria pseudonaviculata* and increase severity of boxwood blight on two *Buxus* cultivars. *Plant Disease*, published as First Look at <https://doi.org/10.1094/PDIS-04-22-0769-RE>.

Abstracts (Presented at Professional Meetings)

1. **Baysal-Gurel, F., Hong, C. X., Daughtrey, M., Luster, D., Hall, C., Weiland, J., Gouker, F., Kong, P., Crouch, J. A., LaMondia, J., Pscheidt, J., Santamaria, L.,**

- Shishkoff, N., and Snover-Clift, K.** 2022. Boxwood Blight Insight Group (BBIG) - An International Consortium. 1890 Research Directors Symposium. Atlanta, GA, April 2 to 5, 2022.
2. Brand, T., Beltz, H., Ehsen, B., Adhikari, U.*, **Daughtrey, M., Luster, D. G., Kong, P., and Hong, C. X.** 2022. Variation of boxwood cultivar susceptibility to the blight pathogens (*Calonectria* spp.) in northern Germany. APS Potomac Division Annual Meeting online March 23 to 25, 2022.
 3. Coop, L., Brittany, B. S., and **Hong, C. X.** 2022. Integrating short and long term risk models for boxwood blight. Integrated Pest Management Symposium, Denver, CO, February 28 to March 3, 2022.
 4. Ghimire, B.*, Pendyala, B., Patras, A., and **Baysal-Gurel, F.** 2022. UV sensitivity of *Botrytis cinerea*, *Calonectria pseudonaviculata*, *Phytophthora vexans* and inactivation of their inoculums using UV-C LED irradiation. 99th Southern division APS hybrid meeting. Chattanooga, TN, March 7 to 10, 2022.
 5. Kodati, S.*, Cowles, R. S., and **LaMondia, J. A.** 2022. Survival of conidia of the boxwood blight pathogen *Calonectria pseudonaviculata* under different relative humidity conditions. APS Annual Meeting in Pittsburgh, PA, August 6 to 10, 2022.
 6. **LaMondia, J. A.** 2022. Integrating research results into a boxwood blight management program. APS Annual Meeting in Pittsburgh, PA, August 6 to 10, 2022.
 7. Li, X. P.*, Omolehin, O., Hemmings, G., **Daughtrey, M., Kong, P., and Hong, C. X.** 2022. Epiphytic bacterial communities of boxwood foliage impacted by fungicides commonly-used for control of boxwood blight. Plant Health 2022 – APS Annual Meeting in Pittsburgh, PA, August 6 to 10, 2022.
 8. Liyanapathirana, P.*, Ghimire, B.*, Simmons, T., Jennings, C., and **Baysal-Gurel, F.** 2022. Comparative performance of fungicides in management of boxwood blight. 10th International IPM Symposium. Denver, CO, February 28 to March 3, 2022.
 9. Liyanapathirana, P.*, Sharma, S., Simmons, T., Jennings, C., and **Baysal-Gurel, F.** 2022. Evaluation of fungicides for the control of boxwood blight. 99th APS Southern Division Meeting. Chattanooga, TN, March 7 to 10, 2022.
 10. **Luster, D.,** Carter, M., McMahon, M., Yang, X.* 2022. Development of a capture-ELISA immunoassay for the boxwood blight pathogens *Calonectria henricotiae* and *C. pseudonaviculata*. Plant Health 2022 – APS Annual Meeting in Pittsburgh, PA, August 6 to 10, 2022.
 11. Ohkura, M.*, Scagel, C. F., and **Weiland, J. E.** 2022. Rapid and scalable DNA extraction and real-time PCR assay for the detection of the boxwood blight pathogen *Calonectria pseudonaviculata*. Plant Health 2022 – APS Annual Meeting in Pittsburgh, PA, August 6 to 10, 2022.
 12. Omolehin, O.*, **Daughtrey, M. L.,** Gray, J., Calabro, J., Yang, X.*, and **Hong, C. X.** 2022. BBIG international boxwood seminars: a successful global and stakeholder partnership. Plant Health 2022 – APS Annual Meeting in Pittsburgh, PA, August 6 to 10, 2022.
 13. Omolehin, O.*, **Kong, P., and Hong, C. X.** 2022. Friend not foe: *Bacillus methylotrophicus*, BP1024, offers potential for biological management of boxwood blight. Plant Health 2022 – APS Annual Meeting in Pittsburgh, PA, August 6 to 10, 2022.

14. **Shishkoff, N.** 2022. The epidemic since 2020: new insights into management of boxwood blight. Plant Health 2022 – APS Annual Meeting in Pittsburgh, PA, August 6 to 10, 2022.
15. **Shishkoff, N.,** and Smallwood, E. 2022. Effect of temperature on lesion area of detached leaves of different boxwood cultivars infected with boxwood blight. Plant Health 2022 – APS Annual Meeting in Pittsburgh, PA, August 6 to 10, 2022.
16. **Weiland, J. E.** 2022. Nursery production decisions on plant spacing and irrigation affect risks for boxwood blight. Plant Health 2022 – APS Annual Meeting in Pittsburgh, PA, August 6 to 10, 2022.

Invited Presentations at Professional Conferences WITHOUT an abstract

1. **Hong, C. X.** 2022. Fighting a good fight against an emerging invasive pathogen. APS Southern Division Annual Meeting in Chattanooga, TN, March 9, 2022.
2. **Crouch, J. A.** 2022. Invasion and expansion of boxwood blight pathogens in the U.S. and globally. APS Southern Division Annual Meeting in Chattanooga, TN, March 9, 2022.
3. **Hall, C.** 2022. Impacts of boxwood blight on the U.S. boxwood market. APS Southern Division Annual Meeting in Chattanooga, TN, March 9, 2022.
4. **Luster, D.** 2022. Development of diagnostic assays for the boxwood blight pathogens *C. pseudonaviculata* and *C. henricotiae*. APS Southern Division Annual Meeting in Chattanooga, TN, March 9, 2022.
5. **Gouker, F.** 2022. Boxwood breeding: selecting for blight resistance. APS Southern Division Annual Meeting in Chattanooga, TN, March 9, 2022.
6. **Daughtrey, M.** 2022. Extending knowledge of boxwood blight and its management to the ornamentals industry. APS Southern Division Annual Meeting in Chattanooga, TN, March 9, 2022.

Invited Lectures

1. **Baysal-Gurel, F.** 2021. Plant diseases. MTSU-Biotechnology class. November 11, 2021.
2. **Baysal-Gurel, F.** 2022. TSU College of Agriculture, Department of Department of Agricultural and Environmental Sciences Host-Microbe Interaction Class - Dr. Korsi Dumenyo. Fungal and Oomycete Diseases - Boxwood Blight Case Study. February 7, 2022.
3. **Baysal-Gurel, F.** 2022. Plant Diseases. 2022 TN Warren County Master Gardener Class. McMinnville, TN. March 29, 2022.
4. **Baysal-Gurel, F.** 2022. Plant diseases. MTSU-Biotechnology class. March 3, 2022.
5. **Baysal-Gurel, F.** 2022. TSU College of Agriculture AGSC 2401 Environmental Sciences 2- Dr. Bill Sutton and Dr. Thomas Byl. Boxwood Blight Identification and Control. April 11, 2022.

Global and Stakeholder Partnerships

International Boxwood Seminar Series

Three webinars were conducted during this project year with speakers from Switzerland, United Kingdom and Canada.

1. Invasion, impact & management of box tree moth (BTM) in Europe. Dr. Marc Kenis, Head Risk Analysis and Invasion Ecology, Centre for Agricultural Bioscience International (CABI) in Switzerland, December 2, 2021.
2. Progress in boxwood blight management in the United Kingdom and New Zealand: 1990s to 2021. Dr. Matthew Crome, Royal Horticultural Society, United Kingdom, March 10, 2022.
3. Biology and management of Volutella blight caused by *Pseudonectria buxi*. Dr. Thomas Hsiang, School of Environmental Sciences, University of Guelph, Canada, June 23, 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 all the hard work behind the scene including coordinating, publicizing, hosting via Zoom.us, recording then posting the recordings at the project outreach website and providing attendance metrics.

International Boxwood Blight Epidemiology Working Group (IBBE) Meetings hosted

- The second IBBE meeting on October 25, 2021 with two invited presentations
 - Predicting boxwood blight infection and establishment risk using CLIMEX, correlative, and DDRP modeling platforms, by Drs. Len Coop and Brittany Barker, Oregon State University.
 - Long-term monitoring of boxwood blight symptom development in an open-air nursery in coastal California - update, by Drs. Wolfgang Schweigkofler and Nilwala Abeysekara, Dominican University of California.
- The third IBBE meeting on February 7, 2022 with one invited presentation
 - What weather conditions make an area a hotspot for boxwood blight? By Dr. Urmila Adhikari, Virginia Tech.
- The fourth IBBE meeting on May 9, 2022 with one invited presentation
 - Attempting to understand boxwood blight epidemiology in landscape plantings through diagnostic sample confirmations, by Dr. Andrew Loyd, Bartlett Tree Experts.

Research Collaboration with German Scientists

1. Brand, T., Beltz, H., Ehsen, B., Adhikari, U., **Daughtrey, M. L., Luster, D. G., Kong, P., and Hong, C. X.** 2022. Multi-year field plantings evaluating boxwood cultivars for susceptibility to the blight pathogens (*Calonectria* spp.) in Northern Germany. Plant Disease published online as First Look at <https://doi.org/10.1094/PDIS-05-22-1102-RE>.
2. Brand, T., Beltz, H., Adhikari, U., **Daughtrey, M. L., 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. Submitted to Journal of Plant Disease and Protection.

Boxwood blight is caused by two fungal pathogens: *Calonectria pseudonaviculata* (*Cps*) and *C. henricotiae* (*Che*). Currently, *Cps* is widespread in Europe, Western Asia, North American 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 higher 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.

These reports made the 16-year (2006 to 2021) field studies conducted in northern Germany 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 current boxwood cultivars and fungicides perform against *Che* under field conditions.

While analyzing and publishing these cultivar performance and fungicide efficacy data were add-ons after the project was approved, they helped develop a better understanding of all field data sets and thus have been critical to improve knowledge of field epidemiology.

Research Collaboration with North Carolina Department of Agriculture and Consumer Services (NCDA&CS) and North Carolina Cooperative Extension (NCCE), as well as Local Farmers

1. 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.** 2022. Three common antidesiccants have minimal impact on boxwood shoot bacterial and fungal communities. Submitted to Phytobiomes.

Working with agencies [NCDA&CS (H. T. Tseng, Chad Taylor and 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 antidesiccants for their blight mitigation potential. This trial was done in western North Carolina, a hotspot of 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 the trial, set it 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 2 to 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 is based.

Looking into how the boxwood foliar microbial community may be affected by antidesiccant products was added to the project after the first treatment in the spring of 2021. This add-on made it possible to integrate antidesiccants 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.

Outreach Website – Boxwood Blight Insight Group (<https://www.boxwoodhealth.org>)

This website is hosted at the HRI and updated monthly by HRI staff to keep stakeholders and the public informed of the project progress.

As content continues to grow in volume, we worked closely with HRI staff to restructure the site to make it more user friendly. Specifically, with timely support from HRI staff, we have a new landing page – Boxwood Gardening for Public and Private Gardeners – initially developed to provide further information for the gardening public at the time of a boxwood article in the *New York Times* on June 1, 2022.

On-Demand at Cultivate '22, A Premier Annual Event for the Entire Horticulture Industry, Hosted by AmericanHort

1. **Baysal-Gurel, F.** 2022. Effect of sanitizers on *Calonectria pseudonaviculata*, causal agent of boxwood blight. BBIG On-Demand session in the tHRIve Knowledge Center at Cultivate'22, hosted by AmericanHort in Columbus, OH on July 17, 2022.
2. **Pscheidt, J.** 2022. Pesticide coverage of boxwood plants with nursery sprayers. BBIG On-Demand session in the tHRIve Knowledge Center at Cultivate'22, hosted by AmericanHort in Columbus, OH on July 17, 2022.
3. **Santamaria, L.** 2022. Tools for training English and Spanish-speaking workers to scout for boxwood blight. BBIG On-Demand session in the tHRIve Knowledge Center at Cultivate'22, hosted by AmericanHort in Columbus, OH on July 17, 2022.
4. **Weiland, J.** 2022. Observations about boxwood blight in the nursery industry. BBIG On-Demand session in the tHRIve Knowledge Center at Cultivate'22, hosted by AmericanHort in Columbus, OH on July 17, 2022.

Partnership with the American Boxwood Society (ABS)

Andrea Filippone, ABS President, worked with **Margery Daughtrey** to contribute a Featured News article to the August 2022 issue of BBIG Newsletter, promoting a systems approach, and facilitated the development and publication of a New York Times article – ‘Why boxwood, a perennial favorite, needs a new approach’, on June 1, 2022.

Partnership with the USDA National Plant Diagnostic Network (NPDN)

Douglas Luster, Margery Daughtrey, and Karen Snover-Clift presented a seminar entitled: ‘BBIG, a team tackling boxwood blight – sharing progress on diagnostics, extension and outreach’, at The Great Plain Diagnostic Network Webinar Series 2022 on March 16, 2022. This webinar was facilitated by **Karen Snover-Clift**.

Partnership with the National Plant Board (NPB)

Margery Daughtrey spoke on boxwood blight at the Central Plant Board meeting in Des Moines, IA. The event was held from April 11 to 14. She was an invited boxwood expert panel member, along with Russ Bullock of USDA Animal and Plant Health Inspection Service and Abigail Weisner of University of Guelph, Canada. **Chuan Hong** advised on the discussion topics and panel selection.

Other Major Extension Products

Twitter.com/boxwoodhealth

This new outreach initiative was instituted in December 2021, aiming to keep stakeholders and the public better informed of the latest research and educational opportunities.

BBIG Monthly Digests

1. **Hong, C. X., Daughtrey, M., and Luster, D.** 2022. BBIG Monthly – January.
2. Omolehin, O.*, **Daughtrey, M., Luster, D., and Hong, C.X.** 2022. BBIG Monthly – February.
3. Omolehin, O.*, **Daughtrey, M., Luster, D., and Hong, C.X.** 2022. BBIG Monthly – March.
4. Omolehin, O.*, **Daughtrey, M., Luster, D., and Hong, C.X.** 2022. BBIG Monthly – April.
5. Omolehin, O.*, **Daughtrey, M., Luster, D., and Hong, C.X.** 2022. BBIG Monthly – May.

6. Omolehin, O.*, **Daughtrey, M., Luster, D., and Hong, C.X.** 2022. BBIG Monthly – June.
7. Omolehin, O.*, **Daughtrey, M., Luster, D., and Hong, C.X.** 2022. BBIG Monthly – July.
8. Omolehin, O.*, **Daughtrey, M., Luster, D., and Hong, C.X.** 2022. BBIG Monthly – August.

The BBIG Monthly was instituted at the beginning of 2022 and 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.

BBIG Quarterly Newsletters (<https://www.boxwoodhealth.org/research-updates>)

1. Yang, X.*, Omolehin, O.*, **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.** 2021. BBIG Newsletter Volume 2, Issue 1, released December 1, 2021.
2. Omolehin, O.*, **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 1, released March 1, 2022.
3. Omolehin, O.*, **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 2, released June 1, 2022.
4. Omolehin, O.*, **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 3, released September 1, 2022.

Boxwood Seminar: Growing the Shrub We All Know and Love Despite Boxwood Blight

This was a day-long roundtable discussion with industry professionals at the Dixon Gallery and Gardens in Memphis, TN on February 18, 2022, with an expert panel of seven including **Lynn Batdorf**, Chris Cooper, **Fulya Baysal-Gurel**, Leslie Hollandsworth, Katy Kilbourne, Booker T. Leigh, and **Bennett Saunders**. Both Batdorf and Saunders serve on the BBIG Advisory Panel.

BBIG Showcased in Major National and International Events and Media

- The First International Boxwood Virtual Workshop – Turkey (<http://iwob2021.erdogan.edu.tr/en/>, October 25 to 27, 2021) – ‘Update on boxwood blight in the United States and Boxwood Blight Insight Group (BBIG)’ presented by **Fulya Baysal-Gurel**.
- The 2022 Association of Research Directors (ARD) Research Symposium (Atlanta, GA, April 2 to 5) – ‘Boxwood Blight Insight Group (BBIG) – An International Consortium’ presented by **Fulya Baysal-Gurel**.
- The New York Times – ‘Why boxwood, a perennial favorite, needs a new approach’, published on June 1, 2022. This publication was facilitated by **Margery Daughtrey** along with the American Boxwood Society.

Field Demonstrations

1. **Weiland, J. E. and Santamaria, L.** 2022. Trained eight nursery employees, 11 nursery inspectors and the lead horticulturist of Oregon Department of Agriculture on how to more effectively scout for boxwood blight in nurseries. These training events were conducted on-site at a nursery with boxwood blight.

Courses

1. **Pscheidt, J.** 2022. Plant Disease Diagnosis (BOT 553). Summer field class for 10 graduate students. Visited ornamental nurseries in Oregon. Boxwood blight was among the diseases seen and discussed.

Interactive Virtual Workshops for both English and Spanish Speakers

1. **Santamaria, L. and Baysal-Gurel, F.** 2022. Virtual Boxwood Disease Scouting Workshop in TN (Spanish), March 24, 2022.
2. **Santamaria, L.** 2022. Scouting for Boxwood Blight and BMPs for Spanish-speaking workers, July 8, 2022
3. **Santamaria, L.** 2022. Scouting for Boxwood Blight and BMPs for English-speaking workers, July 8, 2022

Consultations

1. **Weiland, J. E.** Advised the lead horticulturist at the Oregon Department of Agriculture on how to improve best management practices and inspection protocols for the Boxwood Blight Nursery Cleanliness Program.
2. **Santamaria, L.** Planning with the Lead Horticulturist at the Oregon Department of Agriculture – Nursery & Christmas Tree Program, to list educational activities that qualify for the training requirements of the Boxwood Blight Cleanliness Program.

Advising Public Gardens on Boxwood Blight Mitigation

1. **Hong, C. X.** 2022. Advised on boxwood blight monitoring and mitigation at the Colonial Williamsburg Foundation in Williamsburg, VA throughout the project year from September 2021 to August 2022.

Citizen Science Projects

1. **Hong, C. X.** 2021. Monitoring the blight pathogen in soil at Longwood Gardens, Kennett Square, PA, Rachel Schnaitman, Associate Director of Horticultural Operations, December 2021.
2. **Hong, C. X.** 2022. Monitoring the blight pathogen in soil at Longwood Gardens, Kennett Square, PA, visited the IPM Lab and worked with Beth Pantuliano, IPM Manager, in checking the bait leaves for spores of the blight pathogen, June 3, 2022.
3. Yoder, K. S., Dunn, R. A., Saunders, J. B., Mays, T. R., **Hong, C. X.**, and Scoggins, H. 2022. Field evaluation of *Buxus* cultivars and selections against boxwood leafminer and boxwood blight.

Reaching Out via BBIG-q@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 7,734 as of August 17, 2022

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 inventory shift to less susceptible cultivars. We also continued the International Boxwood Seminars, with speakers from Switzerland, United Kingdom, and Canada.

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

We previously identified a protein target by proteomic analysis for generation of antibodies for serological detection of *Calonectria* spp. in infected boxwood leaves. This highly abundant protein target is unique to the boxwood blight fungi, *Cps* and *Che*. Polyclonal antibodies (pAbs) raised against a partial amino acid sequence of the protein were tested using double antibody sandwich enzyme-linked immunosorbent assays. One pAb efficiently captured its corresponding protein and detected the target antigen in extracts of *Cps*- and *Che*-infected leaves, and did not react with extracts of healthy leaves or leaves expressing *Volutella* (*Pseudonectria buxi*), *Fusarium* spp., *Phytophthora* root rot, or *Macrophoma* (*Dothiorella candollei*) symptoms. The assay detected the pathogen in infected leaves from 22 boxwood cultivars inoculated with *Cps* or *Che* (**Table 1**), although variation in reaction strength was noted between small- and large-leaved cultivars.

Table 1. Data from capture ELISA testing reactivity of *Che*- or *Cps*- inoculated *Buxus* cultivars and control tissue with pAb 12239-1. Results were verified in two separate experiments with leaves harvested and extracted at 10 dai. Number of + signs indicated –fold response above background (negative control = uninoculated leaf samples).

	Arborescens	Buxus Harlandii	Dee Runck	Franklin's Gem	Freedom	GH Phillips	Go Dream	Green Beauty	Green Mound	Green Mountain	Green Velvet	Indy	Jim Smoller	John Baldwin	Justin Brower	Little Missy	Richard	Sprinter	Suffruticosa	TM10	Vander Valley	Winter Gem
<i>Che</i>	+++	+	++	+++	+++	++	+++	+	+++	+++	++	++	+++	++	++	++	+	+	+++	++	++	++
<i>Cps</i>	++	+	+++	++	+	+++	+++	++	++	++	+++	+	+++	++	++	+++	++	+	+	++	+++	+++
Neg Control	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

Overall results from 2021-2022 indicate that the ELISA assay sensitivity was acceptable with inoculated, heavily infected leaves late in the infection cycle; further testing is needed to measure the sensitivity earlier in the infection cycle, including pre-symptomatic leaves. Additional testing with a genetically diverse collection of *Cps* and *Che* isolates is ongoing.

A contract was initiated in May 2022 to generate monoclonal antibodies (mAbs) against two peptides with amino acid sequences derived from the *Calonectria* target protein. Antibodies will be tested for specificity and sensitivity, and we anticipate that a combination of pAbs and mAbs will be suitable for development of a lateral flow device for detection of *Cps* in the field.

1.2 Oregon nursery survey

All nurseries scheduled to be surveyed in 2021 are complete as well as three of the nurseries scheduled for 2022. All but one nursery had boxwood blight. Data were collected on symptom presence, cultivar affected, plant size and spacing. Preliminary results show that the unusually wet and cool spring (2022) in Oregon led to new infections and outbreaks starting in mid-March at several nurseries. These observations challenged the current boxwood blight infection risk model 2.1, which predicted low infection risk for the location at the same period. In addition, symptoms of leaf spot, defoliation, and stem lesions continue to be abundant on boxwood cultivars that are considered more resistant to boxwood blight, including Winter Gem, Green Beauty, and Wintergreen. The most commonly infected cultivars continue to be Winter Gem, Green Gem, Green Mountain, and Green Velvet, which are the cultivars produced in the highest numbers.

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

An experiment was conducted to evaluate the growth of 15 Oregon *Cps* isolates at six temperatures from 5 to 30°C. In addition, a growth chamber experiment was conducted to explore infection at different temperatures on a resistant (Winter Gem) and a susceptible (Green Velvet) boxwood cultivar at 15°C (average Oregon fall/spring temperature) and 25°C (optimum temperature for growth and infection reported in the literature). Results showed that the temperature optima for Oregon *Cps* isolates are dependent upon the substrate on which they are grown. On potato dextrose agar, the optimum temperature for growth is the same as reported in the literature previously (25°C). However, on malt extract agar, Oregon *Cps* isolates grow better at cooler temperatures (15-20°C). In addition, disease is much more severe when inoculated plants are grown at 15°C than at 25°C. At 25°C, Green Velvet developed severe disease, while Winter Gem only had very mild disease. However, at 15°C, Winter Gem developed severe blight. These findings highlight the importance of cool temperatures to the biology of the pathogen in Oregon.

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

These studies have been on hold due to the COVID-19 pandemic, restricted access to the lab facility, and the relocation of the project lead (Crouch) from the USDA-ARS Beltsville, MD to a new laboratory at USDA-ARS Frederick, MD. New laboratory facility and required USDA-APHIS plant pathogen permits are being set up, and a new USDA-ARS Biologist was added to support the project to begin in September 2022. In addition, a postdoctoral research associate was recruited to lead the project research, tentatively scheduled to start in September 2022 after security clearances are obtained.

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

We tested the reaction of six boxwood cultivars (*Buxus sempervirens* ‘Suffruticosa’ and ‘Vardar Valley’, ‘Justin Brouwers’, *Buxus* x ‘Green Velvet’, *B. microphylla* ‘Little Missy’, and *Buxus* ‘NewGen Independence®’) to *Cps* and *Che* at four temperatures to determine, first, if cultivars are affected differently by the two pathogen species, and second, if the cultivars behaved differently at different temperatures.

For each cultivar, we placed five boxwood leaves top-down in each of three Petri dishes lined with moist filter paper and sprayed them with a combined conidial suspension of three *Cps* isolates or three *Che* isolates at 8000 conidia/mL (or water alone for controls). Inoculated leaves in dishes were incubated in growth chambers set at 12, 18, 24 or 30°C for six days, then leaves were measured for incidence and % lesioned area; these were averaged over each plate. The experiment was repeated three times. Analysis of variance and Waller-Duncan k-ratio t-test were carried out in R to provide grouping for statistical differences.

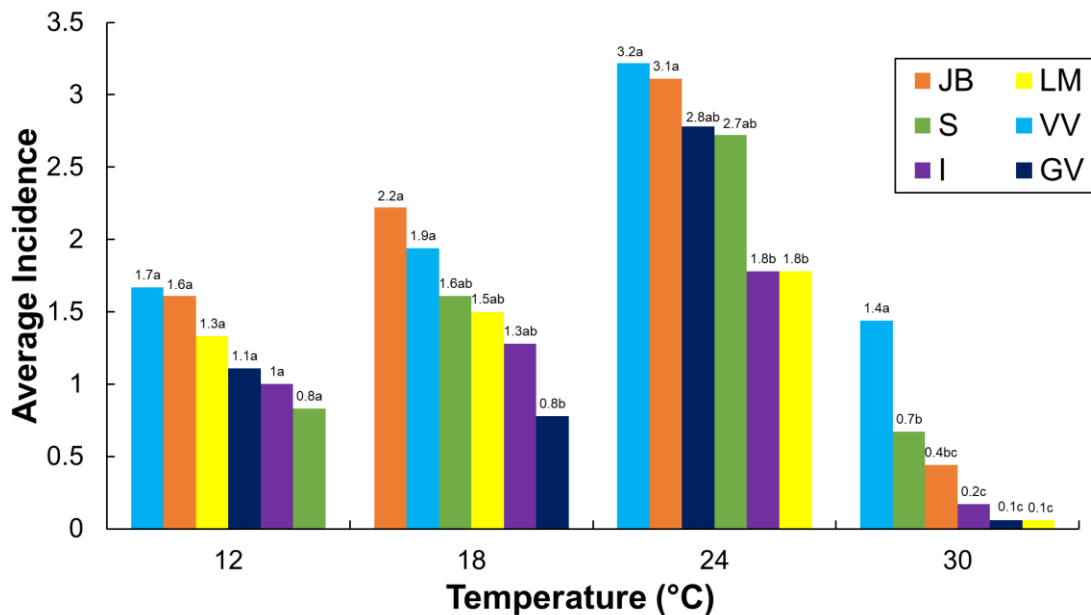


Figure 1 Disease Incidence of *Calonectria henricotiae* (*Che*) & *C. pseudonaviculata* (*Cps*) on 6 *Buxus* cultivars at 4 temperatures. JB = ‘Justin Brouwers’, LM = ‘Little Missy’, S = ‘Suffruticosa’, VV = ‘Vardar Valley’, I = ‘NewGen Independence’, GV = ‘Green Velvet’. Waller-Duncan k-ratio *t*-test was performed to provide grouping using ‘agricolae’ in R.

Different disease incidences were observed among cultivars at some temperatures but not between *Cps* and *Che*. For combined incidence data for both pathogens, ‘Justin Brouwers’ and ‘Vardar Valley’ had the highest incidence overall, but ‘Vardar Valley’ had noticeably higher incidence at 30°C than any other cultivar (**Figure 1**).

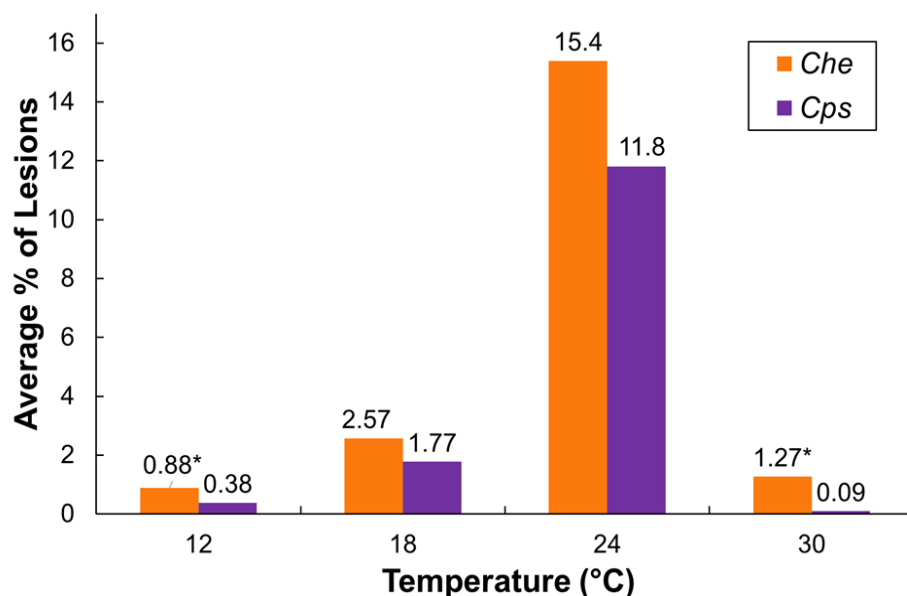


Figure 2 Average % lesions caused by *Calonectria henricotiae* (*Che*) & *C. pseudonaviculata* (*Cps*) on 6 *Buxus* cultivars at 4 temperatures. An * indicates significantly higher % lesion areas caused by *Che* than *Cps* at 12 and 30°C ($P < 0.05$) based on analysis of variance (ANOVA).

Different lesion areas were seen between two pathogen species (**Figure 2**) and among six cultivars (**Figure 3**) at some temperatures. For example, at 12°C, *Che* produced significantly larger lesions than *Cps* ($P = 0.03$). At 30°C, *Che* also produced significantly larger lesions than *Cps* ($P = 0.047$). No significant difference in percentage of lesions was observed among cultivars at lower temperatures (12 and 18°C). At 24°C, ‘Independence’ and ‘Little Missy’ had significantly fewer lesions than other cultivars.

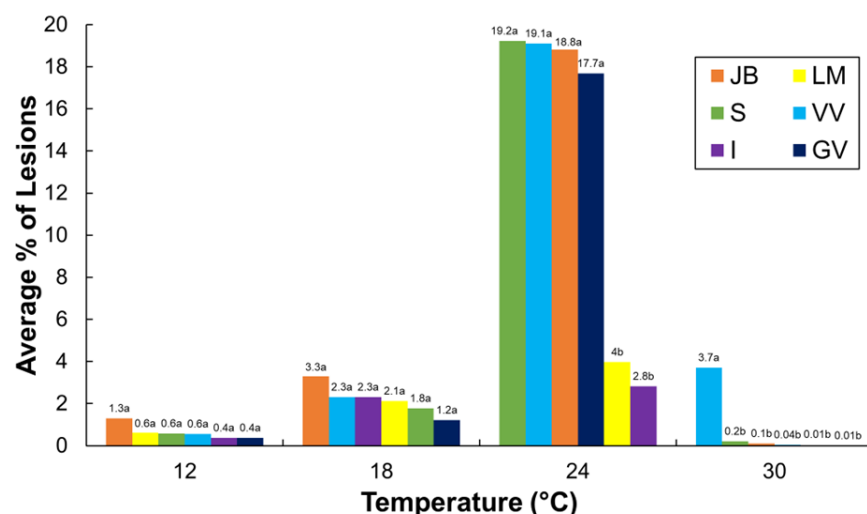


Figure 3 Average % lesions caused by *Calonectria henricotiae* (*Che*) & *C. pseudonaviculata* (*Cps*) on 6 *Buxus* cultivars at 4 temperatures. JB = ‘Justin Brouwers’, LM = ‘Little Missy’, S = ‘Suffruticosa’, VV = ‘Vardar Valley’, I = ‘NewGen Independence’, and GV = ‘Green Velvet’. Waller-Duncan k-ratio t -test was performed to provide grouping using ‘agricolae’ in R.

Based on these results, it is possible that temperature contributes to the observed variable response of boxwood cultivars. ‘Vardar Valley’ had greater incidence and larger lesions at 30°C, suggesting it would be more susceptible in hotter climates. Other cultivars with resistance at higher temperatures did not differ in susceptibility at 12-18°C. There was slightly more severe disease at high temperatures (30°C) with *Che*.

1.6 Bridging the blight knowledge gaps - field epidemiology

We collaborated with a number of scientists across the country including Dr. Len Coop of Oregon State University, Dr. Wolfgang Schweigkofler of Dominican University of California, and Dr. Marc Cubeta of North Carolina State University in the case study of the blight epidemiology. Specifically, we have developed a general framework for relating boxwood blight epidemics to the local weather conditions in North Carolina, Connecticut, California and Oregon, representing four blight development and climatic patterns. We are currently evaluating this framework with the disease and weather data collected by German scientists as they evaluated different boxwood cultivars and different fungicides under field conditions since 2006. In the meanwhile, we made the multi-year field cultivar evaluation data collected in *Che*-dominated northern Germany available to American stakeholders and the international communities through a research publication in *Plant Disease*.

We worked with Drs. Brittany Barker and Len Coop of Oregon State University and developed process-based and correlative models to predict the potential distribution of *Cps* within the United States and globally. The predicted *Cps* global distribution helps to guide the development of national phytosanitary protocols for preventing further spread of this pathogen, to prioritize global surveillance efforts for more effective early detection, and to plan eradication, containment and management where accidental introductions occur.

We also worked with Dr. Andrew Loyd of Bartlett Tree Experts, visualizing their boxwood blight positive diagnoses across the country and charting strategies to analyze them and develop a better understanding of the blight epidemiology at local and national scales.

The International Boxwood Blight Epidemiology Working Group continued to expand and provide a powerful platform for sharing the latest research, brainstorming on new ideas and future directions, and coordinating collaborative studies. Specifically, we hosted three Zoom meetings on October 25, 2021, February 7, and May 9, 2022, with the next meeting scheduled for October 17, 2022.

1.7 Identifying critical control points (CCPs) at production nurseries

We conducted the survey during early summer 2022 to determine growers’ boxwood production flow, baseline knowledge of boxwood blight disease, and knowledge and practice of boxwood blight best management strategies. The survey also included questions related to the estimation of the current economic impact of boxwood blight disease and management techniques. The survey results will be analyzed at the end of summer 2022.

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

2.1 Evaluating antidesiccants for blight mitigation

Three antidesiccant products (TransFilm, Vapor Gard, and Wilt-Pruf) evaluated under field conditions during the first year all have moved into the second phase of field evaluation with increased treatment frequency to monthly or every other month starting in May 2022 (**Figure 4**). Also included in this study were a fungicide standard, Concert II, and a nontreated control. This

new trial is being done with two boxwood cultivars at two field sites in western North Carolina that have a history of boxwood blight. A total of ten treatments were included at each site with seven replicate plants per treatment. Treatments at each site were arranged in a randomized complete block (RCB) design.



Figure 4 The Virginia Tech team worked together – preparing antidesiccant solutions and applying to American boxwood using a customized sprayer boom with mists from three directions for better coverage – at the Lowgap research site in May 2022

As in the first phase, we continued to work with collaborators at NCDA&CS and NCCE, and two collaborating growers. NCDA&CS and NCCE collaborators helped to identify a field with a lightly blighted Vardar Valley boxwood crop near Elkin, NC, applied the fungicide every 3 weeks, and assisted in field preparation for the trial, then conducted monthly antidesiccant treatments and blight evaluation (**Figure 5**). Likewise, the collaborating grower at Lowgap, NC prepared the field, planted and maintained the American boxwood for the trial and applied fungicide treatments every 3 weeks. He also assisted in monthly antidesiccant treatments and blight evaluation. These American boxwood were purchased from a local nursery free of boxwood light by the NCCE collaborator and held in an isolated area near her office for about a month before being taken to the field for planting at the Lowgap site.

The first antidesiccant and fungicide treatments at both research sites were applied in late May and the same treatments have been repeated monthly as scheduled. Likewise, blight incidence was assessed immediately before the monthly treatments starting in May 2022. As of July 20, up to 30% leaves were blighted at the site near Elkin and a reduced incidence at the Lowgap site.



Figure 5 Some of the NCDA&CS and NCCE collaborators evaluating blight severity at Ronda research site

2.2 Further developing biocontrol agents (BCAs) into final products

The research advancements under this objective during the second project year are highlighted by the initiation of the second phase of field evaluation, plus two publications and one presentation.



Figure 6 Preparing and applying a suspension of biological control agents and evaluating blight severity at the research sites in Western North Carolina

First, after carefully analyzing the data from two field trials with plants treated twice in 2021, we selected and moved four BCAs – *Pseudomonas lactis* SW, *P. protegens* 13A3, *Burkholderia* sp. SSG and *Bacillus methylotrophicus* BP1024, plus one combo of SSG and *Trichoderma koningiopsis* Mb2, into the second phase of the field evaluation, increasing treatment frequency to a monthly basis beginning in May 2022 (**Figure 6**). Also included in this new evaluation were

a fungicide standard – Concert II – and a nontreated control. Seven treatments were included in this trial with seven replicate plants per treatment, arranged in an RCB design. This biological control trial is being done in different portions of the same field where the antidesiccant trials are undertaken. As in the antidesiccant trials, the biological control trials are done in collaboration with NCDA&CS and NCCE and the growers at the two research sites. Also, the first treatments were applied in late May and the same treatments have been repeated monthly. Additionally, a similar level of blight incidence was observed on the nontreated control plants at both sites.

Second, we demonstrated that SSG, a boxwood endophyte, did not survive well on the foliage surface. This study was published in the *Journal of Environmental Horticulture*. This study suggests that improving SSG's survival is critical to realize the full potential of this bacterial strain as a broad-spectrum and powerful BCA and possibly as a biofertilizer as well.

Third, we demonstrated that SSG induces various defense primings in boxwood plants through cDNA transcriptome, expanding the understanding of the mechanisms by which this broad-spectrum BCA controls boxwood blight and other diseases.

Fourth, we conducted more *in-vitro*, *in-vivo* and *in-planta* studies in the lab and greenhouse and demonstrated that BP1024 has the potential to control the blight and to promote boxwood growth. Some of these studies were presented at the American Phytopathological Society Annual Meeting – Plant Health 2022 in Pittsburgh, PA.

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



Figure 7 Foliar infection in non-inoculated (left) and inoculated cuttings of *B. sempervirens* ‘Suffruticosa’ as indicated by sporulation/defoliation on leaves following spray inoculation in plastic chambers in replications A through J. Modifications of this methodology will be used to evaluate SAR and nutrient effects on boxwood in 2022

We have propagated enough *Buxus sempervirens* ‘Suffruticosa’ now to allow two trials with this highly susceptible cultivar, in order to test SAR inducers on entire plants in small pots. We will use *Buxus* ‘Green Mountain’ for evaluating the levels of Ca and N nutrients for their effect on boxwood blight in cuttings with the different nutritional backgrounds. We have plastic chambers in which plants/cuttings can be held at high relative humidity after inoculation. Our

fall 2021-winter 2022 trial on fungicide effects was successful using higher inoculum level and a more susceptible boxwood cultivar than used previously (**Figure 7**). No treatment effects were observed in our first runs because there were so few points of infection, even in the inoculated controls. We will try to keep the inoculum level high but to use the moderately susceptible ‘Green Mountain’ for this year’s trials of nutrient effects. We hypothesize that higher nitrogen may increase the susceptibility of the boxwood tissue to disease, whereas higher calcium may reduce disease susceptibility.

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

A second round of experimental treatments has been completed to test the effects of silicon (Si) treatment on boxwood blight incidence and influence on boxwood growth as a supplementary micronutrient. Three year-old plants of three cultivars, *B. microphylla* ‘Little Missy’, *B. sempervirens* ‘Suffruticosa’, and *B. sempervirens* ‘Elegantissima’ were treated and evaluated on the effect of continuous Si soil treatment for eight weeks. We tested Si concentrations of 0, 40, 80, and 100 mg L⁻¹ with YarVita Actisil (Bio Mineral N.V., Belgium), whose active compound is choline-stabilized orthosilicic acid. Treatments were applied over an 8-week period three times a week. Plant height was measured before and after treatments and leaves and stems have been harvested for analyzing Si content using variable pressure scanning electron microscopy with x-ray microanalysis. Treated plants are currently being evaluated for disease symptoms through artificial inoculations and leaf and stem tissue will be sent for ICP-AES metals analysis. This second experiment used 20 to 50 times the concentration of Si treatment compared to the previous experiment. Initial results show improved growth for both *B. sempervirens* compared to *B. microphylla* and initial assay results continue to show reduced severity of blight symptoms for the *B. sempervirens* and no difference for the *B. microphylla* cultivar. Results also indicated a phytotoxic effect for the highest concentration of treatment at 100 mg L⁻¹ for all three 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.

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

Understanding the epidemiological factors governing disease development is foundational for integrating recent innovations into boxwood blight best management practices (BMPs). We evaluated the effect of leaf wetness period and temperature on infection and sporulation of three boxwood cultivars under controlled conditions to develop predictive models for disease development. Specifically, we conducted detached leaf assays between 18 and 27°C, and various leaf wetness periods (lwp), with the cultivars *B. sempervirens* ‘Suffruticosa’, *Buxus* × ‘Green Velvet’, and *B. microphylla* var. *japonica* ‘Winter Gem’. Detached leaves were inoculated with 200 conidia in suspension and disease incidence was recorded at 3 to 13 days post inoculation (dpi). Cultivar, lwp, temperature, and most of the interactions significantly influenced disease development. A minimum of 5 h of leaf wetness was required for any infection. Infection incidence increased most rapidly between 12 and 15 h and continued to increase to about 21 h of leaf wetness. Temperatures between 21 and 25°C were optimal for disease development. There was about a 7-day lag between appearance of lesions and maximal sporulation. The two less-susceptible cultivars had fewer lesions than ‘Suffruticosa’ under the same infection conditions; in addition, *Cps* sporulated on infected Winter Gem boxwood leaves later and at a smaller

proportion. Response surfaces were developed for each cultivar to predict the infection percentage using the lwp and dpi.

We have collaborated with boxwood breeders to screen approximately 150 cultivars, lines, or germplasm accessions for boxwood blight resistance.

A detached leaf assay was conducted to understand the duration of the protection offered by the combination of fungicides and SAR initiation with different modes of action on cultivars with varied susceptibility levels. Initially, *B. microphylla* ‘Tide Hill’ and *B. sinica* var. *insularis* and *B. sempervirens* ‘Justin Brouwers’ plants were sprayed with TopBuxus (TB), Actigard (SAR), and chemical fungicides (FC) (chlorothalonil and propiconazole) in all possible combinations. The leaves were collected at 5, 12, 19, 26-days after the plants were treated. These leaves were inoculated with a drop (50µL) of spore suspension (200 conidia/50µL). Infection and sporulation data were collected after 7 days of incubation at conducive temperatures and humidity levels (Temp 25°C and RH >95%). Our study showed that there was no significant difference between all the combinations (FC, TB, FC+ SAR, FC+TB, TB + SAR, and FC+TB+SAR) except SAR alone in terms of infection. In terms of sporulation, there were significant differences between treatments. The factors of cultivar, treatment, days-post-treatment (DPT), and interaction of cultivar and DPT were significant on both infection and sporulation. We found that all these combinations provided a short duration, a maximum of 12-days, of protection for highly susceptible cultivars, whereas they are providing significant protection on less susceptible cultivars for, up to 26 days.

Scientists in Connecticut investigated the spread of boxwood blight in a simulated landscape under conducive natural conditions. We used strict sanitation to greatly reduce or eliminate spread by contact. Movement of the pathogen from an infected source plant was limited to one adjacent plant, likely spread by means of water splash. Plants were mulched with composted hardwood chips and mulching likely was primarily responsible for limiting spread. Boxwood cultivar susceptibility and fungicide spray programs influenced the incidence of spread and severity of disease. This experiment demonstrates that boxwood blight can be controlled in a landscape by following best management practices.

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

We took over five hundred pictures of symptoms on 12 cultivars for use in extension education materials and to use with machine learning programs designed to recognize boxwood blight symptoms.

Objective 3: To build resilience into boxwood production and plantings

3.1 Developing more resistant boxwood cultivars

This year’s breeding season produced 30 unique controlled cross pollinations with ripening fruit still being monitored for harvest. This was also the first attempt at using previously generated hybrids from the USNA breeding program to generate BCF1 and F2 populations. Last season plants have been growing and transplanted into individual pots for future evaluation. Additional germplasm of 50 *B. sempervirens* cultivars received from Longwood Gardens were screened for boxwood blight susceptibility using a detached leaf assay with multiple *Cps* isolates. All 50 genotypes were found to be highly susceptible to boxwood blight. Additional evaluation was conducted on F1 hybrids that have been growing in the USDA nursery for at least five years, and 15 interspecific *Buxus* hybrids were selected for advanced selection and

propagation. We have rooted at least 70% of these. Once enough plants have rooted, they will be sent to cooperating research partners for field trialing, landscape evaluation, and boxwood blight resistance in field evaluations.

3.2 Cataloging and manipulating boxwood microbiomes against Cps

Major discoveries from the microbiome study included: 1) the differential tolerance to *Cps* of English boxwood plants is associated with the complexity of the culturable fungal and bacterial endophyte community (<https://doi.org/10.3390/plants10112244>), and with foliar endophyte abundance, microbiota core and networks; 2) well established English boxwood garden soil in all five states surveyed (CA, IL, NY, SC, VA) is suppressive to *Cps* (<https://doi.org/10.3390/microorganisms10081514>); 3) foliar bacterial and fungal communities are insensitive to three antidesiccants (Transfilm, Vapor Gard, Wilt-Pruf) commonly used for protecting boxwood crops from environmental stresses and damage including heat, frost and freeze, drought at production and in gardens/public spaces; and 4) foliar fungal communities are more sensitive to selected fungicides (Banner Maxx, Daconil, and Concert II) than bacterial communities on the surface and inside of boxwood shoots. These findings are foundational for developing a systems approach to boxwood health and production while enabling growers and the public to utilize the full potential of selected antidesiccants and fungicides for disease mitigation and other horticultural applications.

Additionally, we are in the process of sequencing shoot samples of different boxwood species, varieties and cultivars collected from both Oregon and Virginia to determine how the microbiome may change with boxwood and geographic location/climatic condition. We also have continued to maintain two English boxwood plantings established on the research farm of Virginia Tech's Hampton Roads Agricultural Research and Extension Center in Virginia Beach, VA in July 2021. These plantings will be used to investigate how fertilization and irrigation regimes may affect the boxwood microbiome and plant health.

3.3 Facilitating adoption of more resistant boxwood cultivars

This objective is not scheduled until year 3 of the project, therefore no progress is being reported on this objective.

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

4.1 Developing baseline production model

To promote the adoption of advanced boxwood blight BMPs within the industry, we have started analyzing the economic dimensions of all recommended changes relative to current standard disease control practices in the industry. Economic engineering is being used to estimate the initial capital investment, production costs, and product prices for the baseline and alternative boxwood nursery production models. The models are being simulated with representative BMPs and proper disease control protocols. The models are also serving as a baseline to determine the socio-economic impacts of our strategies to reduce production and post-production shrink. We have collected and summarized current BMPs associated with boxwood production on the East and West coasts by visiting with growers and project team members to determine equipment usage, input materials, and labor hours used during boxwood production using current (traditional) production practices. The spreadsheet model of the current production system for boxwood in container nurseries in the eastern versus western regions of

the U.S. has been built and is currently under review by the project team. Boxwood blight mitigation strategies (BMP's) will be analyzed according to objective 4.3 below.

4.2 Analyzing cost/benefit of changes in blight mitigation practice

Due to COVID, this objective has been delayed until year 3 of the project. Meetings will be held with all team members to (a) identify the appropriate economic cost parameters to assess and (b) develop data collection protocols to ensure consistent and compatible data is acquired during the activities associated with each objective. Likewise, we will work with the biology teams to analyze the cost/benefit of new diagnostic (2.1.1) and blight mitigation tools including antidesiccants (2.2.1), biological control (2.2.2), self-defense enhancers (2.2.3, 2.2.4) and tech integration (2.2.5).

4.3 Partial budgeting: An economic engineering approach

This objective is not scheduled until year 3 of the project, therefore no progress is being reported on this objective, but it should begin next year. Specifically, partial budgeting modeling procedures will be 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 compares 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, this project requires 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 are not considered in this portion of the analysis. Thus, the technique of partial budgeting examines only the effect of the proposed change in practice, assuming all other aspects of the value chain remain unchanged.

4.4 Performing sensitivity tests

This objective is not scheduled until year 3 of the project, therefore no progress is being reported on this objective.

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

This objective is not scheduled until year 3 of the project, therefore no progress is being reported on this objective.

4.6 Assessing the overall project impact

This objective is not scheduled until year 4 of the project, but an initial analysis of the introduction and subsequent spread of boxwood blight since its detection in the U.S. has been conducted and published in the *Journal of Environmental Horticulture*. This provides a baseline level of boxwood blight to assess the progress of our various projects.

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

Delivering research and innovations to the end user has been an integral part of this project as detailed below. To best serve the horticulture industry, public/private gardeners and other

stakeholders, we instituted tweets on the latest research and educational programing in December 2021 and a new BBIG monthly digest, in addition to the quarterly project newsletter – BBIG Newsletter. We also partnered with AmericanHort/HRI, ABS, NPDN, NPB, and APS to reach out to different stakeholders.

5.1 Translating research into products, protocols, and recommendations

BBIG Newsletter

We published four BBIG Newsletters in November 2021, February, May and August 2022, respectively. Each issue began with Featured News, followed by Featured Research, Extension Highlight, and Project Meeting Brief, Recent Publication, etc. as exemplified by Volume 2, Issue 4 (**Figure 8**). Featured News was about an ongoing hot topic, mostly contributions from our collaborators. 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 Highlight covered several topics – major extension activities and accomplishments and quarterly BBIG Boxwood Seminar recap, plus diagnostic tips. Each issue also included a quick summary of our quarterly deliverables.

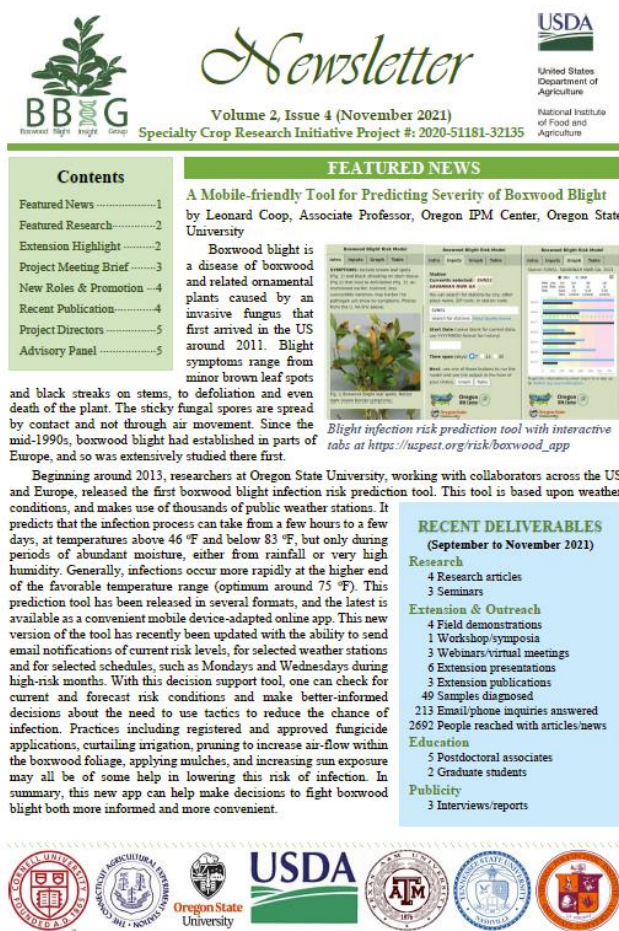


Figure 8 A snapshot of the front page of BBIG Newsletter Volume 2, Issue 4 (November 2021)

5.2 Delivering to end users

We took several new initiatives while using existing venues and platforms for expedited and mass dissemination of research results from this project directly to end users.

New Initiatives

Tweets at Twitter.com/boxwoodhealth

This was initiated in December 2021 for broadening audience reach and instant dissemination of the latest research publications and upcoming educational programs.

BBIG Monthly

This monthly digest was instituted in January 2022 for highlighting the project activities and accomplishments.

Partnership with New York Times

With support from the American Boxwood Society, we worked with the garden editor of the New York Times in publishing an article entitled ‘Why Boxwood, a Perennial Favorite, Needs a New Approach’ on June 1, 2022.

Boxwood Gardening at Home

Synchronized with the publication of the New York Times article, we worked with our stakeholder partners – AmericanHort and HRI – and added a new landing page: **Boxwood Gardening at Home** (Figure 9) to the Boxwood Blight Insight Group website (<https://www.boxwoodhealth.org>). Addition of this new landing page resulted in a surge of page views to 1,400 within the first 22 days of June compared to a monthly average of 430. This also marked the beginning of restructuring this website to get better organization of educational materials we have developed over the first 2 years, helping users to navigate the site and locate the information they seek.

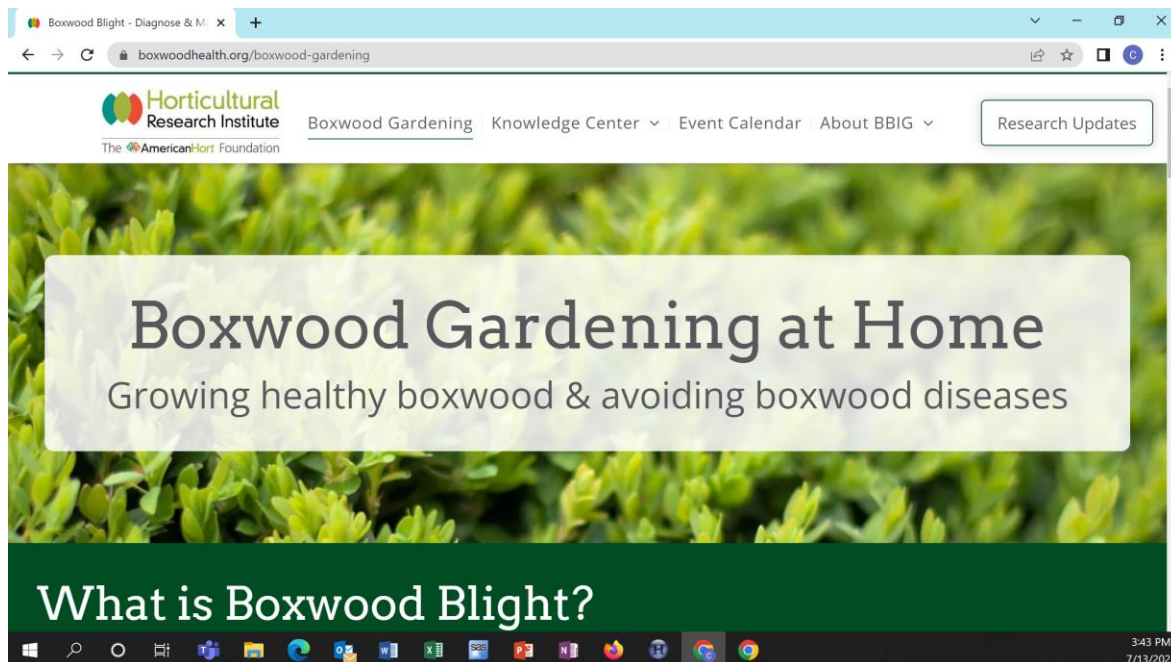


Figure 9 New landing page for Boxwood Gardening at Home at the BBIG website

Meetings with the Advisory Panel (AP)

- Quarterly meetings with AP members, all of whom are leaders in the nursery industry and public gardens, were held via Zoom on the first Friday of September, December, March, and June.
- The first annual meeting with AP was held in two parts, on October 5 and December 3, 2021.

International Boxwood Seminar Series

We continued the success of the quarterly Seminar Series this past year with three new webinars by Dr. Marc Kenis of Switzerland on December 2, 2021, Dr. Matthew Cromeey of United Kingdom on March 10, 2022, and by Dr. Thomas Hsiang of Canada on June 23, 2022, respectively (**Figure 10**) with a fourth one scheduled for September 7, 2022 by Dr. Len Coop of Oregon State University. To meet the needs of our stakeholders, we expanded the coverage of this Series to include box tree moth, a new invasive insect that is threatening the boxwood crops and plantings in the U.S., and Volutella blight, a common fungal disease on boxwood (**Figure 10**).

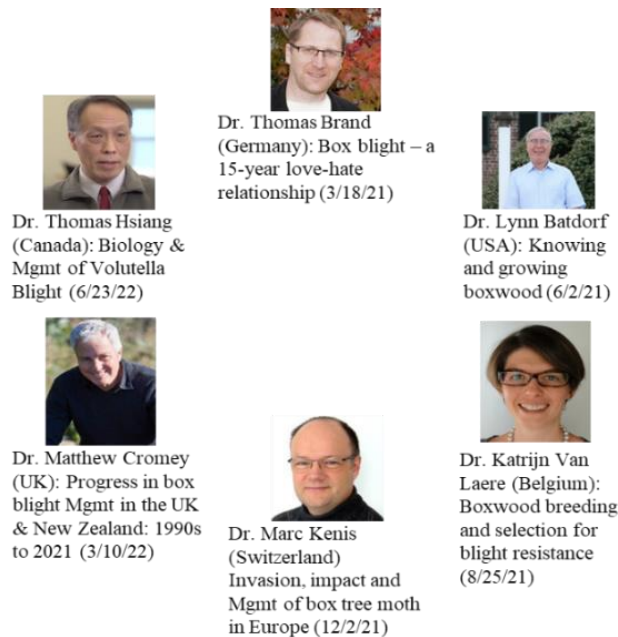


Figure 10 Presenters and presentation titles of six webinars to date

These webinars reached all sectors of the horticulture industry, arborists, public garden managers, private gardeners/consumers, as well as first responders (diagnosticians, regulatory personnel at both state and federal levels), research and extension communities across the U.S. (**Figure 11**). According to HRI, our partner and the hosting institution of the Seminar Series, a total of 1,094 people attended these six webinars. In addition to the attendees from the U.S., there were attendees from 19 foreign countries in five continents. Thus, the U.S. stakeholders and international communities were both well-served by the webinars. This Series set a new example of global and stakeholder partnership in addressing new and emerging plant biosecurity issues like boxwood blight.



Figure 11 Attendee groups of the International Boxwood Seminar Series

Examples of Outreach Programs Targeting at Different Stakeholder Groups

The horticulture industry and the public

- BBIG On-Demand session in the tHRive Knowledge Center at Cultivate'22 in Columbus, OH on July 17, 2022 (**Figure 12**). The Extension team leadership partnered with the AmericanHort/Horticultural Research Institute (HRI) leadership in planning this national event that provided the BBIG Team a platform to present and connect to various sectors of the horticulture industry. This session consisted of four presentations by BBIG Project Directors - **Fulya Baysal-Gurel**, **Jay Pscheidt**, **Jerry Weiland**, and **Luisa Santamaria**. Their presentations were featured in *Nursery Management*, a popular national trade magazine for the horticulture industry.

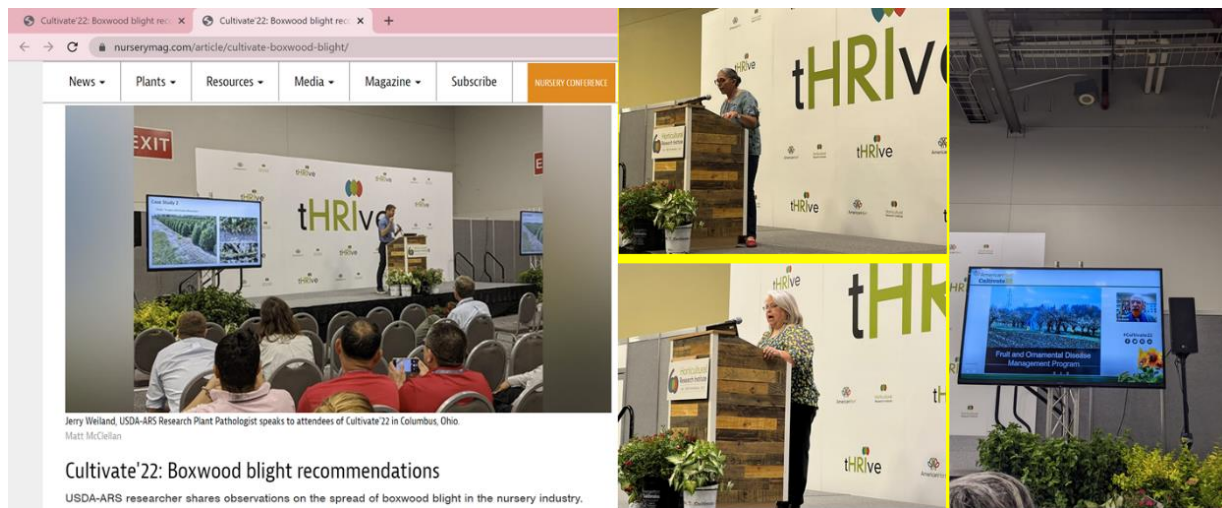


Figure 12 BBIG showcase at the national tradeshow Cultivate'22 in Columbus, OH on July 17, further extending our reach to the industry

- A day-long roundtable discussion with industry professionals entitled: Growing the Shrub We All Know and Love Despite Boxwood Blight, at the Dixon Gallery and Gardens in Memphis, TN on February 18, 2022 (**Figure 13**).



Figure 13 Discussion panel included three BBIG Team members: Lynn Batdorf and Bennett Saunders (AP members) and Fulya Baysal-Gurel (Co-Project Director), and Katy Kilbourne (BBIG Collaborator, not shown)

First responders

- In collaboration with the National Plant Diagnostic Network, **Douglas Luster**, **Margery Daughtrey**, and **Karen Snover-Clift** presented a seminar entitled: ‘BBIG, a team tackling boxwood blight – sharing progress on diagnostics, extension and outreach’, at The Great Plains Diagnostic Network Webinar Series 2022 on March 16, 2022.
- **Margery Daughtrey** presented an invited seminar entitled: ‘A team tackling boxwood blight’, at the Central Plant Board meeting held from April 11 to 14 in Des Moines, IA.

Research and extension communities

- ‘Ornamental Production Challenges and Perspectives: A Case Study of Boxwood Blight’, at the American Phytopathological Society (APS) – Southern Division Annual Meeting in Chattanooga, TN, March 9, 2022
- ‘Epidemiology, Diagnosis and Management of an Emerging Disease – Boxwood Blight’, at Plant Health 2022 – APS Annual Meeting in Pittsburgh, PA, August 8, 2022 (**Figure 14**). At least 65 people attended this symposium.



Figure 14 BBIG team gathering after the symposium – Epidemiology, Detection and Management of an Emerging Disease – Boxwood Blight, at Plant Health 2022 – APS Annual Meeting in Pittsburgh, PA, August 8, 2022

- Hosting three virtual meetings of International Boxwood Blight Epidemiology Working Group via Zoom.us on October 25, 2021, February 7 (**Figure 15**) and May 9, 2022, respectively.

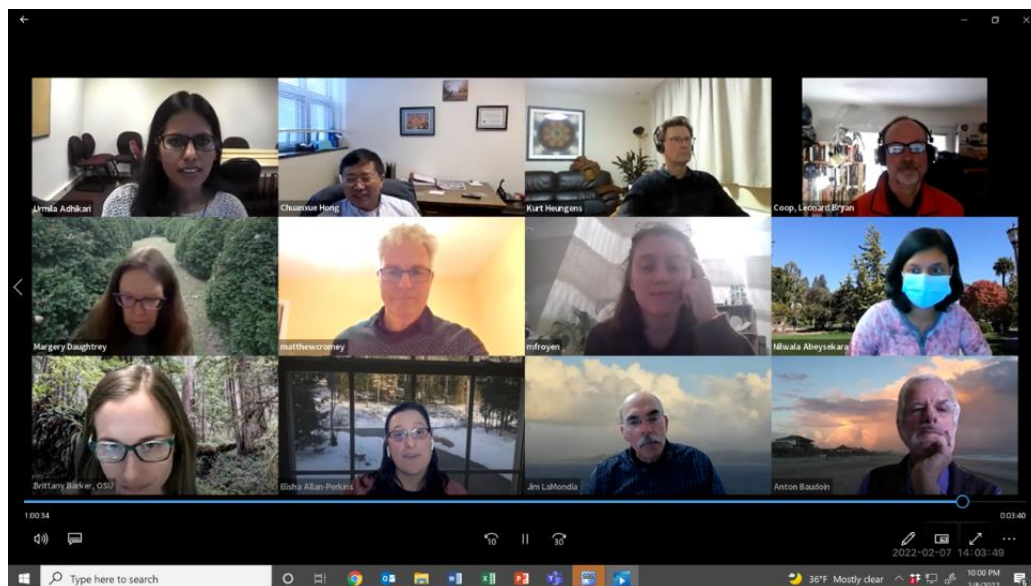


Figure 15 A screenshot of the third IBBE Working Group meeting on February 7, 2022

Other Conventional Means of Technology Transfer

- Publishing nineteen refereed journal articles.
- Giving sixteen presentations at annual meetings of the APS and other professional conferences.
- Providing one course and five invited lectures.
- Conducting one field demonstration.
- Performing three interactive virtual workshops for both English and Spanish speaking workers.
- Two consultations.
- Directly working with and advising public gardens on boxwood blight mitigation.

Other Nonconventional Means of Technology Transfer

- Directing three citizen science projects on the blight pathogen monitoring at infested sites and on field trials evaluating boxwood cultivars.
- Showcasing in major national and international events.
- Reaching the extension communities and large growers and landscapers through open access publications in *Plant Health Progress* and other journals.
- Continuing to use Google Group (bbig-g@vt.edu), along with other local and national networks, for dissemination of the project monthly digest – BBIG Monthly and quarterly BBIG Newsletters, as well as the latest research and extension publications, and boxwood health related news. A total of 7,734 boxwood enthusiasts were reached via the BBIG group alone as of August 24, 2022.

5.3 Documenting project impacts

This project has resulted in many significant impacts on boxwood blight mitigation during the second project year from September 1, 2021 to August 31, 2022.

Changes in Knowledge

- Our extension programs, BBIG Monthly digest, and Quarterly BBIG Newsletters, the BBIG website at www.boxwoodhealth.org, and the International Boxwood Seminar Series, have enabled growers, retailers, landscapers, arborists, garden managers and other horticulturists, as well as regulatory personnel, diagnosticians, and extension specialists and agents 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.
- Our research and innovation have advanced the science of boxwood blight biology, epidemiology, and diagnostic and mitigation technology. For example, preliminary evidence shows that spacing plants further apart reduces the chance for the pathogen to spread from an infected plant to nearby healthy plants. Decreasing irrigation frequency reduces infection and disease severity. Thus, critical changes in the way nurseries grow boxwood (increased plant spacing and decreased irrigation) can reduce the chances for boxwood blight outbreaks and pathogen movement within the nursery. Results from our research have also been used to strengthen existing best management practices for nursery cleanliness programs and to help nursery inspectors learn to more effectively

scout for boxwood blight during scheduled nursery inspections. These advancements will continue to change blight diagnostics and improve disease mitigation in both the landscape and the nurseries, thereby benefiting the horticulture industry and consumers for years to come.

- The breeding of disease-tolerant boxwood cultivars is important for reducing the incidence of boxwood blight in Connecticut while providing landscapers with cultivars that have desired traits. In order to better assess boxwood tolerance to blight among cultivars, Dr. LaMondia is collaborating with researchers across the United States to create a standard protocol for boxwood evaluations that can be conducted across institutions. This will include rating cultivars against previously tested plants to assign a scaling of 1 (most susceptible) to 5 (least susceptible). The evaluation program began in 2018 and has grown in subsequent years to include hundreds of selections from multiple breeding programs being assayed at the Valley Laboratory and at other institutions in the United States. These ratings will be used by plant breeders to assess tolerance and by nurseries to convey susceptibilities to consumers. We observed a very good range of responses in percent leaf infection, leaf and stem lesions per plant and percent defoliation. The very susceptible varieties such as *B. sempervirens* ‘Suffruticosa’ were severely diseased and this cultivar was eventually killed. There is significant potential for the development and release of blight resistant boxwood cultivars in the future.

Changes in Practice and Condition

- The nation’s boxwood production has shifted from severely affected states to less affected states or regulated states as demonstrated by the Economic Team.
- Many growers have shifted their boxwood inventory to less susceptible cultivars, a practice that moves the nation onto a fast track to sustainable boxwood production and gardening.
- 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 and eradicating the disease where such introduction occurs, as exemplified by the public gardens we have worked with.
- Boxwood stakeholders also have learned through Citizen Science Projects and set up their own facilities and programs to monitor the blight pathogen in infested soil. Although these efforts began at individual production facilities and gardens, they were successful for limiting pathogen spread and suppressive to pathogen survival, infection, and reproduction— and thus contributed to the blight mitigation and containment at local, regional, and national levels.

Other Impacts

This project has set a new example for fighting a good fight together against invasive species and destructive diseases through stakeholder partnership and international collaboration.

- AmericanHort and HRI are hosting the project outreach website with a knowledge center and helped us develop a user-friendly interface. 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, AmericanHort and HRI are playing 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 have played key roles in every important step of the ongoing 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.
- In addition to sharing their expertise through the International Boxwood Seminar series, researchers in Belgium, Germany, and United Kingdom are collaborating on several important project components including biocontrol and field epidemiology. These collaborations accelerated understanding of the blight biology.
- Again, we also had extraordinarily fine support from our Advisory Panel members. In addition to providing feedback at our quarterly meetings, some individuals have shared additional time and talent. For example, **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. **Lynn Batdorf** and **Bennett Saunders** along with **Fulya Baysal-Gurel**, Leslie Hollandsworth, Katy Kilbourne, and Booker T. Leigh, shared their expertise in a day-long Boxwood Seminar – Growing the Shrub We All Know and Love Despite Boxwood Blight at the Dixon Gallery and Gardens in Memphis, TN on February 18, 2022. **Bennett Saunders** and **John Keller** joined the BBIG team for the On-Demand session at Cultivate’22 in Columbus, OH on July 17, 2022. Staff from Saunders Brothers nursery worked directly with postdoc Dr. Xiaoping Li to collect boxwood samples for the microbiome studies.

5.4 To prepare a new generation of scientists and educators ready to tackle increasingly complex issues facing the nation’s specialty crop industry in a global economy

Nine postdocs and two graduate students were recruited to work with the BBIG team on boxwood blight mitigation. In addition to planning and executing the proposed research activities, analyzing data, and writing for industry and academic publications, all postdocs and students actively participated in translating research into operational protocols and educational materials, delivering to the end user, and documenting project impacts. They also were regularly interacting with the Advisory Panel via Zoom meetings and email exchanges, gaining an appreciation of how to work with industry partners. This project also provided the team, especially early career scientists, with a variety of other professional training opportunities.

- Two postdocs (Dr. Xiao Yang and Dr. Olanike Omolehin) have had experience as Project Manager, with extra responsibilities for handling communications via Teams, Zoom and email, and assembling reports. Coaching is provided by the Project Leaders as needed. This leadership experience helped Dr. Yang secure the position of Diagnostician and Manager of Plant and Pest Diagnostic Clinic at Clemson University.
- Dr. Olanike Omolehin, the Postdoctoral Applied Plant Pathologist, continued to coordinate field trials evaluating antidesiccants and biocontrol agents with NCDA&CS, NCCE and collaborating growers, and became a point of contact for the collaboration with the Flanders Research Institute for Agriculture, Fisheries and Food in Belgium. PDs advised and supported as needed. She also coordinated with several co-PDs and one

Advisory Panel member the study analyzing and promoting boxwood inventory shift to more resistant cultivars in the U.S.

- Dr. Xiaoping Li, Postdoctoral Microbiome Scientist, coordinated boxwood sampling with the USDA ARS Horticultural Crop Research Lab in Corvallis, OR and collaborating growers in Virginia for the microbiome studies. He also directly worked with the growers taking samples. PDs helped him to make connections with collaborating PDs and growers, advised on planning, and provided other support as needed.
- Dr. Urmila Adhikari, Postdoctoral Epidemiologist, served as a point of contact person for collaboration with German scientists, re-analyzing their data of field trials evaluating fungicides, boxwood cultivars and other blight management strategies from the epidemiology perspective. She also coordinated the International Working Group of Boxwood Blight Epidemiology, with PDs' support as needed. This research and leadership experience helped her win a Research Associate position at the NSF Center for Integrated Pest Management of North Carolina State University in April 2022.
- Dr. Mana Ohkura, Postdoctoral Scholar, assisted with the Oregon nursery survey and refined molecular methods for detecting *Cps* from nursery samples. In addition, she led a research project to evaluate the effect of irrigation and plant spacing on boxwood blight. She wrote an outreach publication in the Oregon Association of Nurseries trade magazine, *The Digger*.
- Dr. Srikanth Kodati, Postdoctoral Scientist, investigated epidemiology and management of boxwood blight at the Connecticut Agricultural Experiment Station.
- Two postdocs on the project coordinated multi-author efforts on two publications on boxwood diseases for the diagnostic community. PDs provided advice and leadership as needed. The first of these publications, a diagnostic guide for boxwood blight disease, has been downloaded over 3,000 times, and was ranked as the second-most-downloaded paper published in the journal for the year of 2021.
- Postdocs and graduate students presented at the quarterly Project Team meetings.
- Postdocs and graduate students were supported to attend the American Phytopathological Society meetings.

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

Please see Objective 5.4 for details.

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 second year of the grant, the ongoing pandemic of COVID-19 has 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. While this has hampered laboratory-based research objectives, significant progress has been made in meeting objectives. PDs have collectively met and exceeded our extension and outreach goals, taking advantage of expanded virtual meeting venues and opportunities. Eight of 13 research goals are at 100% completion or ahead of schedule, while the remaining five research goals are at or below 15% completion due to COVID-19-related restrictions on facility occupancy and/or travel, particularly at USDA locations. University programs have also faced reduced lab access and in some cases, requests from upper administration to cut back on planned activities and spending. Quarantine periods have been necessary on short notice when staff became ill with COVID-19. Despite these restrictions, our team has shown resilience and, as a result, all project objectives appear to be achievable within the granting period.

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; 2) Dr. Karen Rane of University of Maryland who connected the team to Kingsville Nursery with a large collection of boxwood plantings which have been a valuable resource for several proposed research objectives (1.1, 1.4, 2.5 and 3.1); and 3) NCCE Collaborator Amanda Taylor 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.