



Annual Report

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Improving boxwood blight mitigation through innovation, economic analysis and education

Together we save boxwood crops and plantings!



August 31, 2024

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



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Key Stakeholder Collaborators

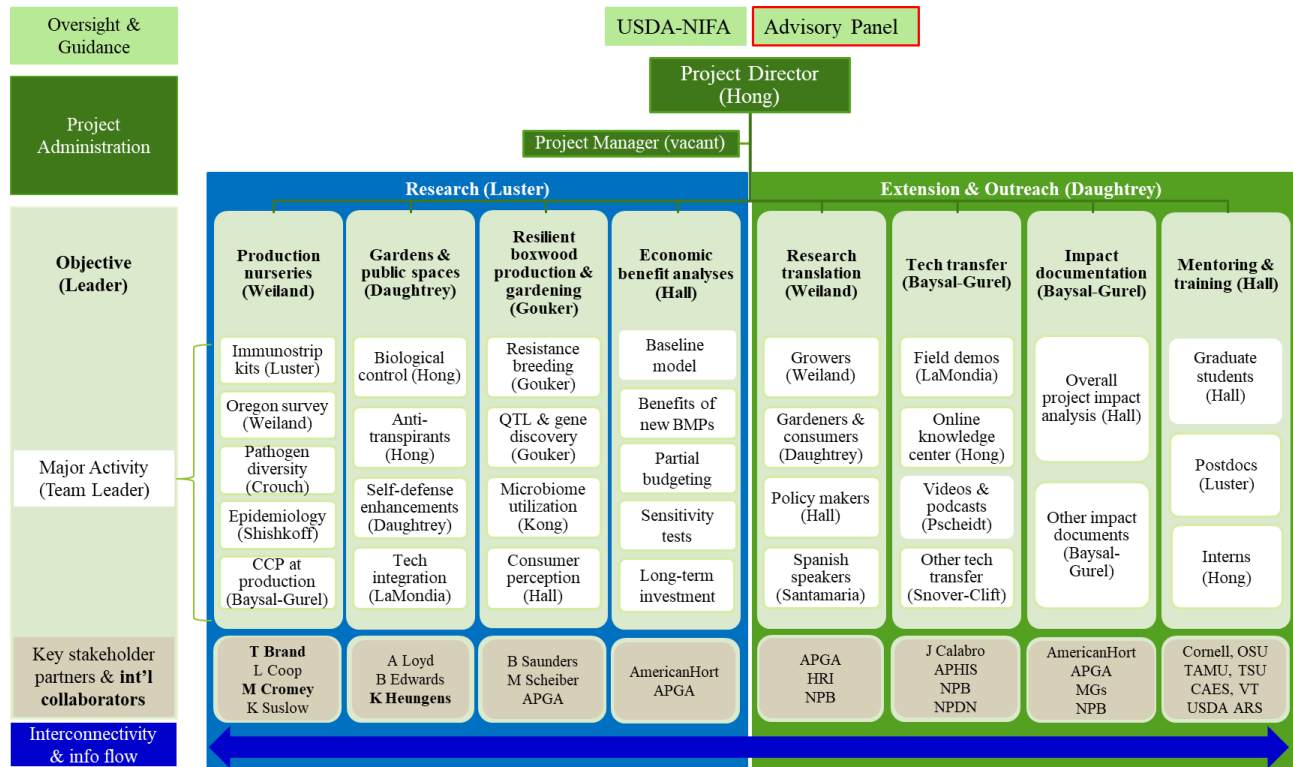
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Project Organization



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; 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, national, and international

Consumers

Master gardeners

Boxwood enthusiasts

Arborists

Diagnosticians

Extension specialists and agents

Regulatory personnel

Conservation biologists

Researchers

Policy makers

PRODUCTS

Research

PhD Dissertations

1. Ghimire, B. 2023. Improving Woody Ornamental Crop Disease Mitigation through Innovation. PhD thesis. Tennessee State University.

Refereed Journal Articles

1. Baysal-Gurel, F., Ghimire, B., and Simmons, T. 2024. Evaluation of fungicides and biorational products for the control of boxwood blight, 2023. Plant Disease Management Report No. 18:OT015. Online publication. The American Phytopathological Society, St. Paul, MN.
2. Ghimire, B., Pendyala, B., Patras, A., and Baysal-Gurel, F. 2024. Evaluating UV-C sensitivity of *Calonectria pseudonaviculata* in model buffer solution using UV-C light emitting-diode system. Plant Disease Open access at <https://doi.org/10.1094/PDIS-03-24-0618-SC>.
3. Kong, P., Ioos, R., and Hong, C. X. 2024. First report of *Calonectria henricotiae* causing box blight in Spain. New Disease Reports. Open Access at <https://doi.org/10.1002/ndr2.12278>.
4. Khaliq, I., Brand, T., Daughtrey, M., Kong, P., and Hong, C. X. 2024. Investigating weather variables driving boxwood blight epidemics: insights from field trials with *Buxus sempervirens* ‘Suffruticosa’ in northern Germany between 2006 and 2020. Plant Pathology Open access at <http://doi.org/10.1111/ppa.13969>.
5. Li, X. P., Weiland, J. E., Ohkura, M., Luster, D. G., Daughtrey, M. L., Gouker, F. E., Chen, G., Kong, P., and Hong, C. X. 2024. Cultivars and production environments shape shoot endophyte profiles of boxwood with different blight resistance. PhytoFrontiers Open access at <https://doi.org/10.1094/PHYTOFR-03-24-0023-R>.
6. Ohkura, M., Beck, B. R., Scagel, C. F., and Weiland, J. E. 2024. The effect of boxwood leaf volatiles on conidial germination of *Calonectria pseudonaviculata*, the causal agent of boxwood blight. Phytopathology (Published online at <https://doi.org/10.1094/PHYTO-12-23-0507-R>).

Abstracts (Presented at Professional Meetings)

1. Daughtrey, M. L., Luster, D. G., Calabro, J., Gray, J., Hall, C., Weiland, E., Baysal-Gurel, F., Gouker, F., Kong, P., Crouch, J. A., LaMondia, J., Pscheidt, J., Santamaria, L., Shishkoff, N., Snover-Clift, K., and Hong, C. X. 2024. Fighting a good fight against an invasive pathogen through the Boxwood Blight Insight Group. The 20th International Plant Protection Congress, Athens, Greece, July 1 to 5, 2024.
2. Ghimire, B., Parajuli, M., Simmons, T., Liyanapathirana, P., and Baysal-Gurel, F. 2023. Enhancing the boxwood blight control using fungicides, antitranspirant, and host plant defense inducer. The 2023 Tennessee Academy of Science Meeting, Memphis, TN, November 18.
3. Gouker, F., and Shao, J. 2024. Chromosome-scale genome assembly of the evergreen shrub, *Buxus sempervirens*. International Plant and Animal Genome Conference 31 (PAG 31). Landscape Trees and Shrubs Workshop, San Diego, CA, USA. January 12.

4. Jennings, C., Ghimire, B., and Baysal-Gurel, F. 2024. Control of boxwood blight using fungicides and biorational products. Plant Health 2024. Memphis, TN, July 27 to 30.
5. Kong, P. and Hong, C. X. 2023. *Burkholderia* sp. SSG – A powerful broad-spectrum biocontrol agent and biostimulant. 7th Partnership in Biocontrol, Biostimulant & Microbiome Congress USA 2023, Raleigh, NC, October 24 to 25.
6. Kong, P., and Hong, C. X. 2024. *Burkholderia* sp. SSG – a safe and powerful broad-spectrum biocontrol agent and biofertilizer. The 20th International Plant Protection Congress, Athens, Greece, July 1 to 5.
7. Omolehin, O., Kong, P., Hemmings, G., Taylor, A., Tseng, H. T., Taylor, C., and Hong, C. X. 2023. Field performance of multiple biocontrol agents against boxwood blight in a disease hotspot of western North Carolina. 7th Partnership in Biocontrol, Biostimulant & Microbiome Congress USA 2023, Raleigh, NC, October 24 to 25.
8. Shishkoff, N. 2024. Differences in boxwood blight symptom expression in different *Buxus* cultivars. American Phytopathological Society – Potomac Division Annual Meeting, Martinsburg, WV. March 27.
9. Shishkoff, N. 2024. Differences in boxwood blight symptom expression at different times and in different boxwood cultivars. Plant Health 24, Memphis, TN, July 29.
10. Stone, C. 2024. Genotypic survey of *Calonectria pseudonaviculata* isolates from Oregon nurseries. American Phytopathological Society – Potomac Division Annual Meeting, Martinsburg, WV, March 27.

Invited Presentations at Professional Conferences WITHOUT Abstract

1. Daughtrey, M. 2024. Introduction: boxwood blight effects and how we are coping. NPDN Symposium – Boxwood Blight Insight Group, January 17.
2. Gouker, F. 2023. Boxwood Breeding at the U.S. National Arboretum. American Boxwood Society Meeting. Pottersville, NJ. September 21-23, 2023.
3. Hong, C. X. 2024. New IPM tools for boxwood blight. NPDN Symposium – Boxwood Blight Insight Group, January 17.
4. Luster, D., Yang, X., McMahon, M. B., Shishkoff, N., Stanley, O., and Carter, M. L. 2024. Diagnostic assays for the boxwood blight pathogens *Calonectria pseudonaviculata* and *C. henricotiae*. NPDN Symposium – Boxwood Blight Insight Group, January 17.
5. Ohkura, M. 2024. Sampling and detection methods for boxwood blight in nurseries. NPDN Symposium – Boxwood Blight Insight Group, January 17.
6. Shishkoff, N. 2024. Boxwood, the plant and its blight symptom expression. NPDN Symposium – Boxwood Blight Insight Group, January 17.
7. Yang, X. 2024. Boxwood blight pathogen morphology and how it differs from other pathogens. NPDN Symposium – Boxwood Blight Insight Group, January 17.

Thesis Defense Seminar

1. Ghimire, B. 2023. Improving woody ornamental crop disease mitigation through innovation. PhD thesis. Tennessee State University, Tennessee State University, McMinnville, TN, October 16

Global and Stakeholder Partnerships

Working with German Scientists on Field Epidemiology while Analyzing and Bringing Multi-year Boxwood Cultivar and Fungicide Evaluation Trial Data to the International Communities

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* is limited to a few central European countries including Germany. Little was known about *Che*, and all published studies were done under lab conditions or controlled environments. Compared to *Cps*, *Che* is more tolerant of high temperatures and less sensitive to some important fungicides, thus, *Che* has the potential to be more adapted to the U.S. weather conditions with fewer control options should it be accidentally introduced into this country.

Following the first observation of boxwood blight in 2004, Dr. Thomas Brand and his colleagues in Germany performed many field trials evaluating boxwood cultivars and fungicides. Through trade journal articles the results of these trials have been communicated to local growers and the public in a timely manner to help manage this destructive disease. These results are equally valuable to growers and horticulturists elsewhere. To bring them to the international communities, we worked with the German scientists – analyzing and publishing the trial data on boxwood cultivar performance in [Plant Disease](#) (2023) and fungicide efficacy in [Plant Diseases and Protection](#) (2023).

We also used these trial data to validate the lab/controlled environments studies evaluating boxwood cultivars and fungicides against the blight disease in the U.S. Specifically, the two multi-year cultivar trials largely confirmed the performance of boxwood species and cultivars in the U.S. studies with a few exceptions. As northern Germany, where both trials were done, was dominated by *Che*, resistant cultivars identified there are also resistant to infection by *Che*. Information on how boxwood cultivars respond to *Che* is important to prepare growers and gardeners in the U.S. and elsewhere for inadvertent introduction of this pathogen. Among the few exceptions were *B. 'Green Velvet'*, *B. microphylla* var. *japonica* 'Compacta' and 'Morris Midget', and *B. microphylla* 'John Baldwin'. Specifically, 'Green Velvet' was rated susceptible in the U.S. studies, but resistant in the Germany trials. Meanwhile, the other three cultivars were rated resistant in the U.S. studies but susceptible in the Germany trials. Nevertheless, these trials, along with all other cultivar evaluation studies and breeding efforts, [enabled growers and gardeners to make informed cultivar selection and build health into new boxwood crops and plantings](#) and [facilitated the further shift to more resistant cultivars in the production inventories](#). Likewise, the other trials expanded the target of fungicide performance from *Cps* to include *Che*, again preparing the environmental horticulture industry and the public in the U.S. and elsewhere for *Che* infection.

Additionally, these trials provided invaluable data for a case study on the field epidemiology – a major knowledge gap in the boxwood blight biology. These data included one or more disease assessments per growing season, depending upon the nature of trials, and on-site weather records – rainfall, relative humidity, temperature, etc. – over a 15-year period from 2006 to 2020. As proposed, we worked with Dr. Brand and delved into these data. Specifically, we focused on *B. sempervirens* 'Suffruticosa', one of the most susceptible cultivars that was used in many trials as a reference cultivar, and the nontreated control in selected fungicide trials in our data analyses. These analyses revealed that the most important contributing weather factors to boxwood blight development in northern Germany was rainfall per rainy day, followed by relative humidity and temperature, documenting for the first time the importance of rainfall and relative humidity in the blight field epidemiology and providing new leads for calibrating the infection risk model. These results were published in [Plant Pathology](#) (2024).

Connecting and Working with Scientists in Southern Europe to Investigate How Widespread and Dominant *Che* Is in Selected Countries

As all known boxwood blight cases in the U.S. to date are caused by *Cps*, the best approach to managing the risk of *Che*, a potentially more damaging pathogen with fewer control options, is to prevent it from entering the U.S. The first and foremost important step to achieve this goal is to know its actual distribution – specifically in Europe. To facilitate the quarantine efforts, we added this distribution investigation to the project.

Although previous literature showed that all *Che* positive countries are in central Europe, we hypothesized that this pathogen is more dominant in southern Europe. This hypothesis was based on *Che*'s tolerance to high temperature. To test this hypothesis, we took the opportunity of attending international conferences and surveyed numerous public gardens and street boxwood plantings in five countries in 2023 and 2024, with the support of our European colleagues.

Specifically, prior to and during attendance at the [12th International Congress of Plant Pathology](#) in Lyon, France (August 2023), we surveyed a number of public gardens and street boxwood plantings in Spain and France, and brought back eleven boxwood samples under an USDA APHIS permit for isolation and identification. All resultant isolates from the samples collected in Madrid and Toledo, Spain belonged to *Che* as identified by PCR with the species-specific primers and DNA sequencing. In contrast, all isolates from Bordeaux, Saint Foy la Grande and Lyon, France, and three isolates provided by Dr. Renaud Ioos of Agence Nationale de Sécurité Sanitaire de l'Alimentation ([ANSES](#)), Laboratoire de la Santé des Végétaux - Unité de Mycologie, Malzéville that had not been identified previously, all belonged to *Cps*. These results were published open access in [New Disease Reports](#), helping to block the movement of *Che* from Spain to the U.S. and other countries that currently are not known to have this pathogen.

Likewise, prior to and during attendance at the [20th International Plant Protection Congress](#) in Athens, Greece (July 2024), we surveyed many public gardens and boxwood plantings in Switzerland, Italy and Greece, and brought back 36 symptomatic samples. Thanks to Dr. Irene Vloutoglou of [Benaki Phytopathological Institute](#) in Athens, Greece, Dr. Vladimiro Guarnaccia of University of Torino, in Torino, Italy, and Dr. Vincent Michel of [Agroscope](#), Federal Department of Economic Affairs – Education and Research EAER in Conthey, Switzerland for helpful discussion. Specific thanks go to Vladi for taking time from his busy schedule and using his personal car to take us to visit three historic sites – [Palace of Venaria](#), [Villa Della Regina](#) and [Palazzina di caccia of Stupinigi](#) with extensive boxwood plantings in Torino and sharing *Calonectria* isolates he recovered from boxwood samples collected in Madeira, Portugal during a family vacation in February 2024. Their discussion and support added productivity to the survey trips. These samples are being processed now to determine whether the boxwood blight pathogen(s) was responsible for the symptoms and how dominant *Che* is in the surveyed gardens and boxwood plantings.

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 (Dr. Hsien 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 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 naturally-infested fields for these trials and 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 2 or 3 weeks – this made the trial manageable, as the research fields were more than 5 hours driving distance from Virginia Beach where the Virginia Tech Team is based. Some of the biocontrol field trial results were presented at the 12th International Congress of Plant Pathology in Lyon, France in August 2023, the 7th Partnerships in Biocontrol, Biostimulants & Microbiome Congress: USA in Raleigh, NC on October 24 to 25, 2023, and the 20th International Plant Protection Congress in Athens, Greece on July 1 to 5, 2024. The antidesiccant field studies provided a basis for further evaluation on the research farm at the Connecticut Agricultural Experiment Station in Windsor, CT.

To make the most out of this collaboration, we also sampled boxwood plants exposed to antidesiccants and evaluated how these products may have affected the phyllosphere microbiome. Among the three film-forming anti-desiccants, Vapor Gard and Wilt-Pruf had greater impact than TransFilm on the microbial communities. Specifically, broader impacts were observed on fungal than bacterial community composition and structure, with most affected fungi being suppressed while bacteria were promoted. These results were published in [BMC Microbiology](#) (2023).

Likewise, we sampled boxwood plants exposed to three fungicide standards along with nontreated controls and examined fungicide impacts on phyllosphere bacterial and fungal communities. The bacteriome analyses revealed that fungicide treatments had strong impacts on epiphytic bacterial community structure and composition. Analysis of compositions of microbiomes with bias correction (ANCOM-BC) and analysis of variance (ANOVA)-like differential expression (ALDEx2) together identified 312 and 1,362 epiphytes with significant change in their abundance due to fungicide treatments in late spring and summer, respectively. Over 50% of these epiphytes were negatively impacted by fungicides. Comparatively, the two chlorothalonil-based contact fungicides had greater impact than the propiconazole-based systemic fungicide. These results were reported in [Microbiology Spectrum](#) (2023). A manuscript reporting the mycobiome study is currently under review by [Scientific Reports](#). These add-on studies provide a foundation for integrating antidesiccants and fungicides into a systems approach to boxwood crop health and production, while utilizing their full potential as horticultural products and looking toward to their future use as blight mitigation tools.

Partnering with AmericanHort/Horticultural Research Institute

AmericanHort is a national trade organization with nearly 16,000 members and its research arm – Horticultural Research Institute (HRI) – has been a key partner in our outreach to the environmental horticulture industry and the public. Together we have developed three major products: an outreach website, the International Boxwood Seminar series, and the BBIG Boxwood Seminar series.

Boxwood Blight Insight Group (BBIG)

The [BBIG website](#) is hosted at the HRI and updated regularly by HRI staff with the contents developed by Project Directors to keep stakeholders and the public informed of the project progress. This website was first released to the public with four tabs – [Research Updates](#), [Knowledge Center](#), [About BBIG](#) and [Event Calendar](#) in November 2020. As the project has

advanced, more and more content has been added to the website. In addition to providing general research and educational updates, new tabs – [Boxwood Gardening](#) and [Box Tree Moth](#) were added in 2021 and 2024, respectively to meet emerging needs for information. To help viewers navigate and locate the information of interest, the website has undergone restructuring twice with many new (sub)sections and more informative section headings under individual tabs. For example, expanded Diagnostic Aids and Management Strategies includes the 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

International Boxwood Seminars

The [International Boxwood Seminars](#) (IBS) Series was launched on March 18, 2021 with Dr. Thomas Brand of Germany as the first speaker. This seminar series aimed to inform the U.S. stakeholders what had already been learned about boxwood blight and its mitigation in Europe and elsewhere where this disease was first discovered about 10 to 15 years before the U.S. This seminar series included eight webinars with invited speakers from seven countries over a 2-year period with the last webinar held on December 21, 2022. These webinars reached all stakeholder groups in every corner of the U.S., with most attendees being ‘multipliers’ – educators and industry leaders. They also attracted attendance from 24 other countries on five continents.

While the BBIG Team developed the program, AmericanHort/HRI took care of all the logistics, including organizing, publicizing, hosting, recording and posting as well as generating the Google analytics, etc. This partnership product continues to have positive impacts through the posting of webinar recordings on the [BBIG](#) website.

BBIG Boxwood Seminars

The [BBIG Boxwood Seminar](#) (BBS) Series was launched on August 23, 2023 following the success of the International Boxwood Seminars. This new seminar series aimed to expedite the technology transfer from the BBIG Team to the field.

- LaMondia, J. 2023. Battling boxwood blight. August 23.
- Gouker, F. 2024. In the lab and the landscape at the National Arboretum: boxwood genomics and AI solutions for blight detection and protection. January 31.
- Baysal-Gurel, F. 2024. Sanitation and nursery practice. May 30.
- Li, X. P. 2024. Tiny but mighty – unlocking the microbiome potential to enhance boxwood resilience to diseases and environmental stresses. July 25.

As with the International Boxwood Seminars, HRI has been doing all the legwork. Starting this year, they also added a survey at the end of each webinar to document the potential

economic impacts. The first survey results indicate that the total economic benefits of Dr. Gouker’s webinar alone was \$2.9 million.

Partnering with the National Diagnostic Network (NPDN) and the National Plant Board (NPB)

The BBIG Team provided two events for plant disease diagnosticians and regulatory staff. BBIG Team members, Margery Daughtrey and Karen Snover-Clift, planned the events with other key team members.

2022 GPDN Seminar Series presentation, *BBIG, a team tackling boxwood blight- sharing progress on diagnostics, extension and outreach.*

The first event (Figure 1) was a 1-hour seminar-style presentation held on March 16, 2022. The presentation was part of the Great Plains Plant Diagnostic Network (GPDN) Seminar Series and was titled, “**BBIG, a team tackling boxwood blight- sharing progress on diagnostics, extension and outreach**”. It featured Doug Luster, the BBIG Co-Director for research, and Margery Daughtrey, the BBIG Co-Director for extension with a brief introduction by Snover-Clift, a fellow NPDN diagnostician. The main goal of this seminar was to provide information about finding extension and outreach products, such as webinar announcements, publication postings, social media posts and newsletter releases, and – the items this audience can’t get enough of – diagnostic guides. The audience also learned this was not a one-way street; the team members were regularly updated with the statistics of diagnostic laboratory submissions. The updates included summary information about the number of boxwood samples submitted to the network’s diagnostic laboratories, the different boxwood species submitted, and the diagnoses, including boxwood blight positives, suspect cases that could not be confirmed, other pathogens found, as well as negative cases.

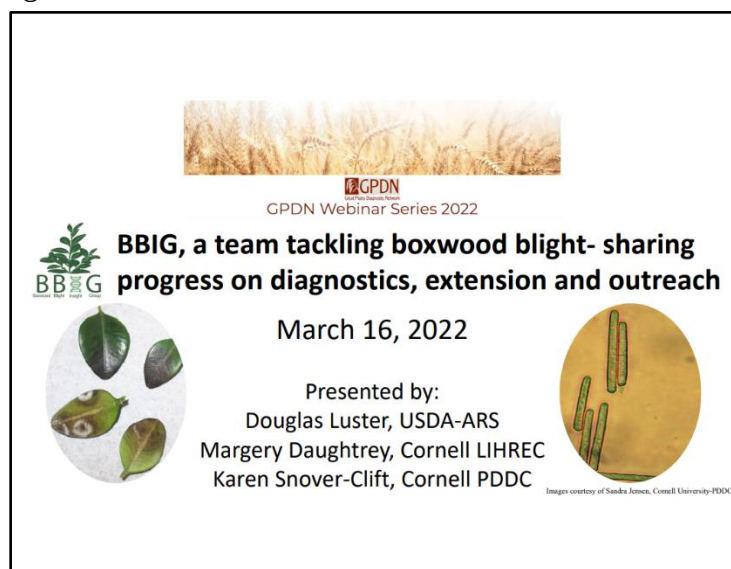


FIGURE 1 THE FIRST PRODUCT OF THE NPDN PARTNERSHIP

2024 NPDN Symposium – Boxwood Blight Insight Group

This symposium (Figure) developed by Margery Daughtrey and Karen Snover-Clift was held on January 17, 2024, and focused on sharing the BBIG team’s findings over the first 3+ years of the project. A key component of the project is timely technology transfer to NPDN diagnosticians and others that protect plants, so those in the field can incorporate the best identification methods and encourage the use of better management strategies.

The symposium included six (6) presenters speaking about their specific area of BBIG project work and their findings. The presentations were 20 mins with 5 minutes for questions and 15 minutes for Q&A at the end. We had **147 people register for the symposium including many members of the National Plant Board (Error! Reference source not found.)**. The NPDN is divided into five regions; this helps the members focus on regional-specific topics, but all

AGENDA

NPDN Symposium: Boxwood Blight Insight Group

January 17, 2024; 1:00pm-4:00pm est



1:00pm **Welcome**-Karen Snover-Clift
Moderator, BBIG NPDN Liaison, Cornell University

1:10pm **Introduction**-Dr. Margery Daughtrey
BBIG Co-Project Leader-Extension, Cornell University

1:20pm **Boxwood blight morphology**-Dr. Xiao Yang
former BBIG Project Contributor, Clemson University

1:55pm **Assay development**-Dr. Doug Luster
BBIG Co-Project Leader-Research, USDA-ARS

2:20pm **Nursery sampling**-Dr. Mana Ohkura
former BBIG Project Contributor, Oregon State University

2:45pm **Break**-10 mins

2:55pm **Boxwood, the plant and blight symptom expression**-Dr. Nina Shishkoff
BBIG Project Contributor, USDA-ARS

3:20pm **Boxwood blight IPM**-Dr. Chuan Hong
BBIG Head Project Leader, Virginia Tech University

3:45pm **Question & Answer Session**

4:00pm **Adjourn**

Note: a more detail agenda is available at: www.NPDN.org

Brought to you by:
The NPDN Professional Development Committee
and
the Boxwood Blight Insight Group (BBIG)



NPDN Symposium: Boxwood Blight Insight Group

January 17, 2024; 1:00pm-4:00pm est



Image credit: Santamaria, Oregon State University Image credit: Daughtrey, Cornell University

BBIG researchers and extension specialists will share findings from their USDA-NIFA-Specialty Crop Research Initiative (SCRI) project, that aims to provide those battling boxwood blight with the knowledge and tools needed to win the war against *Calonectria pseudonaviculata*.

Brought to you by:
The NPDN Professional Development Committee
and
the Boxwood Blight Insight Group (BBIG)



FIGURE 2 THE SECOND MAJOR PRODUCT OF THE NPDN PARTNERSHIP

members are also very active nationally in program area committees and other projects such as professional development workshops. Participants indicated if they were from a region or the USDA and what type of position they served in.

Snover-Clift developed a survey to get feedback after the symposium and received 56 completed surveys back (**Error! Reference source not found.**). Fifty-five (55) attendees said the symposium was helpful and many gave high remarks, one said “perfect” and another said, “It was excellent”. The following tables and graphs contain the results of the feedback.

Regions:	Number participants:	Participant position type:	Number of participants:
GPDN	13	Diagnostician/Lab staff	62
NCPDN	26	Extension Educator	8
NEPDN	59	Dept of Agriculture Staff	60
SPDN	28	Forestry Staff	6
WPDN	16	Student	1
USDA	5	Grower-Nursery staff	3
		USDA	4
		other	3
	147		147

FIGURE 3 DEMOGRAPHICS OF THE 2024 NPDN SYMPOSIUM ATTENDEES

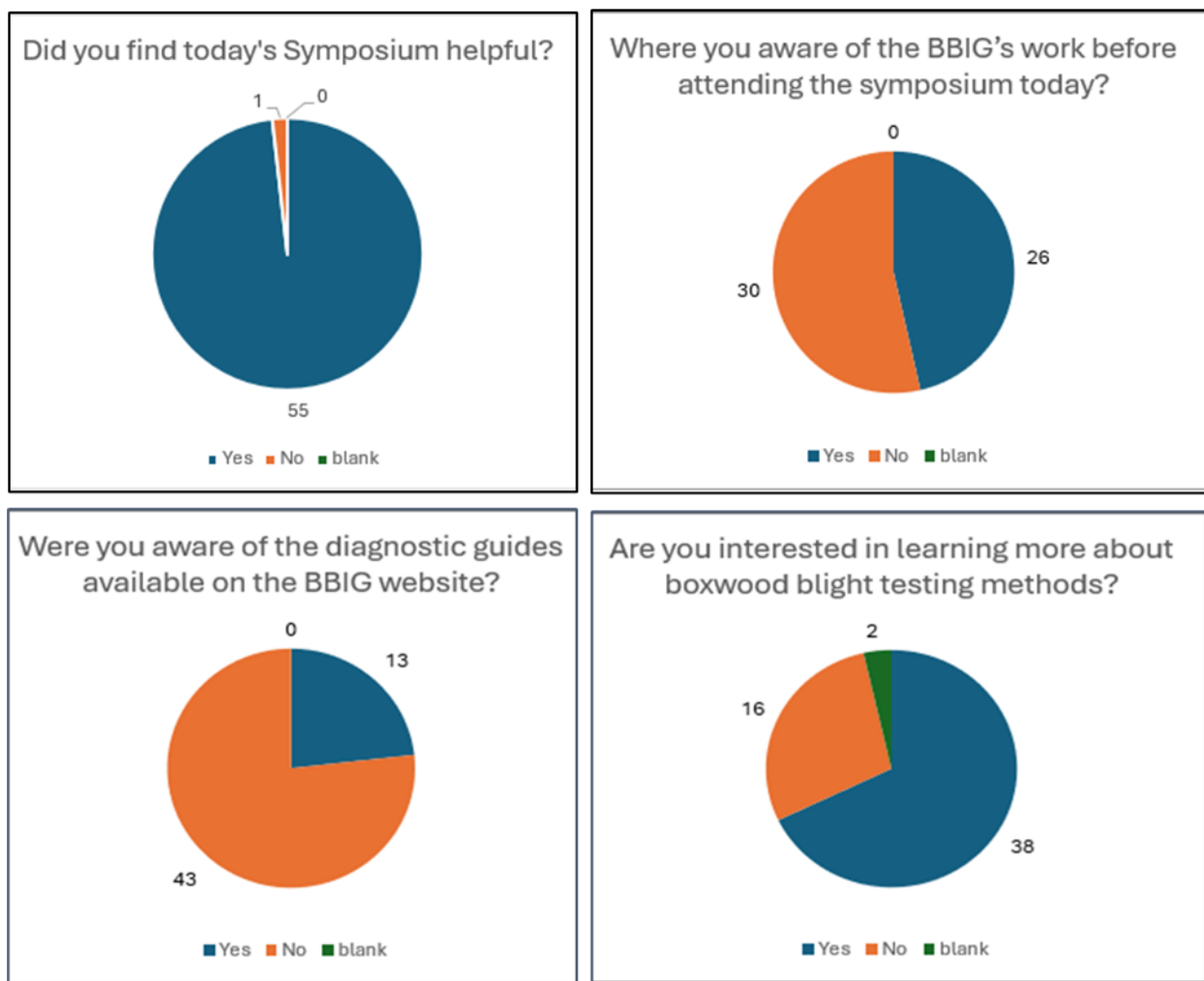


FIGURE 4 SUMMARY OF THE 2024 NPDN SYMPOSIUM SURVEY RESULTS

Other Major Extension Products

[BBIG Monthly Digests](#)

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

1. 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., Snover-Clift, K., and Westrick, N. 2023. [BBIG Monthly – September 2023](#), 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., Snover-Clift, K., and Westrick, N. 2023. [BBIG Monthly – October 2023](#), released October 2.
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., Snover-Clift, K., and Westrick, N. 2023. [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., Snover-Clift, K., and Westrick, N. 2023. [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., Snover-Clift, K., and Westrick, N. 2024. [BBIG Monthly – January 2024](#), released January 2.
 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., Snover-Clift, K., and Westrick, N. 2024. [BBIG Monthly – February 2024](#), 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., Snover-Clift, K., and Westrick, N. 2024. [BBIG Monthly – March 2024](#), 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., Snover-Clift, K., and Westrick, N. 2024. [BBIG Monthly – April 2024](#), released April 1.
 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., Snover-Clift, K., and Westrick, N. 2024. [BBIG Monthly – May 2024](#), 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., Snover-Clift, K., and Westrick, N. 2024. [BBIG Monthly – June 2024](#), released June 3.
 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., Snover-Clift, K., and Westrick, N. 2024. [BBIG Monthly – July 2024](#), released July 1.
 12. Snover-Clift, K., 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., Westrick, N., and Hong, C. X., 2024. [BBIG Monthly – August 2024](#), released August 14.

[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., Snover-Clift, K., and Westrick, N. 2023. [BBIG Newsletter Volume 4, Issue 4](#), released November 22.
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., Snover-Clift, K., and Westrick, N. 2024. [BBIG Newsletter Volume 5, Issue 1](#), released February 23.
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., Snover-Clift, K., and Westrick, N. 2024. [BBIG Newsletter Volume 5, Issue 2](#), released May 31.
 4. Snover-Clift, K., 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., Westrick, N., and Hong C. X. 2024. BBIG Newsletter Volume 5, Issue 3, released August 30.

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

1. Pscheidt, J. 2024. Boxwood (*Buxus* spp.) – boxwood blight. Online at the 2024 Pacific Northwest Plant Disease Management Handbook.
2. Gouker, F. 2024. "Help is on the way — don't give up on box just yet". Flora Culture International Magazine. March 2024.
3. Hong, C. X. 2024. BBIG – the boxwood blight buster. Arborist News (August issue):26-28.

Extension Presentations (Excluding those presented at the quarterly and annual meetings with Advisory Panel)

1. Baysal-Gurel, F. 2023. Boxwood diseases. MTSU-Plant science class. October 4.
2. Baysal-Gurel, F. 2024. Dealing with diseases in the landscape. Warren County Master Gardener's class. McMinnville, TN, April 16.
3. Baysal-Gurel, F. 2024. Boxwood Blight Insight Group (BBIG): sanitation and nursery practices. BBIG Boxwood Seminar series. Virtual. May 30.
4. Baysal-Gurel, F., Ghimire, B., Liyanapathirana, and P., Parajuli, M. 2023. Boxwood blight. The Horticultural Inspection Society - Southern Chapter Meeting, McMinnville, TN, September 20.
5. Daughtrey, M. 2024. Certified Nursery and Landscape Professionals Training, New York State Nursery and Landscape Association, Inc., Riverhead, NY, February 7, 2024.
6. Daughtrey, M. 2024. Diseases of broadleaved trees and shrubs. Branching Out IPM Scouting Skills Workshop, Cornell University, February 8.
7. Daughtrey, M. 2024. Disease update 2024: boxwood, beech and more. Tree Conference, Long Island Arborists Association, February 13.
8. Daughtrey, M. 2024. A mish-mash of maladies. Long Island Horticulture Conference, Upton, NY. March 5.
9. Daughtrey, M. 2024. Stay poised for plant disease management. Pests, plagues and pollinators, New York State Nursery and Landscape Assn. Virtual. March 21.
10. Ghimire, B. 2023. Boxwood blight. Bartlett Tree Experts. Charlotte, NC, September 18.
11. Gouker, F. 2024. In the lab and the landscape at the National Arboretum: boxwood genomics and AI solutions. BBIG Boxwood Seminar series. Virtual. January 31.
12. Hong, C. X. 2023. Boxwood Blight and other signature programs. The 2023 International Plant Propagator Society – Southern Region Annual Conference Attendee Tour of Hampton Roads Agricultural Research and Extension Center, Virginia Beach, VA, October 23.

13. Hong, C. X. 2023. Boxwood Blight and other signature programs. Old Dominion University/Biology Department Faculty Tour of Hampton Roads Agricultural Research and Extension Center, Virginia Beach, VA, November 6.
14. Hong, C. X. and Simmons, G. 2024. Protecting boxwood against blight and box tree moth. U.S. Environmental Protection Agency Integrated Pest Management Webinars. March 5.
15. Hong, C. X. 2024. Boxwood blight and other signature programs. Statewide Annual Master Gardener College. June 7.
16. Li, X. P. 2024. Tiny but mighty: unlocking the microbiome potential to enhance boxwood resilience to diseases and environmental stress. BBIG Boxwood Seminar series. Virtual. July 25.
17. Li, X. P., and Hong, C. X. 2024. Understanding the impacts of fertilization and irrigation on boxwood microbiome and crop health. 2024 Field Day of the Virginia Nursery and Landscape Association. Virginia Beach, VA, July 25.
18. Likins, M. 2024. Field Demonstration to Six Volunteer Clinicians from Chesterfield County Plant Pathology, Entomology and Plant Science Laboratory at a Research Garden in Richmond, VA, April 18.
19. Pscheidt, J. 2024. Smart spraying grapes leads to new boxwood blight management. Farwest Show. Portland, OR, August 21.
20. Pscheidt, J. 2024. Transition from an intelligent sprayer to drenching boxwood plants. Oregon Nursery Research Summit. February 1.
21. Santamaria, L. 2024. Integrating extension and research to serve the nursery industry. Oregon Nursery Research Summit. February 1.
22. Santamaria, L. 2024. Growing Awareness: Enhancing Plant Health in Nurseries Through Education. Farwest Show - Nursery Science Summit. Portland, OR, August 22.
23. Santamaria, L. 2024. Advancing Boxwood Blight Monitoring Techniques. In Spanish. Farwest Show – Seminar Session. Portland, OR, August 22.
24. Shishkoff, N. 2024. Boxwood blight – box cultivars, climate, and disease control. Cultivate'24. Columbus, OH, July 14.
25. Weiland, J.E. 2024. Nursery plant diseases: what have we learned? where do we go from here? Oregon Nursery Research Summit. February 1.
26. Weiland, J.E. 2024. Production decisions influence risks for boxwood blight outbreaks. Farwest Show. Portland, OR, August 21.

BBIG Showcased in Major National and International Events and Media

One BBIG scientist was invited to speak at Cultivate'24 in Columbus, OH on July 14, 2024, while two spoke at the 20th International Plant Protection Congress in Athens, Greece, July 1 to 5, 2024. BBIG scientists were also invited to speak at the 7th Partnership in Biocontrol, Stimulants & Microbiome Congress – USA in Raleigh, NC on October 24 to 25, 2023. Additionally, they presented research results at the annual meeting of the American Phytopathological Society in Memphis, TN on July 26 to 30, 2024.

Consultations

Weiland, J. E. 2024. Consultations with five nurseries to assess boxwood samples for boxwood blight and to diagnose a new outbreak. January to August.

Hong, C. X. 2024. Consultations with numerous nurseries and public gardens on boxwood blight mitigations. September 2023 to August 2024.

Westrick, N. W. 2024 Consultation with nursery in the protocols for establishing in-house boxwood blight resistance evaluations. June.

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 15,054 as of August 30, 2024.

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 in the U.S. and 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 conducted and concluded the International Boxwood Seminars and launched another seminar series – BBIG Boxwood Seminars.

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 an abundant protein target for generation of antibodies to apply in serological detection assays for *Calonectria* in infected boxwood leaves. This protein target is unique to both of the boxwood blight fungi, *Cps* and *Che*. A contract was initiated 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 and results indicate that two of the mouse mAbs are similar in sensitivity to previously tested rabbit antibodies (see data in 2022 Annual Report, Objective 1, Subobjective 1.1). We have contacted two commercial contractors for quotes to manufacture lateral flow devices using either or both sets of mouse and rabbit antibodies. Once manufactured, the sensitivity and specificity of the lateral flow devices will be evaluated 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 to be submitted in 2025. 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 5) and watered more

frequently. Observations and information gained from the survey were used to train Oregon Department of Agriculture (ODA) nursery inspectors how to better scout for disease and to improve the stringency of the state's [Boxwood Blight Cleanliness Program](#). A scouting video developed by Dr. Pscheidt and Dr. Weiland is used as one of the requirements for nurseries wishing to participate in the program: [Scouting for Boxwood Blight](#) (English); [Monitoreando el Tizon de Boxwood](#) (Spanish).

A [real-time PCR method](#) was developed and published in 2023 to detect *Cps* from boxwood samples collected at nurseries. This method was used to confirm results from field samples assessed by moist chambers and to check for false negatives.

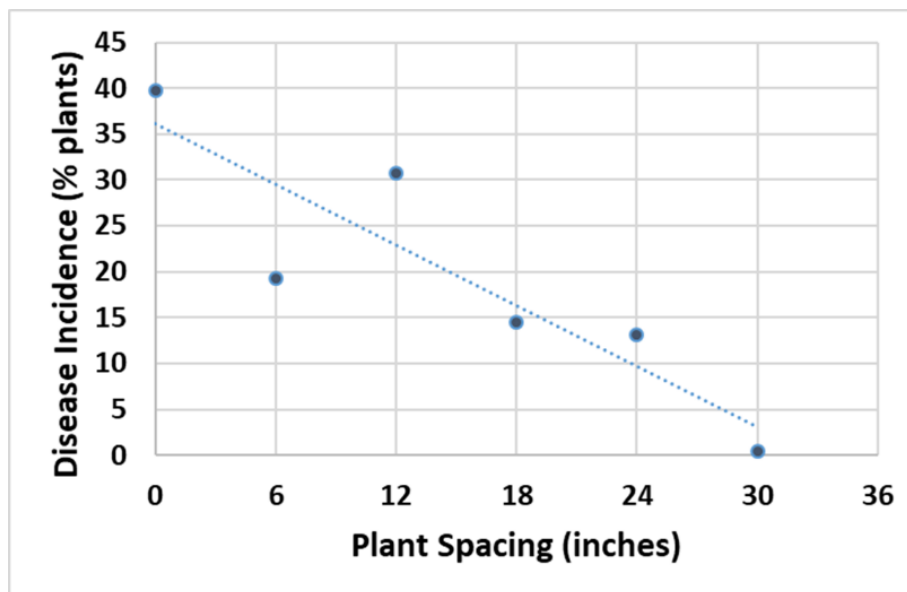


FIGURE 5 BOXWOOD BLIGHT INCIDENCE DECREASED WITH INCREASING PLANT SPACING

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

A completed investigation into the effect of temperature on boxwood blight severity for two different cultivars was published in [Plant Disease](#) (2022). Results showed that boxwood blight is much more severe at cooler temperatures in Oregon and that the resistant cultivar Winter Gem can develop severe disease symptoms at 15°C while remaining almost completely disease free at 25°C (Figure 6). Results were used to update boxwood blight disease forecasting and mapping apps developed by Dr. Coop ([MyPest Page](#)) and Dr. Barker ([Boxwood Blight Risk Mapping Tool for Western OR and WA](#)).

Experiments to evaluate latent infection were completed. In the first experiment, boxwood plants (leaves and stems) were inoculated and disease was allowed to develop. Symptomatic and asymptomatic branches were then sampled for four consecutive months and real-time PCR was used to evaluate the samples for *Cps* presence. The pathogen was only isolated from tissues where lesions were present and never from asymptomatic tissues, suggesting 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. In the second experiment, roots were inoculated with *Cps*, then assessed for

symptoms and assessed for pathogen presence using real-time PCR. No symptoms were observed, but *Cps* was detected on surface-sterilized inoculated roots, suggesting that the pathogen was present inside the roots. This suggests that asymptomatic, latent infection may be an issue for roots. Conclusions will be reported in the manuscript being prepared for Objective 1.2.



FIGURE 6 BOXWOOD BLIGHT SEVERITY INCREASED AS TEMPERATURE DECREASED ON INOCULATED PLANTS OF THE RESISTANT CULTIVAR WINTER GEM

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

After delays in research in previous years, a skilled post-doctoral researcher, Dr. John Dobbs, joined the research team in January 2024. Experiments to study pathogen colonization and phenotypic diversity have been initiated.

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

Research continued 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 *B. sempervirens* ‘Suffruticosa’ (Figure 7) and ‘Justin Brouwers’, *B. microphylla* ‘Little Missy’, and *B. ×* ‘Green Mountain’. The initial analyses of these research data showed that *Che* appeared more aggressive than *Cps*, causing more infections on tested boxwood cultivars.

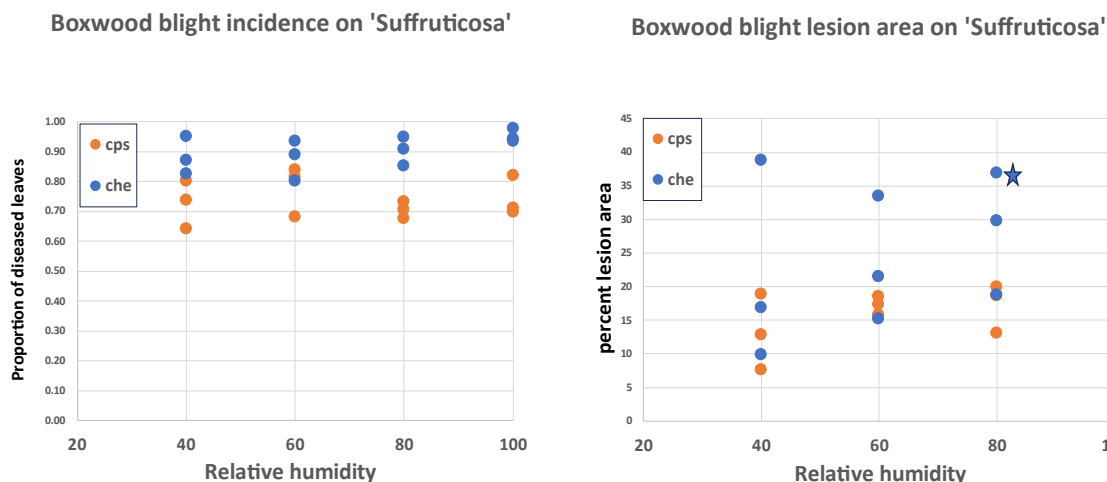


FIGURE 7 DISEASE INCIDENCE AND DISEASE SEVERITY FOR *BUXUS SEMPERVIRENS* ‘SUFFRUTICOSA’ INOCULATED WITH EITHER *CALONECTRIA PSEUDONAVICULATA* (CPS) OR *C. HENRICOTIAE* (CHE) AT DIFFERENT RELATIVE HUMIDITIES. STAR SYMBOLS INDICATE SPORULATION WAS OBSERVED

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 increased with increasing mean rainfall per rainy day, temperature, and relative humidity. These discoveries are published in [Plant Pathology](#) (2024) – providing new leads to calibrate the boxwood blight infection risk model which currently focuses on the temperature during wet hours to predict boxwood blight infection. Meanwhile, we helped the German collaborators to analyze and publish their 15-year field research evaluating boxwood cultivar performance in [Plant Disease](#) (2023) and fungicide efficacy in [Journal of Plant Diseases and Protection](#) (2023), making this practical information available to the US stakeholders and rest of the World. As the research farm where these field trials were conducted has been dominated by *Che*, these three research publications are important to prepare the U.S. stakeholders for inadvertent introduction of this pathogen that has the potential to be more adapted to the weather conditions in this country with few disease management options.

In the second case study, we analyzed 4-year field data of weekly monitoring boxwood blight dynamics with detector plants in Lamsburg, 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. This case study demonstrated that blight pressure was higher in late summer or early fall than other seasons (Figure 8).

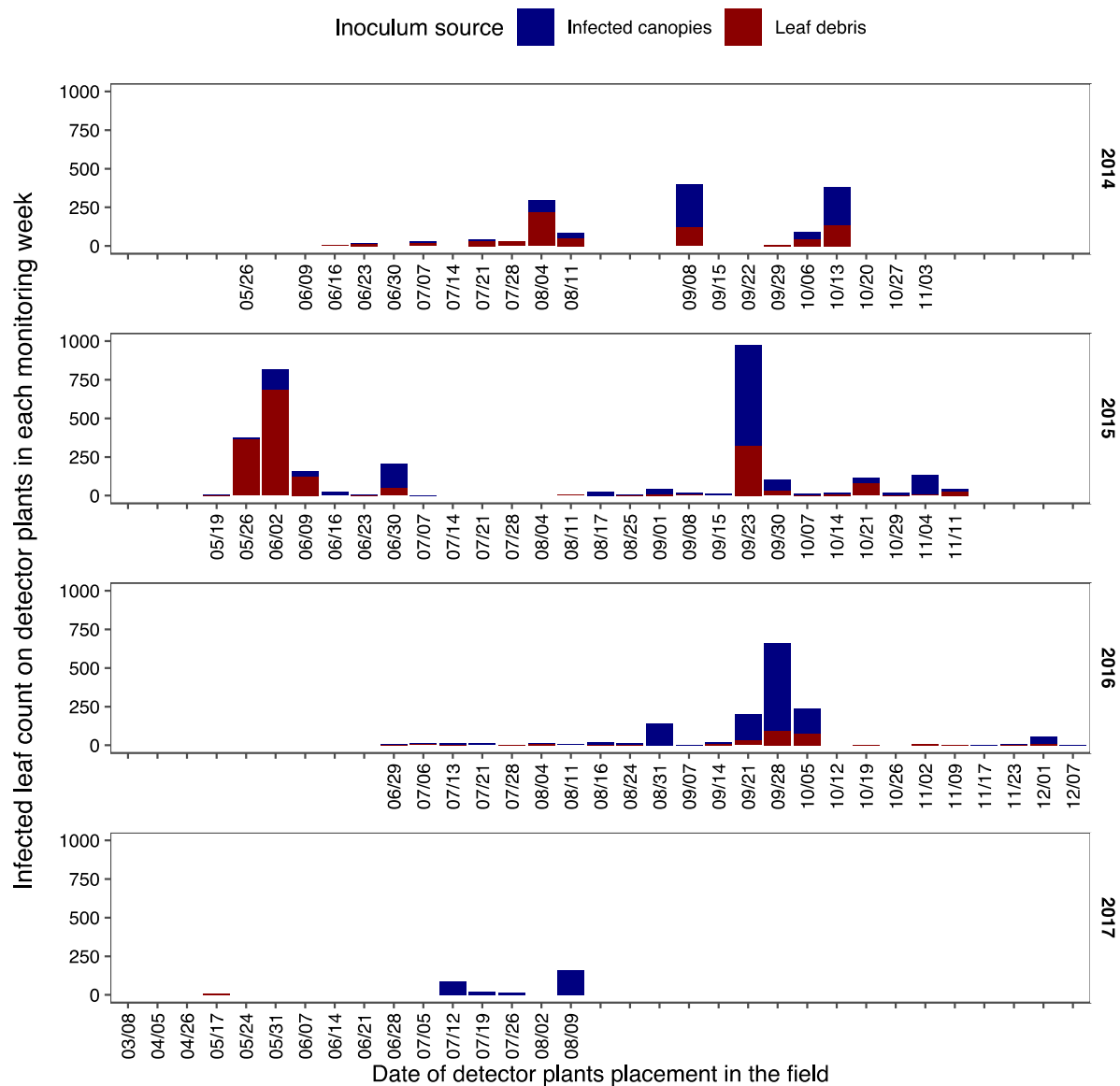


FIGURE 8 INFECTED LEAF COUNT RECORDED ON TWO-YEAR-OLD DETECTOR PLANTS *BUXUS SEMPERVIRENS* ‘SUFFRUTICOSA’ (2014) AND ‘JUSTIN BROUWERS’ (2015–2017) DURING EACH MONITORING WEEK

Relating these disease observations to the weather data recorded on-site through a weather station uncovered that rainfall, high relative humidity outside rainy periods, and optimal temperatures during periods of prolonged leaf wetness had a significant positive effect on the blight development. Disease epidemics were especially pronounced when optimum temperatures (18 to 22°C) coincided with prolonged wet periods. 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 9).

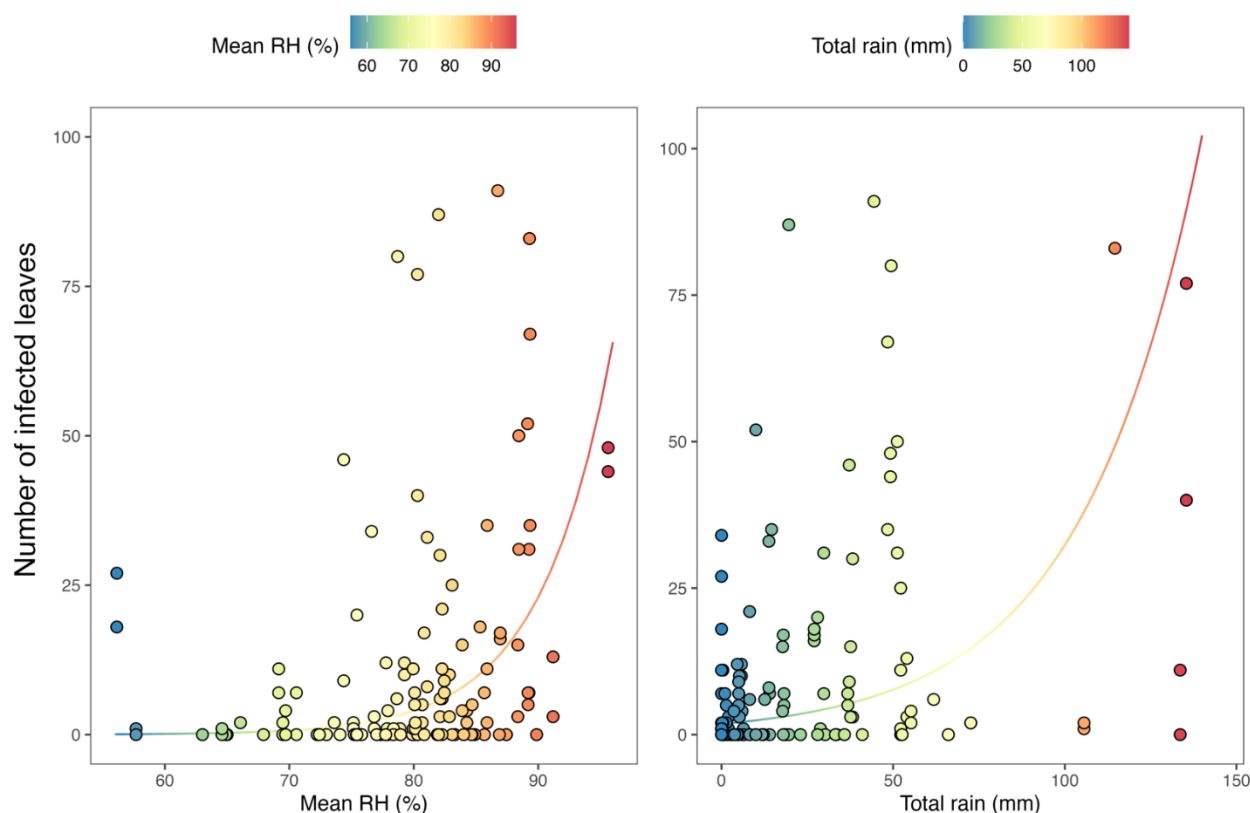


FIGURE 9 GENERALIZED LINEAR MIXED MODEL FIT SHOWING THE EFFECT OF TOTAL RAINFALL AND MEAN RELATIVE HUMIDITY OUTSIDE OF RAINY PERIODS ON THE NUMBER OF INFECTED LEAVES OF DETECTOR BOXWOOD PLANTS IN LAMSBURG, VA (2014) AND LOWGAP (2015-2017)

Additionally, the weekly blight monitoring data were used to validate the infection risk model on two accounts. First, three sets of leaf wetness data recorded directly with sensor or estimated by FLLW or FoxLW heuristic algorithms were evaluated. These analyses indicated that infection risk forecasts using leaf wetness estimated with a leaf wetness sensor were more closely aligned with blight observations from the field. Second, three degree-hour thresholds (56, 150 and 250) were compared for their impact on the disease forecast accuracy. These analyses indicated that the 250-degree hours is the best degree hours threshold for boxwood blight infection risk prediction. These analyses underline the importance to further calibrate and improve the infection risk model.

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

Previous study documented the key components, practices, and procedures in boxwood production that could influence the spread of boxwood blight in nurseries. The sampling plan has been finalized according to growers' boxwood production flow diagram to be able to specify the critical control points in the production process. The sampling has been conducted in representative Georgia and Tennessee nurseries. *Volutella* stem blight and *Phytophthora* spp. were prevalent in studied Georgia and Tennessee nurseries. The team also evaluated UV-C LED irradiation (263-287 nm) as a disinfection method by examining its effectiveness in inactivating conidia and determining the UV-C sensitivity. The findings from this research published in [Plant](#)

[Disease](#) (2024) indicate that UV-C irradiation could be a viable option for disinfecting tools, equipment, and possibly propagation cuttings in nurseries.

1.8 Surveying public and private gardens and street boxwood plantings for Che in southern European countries (Kong/Hong)

As *Che* has not been seen in the U.S., the most effective approach to its risk mitigation is to prevent this pathogen from entering this country. To support this approach, we used the opportunity of attending international congresses in Europe to survey public and private gardens and street boxwood plantings. Specifically, we surveyed Spain and France in 2023 in collaboration with Spanish and French authorities of plant protection and colleagues of plant pathology and discovered that *Che* was the only pathogen isolated from all Spanish boxwood samples as described in [New Disease Reports](#) (2024). In 2024, we continued the survey in Switzerland, Italy and Greece, in collaboration with the local plant pathologists and/or Plant Protection authority. We are currently analyzing 36 samples collected from these countries. This add-on investigation is important to prepare Plant Protection Quarantine authority and other stakeholders in the U.S. and elsewhere this pathogen is not known to be present and invest the limited resources where most needed.

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

2.1 Evaluating antidesiccants for blight mitigation (Hong)

We have completed this proposed study with the second phase of antidesiccant evaluation at two sites in western North Carolina. Two cultivars - Vardar Valley and American were included in these field trials 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 was less reduction than seen from treatment with 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 added to these trials indicated that Vapor Gard and Wilt-Pruf had more impact 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. These discoveries published in [BMC Microbiology](#) (2023).

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 completed in 2022 with the last treatment in September 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 (Figure 10). Their control efficacies were only slightly lower than those by the fungicide standard – Concert II.

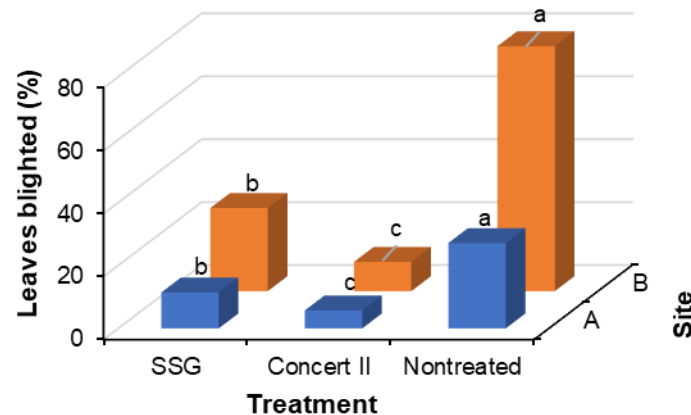


FIGURE 10 BOXWOOD BLIGHT DEVELOPMENT ON VARDAR VALLEY AND AMERICAN BOXWOOD PLANTS TREATED MONTHLY WITH THE BEST PERFORMING BIOLOGICAL CONTROL AGENT – *BURKHOLDERIA* SP. SSG OR CONCERT II IN COMPARISON TO NONTREATED CONTROL PLANTS AT SITES A AND B

In addition to BCAs evaluated in the field trials, 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 11).

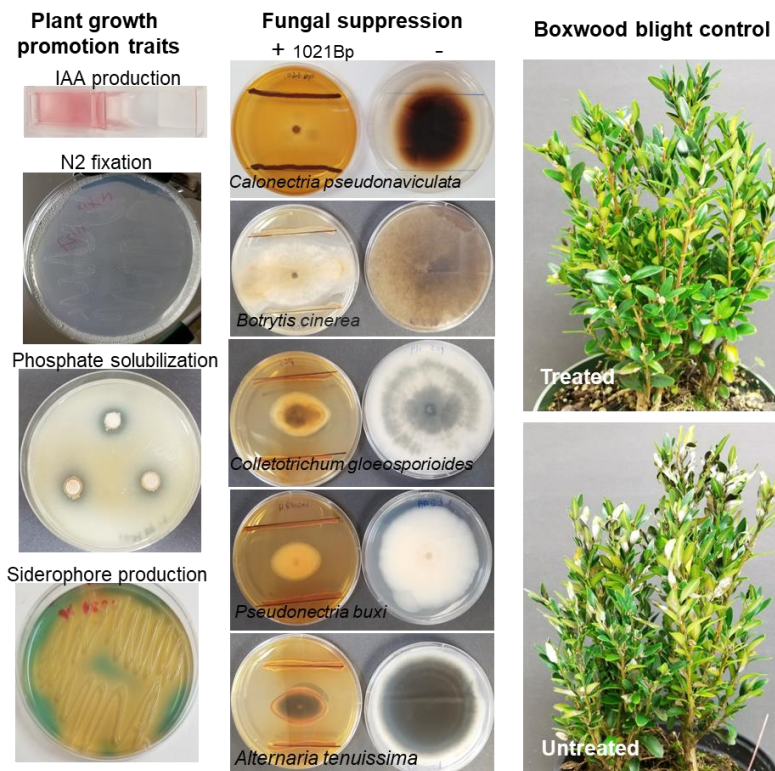


FIGURE 11 *PSEUDOMONAS EUCALYPTICOLA* 1021BP FROM BOXWOOD SHOWS TRAITS OF PLANT GROWTH PROMOTION, FUNGAL SUPPRESSION AND BOXWOOD BLIGHT CONTROL

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

Buxus sempervirens ‘Green Mountain’ (moderately susceptible) and ‘Independence’ (highly resistant) have been propagated and grown into small plants suitable for systemic acquired resistance and selective fertilization treatments to be made in early fall 2024.

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

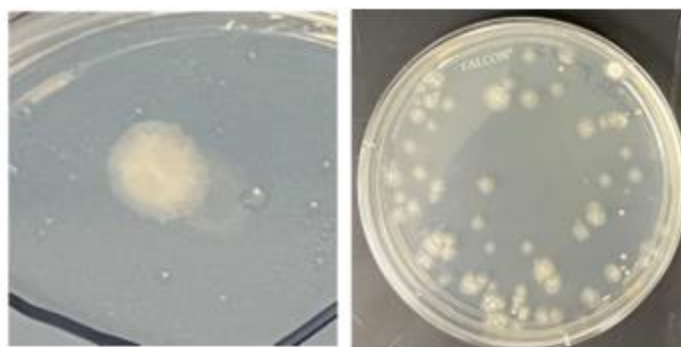
Two independent experiments of 10-week treatments were performed to evaluate the effects of Si treatment on boxwood blight incidence. Two-year old plants of two cultivars, *B. microphylla* ‘Little Missy’ and *B. sempervirens* ‘Suffruticosa’ were treated and evaluated on the effect of continuous Si soil treatment. Treatments were applied during active root growth, at concentrations of 0, 2.5, 5, and 10 mg/L 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 for both cultivars. These experiments are demonstrating a significant effect of Si reducing incidence of boxwood blight on more susceptible cultivars in addition to enhancing plant growth. Additional observations show increased growth during the second year after Si application compared to nontreated control plants.

A third phase of UV-C light treatment has been completed to evaluate its potential for boxwood blight mitigation. We have assessed use of UV-C light as a non-fungicidal option to suppress the boxwood blight pathogen by testing timing, dosage, cultivars, and *Cps* strains using detached leaf assays. A UV-C light meter with a data logger allowed us to capture 20 different measurements over the course of various selectable time intervals. We set our equipment to record the intensity every 60 seconds, which was repeated until it had acquired 20 different data points. The intensity of 3.77 mW/cm² at 30 cm was used to calculate the different doses, the formula for UV-C dosage is: Intensity (mW/cm²) x Time (seconds) = Dose (J/m²). The dosages selected were 150, 350, 500, 1000, and 2000 J/m², respectively. Environmental conditions in the growth chamber setup were set to keep the environment at 23.5°C, 70% humidity, and a 12-hour day-night schedule with lights scheduled on at 5:00 AM and off at 5:00 pm. The summary of experiments given in **Table 1**.

Table 1 UV-C Treatment of fungal cultures

Experiment #	UV-C doses (J/m ²)	Purpose of the experiment
1	100, 350, 500, 750, 1000	Determine optimal dose for fungal growth suppression, and the effect of dark treatment (4h and 24h) and continuous light.
2	150.8, 942.5, 1394, 2073	Determine optimal dose for fungal growth suppression, and the effect of dark treatment (4h and 24h) and continuous light.
3	148, 444, 925, 1369, 2035, 11100 (5min)	Mock-experiment without inoculation of fungus to see effects of irradiation on leaves

The first set of experiments were to assess suppression of fungal growth of pure cultures using spread inoculation on PDA plates. For spreading, 25µl and 50µl conidia (10⁴ spores/ml) were spread on PDA, while 5, 10 and 25 µl of conidia were spotted on plates. After 4 days, colonies and mycelial growth was observed in both type of inoculation (Figure 12).

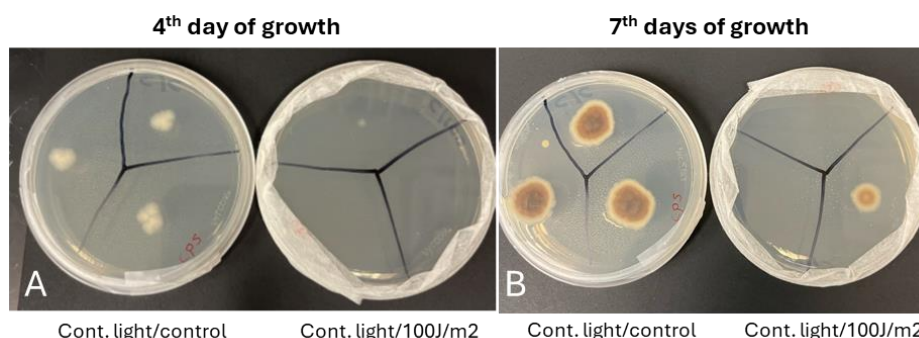


A

B

FIGURE 12 25µL OF CONIDIA OF *CPS* STRAIN INOCULATED AS SPOT (A) AND SPREAD (B) YIELDED MYCELIA AFTER 4 DAYS ON POTATO DEXTROSE AGAR.

Starting from 100 J/m², all doses suppressed fungal growth on potato dextrose agar (PDA) on 4th day of growth in first and second experiments (Figure 13). Inhibition of mycelial growth was similar among continuous light and dark applications, which were more observable on the 7th day after treatment, especially compared with nontreated controls (Figure 14).



Cont. light/control

Cont. light/100J/m2

Cont. light/control

Cont. light/100J/m2

FIGURE 13 GROWTH OF CPS IN CONTROL AND UV-C TREATED PLATES AFTER 4 (A) AND 7 DAYS (B).

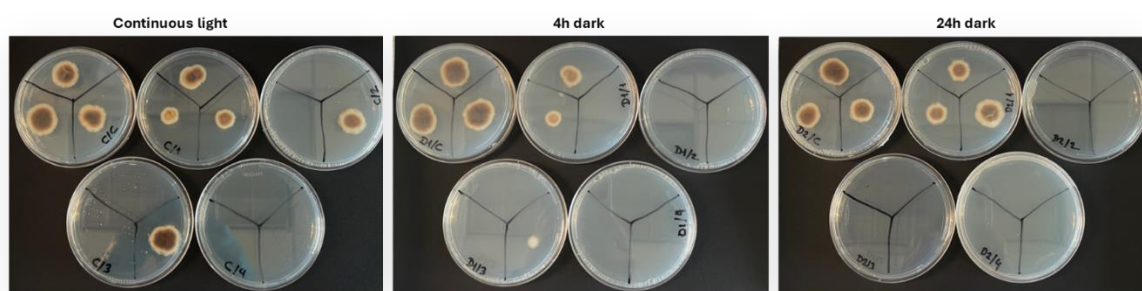


FIGURE 14 GROWTH OF CPS AFTER SEVEN DAYS UNDER CONTINUOUS LIGHT, 4 HOURS OF DARK INCUBATION, AND 24 HOURS OF DARK INCUBATION AFTER UV-C TREATMENT ALONG WITH CONTROL PLATES THAT RECEIVED NO UV-C TREATMENT

UV-C treatment beyond the maximum dosage of 1,000 J/m² was also tested to see if there was greater suppression of fungal growth. Significant differences were observed between nontreated controls and different dosages of UV-C with greater suppression of fungal growth

seen with dosages beyond 942 J/m² as well as significant suppression of growth of cultures incubated for 4 or 24 hours in the dark after treatment compared to cultures that received continuous light (Figure 15).

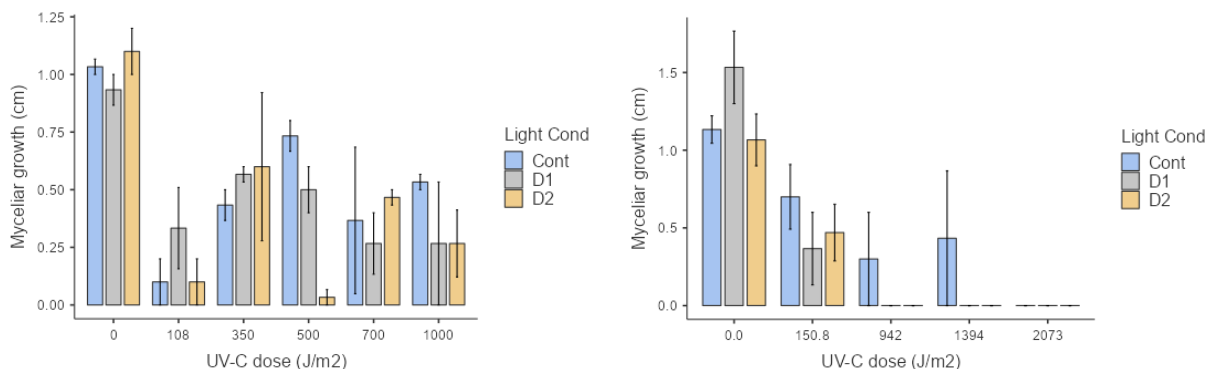


FIGURE 15 SUPPRESSION OF FUNGAL (*Cps*) GROWTH BY UV-C TREATMENTS. A 2-way ANOVA SHOWED THAT EACH DOSE OF UV-C SIGNIFICANTLY INHIBITED GROWTH COMPARED TO CONTROL ($P < 0.001$) ($N = 3$).

Using the same UV-C dosages from the cultured plates of *Cps*, we tested the phototoxic effects of UV-C light on the host plant using detached leaf assays and have not found any noticeable differences between UV-C treatment intensity with dosages ranging from 150 J/m² to 2,000 J/m².

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 U.S. 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 on different cultivars were collected. In addition, photographs of symptoms were taken for cultivars ‘Justin Brouwers’, ‘Golden Dream’, ‘Green Velvet’, ‘Richard’, ‘John Baldwin’, and ‘Dee Runk’ (Figure 16) at 24, 48, 72, 96 and 120 hr after inoculation.

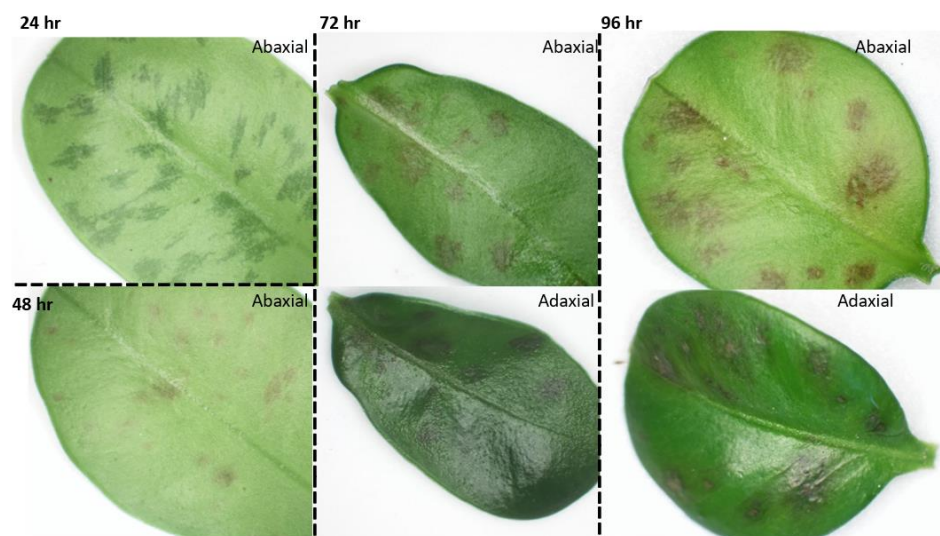


Figure 16

FIGURE 16 *BUXUS SEMPERVIRENS* ‘DEE RUNK’ INOCULATED WITH *CALONECTRIA HENRICOTIAE* AND PHOTOGRAPHED 24, 48, 72 AND 96 HR LATER

2.7 Assessing the potential of selected antidesiccants and nanocopper to provide more environmentally sustainable control of boxwood blight (Westrick)

Antidesiccants are a type of protective coating which can be applied to evergreen plants with the primary goal of protecting tissue from drought and winter damage while providing a potentially valuable control mechanism for boxwood blight. Similarly, modern preparations of nanocopper have been developed at the Connecticut Agricultural Experiment Station which demonstrate high antifungal efficacy with a much lower impact on field soil than traditional copper fungicides. Field trials of both the moderately susceptible variety Green Velvet (GV) and the highly resistant variety Winter Gem (WG)

have been established in Windsor, CT and have been inoculated with *Cps* conidia. Treatments of the antidesiccants (Wilt-Pruf 15% Transfilm 7.5%), nanocopper, and propiconazole will be applied throughout Summer 2024 and relative levels of disease will be recorded. Additionally, 125 GV and WG plants have been established in 1-gallon pots for a replicate of this trial in the greenhouse Fall 2024. The greenhouse trial will be repeated Spring 2025.

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 NCBB1 were used to isolate RNA and submitted to GeneWiz for Illumina 2×150 bp RNA-sequencing. Tissue was harvested at 8 different timepoints – 4, 6, 8, 12, 24, 48, 72 and 96 hours after inoculation of either *Cps* or *Che* conidia.

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, and 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 of 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.

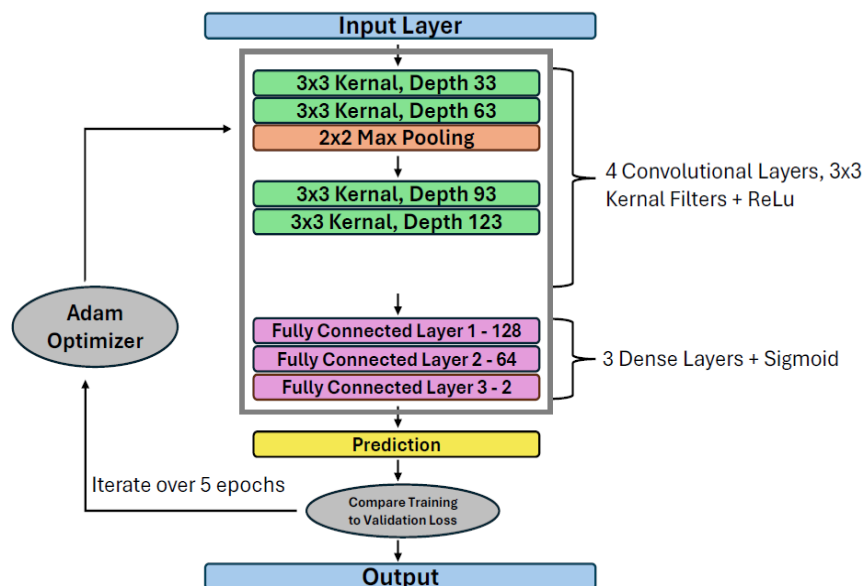


FIGURE 17 BLOCK DIAGRAM SHOWING THE USED DATASETS AND DIFFERENT APPLIED METHODS FOR CREATING THE CUSTOMIZED CONVOLUTIONAL NEURAL NETWORK (CNN)-BB TRAINING FRAMEWORK.

An automated phenotyping has been developed for detection 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. We also included a training set of naturally infected plants in the landscape and from commercial nurseries. 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. We trained a Convolutional Neural Network (CNN) model to detect boxwood blight on stem and leaf images from artificial inoculations in controlled laboratory settings and from naturally infected plants in the nursery, field, and garden landscapes and achieved 98% accuracy on a separate set of test images. The custom model named, the CNN-bb (Figure 17), demonstrates superior performance in detecting boxwood blight lesions on leaf images, in controlled laboratory settings and natural environments relative to manual/visual detection. The utilization of Grad-CAM heat maps provides valuable insights into the regions of interest within images, enhancing the interpretability of the CNN model's decisions and facilitating informed decision-making in disease management strategies (Figure 18). By incorporating data augmentation techniques such as flipping, rotations, and translations, the study ensures the robustness of the machine learning models, improving generalization across diverse environmental conditions where boxwood blight can be found. A manuscript describing this research has been submitted to a journal and is currently under peer review.

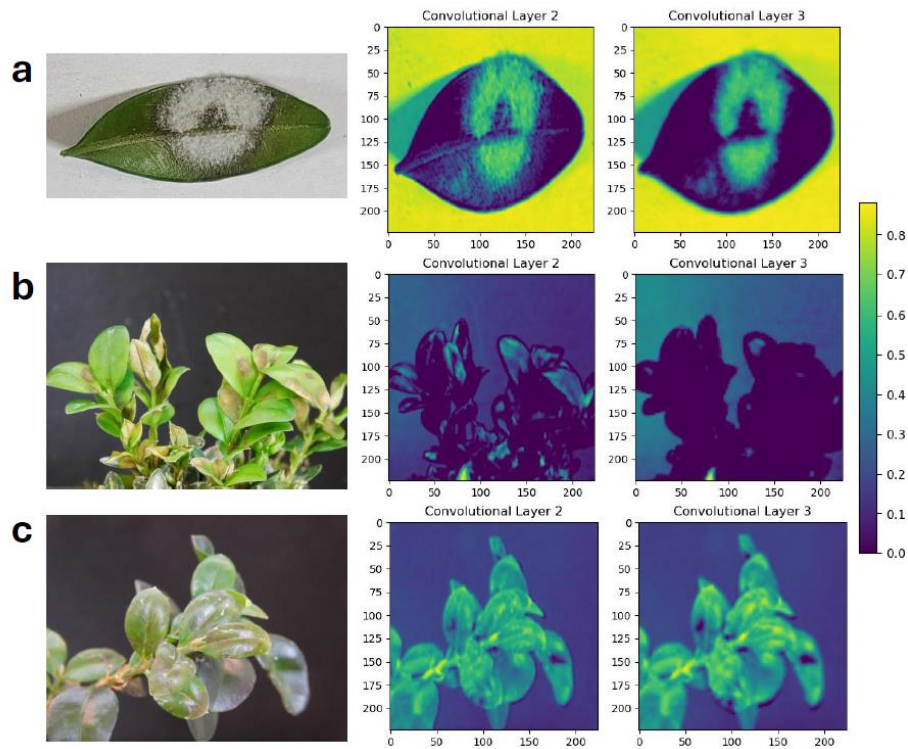


FIGURE 18 COMPARISON OF TEST SET OUTPUT FOR BLIGHT-INFECTED BOXWOOD PLANTS USING THE TRAINED CONVOLUTIONAL NEURAL NETWORKS (CNN). THE ORIGINAL IMAGE IS SHOWN ON THE FAR LEFT FOR A) AN INOCULATED SINGLE LEAF AND B-C) A WHOLE PLANT WITH LESIONS.

Development of efficient procedures in boxwood for protoplast isolation, transfection and CRISPR-Cas9 genome editing was tested. CRISPR-Cas technologies that allow precise modifications in plant genomes and genome editing are excellent tools for crop improvement and basic research. These technologies depend on the delivery of editing components into plant cells and the regeneration of fully edited plants. In vegetatively propagated plants, such as boxwood, protoplast culture provides one of the best avenues for producing non-chimeric and transgene-free genome-edited plants. Boxwood genotypes with various leaf morphology at different growth stages were assessed to identify cellular competency for protoplast isolation. Tested genotypes within the *Buxus* genus were sampled from cultivars and hybrids available at the U.S. National Arboretum Boxwood Collection. Preliminary results revealed the effects of both genotypic differences and sampling time (Figure 19) for efficient protoplast isolation. While in most experiments, cells were not obtained after enzymatic incubation with various durations, in two genotypes (*Buxus riparia* ‘Nana’ and *B. microphylla* ‘Wintergreen’), viable protoplasts were recovered.



FIGURE 19 LEAF MORPHOLOGY WITHIN THE GENUS BUXUS. LEAVES ARE CHARACTERIZED BY THEIR SIMPLE, OVATE SHAPE, GLOSSY TEXTURE, AND OPPOSITE ARRANGEMENT. TOP LEAVES ARE MATURE, BOTTOM LEAVES ARE EMERGING FRESH LEAVES. (RIGHT) LEAVES SAMPLED FROM *B. riparia* “NANA”

Boxwood mesophyll protoplast isolation was carried out based on a modification of a protocol used in Yoo et al 2007. In our study shorter periods than 16-h of enzymatic duration did not yield enough protoplasts (10^7 - 10^8 per gram of fresh weight (g FW)). Enzymatic incubation time, addition of pectolyase, and sampling strategy were critical parameters. The maximum yield (2×10^7 /g FW) of viable (>90%) protoplasts was reached when young, newly emerged green leaves of ‘Wintergreen’ were digested in enzymatic solution including 0.02g Pectolyase Y-23 (Phytotech) in addition to cellulase and macerozyme. At the end of the digestion reaction, cells were filtered and were transfected using PEG- CaCl_2 using a fmv-EYFP vector on which YFP is driven by figwort mosaic virus promoter. Plates were incubated in the dark for 12 h to check GFP/YFP signal of protoplasts. From this we observed YFP signal in > 40% of cells (Figure 20). This is the first report of an efficient protocol for isolating protoplasts from boxwood plants. Additional research is underway to streamline the multiplex genome editing in boxwood using additional vector constructs and optimization of media compositions for cell regeneration.

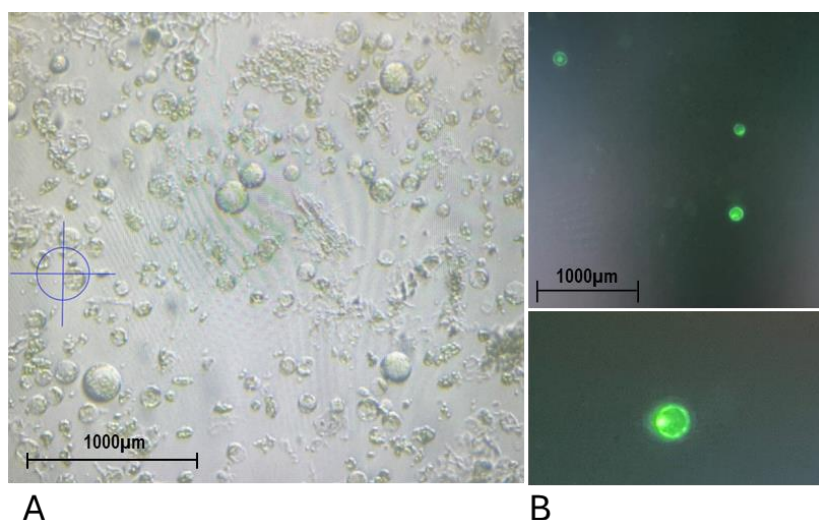


FIGURE 20 A. BRIGHTFIELD IMAGE OF WINTERGREEN PROTOPLASTS AFTER WASH WITH W5 BUFFER; B. CELLS EXPRESSING YFP IN TRANSFECTED PROTOPLASTS VISUALIZED UNDER GREEN FILTER AFTER 12H INCUBATION WITH FMV-EYFP (ZEISS AXIO ZOOM V16).

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

The boxwood microbiome studies continued to make several important discoveries in the fourth year. First, ten core microbiomes (three bacterial and seven fungal genera) were identified from shoot internal tissue of two boxwood species/four cultivars at two nurseries in Oregon and Virginia. These core microbiomes may have conservative functions in support of boxwood's low-maintenance nature. Second, several microbial indicators were identified to be associated with boxwood blight resistance. Particularly, a greater number of indicators was observed in the blight-susceptible than tolerant cultivars, suggesting strict regulation of the microbial colonization may be operating inside a host with higher disease resistance. These results are foundational to developing microbiome-based breeding programs to enhance boxwood resilience to diseases. Third, fungicides Daconil and Concert II with chlorothalonil as primary active ingredient (a.i.) had greater suppressive effects on surface-colonizing fungal communities than Banner Maxx with propiconazole as the sole a.i. Fourth, a range of fungal genera on the shoot surface became less sensitive to Daconil late in the season, providing the first (microbiome) evidence demonstrating development of fungicide resistance to a multisite contact fungicide. Specifically, the genus *Colletotrichum*, with species that are responsible for the emerging boxwood dieback disease, was even promoted two weeks later. These results challenge a long-time assumption that multi-site fungicides like chlorothalonil are at low risk for developing resistance and help understand the rising health issues associated with boxwood due to increasing and repeated use of fungicides.

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 best management 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. A journal article has just been submitted summarizing the added costs of BMPs designed to mitigate boxwood blight occurrence.

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 were total labor and materials (plant protection products) costs, as well as the level of plant shrink (dumps, scrap, culls).

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 takes place in the final year. The project 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

Since the second annual meeting in February 2023, we have 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’ ([Saunders Genetics](#)), ‘Little Missy’ and ‘Littler Mister’ ([Star Roses & Plants](#)), BetterBuxus® ‘Renaissance’, ‘Babylon Beauty’, ‘Heritage’, and ‘Skylight’ ([Herplant](#)) – 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 a fungicide drench study, the Weiland lab is participating in an IR-4 Project trial to evaluate new fungicides for efficacy against boxwood blight. In addition, they are collaborating with Dr. Shishkoff in evaluating East Coast and Oregon *Cps* isolates for sensitivity to currently registered fungicides to see if fungicide resistance may have developed.

5.2 Delivering to end users

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 third annual meeting with AP and stakeholders was held online via Zoom on May 7, 2024. Individual teams, including major partners like AmericanHort (Figure 21), reported their progress over the past year and their plans for the remainder of the project period.



FIGURE 21 NICOLAS LEAS OF AMERICANHORT REPORTING THE OUTREACH WEBSITE PERFORMANCE DURING THE THIRD ANNUAL MEETING ONLINE VIA ZOOM

BBIG outreach website

The BBIG website was first released to the public in November 2020 and it has since undergone several major updates and restructuring. Two new tabs have been added with [Boxwood Gardening](#) in 2022 and [Box Tree Moth](#) in 2024 (Figure 22) to meet the emerging needs of the industry and the public for information on the respective topics. We are currently developing new content in Spanish for Spanish speakers, a critical component of the workforce in the environmental horticulture industry. We also have added many sections and subsections to individual tabs – especially the Knowledge Center – to accommodate increasing contents and postings while helping viewers navigate and locate the information of interest as quickly as possible.

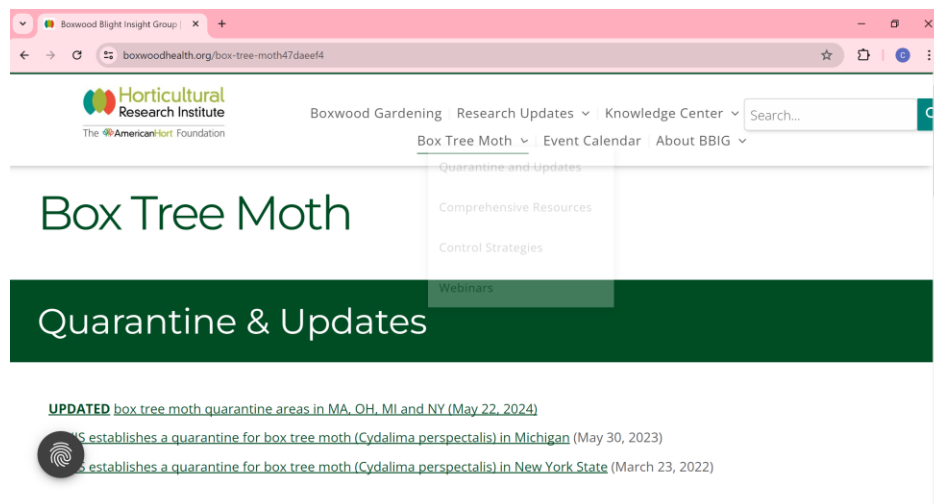


FIGURE 22 BOX TREE MOTH - A NEW TAB OF THE BBIG OUTREACH WEBSITE TO MEET STAKEHOLDERS' EMERGING NEEDS FOR INFORMATION ON THIS NEW INVASIVE PEST

International Boxwood Seminar Series

We analyzed the attendance metrics of the International Boxwood Seminars. Overall, this Seminar series reached 46 states in the U.S., plus the District of Columbia (figure 23) 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 posted 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.

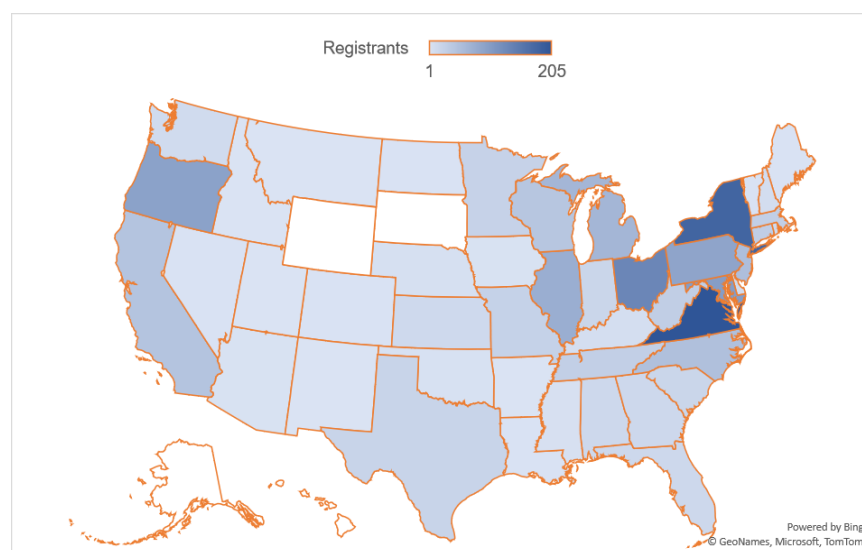


FIGURE 23 NATIONAL REACH TO FORTY-SIX STATES PLUS WASHINGTON DC ACCOMPLISHED BY THE INTERNATIONAL BOXWOOD SEMINARS

BBIG Boxwood Seminar Series

Building upon the success of the International Boxwood Seminars Series, we launched a new Seminar Series – BBIG Boxwood Seminars on August 23, 2023 to expedite the technology transfer from this project to the end users. Dr. James LaMondia was the inaugural speaker, followed by Dr. Fred Gouker (January 31, 2024), Dr. Fulya Baysal-Gurel (May 30, 2024) and Dr. Xiaoping Li (July 25, 2024).

One major initiative with this new Seminar Series was a survey at the end of each webinar to better document the impact of the Seminars. This was instituted in 2024, with the webinar presented by Dr. Fred Gouker. According to the feedback received from attendees, the financial benefits of that webinar alone were estimated to be more than \$2.9 million.

BBIG Newsletter

As in year three of the project, we published four BBIG Newsletters in November 2023, February, May and August 2024, respectively. Each issue began with Featured News, followed

by Featured Research, Extension Highlights, and Project Meeting Brief, Recent Publications, etc. as exemplified by Volume 5, Issue 2 (Figure 24).

Starting in Volume 5, Issue 1 (2024), the Featured News section featured and will continue to feature one or more major products of the BBIG project. At the same time, a new section – Advisory Panel Spotlight – was added to the Newsletter to bring to light the Panel members who have been an integral part of the BBIG team and have worked hard behind the scenes.



FIGURE 24 A SNAPSHOT OF THE FRONT PAGE OF BBIG NEWSLETTER VOLUME 5 ISSUE 2 (MAY 2024)

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 25).



FIGURE 25 EXAMPLE OF BBIG MONTHLY - JULY 2024

Tweets at X.com/boxwoodhealth

Dozens of tweets via the social media “X” platform were used to instantaneously share the latest research, innovations and best management practices as well as boxwood related news with per tweet views as high as 1,239.

Conventional means of technology transfer

- Organized and hosted one virtual National Plant Diagnostic Network Symposium – Boxwood Blight Insight Group via Zoom.us on January 17, 2024.
- Reached out to the science and educational communities by publishing six 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 three newsletter and trade journal articles, and 26 additional educational presentations.

Examples of 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 digest, BBIG Monthly, and quarterly BBIG Newsletters, as well as the latest research and extension publications, and boxwood health related news. A total of 15,054 boxwood enthusiasts were reached via the BBIG group alone as of August 30, 2024.

5.3 Documenting project impacts

This project has significantly improved boxwood blight mitigation in many ways.

Changes in knowledge

- Enabled growers, retailers, landscapers, arborists, first responders, and extension specialists and agents, garden managers and other horticulturists 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 the 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 the microbiome in a systems approach to boxwood health and production. Third, repeated fungicide applications reduced the sensitivity to chlorothalonil in a wide range of epiphytic fungal genera, providing the first microbiome evidence indicative of fungicide resistance to a multi-site contact chemistry. Fourth, 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 major innovations. These included: 1) identification of boxwood cultivars, including many previously not evaluated cultivars and accessions, with resistance to both boxwood blight and/or leafminer, in collaboration with Saunders Genetics, LLC, 2) effective sanitizers and improved sanitation practices, 3) ‘herbal distancing’ – demonstrating that spacing is critical to blight mitigation, 4) an effective blight scouting protocol – when, where and how, 5) a rapid and scalable DNA extraction and real-time PCR assay from boxwood tissues for detection of the blight pathogen *Cps*, and 6) new chemistries – both fungicides and antidesiccants, and a new application method – fungicide drench for blight mitigation that substantially extends the chemical control period from a couple of weeks to 20 weeks.

- Filled several major knowledge gaps in the blight biology. These included: 1) 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, 2) discovered *Che* in Spain, aiding quarantine effort to better prevent this pathogen from entering the U.S., 3) demonstrated rainfall and relative humidity are important contributing weather factors to boxwood blight development and spread through two case studies, and 4) showed that cooler temperatures lead to increased disease severity and loss of boxwood blight resistance in Oregon nurseries. This led to improvements in disease forecasting and mapping models as well as improvements in the timing of scouting and fungicide application activities.
- 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.
- Three scenarios commonly used for nursery production of Boxwood (*Buxus* L. spp., Buxaceae) in #3 containers in the U.S. were modeled based on published best management practices and grower interviews. Detailed inventories of material inputs, equipment use, and labor were developed from the production protocols for each of those scenarios and a partial budgeting analysis was conducted to determine the impact of individual components on the economic costs of the finished shrubs at the nursery gate.
- The total variable costs of each plant from Scenario A (from propagation tray to #1 container to #3 container) were \$8.98. Scenario B (propagation tray to the field and back to #3 container) resulted in variable costs of \$9.19 and takes a year longer in production than the other two models. Scenario C (propagation tray to #1 container bumped up into a #2 container and finally to a #3 container) incurred variable costs of \$11.26 per plant.
- Labor comprised the greatest share of variable costs in each of the three scenarios, while containers, transplants (including transplanting labor), irrigation, and fertilization inputs and associated activities accounted for the greatest portion of materials costs in each scenario. Pruning, assembling orders and loading trucks, applying plant protection products, and chlorination were other important components of variable costs of each scenario.
- The findings of this research are critical to our understanding of the boxwood market and issues affecting the green industry from boxwood blight. Participants in the green industry now have access to data to assist them in making strategic decisions regarding future investments to mitigate the effect of boxwood blight in their respective businesses. In addition, policymakers have better information to inform their decisions regarding efficient allocation of resources in combating this disease.

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 the event of accidental pathogen introduction as shown by all nurseries visited during the [Oregon Nursery Tour](#). Plants are moved further apart from each other earlier in the production cycle to minimize pathogen movement on splashed irrigation or rain water. Also observed during the same tour were wide adoption of: 1) a closed-loop system by not allowing outside boxwood materials to enter the 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. To further promote this transition, we are planning to use what we have learned to update the [Boxwood Health Best Management Practices for Production and Landscape](#) (Version 3) by the Horticultural Research Institute and endorsed by AmericanHort, National Plant Board and the National Association of Landscape Professionals.

Other impacts – fostering global and stakeholder partnerships

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 add updates 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, in addition to providing a lecture on [Reducing boxwood stresses in the landscape](#). **Bennett Saunders** presented at the third annual meeting the selection and public adoption of NewGen Boxwood cultivars. 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. **Mike Gaines** developed and presented his insights on how to successfully manage boxwood blight in the landscape on December 1, 2023 which was much needed and timely to help landscaping professionals, public gardener managers and home gardeners deal with this destructive disease. This talk was first presented to the project teams including Project Directors, their associates and other Advisory Panel members. The recording of this presentation is posted in the [Knowledge Center](#) of the BBIG website for access by all landscaping professionals and other interested parties.

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

To date this project has educated/trained eleven postdocs, three graduate students and five interns. 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 and giving webinars to the national and international audience. These professional development opportunities have helped seven postdocs land a dream job, two graduate students and four interns successfully enter the next steps of their professional development – landing a job and enrolling a dream university or graduate program. Some of their professional development activities and achievements are highlighted below.

- John Dobbs, a post-doctoral research associate with the USDA-ARS Foreign Disease/Weed Science Research Unit in Fort Detrick, MD joined the team in January 2024 after completing his Ph.D. at Colorado State University, where he studied forest and tree health. John has experience in bioinformatics, primer design and testing, field diagnostic experience, and tree pathogen inoculations, along with whole genome sequencing, assembly, and annotation experience from multiple platforms.
- Filiz Gurel, a Post-Doctoral Research Associate with the USDA-ARS U.S. National Arboretum Floral and Nursery Plant Research Unit, coordinated work to test the effects of UV-C treatment on the growth suppression of Boxwood Blight strains and continued previous experiments by diversifying whole plant assays. She also examined Boxwood genotypes for their responsiveness in cell culture establishment and optimized a protoplast isolation and transformation system for select boxwood genotypes.
- 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, resulting in a 2024 publication in Plant Pathology.
- 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 three high-impact journals – Microbiology Spectrum, BMC Microbiology, and PhytoFrontiers. Xiaoping also was invited to give a webinar on the boxwood microbiome on the BBIG Boxwood Seminar series to national and international audience.
- 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. Tennessee Department of Agriculture has named Dr. Prabha Liyanapathirana as the new state plant pathologist starting from June 2024. As the state's plant pathologist, she is responsible for diagnosing plant diseases, educating plant inspectors and stakeholders on plant diseases, and collaborating with plant pathologists on existing and emerging plant diseases of regional, national, and international significance.
- 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. Dr. Ghimire landed a dream job – Plant, Disease and Insect Diagnostician at Bartlett Tree Experts immediately after graduation from Tennessee State University.
- Taylor Phelps, a graduate student at Virginia Tech, is investigating and defining the conditions at which contaminated pruners may spread the blight pathogen from diseased

plants to healthy plants. He also interviewed two Advisory Panel (AP) members and produced articles for the AP Spotlight section of BBIG Newsletter.

- 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 more effective at delivering disease-controlling concentrations to boxwood canopies than spraying directly onto foliage.

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 continue to improve upon 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 fourth 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 hampered the initiation of laboratory-based research objectives and hiring of qualified research staff. We requested and have been granted a 1 year no-cost extension to achieve proposed project objectives while we also continue to achieve complementary, unplanned objectives as needed.

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) NCCE Collaborator Amanda Taylor, who purchased American boxwood plants from a local nursery for both antidesiccant and biocontrol studies at one research site; and 3) Syngenta provided three products – Banner Maxx, Daconil, and Concert II as fungicide standards for the field trials evaluating antidesiccants and biocontrol agents. These and other much-appreciated demonstrations of support added quality, productivity and efficiency to this project.