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, 2025

Boxwood **B**light **I**nsight **G**roup (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 for Program Improvement Through Global Engagement

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PROJECT PARTICIPANTS

Project Leadership Team

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Olanike Omolehin, Virginia Tech (Project Manager, November 2021 to September 2022)
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Xiao Yang, Clemson University
Keith Yoder, Saunders Genetics, LLC

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Thomas Brand, Landwirtschaftskammer Niedersachsen, Germany
Matthew Cromey, Royal Horticultural Society, United Kingdom
Björn Ehsen, Research and Teaching Institute for Horticulture, Germany
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Vladimiro Guarnaccia, University of Torino, Italy
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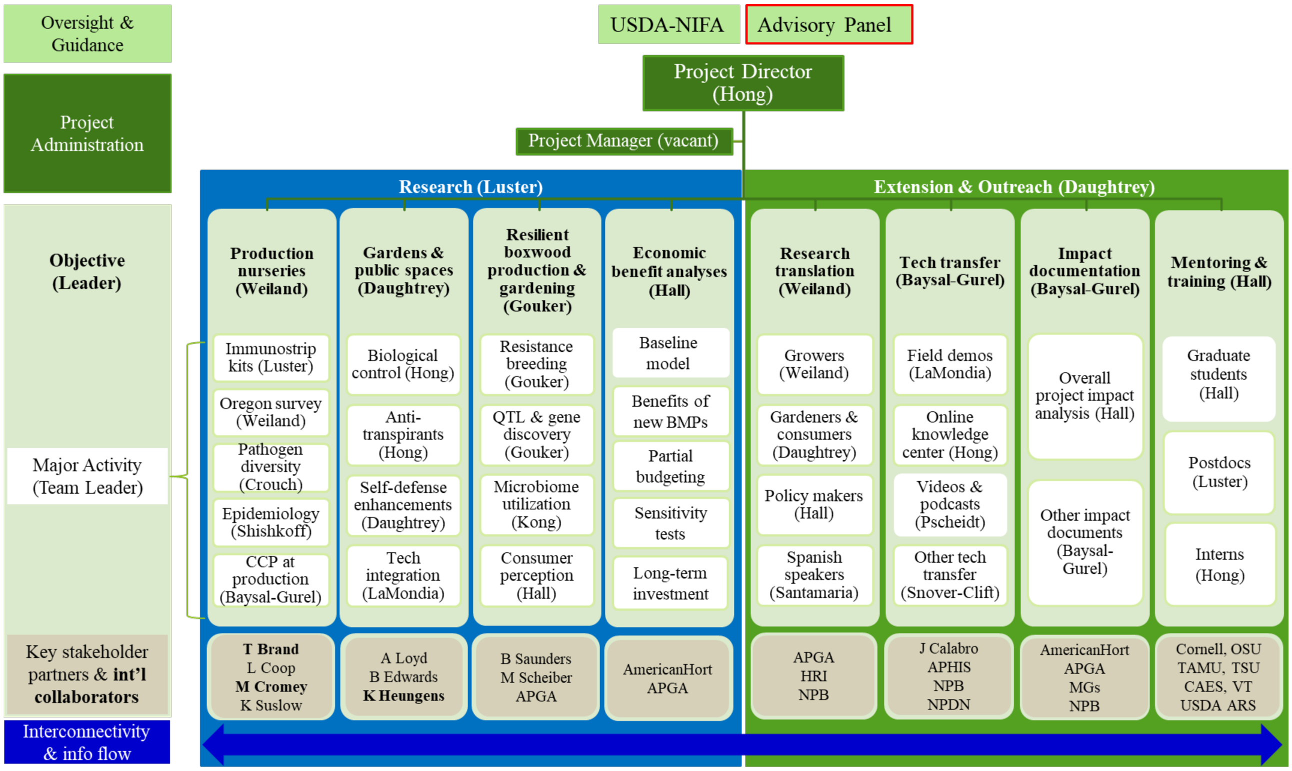
Katrijn Van Laere, Flanders Research Institute for Agriculture, Fisheries and Food, Belgium
Irene Vloutoglou, Benaki Phytopathological Institute, Athens, Greece

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; USDA ARS, USDA Agricultural Research Service; and VT, Virginia Tech

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 Products

(excluding those already reported in previous years)

Refereed Journal Articles

1. Daughtrey, M., Gray, J., Calabro, J., and Hong, C. X. 2025. Fighting against invasive species through global and stakeholder partnerships - a case study of boxwood blight. Plant Disease: First Look at https://apsjournals.apsnet.org/doi/10.1094/PDIS-01-25-0210-SC.
2. Ghimire, B., Parajuli, M., Simmons, T., Liyanapathiranage, P., and Baysal-Gurel, F. 2025. Evaluation of fungicides, host-plant defense inducer, and anti-transpirant in management of boxwood blight. HortTechnology 35:101-115. Open access at: https://doi.org/10.21273/HORTTECH05541-24.
3. Hall, C. 2024. Costs associated with mitigating boxwood blight during nursery production in the U.S. Journal of Environmental Horticulture 42(2):165-172, Open access at: https://doi.org/10.24266/0738-2898-42.4.165).
4. Khaliq, I., Avenot, H. F., Baudoin, A., Coop, L., and Hong, C. X. 2024. Epidemiology of boxwood blight in western North Carolina and Virginia and evaluation of the boxwood blight infection risk model. Scientific Reports 14:26829. Open access at: https://www.nature.com/articles/s41598-024-76443-5.
5. Kong, P., Guarnaccia, V., Carter, C., and Hong, C. X. 2024. First report of *Calonectria henricotiae* causing boxwood blight in Switzerland and Italy. New Disease Reports 50(2): e70006. Open access at: https://DOI.org/10.1002/ndr2.70006.
6. Kong, P., and Hong, C. X. 2024. Evaluation of 1021Bp, a close relative of *Pseudomonas eucalypticola*, for potential of plant growth promotion, fungal pathogen suppression and boxwood blight control. BMC Microbiology 24:346. Open access at: https://doi.org/10.1186/s12866-024-03497-w.
7. Kong, P., Li, X. P., and Hong, C. X. 2025. Sporulation dynamics among US *Calonectria pseudonaviculata* isolates on infected boxwood leaves. Plant Pathology First Look at https://doi.org/10.1111/ppa.70019.
8. LaMondia, J. A., Cowles, R. S., and Shishkoff, N. 2025. The effects of sanitizers on *Calonectria pseudonaviculata* and *C. henricotiae* conidia and microsclerotia viability. Journal of Environmental Horticulture 43(2):83–90 open access at https://doi.org/10.24266/0738-2898-43.2.83.
9. Li, X. P., Hemmings, G., Omolehin, O., Tseng, H. T., Taylor, A., Taylor, C., Kong, P., Gouker, F., and Hong, C. X. 2025. Mycobiome of low maintenance iconic landscape plant boxwood under repeated treatments of contact and systemic fungicides. Scientific Reports 15, 30150. Open access at https://doi.org/10.1038/s41598-025-07593-3.
10. Li, X., and Hong, C. X. 2025. Evaluation of biochar liquid extract for control of boxwood blight. Plant Health Progress. Abstract at: https://doi.org/10.1094/PHP-12-24-0143-PDMR.
11. Li, X., 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 4: 602-615. Open access at https://doi.org/10.1094/PHYTOFR-03-24-0023-R.

12. Likins T., Davis B., Anderson P., Gillis B., and Hong C. X. 2025. Flutriafol drench provides season-long protection of boxwood plantings pre-infected by *Calonectria pseudonaviculata* in the Mid-Atlantic. Plant Disease. Abstract available at: https://doi.org/10.1094/PDIS-12-24-2731-SC.
13. Rolfe, K. J. and Weiland, J. E. 2025. Efficacy of fungicides for control of boxwood blight in Oregon, 2024. Plant Health Progress. Abstract at https://doi.org/10.1094/PHP-03-25-0086-PDMR.
14. Sacher, G., and Pscheidt, J. 2025. Detached leaf assays reveal long-term efficacy of the systemic fungicide flutriafol against boxwood blight. Plant Disease: First Look at https://apsjournals.apsnet.org/doi/abs/10.1094/PDIS-11-24-2508-RE.

A complete list of the research publications from this project to date with weblinks is posted under the Research Updates of the BBIG website.

Abstracts (Presented at professional meetings)

1. Daughtrey, M. L., Luster, D. G., Calabro, J., Gray, J., Hall, C., Weiland, J. E., Baysal-Gurel, F., Gouker, F., Kong, P., Crouch, J. A., LaMondia, J., Pscheidt, J., Santamaria, L., Shishkoff, N., Snover-Clift, K., Westrick, N., Brindisi, L., and Hong, C. X. 2025. Fighting a great fight against boxwood blight. Plant Health 2025 – Annual Meeting of American Phytopathological Society, Honolulu, HI, USA, August 2 to 5.
2. Hong, C. X., Kong, P., Brand, T., Guarnaccia, V., Ioos, R., Daughtrey, M., and Luster, D. 2025. Ever-expanding international collaborative research safeguards boxwood crops and plantings. Plant Health 2025 – Annual Meeting of American Phytopathological Society, Honolulu, HI, USA, August 2 to 5.
3. Li, X. P., Weiland, J. E., Ohkura, M., Luster, D. G., and Hong, C. X. 2024. Rhizosphere microbial composition and mycorrhizal fungi associated with boxwood blight resistance? International Phytobiomes Conference, St. Louis, MO, USA, November 19 to 21.
4. Snover-Clift, K., Daughtrey, M., Luster, D., Hong, C., Hall, C., Weiland, J., Baysal-Gurel, F., Gouker, F., Kong, P., Crouch, J., LaMondia, J., Pscheidt, J., Santamaria, L., Shishkoff, N., Ohkura, M., and Yang, X. 2024. BBIG - A team battling boxwood blight to ensure this iconic woody ornamental lives on. NPDN National Meeting, Portland, ME, USA, September 12.

Presentations at Professional Meetings (without an abstract)

1. 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. 23rd Ornamental Workshop, Raleigh, NC, USA, October 22 to 24.
2. Ghimire, B., Pendyala, B., Patras, A., and Baysal-Gurel, F. 2024. Evaluating UV-C sensitivity of *Calonectria pseudonaviculata* using light-emitting-diode system. 23rd Ornamental Workshop on Insects and Diseases, Raleigh, NC, October 22 to 24.
3. Shishkoff, N. 2024. Difference in boxwood blight symptom expression in different boxwood cultivars. 23rd Ornamental Workshop on Insects and Diseases, Raleigh, NC, USA, October 22 to 24.
4. Shishkoff, N. 2024. Symptoms of boxwood blight from 24-96 hr on different boxwood cultivars. 23rd Ornamental Workshop on Insects and Diseases, Raleigh, NC, USA, October 22 to 24.

Global and Stakeholder Partnerships

Working with German Scientists to Analyze Multi-year Boxwood Cultivar and Fungicide Evaluation Trial Data

Calonectria pseudonaviculata (*Cps*) and *C. henricotiae* (*Che*) – two blight pathogens – were first detected in Germany in 2004 and 2005, respectively. *Che* used to be a subgroup of *Cps* sensu lato, and this subgroup was elected to be a separate, new species in 2016. This subgroup or newer pathogen has higher thermotolerance and reduced sensitivity to three important fungicide chemistries – tetraconazole (FRAC 3), kresoxim-methyl and trifloxystrobin (FRAC 11). *Che* has not been seen outside of Europe – not in the U.S. Little else was known about *Che* when this project started in 2020. Even with *Cps*, all published epidemiology studies were done under lab conditions or controlled environments.

Following the first observation of boxwood blight in 2004, Dr. Thomas Brand and his colleagues in northern Germany performed many field trials evaluating boxwood cultivars and fungicides in the context of *Cps*. This collaboration was initially proposed to work together and analyze the field trial data from the epidemiology perspective to develop a better understanding of the disease's behavior in a real world environment. It has since expanded in multiple directions, resulting in several major research and extension products of global impact.

1. Analyzed and brought the field performance data to American stakeholders and the international communities through two articles in the internationally-recognized journals
   a. The Plant Disease (2023) article reported on two multi-year boxwood cultivar evaluation trials
      i. Confirmed the U.S. studies that had used detached leaves, cuttings, and container-grown plants under controlled environments, with a few exceptions. Among the 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.
      ii. Demonstrated that cultivar resistant to *Cps* are also resistant to *Che*.
   b. The Plant Diseases and Protection (2023) article reported on multiple fungicide evaluation trials
      i. Validated the results of early fungicide evaluations under controlled environments the in the U.S. and fiend settings elsewhere.
      ii. Identified three additional major fungicide chemistries – myclobutanil, propiconazole (FRAC 3) and azoxystrobin (FRAC 11) – to which local *Che* population also had reduced sensitivity.
      iii. Demonstrated that repeated use of some fungicide chemistries led to their efficacy drop over time.

Both publications also noted that *Che* quickly outcompeted *Cps* under local environments, highlighting the importance of determining its actual distribution and triggering boxwood planting surveys in southern Europe with warmer weather than northern Germany. These publications enabled growers and gardeners to make informed cultivar and fungicide selection in blight mitigation planning while better preparing them for inadvertent introduction of *Che*.

2. Developed a better understanding of boxwood blight field epidemiology as published in Plant Pathology (2024). This article 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, followed by relative humidity and temperature, documenting for the first time the importance of rainfall and relative humidity in the blight field epidemiology, helping set up blight mitigation strategies while providing new leads for calibrating the infection risk model.
3. Conducted new field studies comparing the boxwood blight development on three cultivars of distinct susceptibility with *B. sempervirens* 'Suffruticosa' being highly susceptible and *B. microphylla* 'Herrenhausen' least susceptible while *B. sempervirens* 'Arborescens' is intermediate. This study was done at the same site in northern Germany over a 3-year period from 2022 to 2024, and each year started with a new planting in April. Boxwood blight development on three cultivars was assessed every 7 days starting in May and continuing through November. Two sets of weather data were collected. First, temperature, relative humidity, precipitation, wind direction and wind speed were recorded through an automatic weather station, 50 m away from the research field. Second, microclimate conditions – temperature, relative humidity and dew point within the canopy of individual cultivars – were also recorded through data loggers. These data are being analyzed for inclusion in the upcoming BBIG focus issue of PhytoFrontiers.
4. Initial review and conversation about the field data led to development of a highly impactful 8-webinar series – International Boxwood Seminars – with Dr. Brand being the inaugural speaker.

Connecting and Working with Scientists in Southern Europe to Survey Historic Gardens and Other Boxwood Plantings for Che

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

To maximize the productivity of this survey, two approaches were taken. First, we learned additional countries with *Che* detection through interactions with colleagues in Europe. Specifically, we learned two new *Che*-positive countries – Austria and Lithuania – from Dr. Brand of Germany. Second, we directly worked with local pathologists and regulatory professionals and surveyed public gardens and other boxwood plantings in France, Greece, Italy, Spain, and Switzerland. The surveys in Spain and France were conducted in conjunction with attending the 12th International Congress of Plant Pathology in Lyon, France (August 2023) while Greece, Italy and Switzerland were surveyed along with attendance at the 20th International Plant Protection Congress in Athens, Greece (July 2024). These surveys uncovered *Che* from Spain in 2023 and from Switzerland and Italy in 2024, resulting in two short communications in New Disease Reports, an open access journal, to make these new discoveries readily accessible to the regulatory professionals as well as the horticulture industry and the public. Specifically,

1. The Spain and France report detailed the survey of a number of public gardens and street boxwood plantings in both countries with eleven boxwood samples brought back to the

lab at Virginia Tech 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*.

2. The Switzerland, Italy and Greece report detailed the survey of many public gardens and boxwood plantings in three countries and analyses of 36 symptomatic samples, highlighting *Che* detection in Switzerland and Italy. The survey in Turino was conducted jointly with Dr. Vladimiro Guarnaccia of University of Turino. The survey in Greece was facilitated by Dr. Irene Vloutoglou of Benaki Phytopathological Institute in Athens, Greece, and while that of Switzerland was facilitated by Dr. Vincent Michel of Agroscope, Federal Department of Economic Affairs – Education and Research EAER in Conthey, Switzerland through helpful discussion.

Additionally, Dr. Vladimiro Guarnaccia shared the *Calonectria* isolates he recovered from boxwood samples collected in Madeira, Portugal during a family vacation in February 2024. These isolations all belonged to *Cps*.

These collaborations expanded the knowledge of *Che* distribution from 6 to 11 countries while adding Portugal to the *Cps* distribution map. Both updated maps are shared widely through the BBIG outreach website to help block the movement of *Che* from Europe to the U.S. and other countries that currently are not known to have this pathogen.

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

The field trials evaluating selected antidesiccants and biological control agents for their blight mitigation potential were done in western North Carolina, a hotspot for the disease since its first observation in the U.S. (2011). Working with NCDA&CS, NCCE, and local farmers (names not shown per the research protocol approved by the Virginia Tech Institutional Review Board) was another add-on to the project. This add-on has enhanced the project productivity and efficiency for the proposed field trials. Among the key collaborators were Dr. Hsien T. Tseng, Chad Taylor, Ginger Hemmings of NCDA&CS, and Amanda Taylor of NCCE. They have been an integral part of the team, identifying naturally-infested fields for these trials, setting up the trials, applying planned treatments and assessing treatment efficacy monthly. One contribution of critical importance was their application of fungicide standard treatments every 2 or 3 weeks – this made these trials 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. Both antidesiccant and biocontrol studies are being written for inclusion in the BBIG focus issue of PhytoFrontiers.

To make the most out of this collaboration, we further expanded the planned research to include evaluation on how boxwood phyllosphere microbiome might be affected by three antidesiccants – TransFilm, Vapor Gard and Wilt-Pruf, and three commonly-used fungicides – Banner Maxx, Concert II and Daconil Weather Stik. This expansion produced a variety of important discoveries and laid 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 blight mitigation tools. Some of these results have been published through three research articles.

1. The BMC Microbiology (2023) article detailed boxwood sampling for analysis of antidesiccant impacts. 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.
2. The Microbiology Spectrum (2023) article reported on boxwood sampling for fungicide impacts on phyllosphere bacterial communities. The bacteriome analyses revealed that fungicide treatments had strong impacts on epiphytic bacterial community structure and composition. Analysis of compositions of microbiome 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 fungicides with chlorothalonil as sole (Daconil) or major active ingredient (Concert II) had greater impact than the propiconazole-based systemic fungicide (Banner Maxx).
3. The Scientific Reports (2025) article detailed boxwood sampling for fungicide impacts on phyllosphere mycobiome and highlighted three major findings. First, fungicide applications strongly affected epiphytic fungal community diversity, structure and many functional groups. Second, Daconil and Concert II suppressed greater numbers of epiphytes than Banner Maxx. Third, many epiphytic genera in the August samples became less sensitive to Daconil treatment after repeated applications of the same product.

Directly Involving Advisory Panel (AP) Members in Project Activities

In addition to providing oversight and advising roles, AP members used their unique expertise sets, positions and networks, and made significant contributions to the project research and extension/outreach programming as exemplified below.

AP Contributions to the BBIG Research

Among the most notable AP contributions to the research were 1) **field evaluation of boxwood cultivars and selections against boxwood blight**, and 2) **analysis of boxwood production inventory shift across the U.S. over a 10-year period from 2011 to 2021.**

The multi-year field trials spearheaded by **Bennett Saunders,** AP member and General Manager of Saunders Genetics, LLC, evaluated 75 boxwood cultivars and selections in Lowgap, North Carolina, a hotspot of boxwood blight. Additionally, 146 cultivars were evaluated in Piney River, Virginia against leafminer (*Monarthropalpus flavus*), a major insect pest in many U.S. regions, especially in the Mid-Atlantic. These trials produced several NewGen boxwood cultivars – ‘SB 300’ (NewGen Freedom®), ‘SB 108’ (NewGen Independence®), and ‘RLH-BI’ (NewGen Liberty Belle®). They also identified a number of other cultivars and selections with resistance to both boxwood blight and leafminer. These trial results, published in the Journal of Environmental Horticulture (2022), filled several major knowledge gaps and they were foundational for developing a systems approach to boxwood health and production. They also lifted the spirits of both the industry and the public about the future potential for this #1 evergreen shrub crop and iconic landscape plant.

Use of less susceptible boxwood cultivars is critical to sustain boxwood production and plantings. The analysis of boxwood production inventory shift was intended to promote the production inventory shift to more resistant cultivars. This analysis involved 17 selected nurseries across seven states and these nurseries accounted for over 10% of the total boxwood production in the U.S. Several AP members shared their inventory data in 2011, 2016 and 2021 and **John Keller**, AP member and Vice President of Monrovia Production Planning, was directly involved in the analysis and result interpretation.

This analysis demonstrated that the nation's boxwood production inventory has shifted dramatically over the 10-year period from 2011 to 2021, as evidenced by changes in the top 10 seller boxwood cultivars. Seven of the top 10 seller boxwood were highly susceptible cultivars in 2011 but this number dropped to three in 2021. Of the most notable new top seller boxwood were NewGen Boxwood 'SB 300' (Freedom) and 'SB 108' (Independence). These two cultivars resistant to both boxwood blight and leafminer were introduced to the market with limited availability in 2019, and within 2 years, Freedom and Independence arose to the #2 and #3 sellers, only behind the ''Winter Gem'. Publication of these results in Plant Disease in 2022 has expedited the inventory shift, fast-tracking the nation to sustainable boxwood production and plantings.

AP Contributions to the BBIG Extension/Outreach

AP contributions to the BBIG extension and outreach are numerous and significant as exemplified below.

First, two AP members were the key players at a day-long roundtable on **Growing the Shrub We All Know and Love despite Boxwood Blight** on February 18, 2022, in the Dixon Gallery and Gardens in Memphis, TN. This educational event was organized by a Project Director – **Dr. Fulya Baysal-Gurel.** The two AP members were **Lynn Batdorf**, the International Authority of Cultivar Registration for *Buxus* L. appointed by The International Society for Horticultural Science and the owner of the Boxwood Guy® LLC., and **Bennett Saunders**, General Manager of Saunders Genetics, LLC.

Second, two webinars were presented by two AP members for landscaping professionals. One webinar – **Boxwood in the Landscape: Success and Shortcomings** – was presented on December 1, 2023 by **Michael Gaines**, President of CW Arborists, and the other – **Reducing Boxwood Stresses in the Landscape** – was presented on May 9, 2024 by Lynn Batdorf.

Third, **Jill Calabro**, AP Chair and Product Development Manager at Valent USA (formerly Director of Science and Regulatory Affairs at AmericanHort), and three Project Leaders – Chuan Hong, Margery Daughtrey and Douglas Luster – are currently serving as guest editors of the **BBIG Focus Issue of PhytoFrontiers – Reclaiming Boxwood From the Blight** – to expedite the publication of the latest research and innovations on boxwood blight.

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. This partnership had resulted in three impactful products: an outreach website, the International Boxwood Seminar series and the BBIG Boxwood Seminar series.

Outreach Website – Boxwood Blight Insight Group (BBIG)

The BBIG website is hosted at the HRI and updated regularly by HRI staff with content 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 advanced, more content has been added to the respective tabs. More importantly, three new tabs – Boxwood Gardening, Box Tree Moth, and Spanish – were added to the outreach website in 2021, 2024 and 2025, respectively, to meet the emerging need for information and these additions were strongly supported by the Advisory Panel. To help viewers navigate and locate the information of interest, the website has undergone restructuring twice, with many new subsections and more informative section headings under individual tabs. For example, the expanded Diagnostic Aids and Management Strategies section includes the following subsections:

- Blight Symptom Library.
- Boxwood Blight Forecasting App and Push Notification.
- Scouting, Diagnostic Guides and Tools.
- Integrated Disease Management.
- Boxwood Blight Resistance.
- Boxwood Blight Maps.
- Boxwood Blight Cleanliness Programs.
- Sanitizers and Sanitation Practices.
- Cultural Control.
- Fungicide Options and Chemical Protection.
- Best Management Practices.
- Boxwood Blight Biology.

This website has become the go-to resource on boxwood blight and box tree moth for the environmental horticulture industry, the public and other stakeholders in the U.S. and across the globe.

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 bring what had already been learned in Europe and elsewhere about boxwood blight and its mitigation to the stakeholders in the U.S., as many European countries had this disease about 10 to 15 years before the U.S. This seminar series included eight webinars with invited speakers from seven countries over a two-year period, and the last webinar was 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. Additionally, they 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 Google analytics, etc. This partnership product continues to have positive impacts across the nation and globally because webinar recordings are posted at the website.

This seminar series set an example of outreach to address new and emerging plant biosecurity and sustainability issues of national and global significance—through international and stakeholder partnerships as detailed in a recent article in Plant Disease (2025).

BBIG Boxwood Seminars

The BBIG Boxwood Seminar (BBS) Series was launched in August 2023, designed for the BBIG Team to expedite the technology transfer from lab to field. During this past project year, the following two webinars were added to the series.

- Shishkoff, N. 2025. Cleaning up boxwood blight with a full spectrum of sanitizers. February 6.
- Kong, P. 2025. Prospecting for biocontrol of boxwood blight, March 11.

Both webinars were well attended, with potential economic impacts in excess of \$1.3M each according to the post-webinar surveys. The recording of both webinars, along with the first four on this Seminar series is posted at the BBIG website for continuing positive impacts.

We plan to continue this impactful Seminar series in the coming year with additional sessions already having been planned and the first scheduled for October 23, 2025. We are also analyzing the metrics data of the attendance to the first six webinars on this series.

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

The BBIG Team provided two outreach programs 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.*

FIGURE 1 THE FIRST PRODUCT OF THE NPDN PARTNERSHIP

The first program (**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.

<u>*2024 NPDN Symposium – Boxwood Blight Insight Group*</u>

This symposium (**Figure 2**) 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.

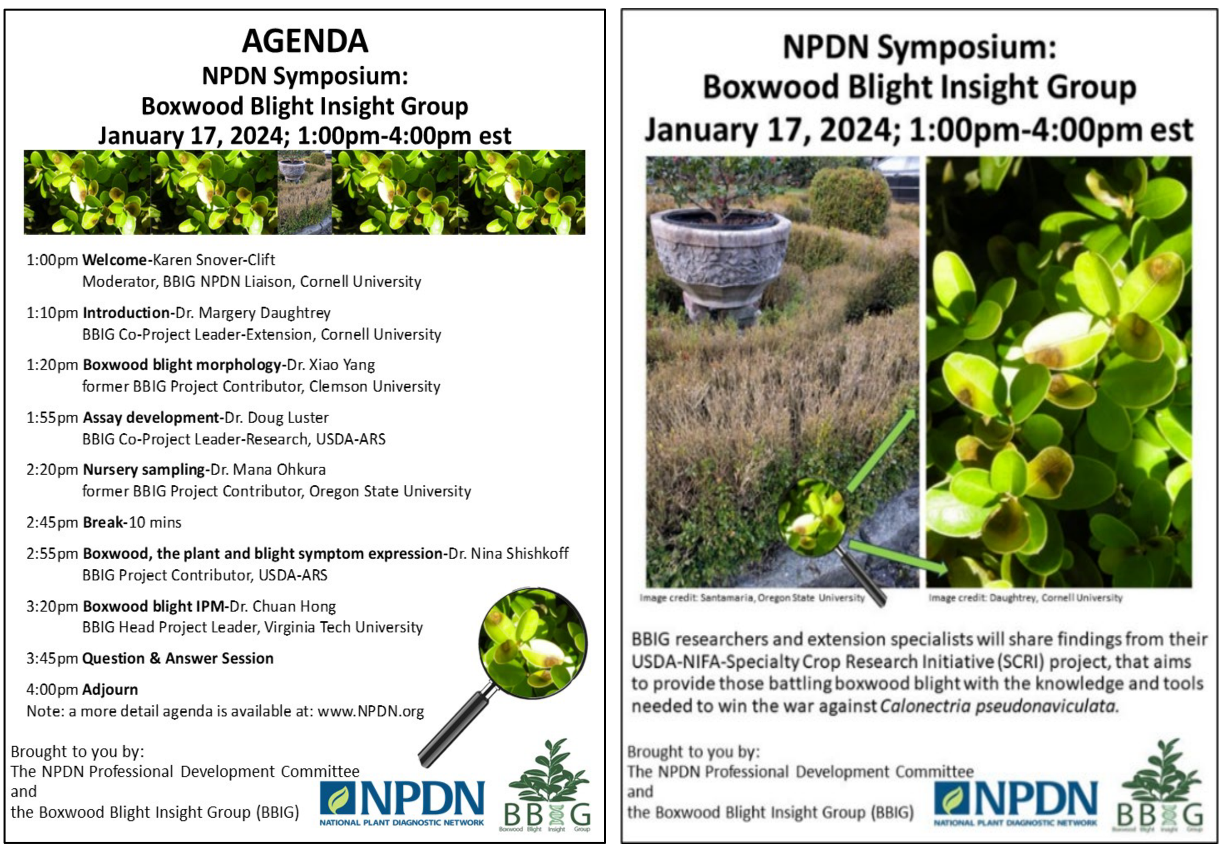


FIGURE 2 THE SECOND MAJOR PRODUCT OF THE NPDN PARTNERSHIP

The symposium included six presenters speaking about their specific area of BBIG project work and their findings. The six presentations were each 20 minutes 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** (**Figure 3**). The NPDN is divided into five regions; this helps the members focus on regional-specific topics, but all 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.

Regions:	Number participants:
GPDN	13
NCPDN	26
NEPDN	59
SPDN	28
WPDN	16
USDA	5
	147

Participant position type:	Number of participants:
Diagnostician/Lab staff	62
Extension Educator	8
Dept of Agriculture Staff	60
Forestry Staff	6
Student	1
Grower-Nursery staff	3
USDA	4
other	3
	147

FIGURE 3 DEMOGRAPHICS OF THE 2024 DNPN SYMPOSIUM ATTENDEES

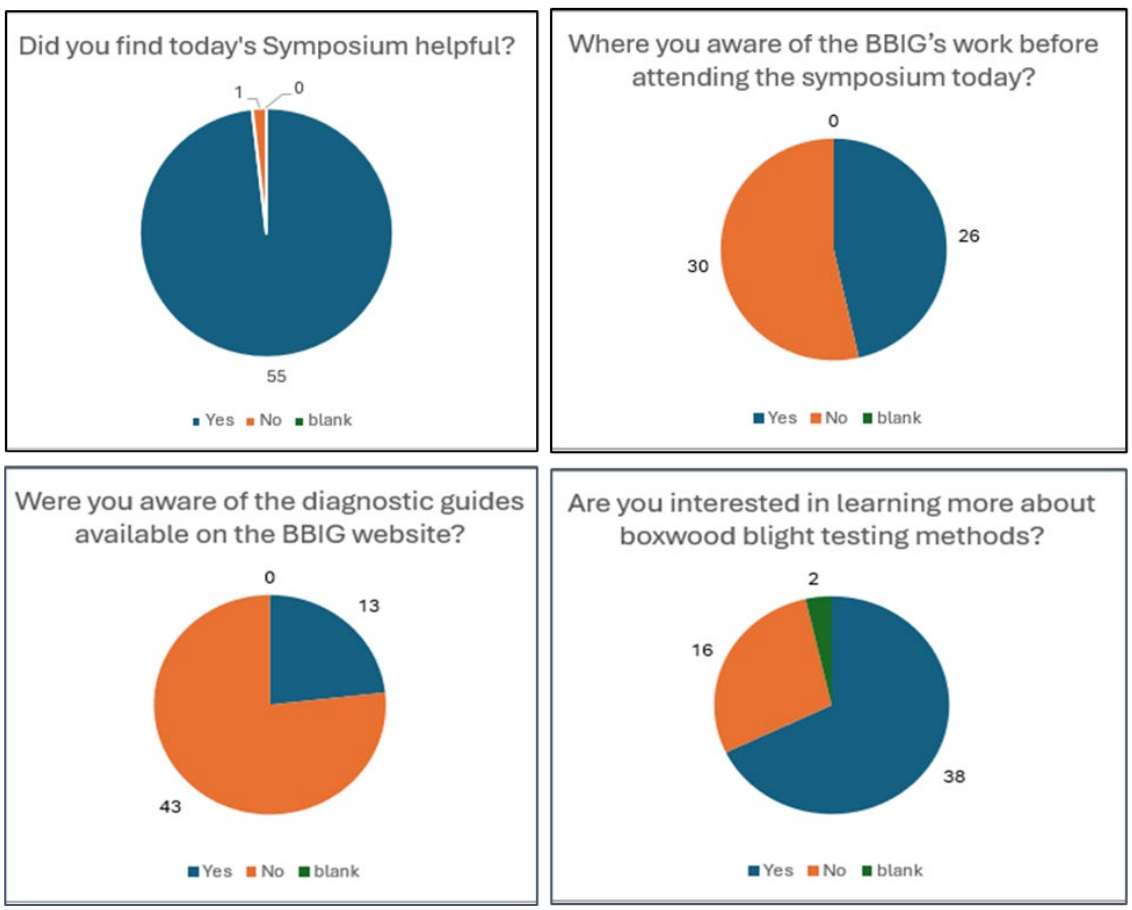


FIGURE 4 SUMMARY OF THE 2024 NPDN SYMPOSIUM SURVEY RESULTS

Snover-Clift developed a survey to get feedback after the symposium and received 56 completed surveys back (**Figure 4**). Fifty-five (55) attendees said the symposium was helpful and many gave high marks: one said "perfect" and another said, "It was excellent". The above tables and graphs contain the results of the feedback.

Partnering with Other Major Stakeholders

In addition to AmericanHort and NPDN, we partnered with many other institutions to broaden and expedite our reach to stakeholders. Following are a few of the most notable.

American Phytopathological Society (APS)

In conjunction with APS annual meetings, we organized one Idea Café – Boxwood Blight Insight Group (BBIG): Fighting a Good Fight against Boxwood Blight – at the Plant Health 2021 Online, and one symposium – Epidemiology, Diagnostics and Management of an Emerging Disease: Boxwood Blight – at the Plant Health 2022 in Pittsburgh, Pennsylvania.

Likewise, in conjunction with the APS Southern Division Annual Meeting in Chattanooga, TN on March 9, 2022, we organized and held another symposium – Ornamental Production Challenges and Perspectives: a Case Study of Boxwood Blight.

We are currently working with APS on a focus issue – **Reclaiming Boxwood from the Blight** on one of its flagship journals – PhytoFrontiers to fast track publication of our latest research and innovations.

International Society of Arborists (ISA)

In response to advice from **Casey Sclar**, AP member and H.O. Smith Endowed Director of the Arboretum at Pennsylvania State University (formerly Executive Director of American Public Gardens Association), we published an introductory article: BBIG – the Blight Buster, on Arborist News (August 2024) to help reach out to arborists and other landscaping professionals in the U.S. and overseas. Also, we were enabled to use the ISA Event Calendar to post our educational events such as the BBIG Boxwood Seminars for the same purposes.

American Boxwood Society (ABS)

We collaborated with ABS in a number of ways. First, Andrea Fillippone, President of ABS and President of F2 Environmental Design and AJF Design, shared her successful organic approach focusing on biologicals to landscape architecture and design through an article – A Holistic Approach to Boxwood in BBIG Newsletter (August 2022). ABS also provides the BBIG Team with a powerful outreach platform – The Boxwood Bulletin, an annual publication of the society for its membership.

U.S. Environmental Protection Agency (EPA) – Integrated Pest Management Program (IPM)

We were invited by the U.S. Environmental Protection Agency (EPA) Integrated Pest Management Program (IPM) to give a webinar on boxwood blight and box tree moth for its On-Demand IPM Seminar series. To best serve the participants, we extended the invitation to Dr. Gregory Simmons, a leading entomologist and researcher on box tree moth at the USDA Animal and Plant Health Inspection Service.

We jointly presented the well-attended seminar Protecting Boxwood against the Blight and Box Tree Moth on March 5, 2024. A total of 1,161 individuals and groups from 49 U.S. states, plus 18 other countries, attended this webinar. Among the most notable were attendees from 78 schools, districts, childcare centers and universities representing over 1.3 million students.

Other Major Extension Products
(excluding those already reported in previous years)

BBIG Monthly Digests

The BBIG Monthly was launched in January of 2022 to keep stakeholders informed of the latest research and innovations, new publications, educational events and programming. A total of 45 editions have been published to date, with the vast majority released on the first business day of the month. Followings are the twelve editions released this past year from September 2024 to August 2025.

1. 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., Brindisi, L., and Hong, C. X. 2024. BBIG Monthly – September 2024, released September 5.
2. 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., Brindisi, L., and Hong, C. X. 2024. BBIG Monthly – October 2024, released October 7.
3. 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., Brindisi, L., and Hong, C. X. 2024.. BBIG Monthly – November 2024, released November 2.
4. 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., Brindisi, L., and Hong, C. X. 2024. BBIG Monthly – December 2024, released December 4.
5. 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., Brindisi, L., and Hong, C. X. 2025. BBIG Monthly – January 2025, released January 10.
6. 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., Brindisi, L., and Hong, C. X. 2025. BBIG Monthly – February 2025, released February 3.
7. 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., Brindisi, L., and Hong, C. X. 2025. BBIG Monthly – March 2025, released March 6.
8. 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., Brindisi, L., and Hong, C. X. 2025. BBIG Monthly – April 2025, released April 2.
9. 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., Brindisi, L., and Hong, C. X. 2025. BBIG Monthly – May 2025, released May 2.
10. 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., Brindisi, L., and Hong, C. X. 2025. BBIG Monthly – June 2025, released June 5.

11. 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., Brindisi, L., and Hong, C. X. 2025. BBIG Monthly – July 2025, released July 7.
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., Brindisi, L., and Hong, C. X. 2025. BBIG Monthly – August 2025, released August 6.

BBIG Newsletters

BBIG Newsletter is a quarterly project digital magazine with the first edition launched in late October 2020. A total of 20 editions have been published to date with the latest four editions detailed below.

1. 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., Brindisi, L., and Hong, C. X. 2024. BBIG Newsletter Volume 5, Issue 4, released December 4.
2. 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., Brindisi, L., and Hong, C. X. 2025. BBIG Newsletter Volume 6, Issue 1, released March 6.
3. 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., Brindisi, L., and Hong, C. X. 2025. BBIG Newsletter Volume 6, Issue 2, released June 12.
4. 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., Brindisi, L., and Hong, C. X. 2025. BBIG Newsletter Volume 6, Issue 3, released September 23.

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

1. Baysal-Gurel, F. 2025. Dealing with diseases in the landscape. Warren County Master Gardener's Class, McMinnville, TN, April 8.
2. Daughtrey, M. 2024. See Spot Run. 2024 Landscape Contractors Association of Long Island Educational Conference, Melville, NY. November 6, 2024.
3. Daughtrey, M. 2025. Beware of bad things that happen to good plants: boxwood, bloomers & trees! Professional Certified Applicators of Long Island Conference, Hauppauge, NY, January 16.
4. Daughtrey, M. 2025. Baffling tree and shrub maladies. New York State Arborists Association. Monticello, NY, January 26.
5. Daughtrey, M. 2025. Potential and persistent plant plagues. New York State Nursery and Landscape Association. Webinar. January 29.

6. Daughtrey, M. 2025. Problems for the nursery industry that cause streaking and wilting. Long Island Nursery and Landscape Association Annual Meeting, Bayard Cutting Arboretum, Great River, NY, January 30.
7. Daughtrey, M. 2025. Understanding plant diseases. Certified Nursery and Landscape Professionals Training Program, New York State Nursery and Landscape Association, Riverhead, NY. February 2.
8. Daughtrey, M. 2025. Can we preserve boxwood in our landscapes? Communities in Bloom Spring Gardening Class. Cornell Cooperative Extension of Erie County. February 22.
9. Daughtrey, M. 2025. Scouting skills for crabapple, maple, and boxwood. Branching Out Webinar, Cornell Cooperative Extension. February 27.
10. Daughtrey, M. 2025. Streaking in the shrubbery. Long Island Horticulture Conference. Cornell Cooperative Extension of Suffolk Co. Brookhaven National Lab, Upton, NY. March 2.
11. Daughtrey, M. 2025. Battling for Boxwood Health. Landscape Design Student and Alumni Organization. New York Botanical Garden. Virtual, April 8.
12. Daughtrey, Margery. The 2025 Pests of the Landscape: Diseases. Urban Forestry Today. University of Massachusetts. Virtual, April 10.
13. Hong, C. X. 2024. An award-winning program – Boxwood Blight Insight Group. New Virginia Cooperative Extension Agents Onboarding Tour at Hampton Roads Agricultural Research and Extension Center, Virginia Beach, VA, October 29.
14. Hong, C. X. 2024. IPM Principles and Strategies for Ornamental Plant Diseases – Boxwood Blight and Phytophthoras. 2024 Integrated Pest Management Workshop, sponsored by USDA, Virginia Tech Pesticide Programs, and Virginia Cooperative Extension, Roanoke, VA, November 6 to 7, 2024.
15. Santamaria, L. 2025. Boxwood blight – update on management & breeding of resistant cultivars. British Columbia Landscape & Nursery Association - Webinar. January 22.

BBIG Showcased in Major National and International Events and Media

One BBIG scientist presented at the International Phytobiomes Conference in St Louis, MO from November 17 to 19, 2024, while several PDs presented at the Plant Health 2025 in Honolulu, HI from August 2 to 5, 2025. Likewise, one PD presented at the NPDN meeting in Portland, ME on September 12, 2024 while two presented at the Ornamental Workshop on Insects and Diseases in Raleigh, NC from October 22 to 24, 2024.

Consultations

Baysal-Gurel, F. 2024-2025. Consultation with 12 Tennessee nurseries to diagnose boxwood diseases.

Hong, C. X. 2024-2025. Consultations with numerous nurseries and public gardens as well as other stakeholders on boxwood blight mitigation.

Weiland, J. E. 2024-2025. Consultations with 30+ Oregon and Washington nurseries to determine prevalence of boxwood blight in the Oregon nursery industry, to collect boxwood blight samples for pathogen isolation, and to help growers manage boxwood blight outbreaks.

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 for efficiently disseminating the latest research and learning opportunities about boxwood blight.

A total of over 15,000 boxwood enthusiasts were reached through this Google group alone during this past year from September 1, 2024 to August 31, 2025. These informational messages were further distributed broadly through recipients' other national and local networks.

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 following major research components, in addition to those detailed above.

- 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, both adding momentum to the national boxwood production and inventory shifts.
- Investigations into flutriafol drench demonstrating its significantly improved coverage and lasting blight control up to 8 months.
- Evaluation of new boxwood breeding lines and accessions for blight resistance and nanocopper technology for blight mitigation as detailed under two new subobjectives 2.7 and 2.8.

Our research and extension programs supported the continual growth (*c.* 25%) of demand and sales for 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. Major accomplishments under each proposed objective are detailed below.

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 are currently validating the antibodies in an ELISA format using field-collected infected material to determine the cost-benefit of funding lateral flow devices for field detection. We have also developed and are currently validating a nucleic acid-based diagnostic Recombinase-Polymerase Assay (RPA) that is isothermal (run at a single temperature) and can be detected using inexpensive commercial lateral flow devices. Preliminary comparison of the antibody vs RPA assays indicates that RPA is more sensitive and may represent a cost-effective method for field diagnosis of the boxwood blight pathogen.

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 2026. 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 spaced more tightly (**Figure 5**) and watered more frequently. Observations about boxwood production practices obtained during the survey were used to design an experiment evaluating how plant spacing (0 or 15 cm between plants) and irrigation (plants irrigated 1, 2, or 3×/day) influenced disease severity and spread. Our results confirmed that boxwood blight was more severe and spread more quickly when plants were placed close together and irrigated more frequently. Results will be published in the BBIG Focus Issue of Phytofrontiers.

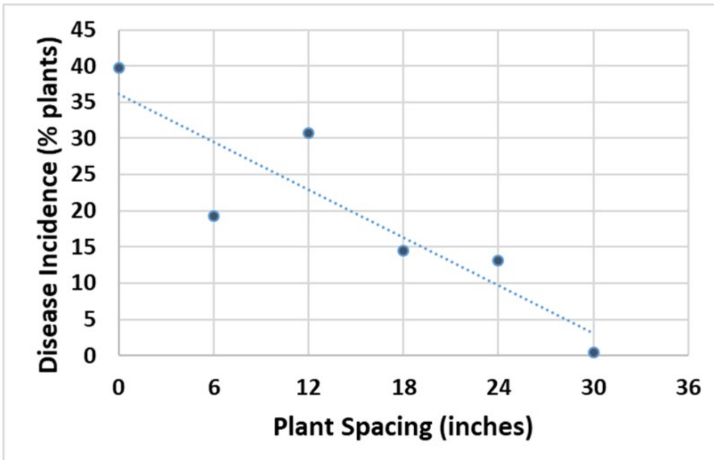


FIGURE 5 BOXWOOD BLIGHT INCIDENCE DECREASED WITH INCREASING PLANT SPACING

Isolates obtained from the survey were used to evaluate *Cps* genetic diversity in Oregon. New genotypes were discovered as reported in Phytopathology (2020), which may impact the ability of Oregon growers to control this disease. Oregon *Cps* isolates collected during the survey are also being evaluated for resistance to commonly used fungicides, as well as in IR-4 Project efficacy trials, to see if new fungicide chemistries (e.g., flutriafol) are effective for boxwood blight control.

A real-time PCR method was developed by Dr. Ohkura (postdoc in Dr. Weiland's lab) and published in Plant Disease (2023) to detect *Cps* from the boxwood samples collected at nurseries. This method was used to confirm results from the nursery samples that were assessed by moist chamber incubation and also to check for false negatives.

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). In addition, tests evaluating the effect of leaf volatiles on *Cps* germination were published in Phytopathology (2024). These results help understand *Cps* biology so that more effective control measures can be developed.

Figure 6 Boxwood blight Severity increased as temperature decreased on inoculated plants of the Resistant Cultivar Winter Gem

Last, experiments to evaluate latent infection were completed. Symptomatic and asymptomatic tissues were collected from inoculated boxwood plants, then tested with real-time PCR for *Cps*. In the canopy, *Cps* was detected only from symptomatic leaves and stems and never from asymptomatic tissues. However, in the roots where no disease developed, *Cps* was detected on asymptomatic, inoculated roots. This could suggest that latent infection could be an issue in the roots. However, it would be unlikely. Rather, it is more likely that mild canopy symptoms were overlooked during the inspection process and resulted in the unintentional movement of infected boxwood plants via nursery trade. Conclusions will be reported in the survey manuscript being prepared for Objective 1.2.

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

Research was conducted on the genetic and genomic comparisons of the boxwood blight pathogens, *Che* and *Cps*. Manuscripts are being prepared for each study.

A study on the genetic diversity of *Cps* in Oregon was evaluated through the sequencing of eleven simple sequence repeat (SSR) loci of 123 isolates collected from ten nursery populations across three counties in Oregon. A total of nine multi-locus genotypes (SSR-MLGs) were identified with 87% of isolates falling into previously identified SSR-MLGs. Five new SSR-MLGs were identified, with one new SSR-MLG occurring at a frequency of 77% among samples collected from a single nursery population. The SSR-MLG G1 occurred at a higher frequency (20%) among Oregon samples than previously reported for studied populations outside of Oregon. *Che* was absent in these ten Oregon nurseries at the time of sampling based on MAT1 idiomorph genetic typing.

To evaluate mycelial growth rate variation in response to temperature, controlled environmental experiments were conducted. Seventy-one isolates, representing each of the nine SSR-MLGs identified, were grown on malt extract agar at five different temperatures ranging from 10 to 30°C. Mycelial growth was measured at 7 and 14 days. Optimal growth for 97% of isolates examined occurred at 20°C, which is cooler than previously reported for isolates collected outside of Oregon. These findings expand our understanding of *Cps* populations in the U.S. and indicate the potential impact of seasonal differences on growth and disease development across the U.S.

Based on a comparative genomic analysis of 10 *Che* and 69 *Cps* previously published, genes were identified that were unique between species and among *Cps* putative populations. *Che* had ca. 1,000 more predicted genes than *Cps.* Among the *Che* unique predicted genes, a copper exporting ATPase that protects the fungus from host response to accumulate high phagolysosomal Cu levels during fungal infection and a carbohydrate-enzyme (CAZyme), a β-1,4-mannanase, likely involved in weakening the plant cell walls and utilizing plant material as a food source, were found. Similarly, among the *Cps* unique predicted genes, a P-type ATPase transporter involved in Cu detoxification was also found. This suggests that Cu detoxification is important for survival of *Che* and *Cps* within boxwood plants.

To identify differentially expressed genes among the two boxwood blight pathogens, three rooted cuttings of a susceptible boxwood cultivar (*B. sempervirens* × *B. microphylla* var. 'Green Velvet') were inoculated with conidia of either *Che, Cps* or sterile water for uninoculated control plants. Symptomatic leaves were collected at 4 and 10 days post-inoculation (DPI). Extracted total RNA was sent to NovoGene for Illumina sequencing on a NovaSeq X Plus instrument. Samples produced a total of 1156.4 Gb of raw data. Among the 26,137 identified genes, 4,229 were differentially expressed in plant. Most were shared between the two species with 838 and 33 genes upregulated in only *Che* or *Cps*, respectively. Four *Cps* genomic clusters were identified due to a compliment of secreted proteins that likely differentiate the groups by virulence, but more research is needed to confirm the significance of these clusters. *Che* produced 27 secreted proteins, 42 unique pathogen-host interaction genes and 70 unique CAZymes in plant. However, *Cps* was only found to produce 1 unique secreted protein, 0 unique pathogen-host interaction genes and 3 unique CAZymes. The results from this study identify potential genes and pathways used by *Calonectria* fungi that may be necessary for high virulence in boxwood plants. These results can be useful in developing rapid detection and effective management strategies for these pathogens.

<u>*1.5 Bridging the blight epidemiology knowledge gaps - cultivar and relative humidity impacts on pathogen sporulation* (Shishkoff)</u>

We knew something about the effect of temperature and leaf wetness on the development of boxwood blight caused by *Cps*, but there were epidemiological variables we did not fully understand. First, we did not know whether the second species of boxwood blight pathogen *Che* behaved similarly to *Cps*. The Foreign Disease Weed Science Research Unit is uniquely equipped to study a foreign pathogen such as *Che* in BioSafety Level 3 containment. All our studies included both pathogens. A second unknown was whether different boxwood cultivars behaved differently at different temperatures and relative humidities, or when infected with *Che*.

Our first test was to rate disease severity for the two pathogens in whole plant tests at different temperatures (12, 16, 20, 24, 28, and 32ºC) using *Buxus sempervirens* 'Justin Brouwers', a highly susceptible cultivar. Statistical analysis showed there was a significantly statistical difference between two pathogen species – *Che* and *Cps* (P<0.05) – at each temperature except 32˚C. Comparatively, *Che* is somewhat more virulent than *Cps* (data not shown), consistent with European studies previously.

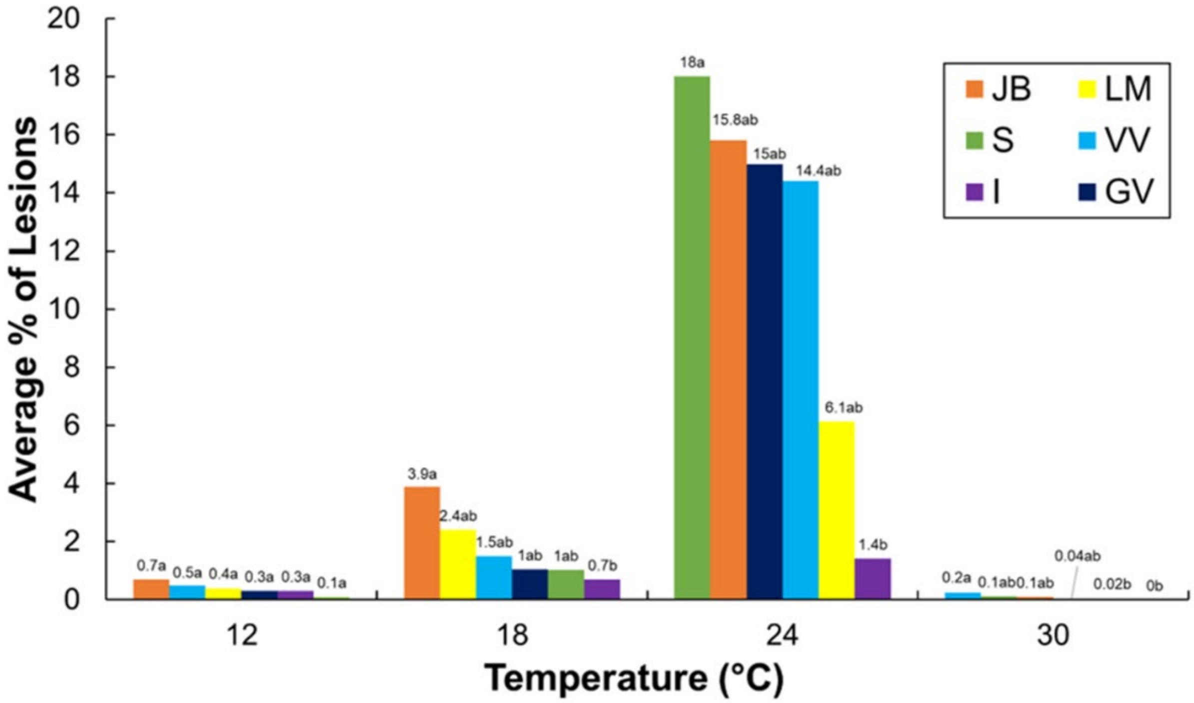


FIGURE 7 NUMBER OF LESIONS CAUSED BY *CPS* ON SIX BUXUS CULTIVARS AT FOUR DIFFERENT TEMPERATURES. JB='JUSTIN BROUWERS', LM='LITTLE MISSY', S='SUFFRUTICOSA', VV='VARDAR VALLEY', I='INDEPENDENCE', AND GV='GREEN VELVET'.

In another experiment, we used detached leaves from 6 boxwood cultivars incubated for 6 days at 12, 18, 24 and 30 ºC in growth chambers set at a 16-h photoperiod. Disease incidence and spore production were measured for each cultivar. 'Justin Brouwers' and 'Vardar Valley' had the highest incidence overall, but 'Vardar Valley' had noticeably higher incidence at 30ºC. No significant difference in disease severity was observed among cultivars at 12°C, but Independence had significantly fewer lesions than Justin Brouwers at 18ºC and Suffruticosa at 24ºC (**Figure 7**).

Based on these results, it is possible to speculate that temperature contributes to the observed variability of the response of different boxwood cultivars at different locations. 'Vardar Valley' had greater incidence and larger lesions at 30°C, suggesting it would be more susceptible in hotter climates. Other cultivars with resistance at higher temperatures did not differ in susceptibility at 12-18°C.

Relative humidity was a difficult thing to study, and we began by developing a relative humidity assay and using one cultivar to test it. We examined the effects of relative humidity on disease severity and sporulation for *Cps* and *Che* on *Buxus* x 'Green Velvet' by inoculating branches then incubating in airtight plastic boxes maintained at relative humidity levels of approx. 40, 60, 80, and 100% using specific ratios of glycerol and water. Datalog readouts demonstrated that while relative humidity could not be kept uniformly at the desired level, it was possible to maintain four discrete humidity levels over 7 days and successfully measure disease severity and sporulation on leaf tissue.

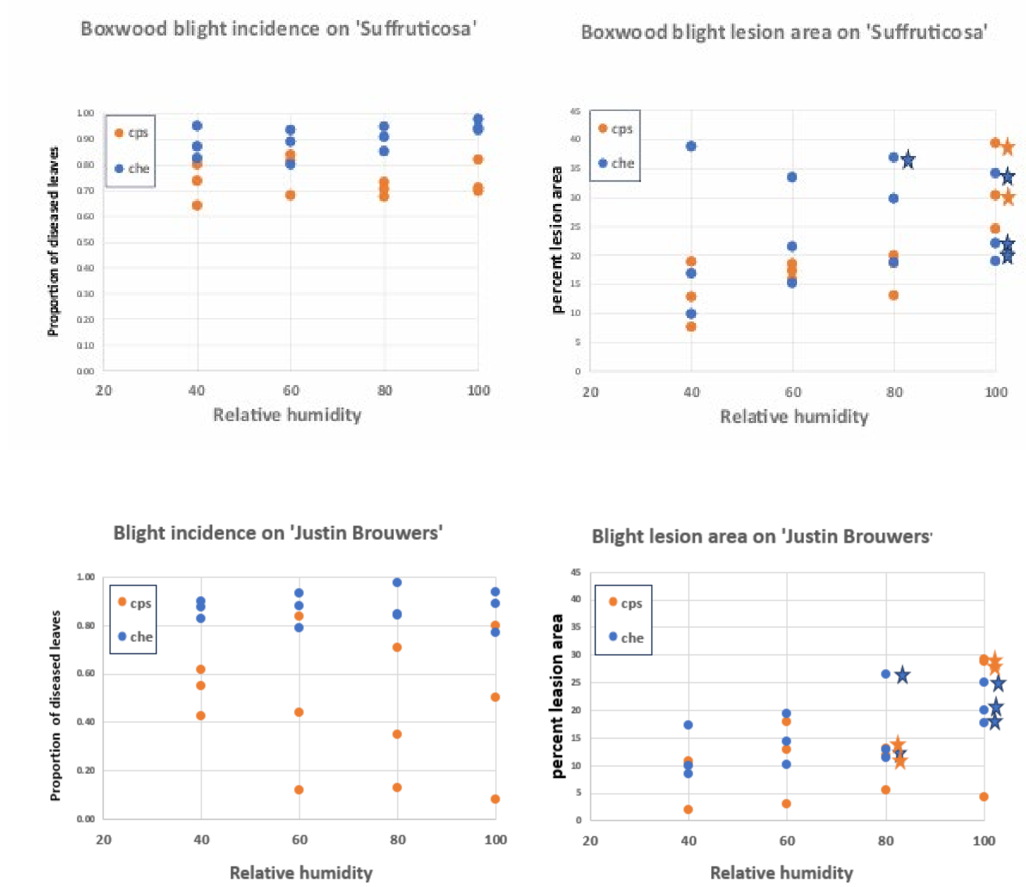


FIGURE 8 DISEASE INCIDENCE AND % LESION AREAS FOR *BUXUS SEMPERVIRENS* 'SUFFRUTICOSA' AND 'JUSTIN BROUWERS' INOCULATED WITH *CPS* OR *CHE* AT DIFFERENT RELATIVE HUMIDITIES. STAR SYMBOLS INDICATE SPORULATION WAS OBSERVED.

In previous studies, *Che* has been reported to be more virulent than *Cps*. However, nothing was known about the comparative degrees of sporulation or the effect of relative humidity on the two pathogen species. The effect of humidity on disease on different cultivars was also unknown. Therefore, at different relative humidities (100%, 80%, 60% 40%) we measured disease severity and sporulation in three replicated trials each on *B. sempervirens* 'Suffruticosa' and 'Justin Brouwers' (**Figure 8**), *B. microphylla* 'Little Missy', and *Buxus* × 'Green Mountain'. Relative humidity appeared to have little effect on disease severity but sporulation was only observed at 80-100% relative humidity. This information can be incorporated in predictive models

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

We investigated the field epidemiology of boxwood blight through three case studies.

In collaboration with German scientists, we analyzed the data from field trials conducted with *B. 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 U.S. stakeholders and the 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, which has the potential to be more adapted to the weather conditions in this country while having fewer disease management options.

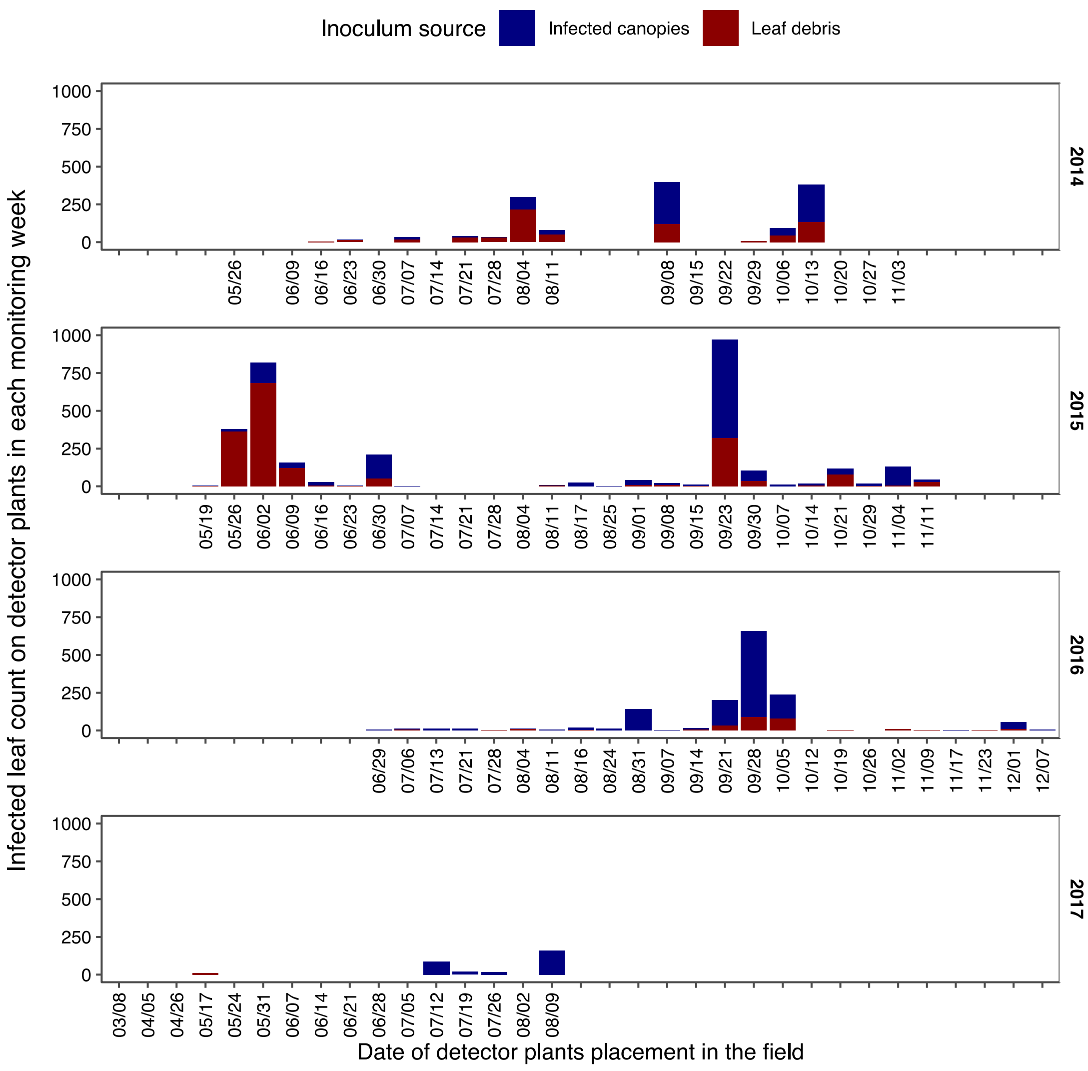


FIGURE 9 INFECTED LEAF COUNT RECORDED ON TWO-YEAR-OLD DETECTOR PLANT *BUXUS SEMPERVIRENS* 'SUFFRUTICOSA` (2014) AND 'JUSTIN BROUWERS' (2015–2017) DURING EACH MONITORING WEEK

In the second case study, we analyzed 4-year field data from weekly monitoring of boxwood blight dynamics with detector plants in Lambsburg, VA (2014) and Lowgap, North Carolina (2015-2017) – hotspots of boxwood blight, where this disease was first discovered and confirmed in the U.S. in 2011. This case study demonstrated that blight pressure was higher in late summer or early fall than other seasons (**Figure 9**).

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 10**).

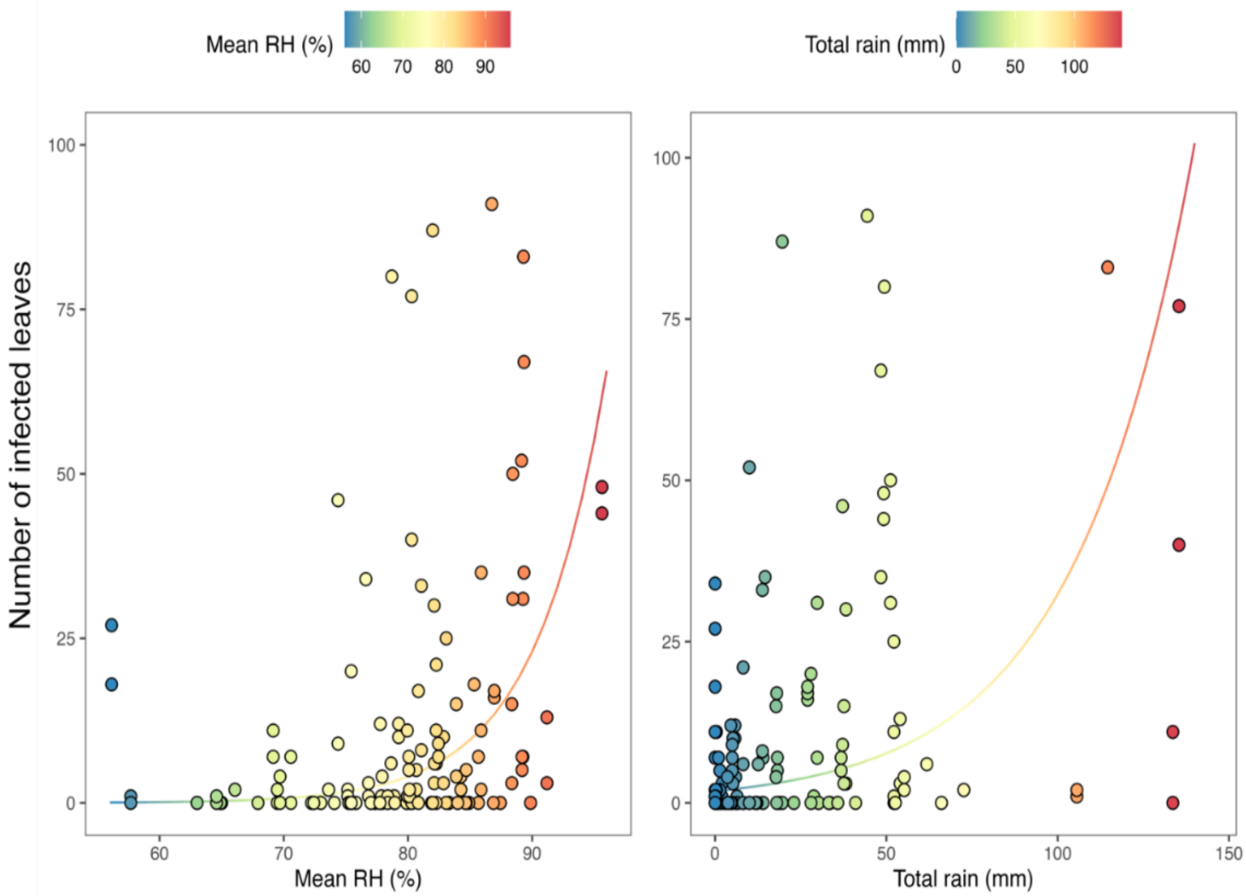


FIGURE 10 GENERALIZED LINEAR MIXED MODEL FIT SHOWING THE EFFECT OF TOTAL RAINFALL AND MEAN RELATIVE HUMIDITY OUTSIDE OF RAINY PERIODS ON THE NUMBER OF INFECTED LEAVES OF DETECTOR BOXWOOD PLANTS IN LAMSBURG, VA (2014) AND LOWGAP (2015-2017)

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 250 degree-hours is the best threshold for boxwood blight infection risk prediction. These analyses underline the importance of further calibrating and improving the infection risk model.

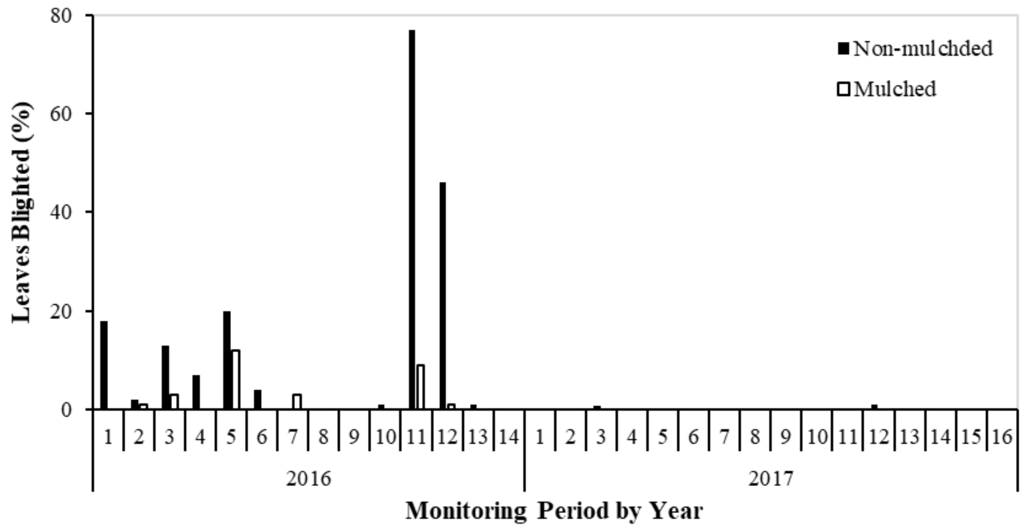


FIGURE 11 BOXWOOD BLIGHT DEVELOPMENT ON DETECTOR PLANTS AFTER 2-WEEK EXPOSURE TO NATURAL INOCULUM IN SOIL ON MULCHED AND NON-MULCHED AREAS DURING MONITORING PERIODS

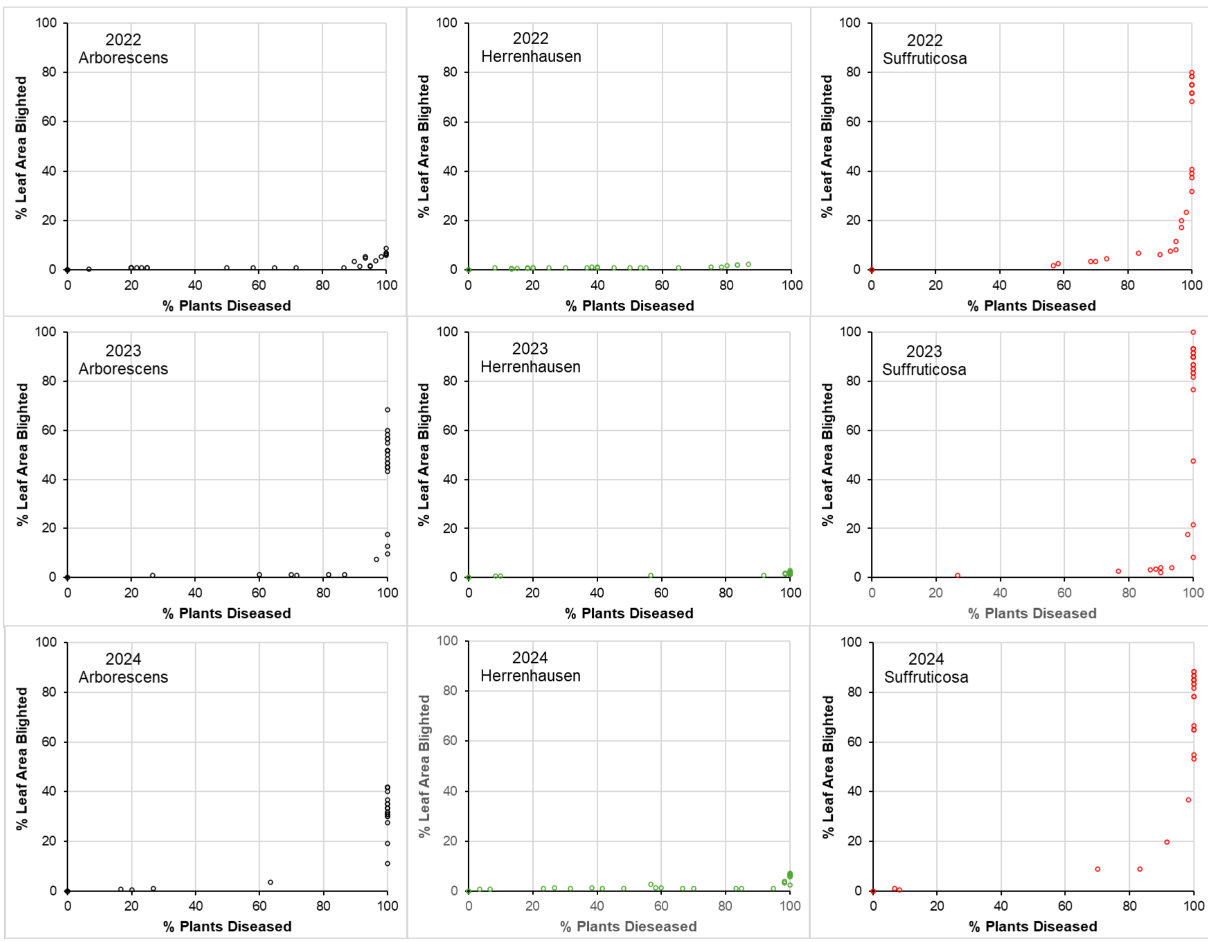


FIGURE 12 BOXWOOD BLIGHT SEVERITY (% LEAF AREA BLIGHTED) IN RELATION TO DISEASE INCIDENCE (% PLANTS DISEASED) ON THREE CULTIVARS IN EACH OF THE THREE STUDY YEARS

The third case study focused on the field data collected during a mulching study in central Virginia from 2016 to 2017 (**Figure 11**). This highlighted the importance of initial inoculum level and cultural practices in the boxwood blight epidemiology, while validating the significant weather parameters identified above. This case study has been written for inclusion in the BBIG Focus Issue of PhytoFrontiers.

This study has further been expanded to include three new yearly trials comparing boxwood blight field epidemiology on three cultivars, with *B. sempervirens* 'Suffruticosa' representing high susceptibility and *B. microphylla* 'Herrenhausen' high tolerance while *B. sempervirens* 'Arborescens' is intermediate. Again, these trials were performed in northern Germany by a collaborating team headed by Dr. Thomas Brand. Data on both disease incidence (% plants diseased) and blight severity (% leaf area affected, including fallen leaves) were collected weekly. As shown in **Figure 12**, blight severity was more sensitive a measurement than disease incidence. The blight severity data are being analyzed in relation to the local weather conditions and will be written up for inclusion in the BBIG Focus Issue of PhytoFrontiers.

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

The critical control points identified were infected plants, contaminated tools, plant debris, personal belongings according to the nursery surveys as published in HortScience (2023). The sampling conducted in representative Tennessee nurseries in 2024-2025 also revealed that Volutella stem blight and *Phytophthora* spp. were prevalent in studied nurseries.

In addition to identifying the critical control points, we did a comprehensive evaluation of sanitizers to help prevent the blight pathogen from entering the nursery production systems. These results were published in Plant Disease (2021). Additionally, we explored and adapted two novel technologies for the same purposes. The investigations into use of UV-C for blight control have been reported through a research article in Plant Disease (2024). Plasma-activated water (PAW) generated using non-thermal atmospheric plasma has gained attention as an innovative antimicrobial strategy for plant disease management. The production of reactive oxygen and nitrogen species in PAW can effectively inhibit phytopathogenic bacteria and fungi. The team started to evaluate the efficacy of PAW in controlling foliar diseases, including boxwood blight and Volutella blight on boxwood.

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

2.1 Evaluating antidesiccants for blight mitigation (Hong)

This study was performed in two phases as proposed. To expedite the process and make the research the most cost-productive, each phase of study was done at two sites with different boxwood cultivars.

The first phase of study was done in 2021 with *B. sempervirens* 'Vardar Valley' was used at site A while *B. sempervirens* 'Justin Brouwers' and *B. microphylla* var *Japonica* 'Green Beauty' at site B. Three selected antidesiccants – Vapor Gard, TransFilm and Wilt-Pruf – were applied at label rates and they were applied twice, with the first treatment on April 12 and the second one on August 25. Also included in this study were three fungicide standards – Banner Maxx (propiconazole), Daconil Weather Stik (chlorothalonil) and Concert II (both propiconazole and chlorothalonil) – and combinations ('Combos') of TransFilm with each of the three fungicides, plus one nontreated control. Three combos were also applied twice – April 12 and August 25, respectively. The three fungicide standards were applied at their respective label rates. Fungicide treatments were first applied on April 12 (the same date as antidesiccants), then repeated at recommended intervals: every 3 weeks for Banner Maxx and Concert II, and every 2 weeks for Daconil until July 5. After a 50-day summer gap or break – skipping treatments on July 19, August 2 and 16 – fungicide treatments resumed on August 25 then continued through the next 2 months. The last application of Daconil was performed on October 25 and Banner Maxx and Concert II on November 5. There were a total of twelve treatments for Daconil (7 before and 5 after the summer break) and nine treatments for Banner Maxx and Concert II (5 before and 4 after summer break). Ten treatments total, each having 7 replicates per cultivar, were arranged in a randomized complete block (RCB) design. Boxwood blight was assessed monthly. Disease data were analyzed using ANOVA Procedure of Statistical Analysis Package v9.4 (SAS Institute, Cary, NC) to determine the level of differences among treatments. Treatment means were separated according to the least significant difference (LSD) at P=0.05. All three fungicide standards reduced blight incidence but none of the antidesiccants or combos did (data not shown).

The second phase of the study was performed in 2022 at the same sites, with site B having a new planting of American boxwood instead. Both TransFilm and Wilt-Pruf were applied at three intervals – monthly, every 2 and 3 months, all starting on May 25 and continued through September 23. Vapor Gard was applied at two intervals – monthly and every 2 months. Also included in the 2022 trials were one fungicide standard – Concert II – and a nontreated control. As in the 2021 trials, each treatment had 7 replicate plants and all treatments were arranged in an RCB design for each cultivar. Likewise, blight assessment was done monthly starting in May and continued through the end of October. Phytotoxicity was observed on boxwood treated with Vapor Gard at both application intervals, thus this product was excluded from efficacy analyses. 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. Neither product applied at 2-month intervals resulted in any blight control. These data, along with those collected from controlled environment studies, are being analyzed and written for inclusion in the BBIG Focus Issue of PhytoFrontiers.

Metagenomic analyses were added to these trials to evaluate antidesiccant and fungicide impacts on phyllosphere microbial communities. For antidesiccant impact, boxwood of both cultivars were sampled three times, on June 16, August 26 and October 18. For fungicide impacts, boxwood shoots were collected over two time periods, with the first starting on May 26 and the second beginning on August 25 as pretreatment samples. Additional samples were collected at 1, 7 and 14 days after fungicide treatment. These samplings revealed the impacts of selected antidesiccants and fungicides on boxwood phyllosphere bacterial and fungal communities; these discoveries are reported through three publications in three journals – BMC Microbiology (2023), Microbiology Spectrum (2023) and Scientific Reports (2025). It is worth noting that the fungicide impact on mycobiome study provided 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 consistently suppressed in the Daconil-treated boxwood samples collected 1, 7 and 14 days after treatment in the spring (May-June), when compared to those pretreatment samples. However, this changed during the second sampling period from late August to early September. The Colletotrichum population was no longer suppressed in the 1- and 7-day posttreatment samples and it actually increased in the 14-day posttreatment samples when compared to the pretreatment samples. These results challenge a long-time assumption that multi-site fungicides like chlorothalonil are at low risk for developing resistance. Overall, these microbiome studies are foundational for developing a true systems approach to boxwood health and production while fully utilizing the potential of these agrochemicals as horticultural products and blight mitigation tools.

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

As with the antidesiccant studies above, this study was done at the same sites in North Carolina under a USDA Animal and Plant Health Inspection Service No.: P526P-21-02753.

The 2021 trials each included six selected BCAs, four combos, one fungicide standard – Concert II and one nontreated control, totaling 12 treatments. Each treatments included 7 replicate plants (**Table 1**). The twelve treatments were arranged in a RCB design. The trial was done in two different sections at site A with Vardar Valley boxwood crops having different levels of blight incidence. All BCA treatments – individuals and combos – were applied twice with the first on May 26 and the second on August 25. *Trichoderma koningiopsis* Mb2 was applied at 4×10^5 conidia/ml while individual bacterial BCAs were applied at 2 to 4×10^9 cells/ml (**Table 2**). Boxwood blight incidence was assessed monthly. Concert II reduced boxwood blight incidence in both October and November but none of the BCA treatments did (data not shown).

TABLE 1 SUMMARY OF PHASED STUDIES INVESTIGATING THE PERFORMANCE OF SELECTED BIOCONTROL AGENTS (BCAS) AND THEIR COMBINATIONS (COMBOS) AGAINST BOXWOOD BLIGHT UNDER PRODUCTION SETTINGS IN WESTERN NORTH CAROLINA

	Phase I (2021) ^{z}		**Phase II (2022)**	
Site	Elkin, NC (SW)	Elkin, NC (NE)	Elkin, NC	Lowgap, NC
Cultivar	Vardar Valley	Vardar Valley	Vardar Valley	American
Treatment total	12	12	7	7
BCA individual	6	6	4	4
BCA combo	4	4	1	1
Fungicide standard	1	1	1	1
Nontreated control	1	1	1	1

^{z} SW=southwest trial, and NE=northeast trial.

The 2022 trial included six treatments – four best performing BCAs and one combo from the previous year, plus a fungicide standard and a nontreated control (**Table 1**). This trial was done at site A with Vardar Valley and site B with American boxwood. The first application of all treatments was applied on May 25. Biocontrol treatments were repeated monthly until September 23 while Concert II repeated at 3-weeks interval until October 5 (**Table 2**). Boxwood blight incidence was assessed right before the first treatment and repeated monthly until late October. All five selected BCAs consistently reduced boxwood blight on both cultivars during the October assessment. Specifically, the best performer was *Burkholderia* sp. SSG (**Figure 13**). BCA control efficacies were only slightly lower than those by the fungicide standard – Concert II.

TABLE 2 BIOCONTROL AGENTS (BCAS) AND THEIR COMBINATIONS (COMBOS) EVALUATED WITH TWO BOXWOOD CULTIVARS AT TWO SITES IN WESTERN NORTH CAROLINA

Species	Isolate	Concentration	2021 [x]	2022 [y]
Trichoderma koningiopsis	Mb2	4×10^5 spores/ml	Twice	
Bacillus velezensis	BP10-2-4	2 to 4×10^9 cells/ml	Twice	Monthly
Burkholderia sp.	SSG	2 to 4×10^9 cells/ml	Twice	Monthly
Pseudomonas lactis	SW	2 to 4×10^9 cells/ml	Twice	Monthly
P. protegens	13A3	2 to 4×10^9 cells/ml	Twice	Monthly
P. protegens	14D5	2 to 4×10^9 cells/ml	Twice	
Combo	SSG+13A3	½ of each component applied alone [z]	Twice	
Combo	SSG+14D5	½ of each component applied alone	Twice	
Combo	SSG+Mb2	½ of each component applied alone	Twice	Monthly
Combo	SSG+SW	½ of each component applied alone	Twice	
Fungicide standard	Concert II	½ of each component applied alone	Every 3 weeks	Every 3 weeks
Nontreated control		-	-	-

[x] Twelve treatments were included in the 2021 trials, with the first application of all biocontrol agents (BCAs) on May 26 and the second application on August 25, while the first application of fungicide standard started on the same date and continued at 3-week intervals.

[y] Seven treatments were included in the 2022 trials, with the first application of all treatments on May 25. Biocontrol treatments were repeated monthly with the last on September 23. Concert II was repeated at 3-week intervals with the last application on October 5.

[z] Concentration of individual components in combos was half of that in single BCA treatments.

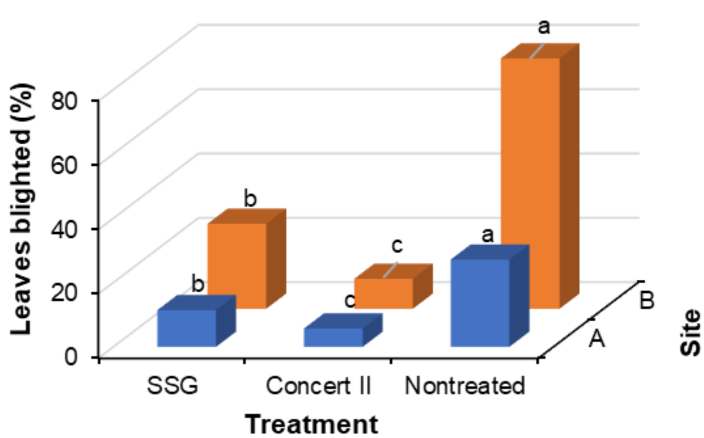


FIGURE 13 BOXWOOD BLIGHT DEVELOPMENT ON VARDAR VALLEY AND AMERICAN BOXWOOD PLANTS TREATED MONTHLY WITH THE BEST PERFORMING BIOLOGICAL CONTROL AGENT – *BURKHOLDERIA* SP. SSG OR CONCERT II IN COMPARISON TO NONTREATED CONTROL PLANTS AT SITES A AND B

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, as published in BMC Microbiology (2024).

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

A question remained regarding whether boxwood's resistance to boxwood blight could be improved through the application of materials with an SAR (systemic acquired resistance) effect. Such materials divert the plant's energy to defense rather than growth, so they would be expected to be more valuable in the landscape than in a nursery setting. We chose to focus on one of the best-studied SAR inducers, acibenzolar-S-methyl (A-S-M), sold as Actigard, because it is registered for some horticultural uses. We also chose to work with boxwood of naturally lower susceptibility to boxwood blight, as these are the plants now being used in the trade, due in part to BBIG's educational efforts. *B. sempervirens* 'Green Mountain' (moderately susceptible) and the NewGen Boxwood® Independence (highly resistant) were propagated from cuttings in 2024 and grown into small plants in 3-inch pots for a 2025 experiment on the effect of applications of an SAR material on susceptibility to *Cps*.

Actigard was evaluated at three per-plant drench volumes of 30, 60 and 240 ml, all at 2.0 oz/100 gal. Also included were Reliant (mono- and di-potassium salts of phosphorous acid, a phosphite which has SAR properties) 0.5% solution drenched at 236 ml/plant, and Propiconazole as Banner MAXX II EC (a standard fungicide used in rotations for boxwood blight management in the landscape), with 8 oz/100 gal as a spray, plus a nontreated/noninoculated control (Water spray), and a nontreated/inoculated control. Chemical treatments were applied on June 26.

Two weeks later, on 10 July, plants were inoculated with 10^4 spores/ml with a spray nozzle, applying approximately 1/2 ml per plant in two pulses. Inoculum was produced on 1/2 strength potato dextrose agar by inoculating each plate with 30 droplets containing spores; spores for plant inoculum were harvested by rubbing gently with a glass rod immediately after flooding each plate with 5 ml of sterile deionized water containing 2 drops of Tween 80/liter. Inoculum was brought up to 125 ml volume to use with a spray nozzle set into a 125-ml flask. Plants were set in individual plastic bags, inoculated, and incubated indoors to avoid high greenhouse temperatures known to be lethal to the pathogen. Temperature ranged from 23-24°C during the trial. Boxwood were arranged in 7 single plant replications of each of the two cultivars for the 7 treatments in a RCB design. Each plant was removed from its bag for an assessment of symptoms of blight 11 days later, on July 21, attempting to separate boxwood blight symptoms from Volutella blight symptoms visually. Plants were rebagged and reinoculated as before on July 22, after 1 ml of water was added to each plastic bag to maintain a high relative humidity. Boxwood were incubated for an additional two weeks and then were removed from bags for a detailed assessment of infection on August 8. Each symptomatic leaf was removed and sporulation was scraped off with a toothpick and examined with a compound microscope at 200X to differentiate sporulation of *Cps* from that of a *Pseudonectria* sp. that causes Volutella blight.

The number of leaves per plant with each of the two pathogens was recorded, as well as the number of cankers bearing sporulation of one of the pathogens. All data were analyzed to determine the difference among the treatments, and treatment were separated according to Tukey's HSD at P=0.05. *Pseudonectria* sporulation was found much more often than *Cps* sporulation in the trial. Foliage of 'Green Mountain' boxwood given propiconazole or the lowest level of A-S-M was less diseased with *Pseudonectria* sp. than the inoculated controls, while treatments of ‘Independence' showed less *Pseudonectria* sp. in the highest rate of A-S-M and propiconazole treatments. *Cps* foliar infection was not significantly different for either cultivar in any treatment. The number of cankers due to *Pseudonectria* were reduced by all but the Reliant treatment for 'Green Mountain', and only by the high A-S-M and propiconazole in Independence boxwood. SAR treatment has thus shown some potential benefit for management of Volutella blight but not boxwood blight on moderate- and low-*Cps*-susceptibility cultivars. Effects of A-S-M treatment rates appear to be somewhat cultivar-specific.

Future studies will include additional exploration of SAR effects on the highly *Cps*-susceptible *B. sempervirens* ’Suffruticosa’ as well as testing for infection predisposition on ‘Green Mountain’ across a gradient of dolomitic lime and fertilizer levels.

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

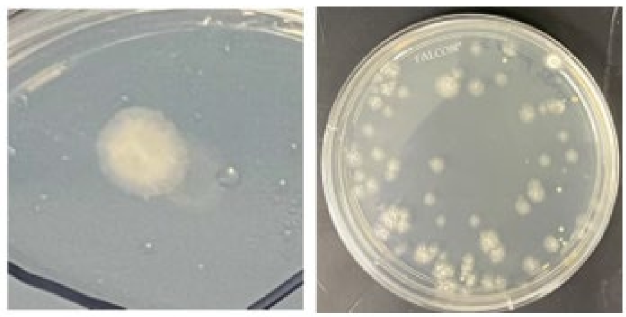
Two independent experiments of 10-week treatments were performed to evaluate the effects of Si treatment on boxwood blight incidence. Two-year old plants of two cultivars, *B. microphylla* ‘Little Missy’ and *B. sempervirens* ‘Suffruticosa’ were treated and evaluated on the effect of continuous Si soil 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 on the *B. sempervirens* but not on 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 demonstrated that Si reduced the 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/cm^2 at 30 cm was used to calculate the different doses, the formula for UV-C dosage is: Intensity (mW/cm^2) x Time (seconds) = Dose (J/m^2). The dosages selected were 150, 350, 500, 1000, and 2000 J/m^2, respectively. The growth chamber was 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 a.m. and off at 5:00 pm. The summary of experiments is given in **Table 3**.

TABLE 3 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 potato dextrose agar (PDA) plates. For spreading, 25µl and 50µl conidia (10^4 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 were observed in both types of inoculation (**Figure 14**).



A B

FIGURE 14 25µL OF CONIDIA OF *CPS* SPOTTED (A) AND SPREAD (B) ON POTATO DEXTROSE AGAR PLATES YIELDED COLONIES AND MYCELIAL GROWTH AFTER 4 DAYS OF INCUBATION

Starting from 100 J/m^2, all doses suppressed fungal growth on PDA on 4th day of growth in first and second experiments (**Figure 15**). 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 16**).

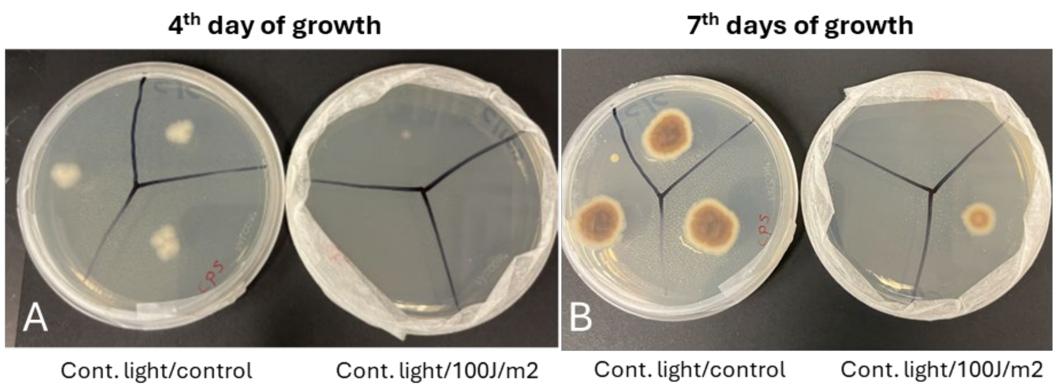


FIGURE 15 GROWTH OF *CPS* IN CONTROL AND UV-C TREATED PLATES AFTER 4 (A) AND 7 DAYS (B).

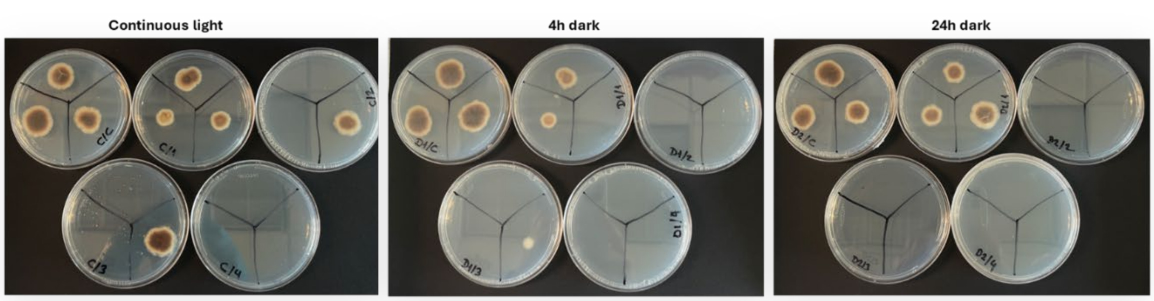


FIGURE 16 GROWTH OF *CPS* AFTER SEVEN DAYS UNDER CONTINUOUS LIGHT, 4 HOURS OF DARK INCUBATION, AND 24 HOURS OF DARK INCUBATION AFTER UV-C TREATMENT ALONG WITH CONTROL PLATES THAT RECEIVED NO UV-C TREATMENT

UV-C treatment beyond the maximum dosage of 1,000 J/m^2 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/m^2 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 17**).

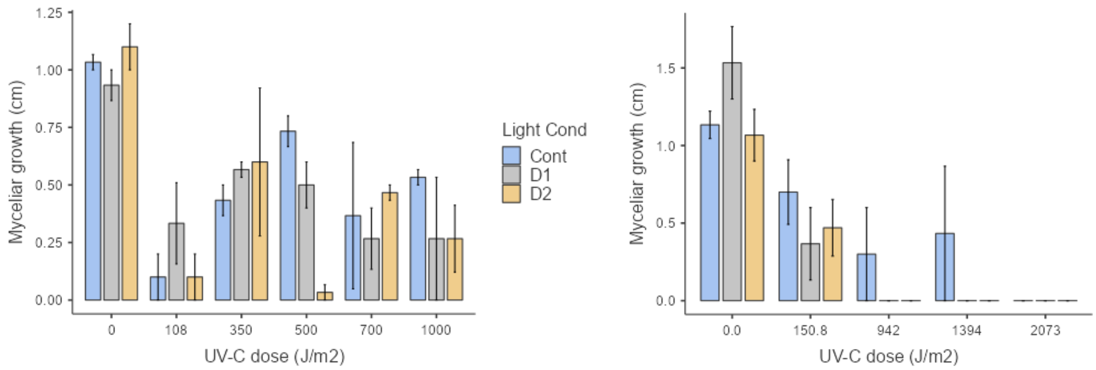


FIGURE 17 SUPPRESSION OF *CPS* GROWTH BY UV-C TREATMENTS. A 2-WAY ANOVA SHOWED THAT EACH DOSE OF UV-C SIGNIFICANTLY INHIBITED GROWTH COMPARED TO CONTROL (P<0.001) (N=3).

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/m^2 to 2,000 J/m^2.

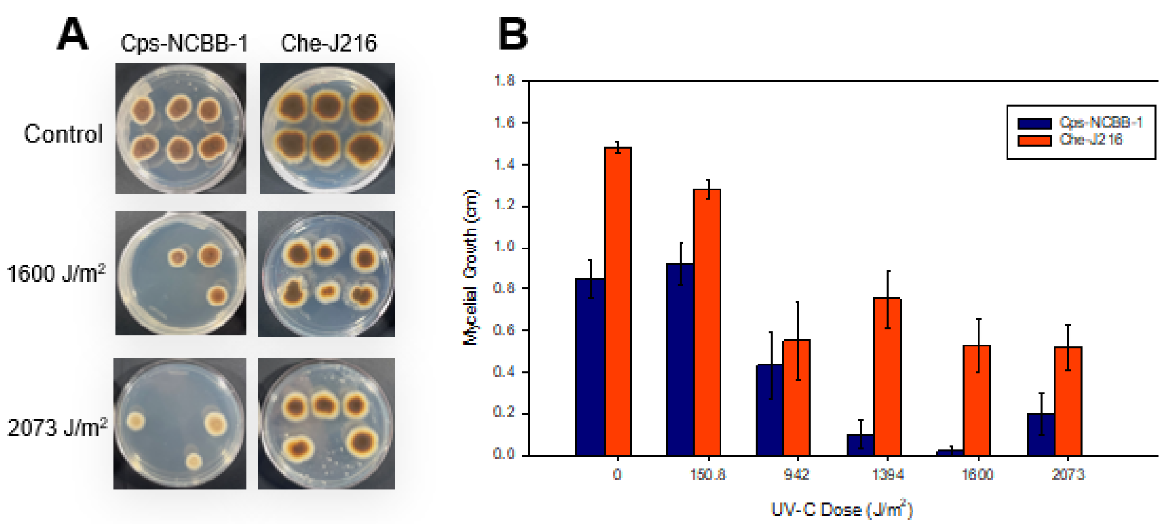


FIGURE 18 MEAN LESION SIZES AND THEIR 95% CONFIDENCE INTERVALS FROM DETACHED LEAF ASSAYS OF EACH CULTIVAR INOCULATED WITH *CPS* OR *CHE*. POINTS REPRESENT THE AVERAGE LESION SIZE MEASURED 7 DAYS AFTER INOCULATION

We recently looked at mitigating the conidia number and further germination of both boxwood blight pathogens – *Cps* and *Che* – which have been shown to be inactivated significantly by UV-C doses of 942-2073 J/m^2 verified by the reduction in both CFU and mycelial growth (**Figure 18**). We also demonstrated that UV-C had no or minimal effects on leaf morphology and quality, which have an impact on the marketability of boxwood cuttings (**Figure 19**). Effectiveness of UV-C was evaluated on detached leaves inoculated by conidia suspension in 2 different seasons. Higher doses than 1600 J/m^2 resulted in smaller brown spot occurrence while there were slight differences in treatments due to leaf age and seasons. Next, we performed a whole plant assay using cuttings sprayed by conidia on abaxial sides and administered 2000 J/m^2 UV-C twice weekly to see the effect of UV-C on symptoms. A four week application of UV-C at dark conditions led to decreased percent disease index (PDI) values in both 'Wintergreen' and 'Suffruticosa' cultivars.

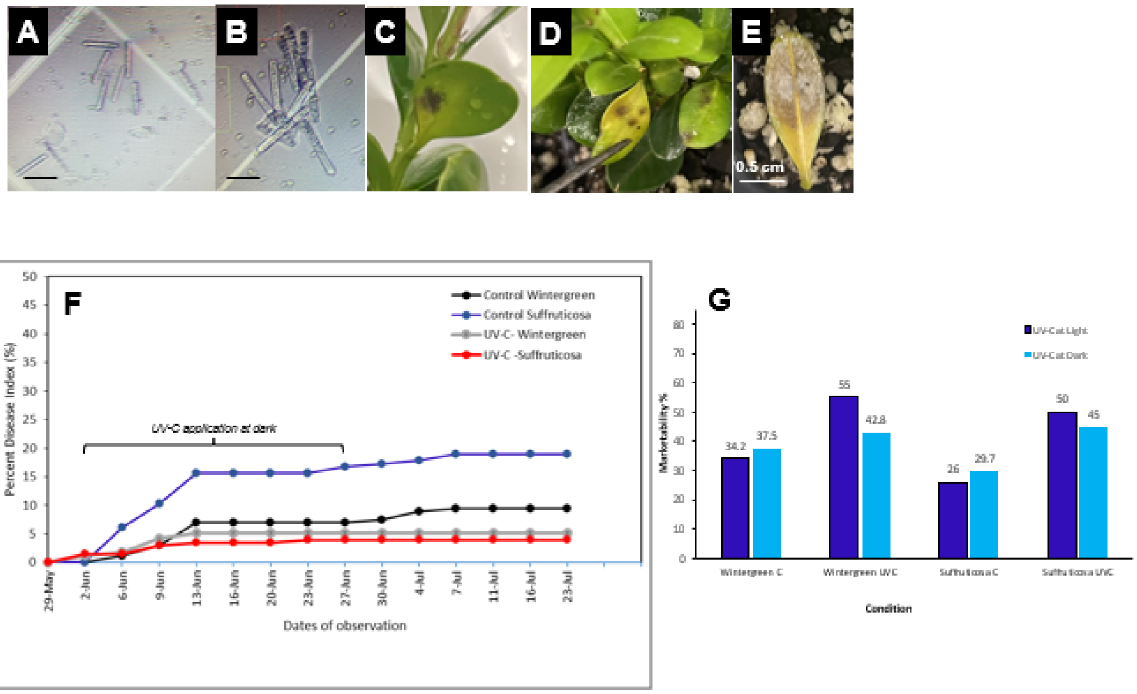


FIGURE 19 IN-PLANTA ASSAY WITH B. SEMPERVIRENS 'SUFFRUTICOSA' AND B. KOREANA 'WINTERGREEN': CONIDIA OF CPS AS INDIVIDUAL (A) AND IN CLUMPS (B) IN SPORE SUSPENSION BEFROE INOCULATION (C-E) AFTER INOCULATION. (F) % DISEASE INDEX (PDI) DURING AND AFTER UV-C APPLICATION. (G) PROPORTIOIN OF PLANTS THAT WOULD BE CONSIDERED MARKETABLE AFTER INOCULATION AND UV-C TREATMENT.

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. *B. microphylla* 'Tide Hill' (less susceptible) and *B. 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)

We noticed that there was no document showing the different paths of spread of the boxwood blight pathogen in the landscape, so we prepared an illustration based on the current knowledge (**Figure 20**) and distributed it widely, in extension documents and in presentations to stakeholders.

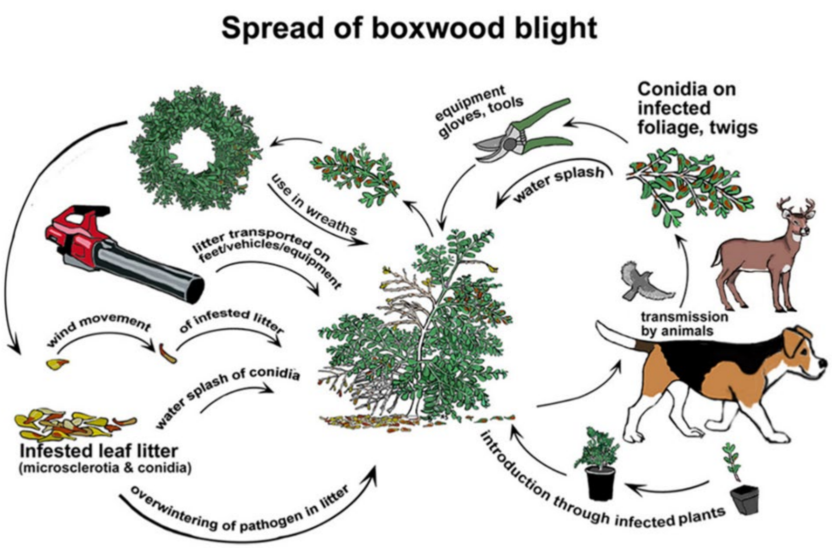


FIGURE 20 THE SPREAD OF BOXWOOD BLIGHT IN THE LANDSCAPE

We also thought it would be useful for workers in the nursery industry to have photographs of the symptoms of boxwood blight on different cultivars and at different times after infection. We took over five hundred pictures of symptoms on 12 cultivars for use in outreach presentations and in extension education materials as illustrated with *B. sempervirens* 'Vardar Valley', *B. harlandii*, and *B. microphylla* 'Golden Dream' (**Figure 21**). They were also prepared for use with machine learning programs designed to recognize boxwood blight symptoms. Photographs of symptoms were taken for cultivars 'Justin Brouwers', 'Golden Dream', 'Green Velvet', 'Little Missy', 'Vardar Valley''Richard', 'John Baldwin', and 'Dee Runk' at 24, 48, 72, 96 and 120 hr after inoculation (**Figure 22**).

FIGURE 21 BOXWOOD BLIGHT SYMPTOM VARIATIONS AMONG BOXWOOD SPECIES AND CULTIVARS INOCULATED WITH *CPS*

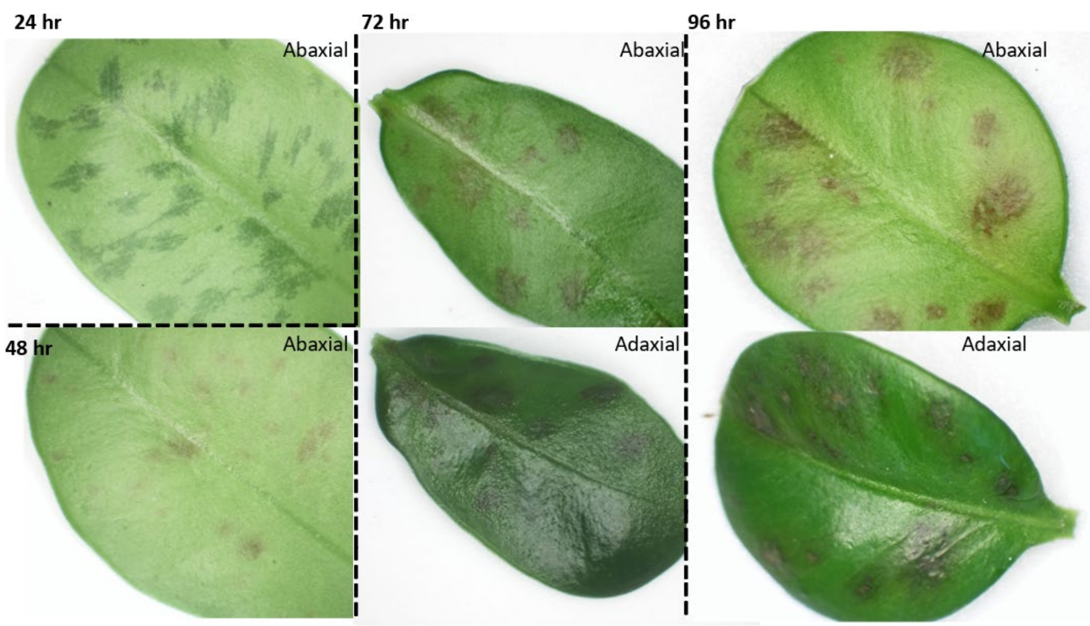


FIGURE 22 *BUXUS SEMPERVIRENS* 'DEE RUNK' INOCULATED WITH *CALONECTRIA HENRICOTIAE* AND PHOTOGRAPHED 24, 48, 72 AND 96 HOURS AFTER INOCULATION

2.7 Assisting breeders in the identification of durable boxwood blight resistant cultivars (Westrick)

Disease resistance is a critical tool in managing boxwood blight, given its capacity to rapidly defoliate and kill susceptible cultivars. Developing and deploying resistant varieties reduces reliance on chemical controls, mitigates disease spread, and supports sustainable landscape and nursery production. Given that importance, we have collaborated with commercial *Buxus* breeders from across the United States to screen genetically novel breeding lines for their susceptibility to this important disease. For the last 6 years we have taken replicates of early breeding lines from these programs and inoculated them in 3-inch pots with *Cps.* Controls including highly resistant (JNI *B. sinica* var. *insularis* 'Wintergreen' and/or NewGen Boxwood® Liberty Belle), and highly susceptible (*B. sempervirens* 'Suffruticosa' and/or *B.* × Green Velvet) accessions were included in all evaluations. Lesion counts and defoliation of all plants were collected several weeks after initial inoculation and the efficacy of the disease screen was determined through the response of control plants. This additional work has resulted in the identification of dozens of promising lines exhibiting resistance levels comparable to or exceeding industry standards such as Wintergreen and NewGen Liberty Belle. This work has been valuable in providing new options for growers seeking durable, high-performing cultivars.

2.8 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 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 were applied throughout Summer 2025 and relative levels of disease are being recorded. Additionally, 125 GV and WG plants have been established in 1-gallon pots for a replicate of this trial in the greenhouse Spring of 2026.

Initial results from the nanocopper treatments show the surprising result that when applied to inoculated susceptible plants (Green Velvet), specific concentrations can give control comparable to the positive control (propiconazole) (**Figure 23**). These results, specifically the suppression of disease at 50 ppm nanocopper but not higher doses, seem to suggest that the disease suppression is not dose-responsive. While initially unintuitive, this finding is conceptually plausible given findings from the Connecticut Agricultural Experiment Station that nanocopper is a potent inducer of the plants defense system and, unlike elemental copper, does not appear to be meaningfully antifungal in in-vitro testing with *Cps.* This suggests that, unlike elemental copper which is a fungicidal protectant, nanocopper may be suppressing disease through the systemic induction of plant defenses. The greater disease seen at high nanocopper concentrations (100-200 ppm) is consistent with results from other pathosystems that over-induction of defenses can "exhaust" plants in a way that can be deleterious for disease management. Future work will focus on the fine-tuning of this nano-copper application rate and the incorporation of other defense inducers, such as phosphite products, into boxwood blight disease management.

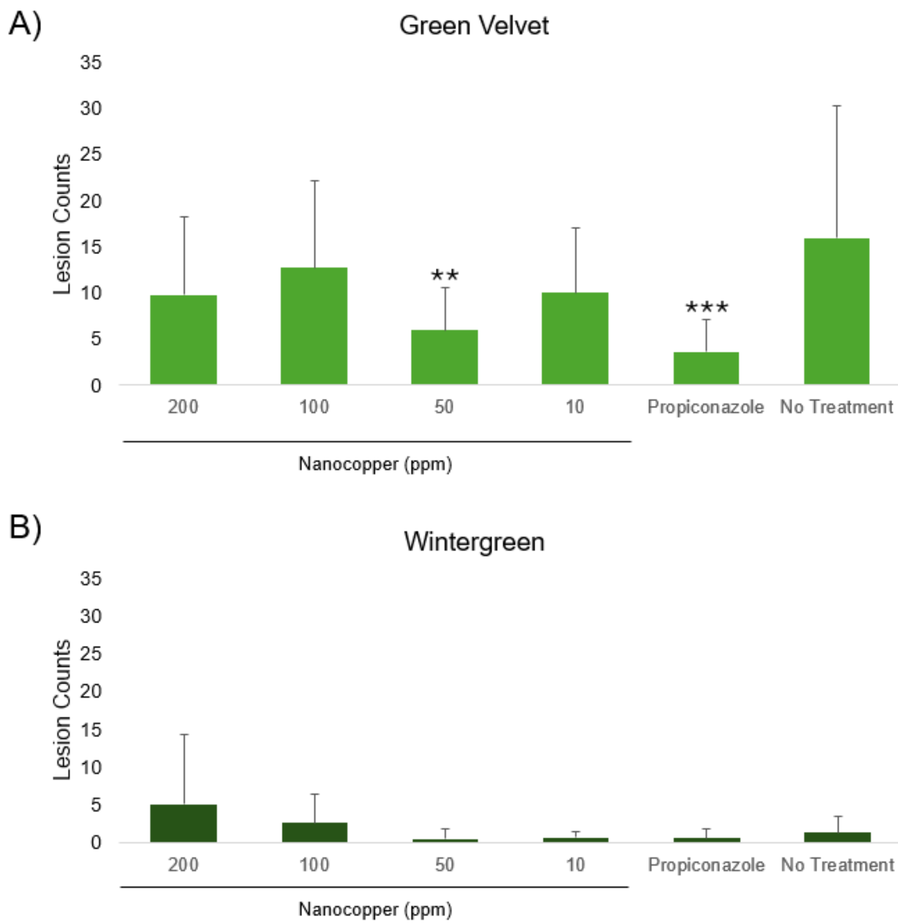


FIGURE 23 NANOCOPPER TREATMENTS TO CONTROL BOXWOOD BLIGHT ON A0 GREEN VELVET, AND B) WINTERGREEN. P-VALUE: *<0.05, **<0.01. AND ***<0.001.

Objective 3: To build resilience into boxwood production and plantings

3.1 Developing more resistant boxwood cultivars (Gouker and Brindisi)

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. Preliminary differential expression analysis has been conducted on the sequencing data showing expressed genes strongly clustered between the two boxwood species and weakly clustered by the blight pathogen - *Calonectria* spp. (**Figure 24**).

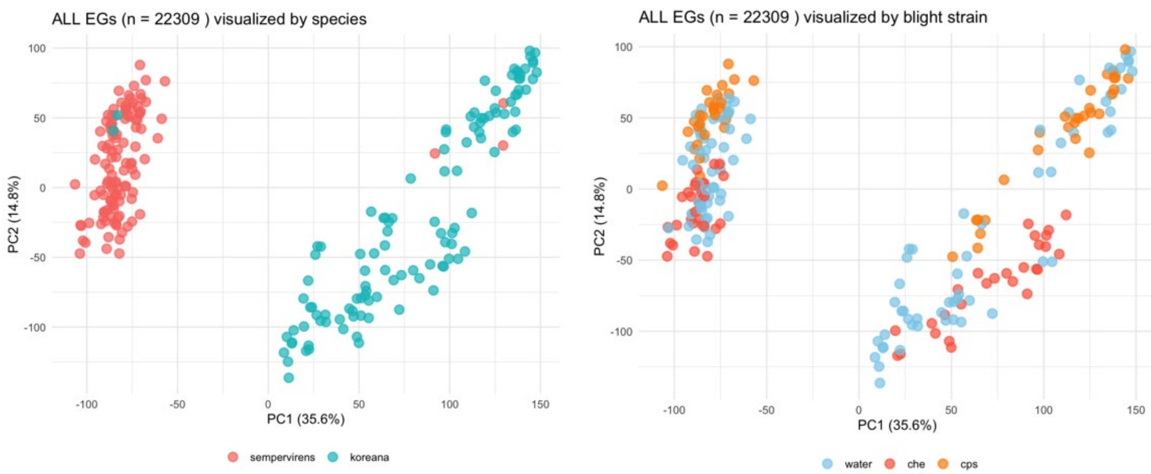


FIGURE 24 PCA PLOTS OF EXPRESSED GENES BY BOXWOOD SPECIES (A) AND THE BLIGHT PATHOGEN SPECIES (B).

So far we have noticed there are two distinct sets of DEGs in common spread across timepoints 2 to 12 hr post inoculation and 48 to 96 hr post inoculation, with common overlap of upregulated and downregulated DEGs between the two species. Further analysis of gene ontology (GO) terms shows several significant genes relating to bacterial and fungal defense responses as well as pathogen-associated molecular pattern recognition. Additional analysis is ongoing to look at candidate genes from specific timepoints with DEG enrichment for defense responses and hormone signaling relating to susceptible (S) genes and defense suppression.

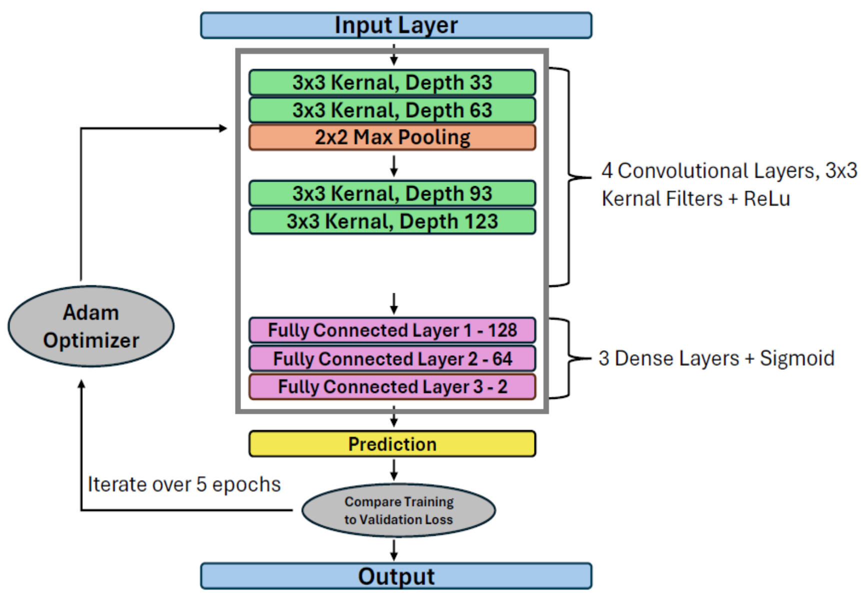


FIGURE 25 BLOCK DIAGRAM SHOWING THE USED DATASETS AND DIFFERENT APPLIED METHODS FOR CREATING THE CUSTOMIZED CONVOLUTIONAL NEURAL NETWORK (CNN)-BB TRAINING FRAMEWORK.

A total of 1.5 TB whole genome sequence data (PacBio and Omni-C sequencing) for *B. sempervirens* ‘Suffruticosa’ and transcriptome data from the 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.

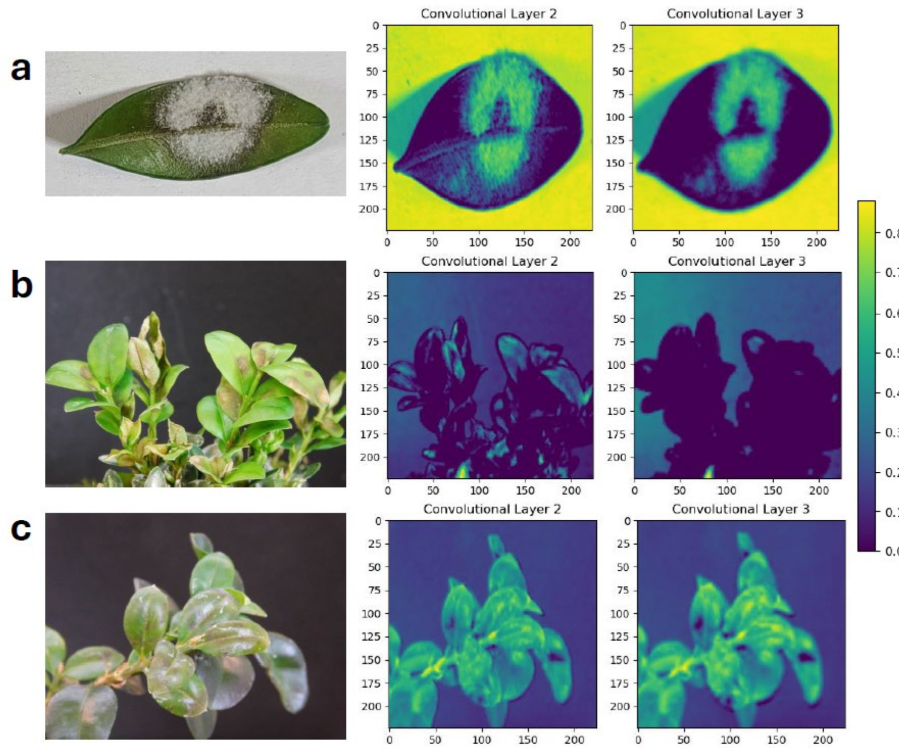


FIGURE 26 COMPARISON OF TEST SET OUTPUT FOR BLIGHT-INFECTED BOXWOOD PLANTS USING THE TRAINED CONVOLUTIONAL NEURAL NETWORKS (CNN). THE ORIGINAL IMAGE IS SHOWN ON THE FAR LEFT FOR A) AN INOCULATED SINGLE LEAF AND B-C) A WHOLE PLANT WITH LESIONS.

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 25**) 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 26**). 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 is currently under peer review.

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 27**) 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 27 LEAVE 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 x 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 28**).

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 composition for cell regeneration.

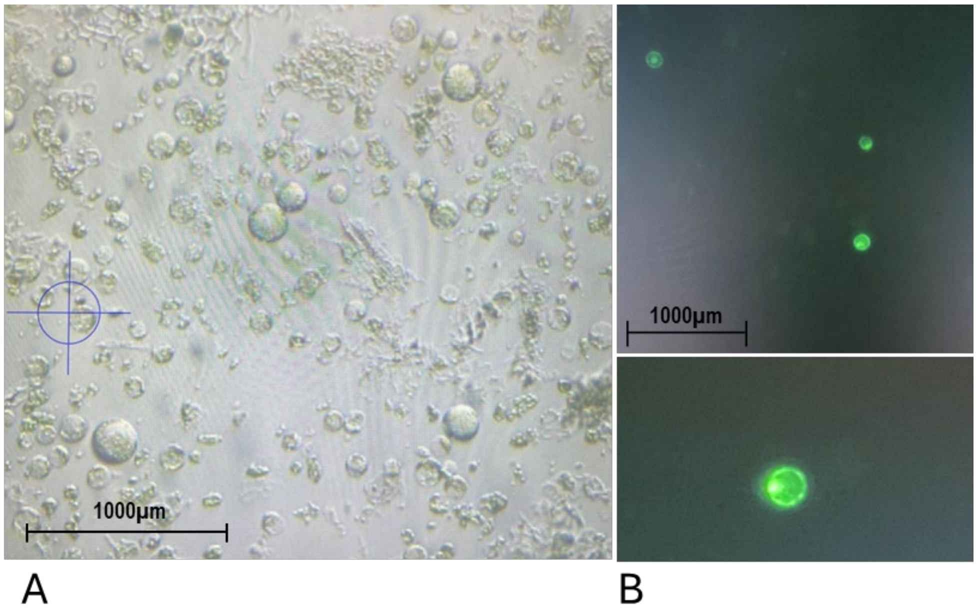


FIGURE 28 A. BRIGHTFIELD IMAGE OF WINTERGREEN PROTOPLASTS AFTER WASH WITH W5 BUFFER; B. CELLS EXPRESSING YFP IN TRANSFECTED PROTOPLASTS VISUALIZED UNDER GREEN FILTER AFTER 12H INCUBATION WITH FMV-EYFP (ZEISS AXIO ZOOM V16).

3.2 Cataloging and manipulating boxwood microbiomes against Cps (Kong)

In addition to the discoveries presented under Obj. 2.1 above, this study examined the phyllosphere microbiome of several major boxwood cultivars across distinct geographic locations, uncovering important evidence supporting boxwood as a low maintenance crop. 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 published in PhytoFrontiers (2024) are foundational to developing microbiome-based breeding programs to enhance boxwood resilience to diseases.

Likewise, a large variety of mycorrhizae and other beneficial microbes were identified from the root zone of multiple boxwood species and cultivars across locations. This finding further supports boxwood as a low maintenance crop as reported in a manuscript being currently considered for publication in BMC Plant Biology.

3.3 Facilitating adoption of more resistant boxwood cultivars (Hall)

This research involving a mixture of experimental methods measured 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 were determined through net present value analysis in objective 4.6.

Nayak and Campbell (2024) provide one of the first comprehensive studies examining U.S. consumer awareness and opinion regarding boxwood shrubs and boxwood blight. Using a national sample of 2,795 adult respondents obtained via a web-based panel, the authors conducted a controlled survey experiment wherein participants viewed images of healthy and blight-infected boxwood before responding to a series of attitudinal and knowledge-based questions. The analysis incorporated descriptive statistics, chi-square tests, t-tests, and a Tobit regression model to quantify how socio-demographic characteristics influence consumer perceptions before and after exposure to information about boxwood blight.

The study found that consumer awareness of boxwood shrubs was highest among suburban residents, likely reflecting their greater exposure to boxwood in city-maintained or commercial landscapes. By contrast, urban respondents expressed the most favorable pre-treatment opinion of boxwood, with a mean rating of 70.79 on a 0–100 scale, compared to 65.05 for suburban and 63.26 for rural residents. After being shown visual evidence of blight and receiving a brief informational treatment, all three groups exhibited significant declines in their overall opinions. The post-treatment ratings declined to 56.41 for urban respondents, 47.50 for suburban, and 46.6 for rural, suggesting that increased awareness of plant disease can substantially alter consumer preferences.

The Tobit regression results further revealed nuanced demographic effects. Younger generations (Gen Z, Millennials, and Gen X) demonstrated less reduction in their post-treatment boxwood ratings compared to Baby Boomers. Men retained more favorable opinions than women after the blight treatment, a finding potentially explained by greater involvement in landscape maintenance activities among men (Bureau of Labor Statistics, 2023). Education also played a role: respondents with graduate degrees rated boxwood more favorably than those with some college education. Notably, Caucasians exhibited a greater negative shift in opinion post-treatment than non-Caucasians, possibly due to higher prior ownership or familiarity with the shrub.

These findings hold practical implications for the horticultural industry, especially as it seeks to manage the dual challenges of disease control and consumer engagement. As the authors note, communication strategies tailored to specific demographic segments—particularly suburban residents and older consumers—could be essential for mitigating negative perceptions and maintaining demand. Additionally, the authors suggest that future work should explore consumer preferences for alternative or disease-resistant cultivars. This is supported by recent findings indicating increased sales of less-susceptible boxwood varieties such as 'Winter Gem', 'Independence', and 'Little Missy' following disease outbreaks (Omolehin et al., 2023).

Overall, the study by Nayak and Campbell (2024) advances our understanding of how public awareness intersects with plant health issues in the ornamental horticulture sector. By combining rigorous statistical methods with a nationally representative sample, the authors offer valuable insights into consumer behavior that can inform both industry practices and extension outreach. Their work underscores the importance of integrating social science perspectives into plant pathology and horticultural marketing to enhance the resilience of the green industry in the face of emerging biotic threats.

Understanding how consumers value plant health traits has become increasingly important as breeders invest in resistance genes to safeguard ornamental crops. Early market research suggested that homeowners intuitively appreciated "trouble free" plants, yet only over the past decade have economists begun measuring the monetary premium that buyers attach specifically to improved disease resistance, whether delivered through conventional breeding or modern biotechnologies. Across this growing body of work, the most robust evidence comes from discrete choice experiments that convert stated preferences into willingness to pay (WTP) estimates; complementary perception surveys illuminate how resistance claims influence likelihood of purchase (LTP) when prices are not shown.

Analyses centered on garden roses provide the clearest benchmark. Using a nationwide online choice experiment that isolated five attributes, Chavez and colleagues estimated that U.S. consumers would pay on average between US $9.90 and $13.40 more per plant for “disease tolerant” cultivars, a premium second only to heat tolerance and markedly higher than that for larger blooms or additional foliage cover (Chavez et al., 2020). A follow on study within the “Combating Rose Rosette Disease” project incorporated eye tracking to verify attribute attention and found comparable WTP values (US $8.46–13.21) for a bundled adaptation trait that emphasized heat, drought, and pathogen resilience; when projected to market scale, a certified RRD resistant cultivar yielded an estimated 26.9 % grower level price lift and annual national welfare gains of about US $35 million even at modest adoption rates (NIFA, 2024). These investigations collectively demonstrate that resistance traits are not a niche concern but a primary driver of value in high profile ornamentals.

Evidence for other woody ornamentals now corroborates the rose findings. An Italian discrete choice experiment on *Abelia × grandiflora* showed that consumers were willing to pay roughly 20 % more—about €1.10 per plant—for stock guaranteed free of quarantine pathogens, whereas sustainable production practices attracted a smaller €0.90 premium (Frem et al., 2024). The latent class model identified a segment in which plant health assurances outweighed all eco labels, underscoring that clear diagnostic information can shift valuation even in a Mediterranean market long accustomed to ornamental imports.

Perception work on boxwood further illustrates how exposure to disease imagery shapes purchase intent. A 2024 national survey revealed that viewing photos of blight symptoms significantly reduced stated liking among Caucasian respondents, while men nonetheless remained more willing to purchase the shrub than women; the authors concluded that marketing campaigns for forthcoming blight resistant cultivars will need demographic targeting to avoid demand erosion (Nayak & Campbell, 2024). Although the study did not attach explicit prices, its Tobit model of LOP points to sizeable elasticities that resistant brands could capture.

Market gatekeepers amplify these signals. In a multi-state survey of 391 landscape maintenance firms, most respondents anticipated greater client satisfaction and higher profits if disease and insect resistant ornamentals became mainstream, and only 4 % feared revenue loss even though such plants could reduce service calls; profitability was perceived to fall only if over 60 % of installed material were resistant (Klingeman et al., 2009). Because many commercial installations arise from landscape professional specifications, their willingness to endorse resistant stock suggests that retail premiums documented in consumer studies can be transmitted through the supply chain with little additional marketing outlay.

The literature evaluating genetically modified (GM) resistance in ornamentals remains thin, partly because few GM cultivars have been commercialized. As mentioned earlier, the only dedicated study surveyed 607 Tennessee Master Gardeners and found that 73 % would purchase GM ornamental plants if environmental or human health benefits were evident, although women reported greater concern about invasiveness and transparency over the gene source (Klingeman and Hall, 2006). Acceptance was thus tied to perceived societal benefit rather than the transformation method itself, implying that explicit disease control claims could offset generic biotech skepticism once regulatory pathways for non food ornamentals become clearer.

Across these works, four explanatory themes recur. First, buyers interpret resistance as a proxy for lower maintenance costs, a motive almost as powerful as environmental altruism when explaining WTP—especially among time pressed householders documented in the rose and Abelia studies (Chavez et al., 2020; Frem et al., 2024). Second, label credibility matters: resistance certified by a third party or presented with transparent diagnostic information raises valuations beyond the same claim offered generically. Third, information processing style affects bids; the rose eye tracking experiment showed that consumers who actively fixated on adaptation icons produced narrower WTP distributions, suggesting less attribute non-attendance. Finally, demographic segmentation—by gender, education, and baseline plant knowledge—consistently moderates both WTP magnitudes and LOP probabilities, warning marketers that uniform messaging risks leaving revenue untapped (Klingeman et al., 2009; Nayak & Campbell, 2024).

For breeders, these findings justify redirecting R&D budgets toward durable resistance genes: a 10–30 % consumer premium can more than offset development costs, especially in perennial lines with extended royalty windows. Retailers and grower brands should combine resistance logos with concise statements about pesticide savings and landscape longevity to convert technical improvements into perceived personal benefit. Extension educators can accelerate adoption by standardizing transparent, benefit centered labelling for future gene edited ornamentals, thereby preempting the trust deficits that complicated first generation GM crops.

However, important gaps remain. Most valuation studies are concentrated in the United States or Western Europe, leaving Asia—where ornamental demand is growing fastest—largely unexamined. Real market experiments that track scanner data or loyalty card purchases are rare but vital for validating stated WTP estimates. Moreover, few experiments decouple disease resistance from broader "adaptation bundles"; isolating single trait premiums will be essential for estimating returns on individual resistance loci, whether introgressed conventionally or via CRISPR, a technology that research scientists use to selectively modify the DNA of living organisms.

Taken together, the literature shows that consumers and landscape professionals already recognize the tangible and intangible benefits of disease resistant ornamentals and are willing to reward those traits financially. Harnessing that latent demand, however, hinges on delivering verifiable resistance claims accompanied by clear narratives about maintenance savings and environmental stewardship.

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, applying this technique required 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 was 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. A draft journal article has been generated addressing this objective.

4.6 Assessing the overall project impact

This part of the project took place in the final year. The project was 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 were then be analyzed as to their implications for policymakers and stakeholders.

Since the Boxwood Blight Insight Group (BBIG) received its USDASCRI award in 2020, four consecutive annual reports trace a clear arc from fundamental science to durable, economywide returns. At the start of the grant period, field surveys confirmed that the United States grows and sells roughly $140.9 million in boxwood each year—a figure that, as stated earlier, swells to well over $1.6-$2.1 billion once allied inputs, landscape services, and retail markups are counted. The pathogen, iculata, *Calonectria pseudonaviculata* threatened to unravel that value chain, yet by 2024 BBIG's integrated program had not only shielded the crop but triggered measurable growth in demand, secured profitable operating margins for producers, reduced environmental externalities, and generated public goods that outlive any single disease cycle.

Economically, the most visible achievement is revenue preservation and expansion. Sector wide nursery interviews in 2021 established a baseline of suppressed but resilient sales; by 2022 those same operations reported a roughly twenty-five percent increase in boxwood shipments compared with pre-project levels, attributing the rebound to the confidence created by BBIG's rapid fire findings on disease spread and management. The 2023 report confirmed that trend, documenting continued unit sales growth even in infection hotspots, while the 2024 stakeholder summary showed that boxwood's share of total broadleaf evergreen sales had returned to its long-term average for the first time since 2015. Taken together, those annual snapshots imply that BBIG has safeguarded several hundred million dollars in farmgate sales and billions more in downstream economic activity that would otherwise have been at risk.

Cost containment has amplified the topline gains. Across four seasons of replicated on farm trials in Oregon, Tennessee, the MidAtlantic and New England, BBIG scientists demonstrated that wider plant spacing, reduced overhead irrigation and strategic canopy ventilation can cut inoculum pressure by more than half without additional capital expenditure. When these cultural adjustments are paired with monthly—rather than fortnightly—applications of biocontrol agents, silicon supplements, or extended fungicide application intervals, growers report trimming chemical purchases by one third and labor devoted to plant protection by as much as forty percent. BBIG's economics team translated those biological results into a partial budget framework that dissects variable costs for both container and field production systems; by 2023 that model had become a de facto decision tool for lenders, insurers, and state cost share programs, guiding millions of dollars in loans and public incentives toward the most cost-effective mitigation options. The research also redirected private investment toward more resilient assets.

Genotype by environment trials confirmed significant resistance in cultivars such as NewGen 'Independence' and 'Freedom', accelerating an inventory pivot that now sees an estimated forty percent of young stock in major producing states composed of tolerant selections. Concurrently, the team's epidemiological mapping spurred a gradual geographic shift away from chronically infested production zones to regions where cooler nights or regulatory stringency keep disease pressure low. Those two structural changes—genetic diversification and regional redistribution—reduce the likelihood of sudden supply shocks that would reverberate through garden center shelves, landscape contractor schedules, and public garden displays.

While the ledger gains are compelling, the environmental dividends are equally noteworthy. By stretching spray intervals and replacing broad spectrum fungicides with narrow spectrum biologicals in trial nurseries, BBIG practices shaved an estimated 13,000 pounds of active ingredient from the production footprint of participating growers between 2022 and 2024. Leaner irrigation schedules saved roughly 180 million gallons of water over the same period—a nontrivial figure in drought prone states such as Oregon and California. Reductions in chemical load and water use translate into healthier soil microbiomes, fewer nontarget effects on pollinators, and diminished nutrient runoff to adjacent waterways, providing ecosystem services that accrue well beyond nursery boundaries.

Societal benefits radiate further. Boxwood anchors the formal designs of historic estates, university campuses, and civic spaces; protecting the crop therefore safeguards the aesthetic and real estate value of settings that draw tourists, students, and residents. The project's diagnostic advances—a five-minute lateral flow strip test and a portable isothermal DNA assay—have shortened regulatory holds at state borders and saved public gardens tens of thousands of dollars in emergency plant removals. Knowledge transfer has been rapid: BBIG's quarterly webinars, best practice videos, and diagnostics library grew from a few hundred early adopters in 2021 to more than 15 000 registered users across forty-six states and two dozen countries by early 2025. That global reach positions U.S. researchers and consultants as net exporters of intellectual property, adding a modest but growing stream of consultancy and licensing revenue to the ledger.

For the public and private landscape users who ultimately select, install and maintain boxwood, BBIG's work translates into concrete operational and cultural gains. Municipal parks departments and state departments of transportation can now specify boxwood with far greater confidence that the plants will establish, thrive, and meet design intent, which lowers replacement budgets and avoids conspicuous gaps in high visibility plantings. Commercial property managers and homeowners' associations, faced with tighter maintenance margins, benefit from reduced pesticide requirements and lower irrigation needs—savings that compound over the decade plus lifespan of a mature hedge. Heritage gardens, many of which rely on boxwood to frame historic vistas or protect rare plant collections from foot traffic, avoid the reputational and financial costs of largescale removals, preserving visitor appeal and ticket revenues. Even individual homeowners gain in that retail garden centers can stock disease free plants year-round, shortening the "out of stock" cycles that once sent consumers toward synthetic substitutes or entirely different plant palettes. Collectively, these gains enhance the visual coherence, ecological performance, and economic efficiency of America's built landscapes, reinforcing the social value of green infrastructure in both public parks and private landscapes.

The BBIG project has proactively invested heavily in human capital. Over the grant period BBIG supported eleven postdoctoral researchers, five graduate students, and multiple undergraduate interns, each cross trained in pathology, economics, and extension communication. Many have already moved into tenure track positions, regulatory agencies, or private sector R &D units, seeding the green industry with professionals fluent in both the biology of plant disease and the economics of risk management—competencies that will compound long after the grant sunsets.

In aggregate, the four years of BBIG work document a virtuous cycle: timely science reduced uncertainty; reduced uncertainty preserved and then expanded market demand; larger and more stable markets justified further private and public investment in best management innovation; and each innovation, in turn, lowered environmental impact and fortified community assets. By 2024 the consortium had not merely averted a potential collapse of the nation's leading evergreen ornamental; it had transformed that crisis into an engine of innovation, cost efficiency and public value, delivering benefits that cascade through nursery ledgers, landscaped spaces, environmental quality, and the very workforce that will steer the green industry's next wave of growth.

The project's outcomes carry clear policy implications for leaders at every level of governance. At the federal tier, BBIG's data show that relatively modest research investments yield outsized economic returns and environmental co-benefits, underscoring the value of maintaining, or even enlarging, Specialty Crop Research Initiative funding lines and streamlining APHIS approval of rapid diagnostics that keep interstate commerce moving. State legislators can leverage the partial budget framework to craft targeted cost share programs—much like existing nutrient management incentives—that reimburse growers for adopting the most cost-effective cultural controls, thereby reducing both fiscal outlay and chemical load. Municipal councils and urban forestry boards can incorporate BBIG's resistant cultivar lists and irrigation guidelines into landscape ordinances, simultaneously lowering long-term maintenance budgets and advancing climate resilience goals. Local extension offices and civic beautification committees can deploy the group's open access training modules to equip community gardeners and volunteer stewards with science-based protocols, increasing plant survival rates while diminishing pesticide use in public spaces. Collectively, these policy actions translate BBIG's research into sustainable regulatory frameworks, smarter public investment strategies, and community scale greening initiatives that lock in the project's economic, environmental, and societal dividends for decades to come.

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, observations from the survey were also used to train Oregon Department of Agriculture (ODA) nursery inspectors on how to better scout for disease and to improve the stringency of the Oregon'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). Likewise, a list of cultivars consistently resistant to both boxwood blight and leafminer was developed to promote their adoption. Some of the most resistant cultivars – NewGen® Boxwood 'Independence' and 'Freedom' (Saunders Genetics), 'Little Missy' and 'Little Mister' (Star Roses & Plants), BetterBuxus® 'Renaissance', 'Babylon Beauty', 'Heritage' and 'Skylight' (Herplant) – were featured in the BBIG Newsletters. Further, a new map of the boxwood blight pathogen *Che* in Europe was developed and shared broadly to help prevent this potentially more destructive blight pathogen from entering this country.

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 fourth annual meeting with AP and stakeholders was held online via Zoom on May 14 and 15, 2025. Individual teams, including major partners like AmericanHort (**Figure 29**), reported their progress over the 4.5-year period and their plans for the remainder of the project period.

FIGURE 29 NICOLAS LEAS OF AMERICANHORT REPORTING THE OUTREACH WEBSITE PERFORMANCE DURING THE THIRD ANNUAL MEETING ONLINE VIA ZOOM

BBIG outreach website

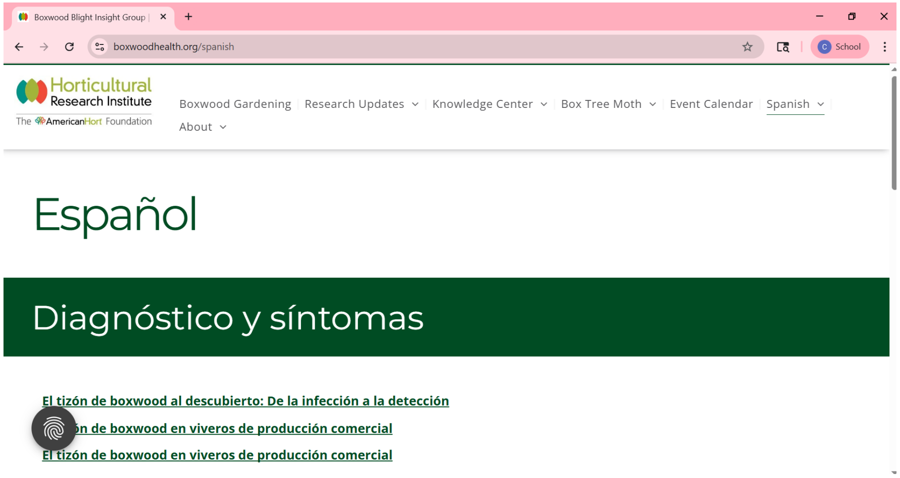


FIGURE 30 SPANISH - A NEW TAB OF THE BBIG OUTREACH WEBSITE TO MEET THE EDUCATIONAL NEEDS OF SPANISH-SPEAKING WORKFORCE IN THE ENVIRONMENTAL HORTICULTURE INDUSTRY

The BBIG website was first released to the public in November 2020 and it has since undergone several major updates and restructuring. Three new tabs have been added with Boxwood Gardening in 2022, Box Tree Moth in 2024 and Spanish (**Figure 30**) to meet the emerging needs of the industry and the public for information on the respective topics. 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.

International Boxwood Seminar Series

This seminar series was launched on March 18, 2021 with Dr. Thomas Brand of Germany as the inaugural speaker and concluded on December 21, 2022. A total of eight webinar sessions were conducted with leading scientists from seven countries as speakers, bringing boxwood blight research already done in Europe to the U.S. stakeholders as intended. This seminar series also covered information needs for another emerging disease – Volutella blight – and an invasive pest – box tree moth.

This series was highly impactful as shown by its attendance metrics. Overall, this Seminar series reached 46 states in the U.S., plus the District of Columbia (**Figure 31**) 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, as reported in a recent publication in Plant Disease.

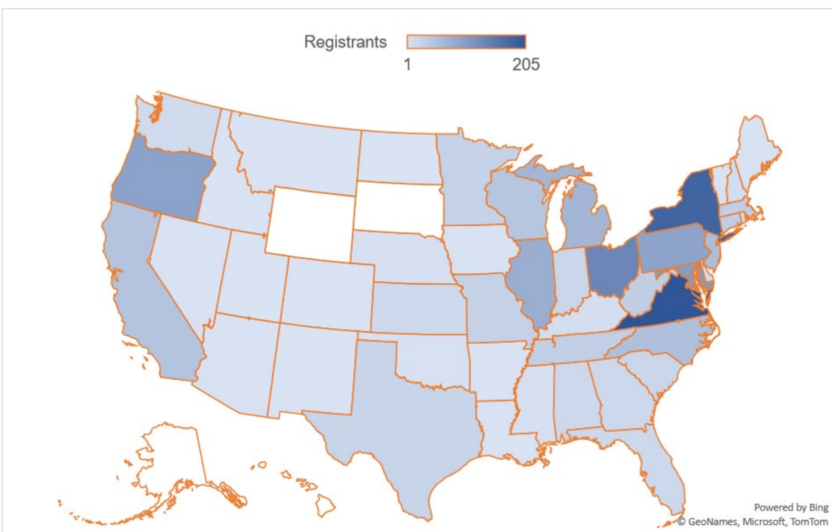


FIGURE 31 NATIONAL REACH TO FORTY-SIX STATES PLUS WASHINGTON DC ACCOMPLISHED BY THE INTERNATIONAL BOXWOOD SEMINARS

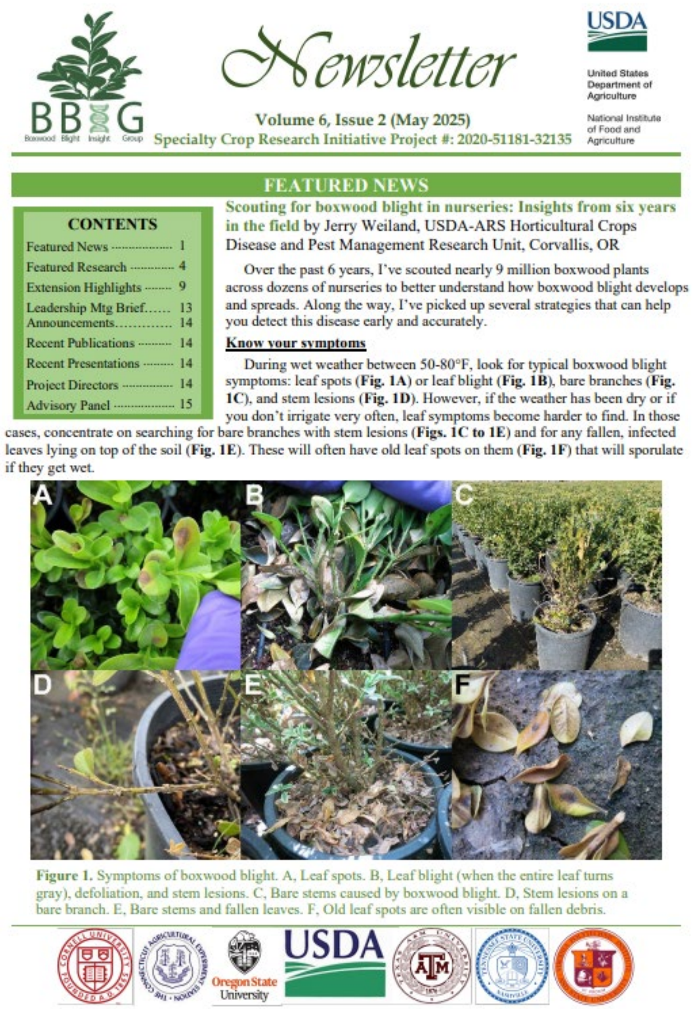
The recording of the International Boxwood Seminar series posted at the BBIG website has been a valuable resource for the industry and other stakeholders since conclusion of the Series and will continue to serve them for years to come.

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, 20024) and Dr. Xiaoping Li (July 25, 2024), Dr. Nina Shishkoff (February 6, 2025) and Dr. Ping Kong (March 14, 2025).

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 the latest five live webinar sessions were estimated to range from $1.3 to $2.9 million each. As with the International Boxwood Seminars, the recording of the BBIG Boxwood Seminars will continue to serve the horticulture industry, the public and other stakeholders for years to come.

BBIG Newsletter


BBIG Boxwood Blight Insight Group

Newsletter

Volume 6, Issue 2 (May 2025)

Specialty Crop Research Initiative Project #: 2020-51181-32135

USDA United States Department of Agriculture National Institute of Food and Agriculture

FEATURED NEWS

CONTENTS

Scouting for boxwood blight in nurseries: Insights from six years in the field by Jerry Weiland, USDA-ARS Horticultural Crops Disease and Pest Management Research Unit, Corvallis, OR

Over the past 6 years, I've scouted nearly 9 million boxwood plants across dozens of nurseries to better understand how boxwood blight develops and spreads. Along the way, I've picked up several strategies that can help you detect this disease early and accurately.

Know your symptoms

During wet weather between 50-80°F, look for typical boxwood blight symptoms: leaf spots (**Fig. 1A**) or leaf blight (**Fig. 1B**), bare branches (**Fig. 1C**), and stem lesions (**Fig. 1D**). However, if the weather has been dry or if you don't irrigate very often, leaf symptoms become harder to find. In those cases, concentrate on searching for bare branches with stem lesions (**Figs. 1C to 1E**) and for any fallen, infected leaves lying on top of the soil (**Fig. 1E**). These will often have old leaf spots on them (**Fig. 1F**) that will sporulate if they get wet.

A B C D E F

Figure 1. Symptoms of boxwood blight. A, Leaf spots. B, Leaf blight (when the entire leaf turns gray), defoliation, and stem lesions. C, Bare stems caused by boxwood blight. D, Stem lesions on a bare branch. E, Bare stems and fallen leaves. F, Old leaf spots are often visible on fallen debris.

Oregon State University USDA

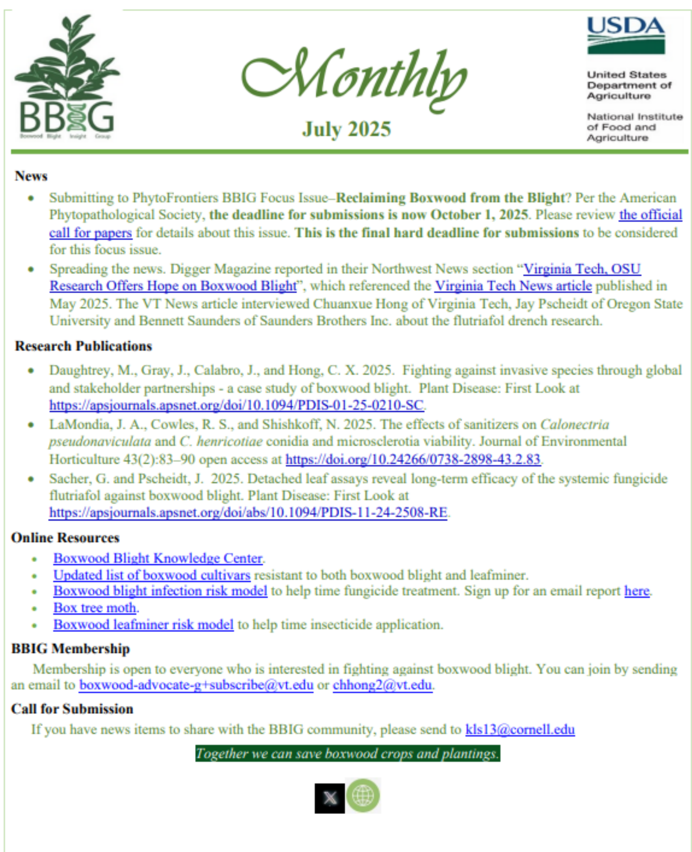
FIGURE 32 A SNAPSHOT OF THE FRONT PAGE OF BBIG NEWSLETTER VOLUME 6 ISSUE 2 (MAY 2025)

BBIG Newsletter is a quarterly digital magazine, serving multiple purposes. First, it is a quarterly project report to the project's Advisory Panel (AP) members, scheduled to be sent to them one week before the quarterly AP meeting. Second, it is also released to the broad BBIG community (bbig-g@vt.edu) with over 400 members globally at the same time to keep the environmental horticulture industry and other stakeholders informed of project development. Third, it is posted at the BBIG outreach website for public access at their convenience. This digital magazine was launched in November 2020 and as of August 5, 2025, it has been published in 19 issues in six volumes. Each issue began with Featured News, followed by Featured Research, Extension Highlights, and Project Meeting Brief, Recent Publications, etc. as exemplified by Volume 6, Issue 2 (**Figure 32**).

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.

BBIG Monthly

This monthly digest was launched in January 2022 to expedite the technology transfer from this project to end users. It 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 33**).


BBIG

Boxwood Blight Insight Group

Monthly

July 2025

USDA

United States Department of Agriculture

National Institute of Food and Agriculture

News

- Submitting to PhytoFrontiers BBIG Focus Issue–**Reclaiming Boxwood from the Blight**? Per the American Phytopathological Society, **the deadline for submissions is now October 1, 2025**. Please review the official call for papers for details about this issue. **This is the final hard deadline for submissions** to be considered for this focus issue.
- Spreading the news. Digger Magazine reported in their Northwest News section "Virginia Tech, OSU Research Offers Hope on Boxwood Blight", which referenced the Virginia Tech News article published in May 2025. The VT News article interviewed Chuanxue Hong of Virginia Tech, Jay Pscheidt of Oregon State University and Bennett Saunders of Saunders Brothers Inc. about the flutriafol drench research.

Research Publications

- Daughtrey, M., Gray, J., Calabro, J., and Hong, C. X. 2025. Fighting against invasive species through global and stakeholder partnerships - a case study of boxwood blight. Plant Disease: First Look at https://apsjournals.apsnet.org/doi/10.1094/PDIS-01-25-0210-SC.
- LaMondia, J. A., Cowles, R. S., and Shishkoff, N. 2025. The effects of sanitizers on *Calonectria pseudonaviculata* and *C. henricotiae* conidia and microsclerotia viability. Journal of Environmental Horticulture 43(2):83–90 open access at https://doi.org/10.24266/0738-2898-43.2.83.
- Sacher, G. and Pscheidt, J. 2025. Detached leaf assays reveal long-term efficacy of the systemic fungicide flutriafol against boxwood blight. Plant Disease: First Look at https://apsjournals.apsnet.org/doi/abs/10.1094/PDIS-11-24-2508-RE.

Online Resources

- Boxwood Blight Knowledge Center.
- Updated list of boxwood cultivars resistant to both boxwood blight and leafminer.
- Boxwood blight infection risk model to help time fungicide treatment. Sign up for an email report here.
- Box tree moth.
- Boxwood leafminer risk model to help time insecticide application.

BBIG Membership

Membership is open to everyone who is interested in fighting against boxwood blight. You can join by sending an email to boxwood-advocate-g+subscribe@vt.edu or chhong2@vt.edu.

Call for Submission

If you have news items to share with the BBIG community, please send to kls13@cornell.edu

Together we can save boxwood crops and plantings.

FIGURE 33 EXAMPLE OF BBIG MONTHLY - JULY 2025

Tweets at X.com/boxwoodhealth

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

Summary of conventional means of technology transfer to date

- Reached out to the science and educational communities by
  - Publishing 66 refereed journal articles with additional research manuscripts being prepared for inclusion in the upcoming BBIG Focus Issue of PhytoFrontiers.
  - Organizing and hosting nine national and international meetings including one idea café and two symposia in conjunction with the APS annual meetings, one National Plant Diagnostic Network Symposium – Boxwood Blight Insight Group, and five meetings of the international boxwood epidemiology working group.
  - Giving 62 presentations at annual meetings of the American Phytopathological Society and other professional conferences.
  - Presenting 23 invited/guest lectures
- Reached out to the environmental horticulture industry and the public through
  - Two On-Demand sessions and eight presentations at Cultivate, an annual educational venue hosted by AmericanHort.
  - 126 presentations on different educational programs, including some Spanish presentations for the Spanish-speaking workforce. The educational programs and presentations were all well attended with the largest attendee count of 1161 from the live EPA IPM On-Demand Webinar session on boxwood blight and box tree moth on March 5, 2024. That webinar recording posted at the EPA website has since continued to serve the industry and the public 24/7.
  - Fifteen extension articles, excluding those published in the BBIG Newsletters.
  - Fourteen workshops and courses including some in Spanish or Spanish and English (**Figure 34**).
  - Three field demonstrations.
  - Three video clips.

FIGURE 34 ON-FARM HANDS-ON TRAINING ABOUT SCOUTING FOR BOXWOOD BLIGHT ON JULY 24, 2025

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.
- Reached out to the broad BBIG communities through 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 64, 692 boxwood enthusiasts were reached via the BBIG group alone as of May 1, 2025.

<u>*5.3 Documenting project impacts*</u>

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

<u>*Changes in knowledge*</u>

- Enabled growers, retailers, landscapers, arborists, first responders, 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' – demonstrated 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, Switzerland and Italy, 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 rainwater. 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 3) 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 occur, 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, France, Germany, Greece, Italy, Switzerland and United Kingdom have been collaborating on several important project components, including biocontrol, field epidemiology and survey of boxwood plantings in southern Europe for *Che*. 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. They also uncovered *Che* in Italy, Spain and Switzerland.
- AmericanHort and HRI continue 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

This project has trained eleven postdocs, five graduate students and six interns. In addition to learning research planning and execution, data analysis and interpretation, and writing for publications, they actively interacted regularly with the rest of the BBIG team (including the Advisory Panel). Through these interactions, they gained an appreciation of how to work with collaborators and industry partners, while honing communication and leadership skills. This project also provided them with a variety of other professional training opportunities – attending professional meetings and giving webinars to the national and international audience, etc. Below are a few examples of their professional development and accomplishment.

- Urmila Adhikari, a Postdoctoral Associate at Virginia Tech, analyzed the field trial data collected by collaborators in northern Germany between 2006 and 2020, bringing this invaluable research to American stakeholders and international communities through two research publications in the internationally-recognized journals. This productive working experience earned her a dream job – Research Associate at the National Science Foundation (NSF) Center for Integrated Pest Management at North Carolina State University in April 2022.
- Ihsan Khaliq, a Postdoctoral Associate at Virginia Tech, did two case studies of field epidemiology of boxwood blight. He analyzed field data collected by collaborators in northern Germany since 2006 and those at Virginia Tech between 2014 and 2017. These

case studies resulted in two research publications that helped him land another postdoc position at Texas A&M University in August 2024.

- Srikanth Kodati, a Postdoctoral Associate at Connecticut Agricultural Experiment Station, explored several technologies including copper nanoparticles for blight mitigation. He also studied the epidemiology of boxwood blight, laying a foundation for integrating existing and emerging disease management technologies. In addition, he actively participated in the project extension activities. These research and extension experiences helped him land a dream job – Pesticide Safety and Crop Protection Extension Educator at University of Connecticut in early 2023.
- Xiaoping Li, a Postdoctoral Associate at Virginia Tech, performed the boxwood microbiome studies in collaboration with the USDA ARS Horticultural Crops Disease and Pest Management Research Unit in Corvallis, OR, North Carolina Department of Agriculture and Consumer Services, and North Carolina Cooperative Extension, as well as local farmers. These collaborative studies have resulted in five full-length publications in high-impact journals, plus one short communication and several co-authorships. Xiaoping also was invited to give a webinar on the boxwood microbiome in the BBIG Boxwood Seminar series to national and international audience. His research publications, along with the extension and leadership experience, earned him an on-campus interview for a tenure-track assistant professor position in summer 2024.
- Prabha Liyanapathiranage, a Postdoctoral Associate, conducted research work on identifying effective fungicide treatments for boxwood blight management. She also assisted diagnosis of the boxwood samples received at the Otis L. Floyd Nursery Research Center in McMinnville TN. In addition, she delivered an oral presentation to the Warren County, TN Master Gardener Association on boxwood diseases. Dr. Prabha Liyanapathiranage was named by Tennessee Department of Agriculture as the new state plant pathologist beginning June 2024.
- Mana Ohkura, a Postdoctoral Research Associate at USDA ARS Horticultural Crops Disease and Pest Management Research Unit, Corvallis, OR, developed a scalable DNA extraction method and a PCR assay to facilitate the nursery survey. She also investigated the impact of the volatiles from boxwood leaves on *Cps* conidial germination. In addition, she assisted in several other projects while actively participating in project extension activities. These research and extension experiences earned her a dream job – diagnostician and manager of Plant Disease Clinic at Oregon State University.
- Olanike Omolehin, a Postdoctoral Associate at Virginia Tech, conducted several field studies evaluating selected antidesiccants and biocontrol agents in western North Carolina, in collaboration with North Carolina Department of Agriculture and Consumer Services and NC Cooperative Extension. She also analyzed the boxwood production inventory shift over a 10-year period from 2011 to 2021. Additionally, she served as the Project Manager coordinating project activities, including project meetings and digital magazines – quarterly BBIG Newsletter and monthly digest. These research and project management experiences earned her a dream job – Data Analyst at IBM in Baton Rouge, LA in August 2022.
- Xiao Yang, a Postdoctoral Research Associate at USDA ARS Foreign Disease/Weed Science Research Unit in Ft Detrick, MD, studied and identified the proteins unique to the boxwood blight pathogens, *Cps* and *Che*, and laid the groundwork for developing robust, serological and DNA-based diagnostic innovations. He also served as the Project

Manager, coordinating project meetings and digital magazine (BBIG Newsletter) before landing a dream job – Director of Diagnostic and Pest ID labs at Clemson University in October 2021.

- Bhawana Ghimire was a graduate student at Tennessee State University. She investigated 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. Additionally, she was 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.
- Gabriel Sacher, a graduate student at Oregon State University, worked on boxwood and rhododendron for his thesis with an emphasis on fungicide management of boxwood blight. 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. He landed a postdoctoral associate job at Pennsylvania State University immediately after graduation from Oregon State University.
- Gloria Chen, an intern at Virginia Tech from Perkiomen School in Pennsburg, PA, worked with the boxwood microbiome research team, learning a variety of metagenomics analysis skills and co-authored one research publication. This research experience helped her enroll at her dream school – University of California at Los Angles.
- John Dobbs, a Postdoctoral Research Associate with the USDA-ARS Foreign Disease/Weed Science Research Unit in Fort Detrick, MD, joined the team in January 2024. He has since been working on pathogen colonization, latency and phenotypic diversity.
- Filiz Gurel, a Postdoctoral Research Associate with the USDA-ARS U.S. National Arboretum Floral and Nursery Plant Research Unit, has been coordinating the assessment of UV-C treatment on the boxwood blight pathogens and continued the 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.

Additionally, two training sessions on Microsoft Teams were organized for all Project Directors, their associates and Advisory Panel members in July 2020, right before the project start, facilitating internal communication within this project and enabling coordination of future projects. Likewise, two webinars were organized for the capacity-building seminar series with one focusing on the plant microbiome in February 2021 and the other on near-infrared spectroscopy and machine-learning in July 2023.

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?

Most collaborating labs have completed their proposed project activities with some well exceeding and concluded their components of the project on August 31, 2025. Only four USDA

ARS labs in Beltsville, MD area plus three at Cornell and Virginia Tech, will continue to make efforts to catch up on research components that were delayed due to the COVID-19 pandemic, while fast tracking the publication of the latest research discoveries and innovations through the BBIG Focus Issue of PhytoFrontiers, in collaboration with the American Phytopathological Society.

CHANGES/PROBLEMS

During the pandemic of COVID-19, four USDA PI's research labs in Beltsville, MD area 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. Three labs, along with two at Cornell and Virginia Tech, requested another 1 year no-cost extension until August 31, 2026 to achieve proposed project objectives. All other collaborating labs have completed all proposed project activities and closed their components of the project on August 31, 2025.

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, who 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.