



Annual Report

USDA Award No.: 2020-51181-32135

Improving boxwood blight mitigation through innovation, economic analysis and education

Together we save boxwood crops and plantings!



September 9, 2021

Boxwood Blight Insight Group (BBIG) is a team of scientists working together on an USDA National Institute of Food and Agriculture – Specialty Crop Research Initiative (SCRI) project, in partnership with stakeholders and international collaborators. This transdisciplinary team and its partners aim to safeguard boxwood—the nation’s #1 evergreen ornamental shrub crop—from blight disease, thus saving an iconic plant featured in American landscapes since 1653.

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PROJECT PARTICIPANTS

Project Leadership Team

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TARGET AUDIENCE

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

PRODUCTS

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

Research

Idea Café

- Boxwood Blight Insight Group (BBIG): Fighting a good fight against boxwood blight, at Plant Health 2021-Annual Meeting of the American Phytopathological Society (APS), August 2, 2021.

Journal Publications

1. Becker, L. E., Adhikari, U., May, T., **Shishkoff, N.**, **Crouch, J. A.**, and Cubeta, M. A. 2021. Evaluation of boxwood cultivars for resistance to boxwood blight, 2020. *Plant Disease Management Reports* 15:OT008. <https://www.plantmanagementnetwork.org/pub/trial/pdmr/volume15/abstracts/ot008.asp>
2. **Bika, R.***, Copes, W., and **Baysal-Gurel, F.** 2021. Comparative performance of sanitizers in managing plant-to-plant transfer and postharvest infection of *Calonectria pseudonaviculata* and *Pseudonectria foliicola* on boxwood. *Plant Disease* published online at <https://doi.org/10.1094/PDIS-03-21-0481-RE>.
3. **Baysal-Gurel, F.**, Jennings, C., and Simmons, T. 2020. Evaluation of fungicides for the control of boxwood blight, 2020. *Plant Disease Management Reports* 15:OT002. <https://www.plantmanagementnetwork.org/pub/trial/PDMR/volume15/>.
4. **Castroagudín, V. L.***, **Shishkoff, N.**, Stanley, O., Whitesell, R., Olson, T., and **Crouch, J. A.** 2021. First report: Co-infection of *Sarcococca hookeriana* (sweetbox) by *Coccinonectria pachysandricola* and *Calonectria pseudonaviculata* causes a foliar disease of sweetbox in Pennsylvania. *Plant Disease* published online at <https://doi.org/10.1094/PDIS-1006-1020-1198-PDN>.
5. **Castroagudín, V. L.***, **Weiland, J. E.**, **Baysal-Gurel, F.**, Cubeta, M. A., **Daughtrey, M. L.**, Gauthier, N. W., **LaMondia, J.**, **Luster, D. G.**, Hand, F. P., **Shishkoff, N.**, Williams-Woodward, J., **Yang, X.***, LeBlanc, N., and **Crouch, J. A.** 2020. One clonal lineage of *Calonectria pseudonaviculata* is primarily responsible for the boxwood blight epidemic in the United States. *Phytopathology* 110:1845–1853. <https://doi.org/10.1094/phyto-04-20-0130-r>.
6. **Castroagudín, V. L.***, **Yang, X.***, **Daughtrey M. L.**, **Luster, D. G.**, **Pscheidt, J. W.**, **Weiland, J. E.**, and **Crouch, J. A.** 2020. Boxwood blight disease: A diagnostic guide. *Plant Health Progress* 21:291–300. <https://doi.org/10.1094/PHP-06-20-0053-DG>.
7. **Gouker, F. E.**, Guo, Y. H., and Pooler, M. R. 2020. Using acetone for rapid PCR-amplifiable DNA extraction from recalcitrant woody plant taxa. *Applications in Plant Sciences* 8:e11403. <https://doi.org/10.1002/aps3.11403>.
8. **Hall, C. R.**, Hodges, A. W., Khachatryan, H., and Palma, M. A. 2020. Economic contributions of the green industry in the United States in 2018. *Journal of Environmental Horticulture* 38:73–79. <https://doi.org/10.24266/0738-2898-38.3.73>.
9. **Hall, C.**, **Hong, C. X.**, **Gouker, F. E.**, and **Daughtrey, M. L.** 2021. Analyzing the structural shifts in U.S. boxwood production due to boxwood blight. *Journal of Environmental Horticulture* (in press).

10. **Hall, C. R.**, and Knuth, M. J. 2020. An update of the literature supporting the well-being benefits of plants: Part 4 – available resources and usage of plant benefits information. *Journal of Environmental Horticulture* 38:68–72. <https://doi.org/10.24266/0738-2898-38.2.68>.
11. **Kong, P.**, Richardson, P., and **Hong, C. X.** 2020. *Burkholderia* sp. SSG is a broad-spectrum antagonist against plant diseases caused by diverse pathogens. *Biological Control* 151:104380. <https://doi.org/10.1016/j.biocontrol.2020.104380>.
12. **LaMondia, J. A.** 2020. Curative fungicide activity against *Calonectria pseudonaviculata*, the boxwood blight pathogen. *Journal of Environmental Horticulture* 38:44–49. <https://doi.org/10.24266/0738-2898-38.2.44>.
13. **LaMondia, J. A.**, and Maurer, K. 2020. *Calonectria pseudonaviculata* conidia dispersal and implications for boxwood blight management. *Plant Health Progress* 21:232–237. <https://doi.org/10.1094/PHP-04-20-0024-RS>.
14. LeBlanc, N., Cubeta, M. A., and **Crouch, J. A.** 2021. Population genomics trace clonal diversification and intercontinental migration of an emerging fungal pathogen of boxwood. *Phytopathology* 111:184–193. <https://doi.org/10.1094/phyto-06-20-0219-fi>.
15. **Sacher, G. O.***, **Weiland, J. E.**, Putnam, M. L., **Crouch, J. A.**, and **Castroagudín, V. L.*** 2020. Confirmation of *Calonectria pseudonaviculata* causing boxwood blight of *Buxus* cultivars in Oregon. *Plant Disease* 104:1862. <https://doi.org/10.1094/pdis-01-20-0078-pdn>.
16. **Shishkoff, N.**, Miller, M. E., and Cubeta, M. A. 2021. Rooting response of boxwood cultivars to hot water treatment and thermal sensitivity of *Calonectria henricotiae* and *C. pseudonaviculata* in diseased boxwood (*Buxus* spp.). *Journal of Environmental Horticulture* 39:1–10. <https://doi.org/10.24266/0738-2898-39.1.1>.
17. Stravoravdis, S., Marra, R.E., LeBlanc, N. R., **Crouch, J. A.**, and Hulvey, J. P. 2021. Evidence for the role of CYP51A and xenobiotic detoxification in differential sensitivity to azole fungicides in boxwood blight pathogens. *International Journal of Molecular Sciences* 22:9255. <https://doi.org/10.3390/ijms22179255>.
18. **Yang, X.***, **Castroagudín, V. L.***, **Daughtrey, M. L.**, Loyd, A., **Weiland, J. E.**, **Shishkoff, N.**, **Baysal-Gurel, F.**, **Santamaria, L.**, **Salgado-Salazar, C.**, **LaMondia, J. A.**, **Crouch, J. A.**, and **Luster, D. G.** 2021. A diagnostic guide for Volutella blight affecting *Buxaceae*. *Plant Health Progress* published online at <https://doi.org/10.1094/PHP-02-21-0052-DG>.
19. **Yang, X.***, McMahon, M. B., Ramachandran, S. R., Garrett, W. M., LeBlanc, N., **Crouch, J. A.**, **Shishkoff, N.**, and **Luster, D. G.** 2021. Comparative analysis of extracellular proteomes reveals putative effectors of the boxwood blight pathogens, *Calonectria henricotiae* and *C. pseudonaviculata*. *Bioscience Reports* 41(3):BSR20203544. <https://doi.org/10.1042/BSR20203544>.

Abstracts (Presented at Professional Meetings)

1. Barker, B., Coop, L., and **Hong, C. X.** 2021. Risk modeling to predict outbreaks and establishment of invasive pathogens: a case study of boxwood blight. Annual Meeting of Ecological Society of America online at [ESA Meetings – The Ecological Society of America](#), August 2–6, 2021.
2. **Bika, R.***, Simmons, T., Jennings, C., and **Baysal-Gurel, F.** 2020. Comparative performance of sanitizers in reducing the plant-to-plant transfer of *Calonectria*

- pseudonaviculata* in boxwood using cutting tool. 130th Meeting of the Tennessee Academy of Science. November 21, 2020.
3. **Bika, R.***, Simmons, T., Jennings, C., and **Baysal-Gurel, F.** 2020. Comparative performance of sanitizers in reducing the plant-to-plant transfer of *Calonectria pseudonaviculata* in boxwood using cutting tool. Northeastern Plant, Pest, and Soil Conference (NPPSC). January 4–7, 2021.
 4. **Bika, R.***, Copes, W., and **Baysal-Gurel, F.** 2021. Efficacy of sanitizers in reducing plant-to-plant transfer of *Calonectria pseudonaviculata* conidia on cutting tool blade. 98th Annual Meeting of the APS Southern Division. February 15–19, 2021.
 5. **Bika, R.*** and **Baysal-Gurel, F.** 2021. Dip application of sanitizers in managing postharvest infection of *Calonectria pseudonaviculata* on boxwood cuttings. Plant Health 2021 – APS Annual Meeting. August 2–6, 2021.
 6. **Hong, C. X.** 2021. Potential of antidesiccants as a physical barrier to prevent infection of boxwood plants by *Calonectria pseudonaviculata* under controlled environments. Plant Health 2021 – APS Annual Meeting. August 2–6, 2021. <https://api.ltb.io/show/ABDSR>.
 7. **Hong C. X., Daughtrey, M. L., Luster, D. G., Hall, C., Weiland, J., Baysal-Gurel, F., Gouker, F. E., Kong, P., Crouch, J. A., LaMondia, J. A., Pscheidt, J. W., Santamaria, L., Shishkoff, N., and Snover-Clift, K.** 2021. Boxwood Blight Insight Group (BBIG): an international consortium to protect a major evergreen shrub crop and iconic landscape plant. Plant Health 2021 – APS Annual Meeting. August 2–6, 2021. <https://api.ltb.io/show/ABDST>.
 8. **Hong, C. X., Daughtrey, M. L., Howle, M., Williamson, M., and Mirmow, N.** 2021. Testing garden soil infectivity at landscape sites once infested by *Calonectria pseudonaviculata* in Eastern United States with detector boxwood plants. Plant Health 2021 – APS Annual Meeting. August 2–6, 2021. <https://api.ltb.io/show/ABDSU>.
 9. **Hong, C. X., Kong, P., Yang, X.*, and Omolehin, O.*** 2021. Developing sustainable solutions to boxwood blight (*Calonectria pseudonaviculata*) with biological control agents. Plant Health 2021 – APS Annual Meeting. August 2–6, 2021. <https://api.ltb.io/show/ABDSU>.
 10. **Kodati, S.***, Allan-Perkins, E., and **LaMondia, J. A.** 2021. In vitro study on the effect of temperature, leaf wetness period, and cultivar susceptibility on boxwood blight incidence. 80th Annual Meeting of the APS Northeastern Division, March 10–12, 2021.
 11. **Ohkura, M.***, Nackley, L. L., Mitchell, J. N., Scagel, C. F., and **Weiland, J. E.** 2021. Social distancing reduces boxwood blight incidence and severity. Plant Health 2021 – APS Annual Meeting. August 2–6, 2021. <https://api.ltb.io/show/ABDST>.
 12. **Shishkoff, N., Daughtrey, M. L., and Smallwood, E.** 2021. Sorting Through the Boxwood Blights. 77th Annual Meeting of the APS Potomac Division, March 10–12, 2021.
 13. **Shishkoff, N., Smallwood, E., and Camp, M. J.** 2021. The effect of *Trichoderma koningiopsis* and *Calonectria* spp. on survival of *Pseudonectria buxi* in boxwood leaf debris incubated at 0, 10, or 20 °C. 77th Annual Meeting of the APS Potomac Division, March 10–12, 2021.
 14. **Shishkoff, N., Smallwood, E., and Camp, M. J.** 2021. Use of *Trichoderma koningiopsis* to reduce viability of *Calonectria* spp. in boxwood leaf litter incubated at 0, 10, or 20 °C. Plant Health 2021 – APS Annual Meeting. August 2–6, 2021. <https://api.ltb.io/show/ABDSR>.

15. **Yang, X.***, Carter, M. L., and **Luster, D. G.** 2021. Development of serological diagnostic assays for boxwood blight disease. Plant Health 2021 – APS Annual Meeting. August 2–6, 2021. <https://my.ltb.io/www/#/stack/ABEBO>.
16. **Yang, X.***, McMahon, M. B., Carter, M. L., and **Luster, D. G.** 2021. Serological and recombinase polymerase amplification-based assays to diagnose boxwood blight. 77th Annual Meeting of the APS Potomac Division, March 10–12, 2021.

Invited Lectures

1. **Baysal-Gurel, F.** 2021. Common Diseases in Woody Ornamental Production-Research Updates. University of Arkansas, Plant Pathology (PLPA) 5404, SUMMER 2021, June 15, 2021.
2. **Daughtrey, M. L.** 2020. Learning plant pathology one disease at a time. Plant Pathology Department Seminar, Ohio State University, October 12, 2020.

Major Extension Products

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

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

1. **Hong C. X., Daughtrey, M. L., Luster, D. G., Hall, C., Weiland, J., Baysal-Gurel, F., Gouker, F. E., Kong, P., Crouch, J. A., LaMondia, J. A., Pscheidt, J. W., Santamaria, L., Shishkoff, N., and Snover-Clift, K.** 2020. Boxwood Blight Insight Group (BBIG) Newsletter Volume 1, Issue 1, released December 6, 2020.
2. **Hong C. X., Daughtrey, M. L., Luster, D. G., Hall, C., Weiland, J., Baysal-Gurel, F., Gouker, F. E., Kong, P., Crouch, J. A., LaMondia, J. A., Pscheidt, J. W., Santamaria, L., Shishkoff, N., and Snover-Clift, K.** 2021. BBIG Newsletter Volume 2, Issue 1, released March 8, 2021.
3. **Hong C. X., Daughtrey, M. L., Luster, D. G., Hall, C., Weiland, J., Baysal-Gurel, F., Gouker, F. E., Kong, P., Crouch, J. A., LaMondia, J. A., Pscheidt, J. W., Santamaria, L., Shishkoff, N., and Snover-Clift, K.** 2021. BBIG Newsletter Volume 2, Issue 2, released May 28, 2021.
4. **Hong C. X., Daughtrey, M. L., Luster, D. G., Hall, C., Weiland, J., Baysal-Gurel, F., Gouker, F. E., Kong, P., Crouch, J. A., LaMondia, J. A., Pscheidt, J. W., Santamaria, L., Shishkoff, N., and Snover-Clift, K.** 2021. BBIG Newsletter Volume 2, Issue 3, released September 3, 2021.

International Boxwood Seminar Series

1. Boxwood blight – A 15-year love-hate relationship. Dr. Thomas Brand, Department Head of Plant Protection Service of Lower Saxony, Germany, March 18, 2021.
2. Knowing and growing boxwood. Dr. Lynn Batdorf, Retired Curator of the National Arboretum Boxwood Collection of U.S. National Arboretum, and International Authority for Boxwood Cultivar Registration, International Society for Horticultural Sciences, June 2, 2021.
3. Boxwood breeding and selection for blight resistance. Dr. Katrijn van Laere, Senior Researcher, Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Belgium, August 25, 2021.

Live Stage at Cultivate'21

(Note: Cultivate is the premier annual event for the entire horticulture industry, hosted by AmericanHort.)

1. **Gouker, F.** 2021. Boxwood breeding at the U.S. National Arboretum. Cultivate'21 Live Stage, hosted by AmericanHort. Columbus, OH, July 11, 2021.
2. **Hong, C. X.** 2021. Boxwood blight research: what's the BBIG deal. Cultivate'21 Live Stage, hosted by AmericanHort. Columbus, OH, July 11, 2021.
3. **LaMondia, J. A.** 2021. Fungicides and management implications for boxwood health. Cultivate'21 Live Stage, hosted by AmericanHort. Columbus, OH, July 11, 2021.

Peer-Reviewed Videos

1. **Baysal-Gurel, F.** 2020. Boxwood blight. 2020 Tennessee Small Farms Expo. Video available at https://www.youtube.com/watch?time_continue=1&v=Xv91WAmyP8s. 162 views as of August 22, 2021.
2. **Pscheidt, J. W., Weiland, J. E., and Santamaria, L.** 2020. Scouting for Boxwood Blight. Corvallis, OR: Oregon State University. Video available at <https://www.youtube.com/watch?v=L-Yd8qCbb0A>. 1,101 views as of August 22, 2021.
3. **Pscheidt, J. W., Weiland, J. E., Santamaria, L., and Valenzuela, L.** 2021. Monitoreando el tizon de boxwood. Corvallis, OR: Oregon State University. (Spanish version of boxwood blight scouting video.) Video available at <https://www.youtube.com/watch?v=v5lCoLhoUss>. 18 views as of August 22, 2021.

Field Demonstrations

1. **Baysal-Gurel, F.** 2021. Boxwood blight mitigation. McMinnville, TN.
2. **Hong, C. X.** 2020. Rebuilding boxwood garden after blight. Midlothian, VA.

Courses

1. **Baysal-Gurel, F.** 2020. Tennessee State University, College of Agriculture, Department of Agricultural and Environmental Sciences Research Methods Class (AGSC 5110-80) - The Environmental Sciences Panel - boxwood blight research, October 19, 2020.
2. **Baysal-Gurel, F.** 2021. Boxwood Blight Identification and Management. Middle Tennessee Landscape & Grounds Virtual Short course, January 12 to 13, 2021.
3. **Baysal-Gurel, F.** 2021. Boxwood blight research. 2021 Fireside chat with the Tennessee State University College of Agriculture Dean and Research Faculty, Tennessee State University, January 22, 2021.

Webinars

1. **Baysal-Gurel, F.** 2020. Boxwood blight. Southern Region Green Industry Webinar hosted by University of Georgia, Center for Urban Agriculture, November 12, 2020.
2. **Daughtrey, M. L.** 2020. Cornell Update on Diseases. New York State Arborists Association Annual Meeting, November 6, 2020.
3. **Daughtrey, M. L.** 2020. Tree health and disease issues. Engaging Communities Through Tree Stewardship Program. Cornell Cooperative Extension of Dutchess County, October 9, 2020.

4. **Daughtrey, M. L.** 2020. Diseases of 2020. Landscape Seminar. Cornell Cooperative Extension of Suffolk County, October 14, 2020.
5. **Daughtrey, M. L.** 2020. Battling garden enemies. Horticultural Alliance of the Hamptons, November 8, 2020.
6. **Daughtrey, M. L.** 2021. The notorious and baffling diseases of boxwood and beech. Nursery and Landscape Session. Long Island Agricultural Forum, January 7, 2021.
7. **Daughtrey, M. L.** 2021. Boxing against boxwood blight. New York State Nursery and Landscape Association, February 10, 2021.
8. **Daughtrey, M. L.** 2021. Defying diseases. 2021 Virtual Tree Steward Program. Cornell Cooperative Extension of Westchester County, March 17, 2021.
9. **Hong, C. X.** 2020. Boxwood blight and other must-know emerging diseases. Eastern Shore of Virginia Master Gardener Training on Plant Disease Diagnosis and Control, November 12, 2020.
10. **Hong, C. X.** 2021. Fighting boxwood blight together. ‘Ornamentals/Nursery’ track at the 66th New Jersey Annual Vegetable Meeting, hosted by Rutgers University, February 22–25, 2021.
11. Jeffers, S. N., and **Daughtrey, M. L.** 2020. Boxwood blight: identification and management. Virtual IPM Symposium, Clemson Cooperative Extension Service, Clemson University, October 22, 2020.
12. **LaMondia, J. A.** 2021. Management of boxwood blight. Chesapeake Green, February 18, 2021.
13. **LaMondia, J. A.** 2021. Identification and management of boxwood blight. Connecticut Department of Environmental Protection, February 24, 2021.
14. **Santamaria, L.** 2021. Tizón de boxwood: Conociendo al enemigo para un mejor manejo de la enfermedad. Manejo integrado de plagas para plantas de viveros (Integrated pest management for nursery crops). Oregon Association of Nurseries, March 2, 2021.
15. **Santamaria, L.** 2021. Scouting for boxwood blight: a new bilingual educational tool in an Interactive Virtual Workshop. SCRI - Boxwood Blight-Advisory meeting. Aurora, OR, June 4, 2021.

Interactive Virtual Workshops for both English and Spanish Speakers

1. **Baysal-Gurel, F.** 2021. Dealing with diseases in the landscapes. University of Tennessee - Tennessee Extension Master Gardener (UT-TEMG) Central Region Virtual Conference/Workshop, June 24, 2021.
2. **Santamaria, L.** 2021. El tizón de boxwood: ¡Monitoreando al culpable! Taller Virtual Interactivo, April 12, 2021.
3. **Santamaria, L.** 2021. Boxwood blight - scouting, symptom identification, and best management practices, May 17, 2021.

Extension Publications in both English and Spanish

1. **Ohkura, M.***, Nackley, L. L., Scagel, C. F., and **Weiland, J. E.** 2021. Social distancing and staying dry keeps boxwood blight at bay. Digger, Part of the Oregon State University (OSU) Growing Knowledge Series at <http://www.diggermagazine.com/call-it-herbal-distancing/>.
2. **Pscheidt, J. W.** 2021. Boxwood (*Buxus* spp.)-Box Blight. In: Pscheidt, J. W., and Ocam, C. M., senior editors. 2021. Pacific Northwest Plant Disease Management

Handbook [online]. Corvallis, OR: Oregon State University.

<https://pnwhandbooks.org/plantdisease/host-disease/grass-seed-stem-rust>.

3. **Santamaria, L.** 2020. El tizón de boxwood en viveros de producción comercial. Oregon Extension Service Publication No.: EM9124-S first published in 2016, reviewed and updated 2020 at <https://catalog.extension.oregonstate.edu/em9124s>.
4. **Santamaria, L.** 2020. Boxwood blight in commercial nurseries. Oregon Extension Service Publication No.: EM9124 first published in 2016, reviewed and updated 2020 at <https://catalog.extension.oregonstate.edu/em9124>.
5. **Weiland, J. E. and Pscheidt, J. W.** 2020. Scouting for boxwood blight. Digger 64(3):25–28. Part of the Oregon State University “Growing Knowledge” series.

Bilingual Poster

1. **Santamaria, L.** 2020. Boxwood Blight in commercial nursery production - El tizón de boxwood en viveros de producción comercial. Oregon Extension Service Publication No.: EM9124-P first published in 2016, reviewed and updated 2020 at <https://catalog.extension.oregonstate.edu/em9124p>.

Advising Public Gardens on Boxwood Blight Mitigation

1. **Hong, C. X.** 2020. Boxwood blight diagnosis and mitigation. Dumbarton Oaks, DC via Zoom, October 26, 2020.
2. **Hong, C. X.** 2020. Composting of blighted plant materials. Longwood Gardens, Kennett Square, PA via Zoom, November 6, 2020.
3. **Hong, C. X.** 2021. Surveying and containment of an accidental introduction. Colonial Williamsburg Foundation, Williamsburg, VA, July 30, 2021.

Citizen Science Projects

1. **Hong, C. X.** 2020. Monitoring the blight pathogen in soil at Longwood Gardens, Matt Taylor, Longwood Gardens, Kennett Square, PA, October to November 2020.
2. **Hong, C. X.** 2021. Monitoring the blight pathogen in soil at Longwood Gardens, Rachel Schnaitman, IPM Manager, Kennett Square, PA, June to July 2021.
3. **Hong, C. X.** 2020. Evaluating boxwood collections on a naturally infested farm in Lowgap, NC, Saunders Genetics, LLC, Piney River, VA, September 2020 to August 2021.

Professional Development

Capacity-Building Seminar Series

Microsoft Teams for improved internal communications by Paige Ghra, Program Coordinator, Collaborative Computing Solutions at Virginia Tech, on July 20, 2020. This 90-minute training session was conducted on the Teams platform and attended by all Project Directors and associates and some advisory panel members.

Plant microbiome and how to study it with high throughput sequencing by Dr. Xiaoping Li, Postdoctoral Microbiome Scientist of Virginia Tech on February 12, 2021. This seminar was conducted via Zoom with a large number of participants including Project Directors, associates, Advisory Panel members, and collaborators. The webinar was recorded. The recording is posted at the outreach website - [Boxwood Blight Insight Group | HRI \(boxwoodhealth.org\)](https://boxwoodhealth.org) under the Knowledge Center tab.

ACCOMPLISHMENTS

What was accomplished under the proposed objectives?

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

1.1 Developing on-site commercial strip kits for pathogen detection

We took two approaches to develop on-site pathogen detection assays, namely a serological approach and a DNA isothermal amplification method. For the serology-based approach, we identified a protein target (12239) by proteomics analysis. This highly abundant protein target is unique to the boxwood blight fungi, *Calonectria pseudonaviculata* (*Cps*) and *C. henricotiae* (*Che*). High abundance and specificity make it a potentially suitable candidate to develop antibodies. Subsequently, two polyclonal antibodies, pAb 12239-1 and pAb 12239-2 (**Figure 1**) against two partial sequences of target protein 12239 and their corresponding recombinant proteins (rProteins) were generated and purified.

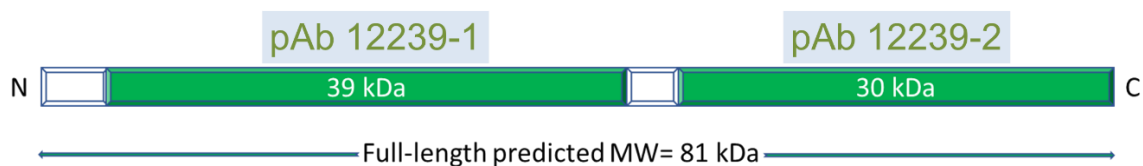


Figure 1. A schematic of the protein target including two polyclonal antibody-targeted partial sequences used for the development of serological diagnostic assays for boxwood blight

We have tested the pAbs using both western blots and double antibody sandwich enzyme-linked immunosorbent assays (DAS-ELISA). The western blot results (**Figure 2**) indicated that both pAbs recognized their respective rProteins and reacted with the target protein in extracts of *Cps*- and *Che*-infected boxwood leaves, but not with extracts of healthy leaves or leaves with *Volutella* blight symptoms.

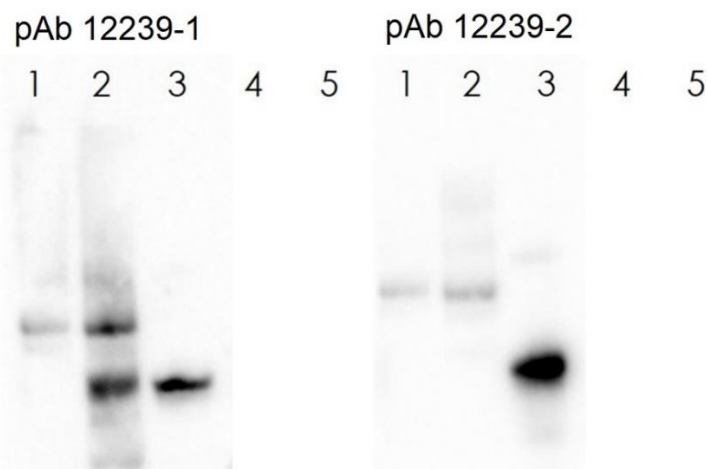


Figure 2. Western blots using pAbs 12239-1 and 12239-2. Lane 1, *Buxus sempervirens* 'Justin Brouwers' leaves infected by *Cps* isolate BB; Lane 2, boxwood leaves infected by *Che* isolate G55; Lane 3, 1 ng of corresponding rProtein; Lane 4, boxwood leaves with symptoms of *Volutella* blight caused by *Pseudonectria* spp.; Lane 5, non-infected boxwood leaves.

The DAS-ELISA result (**Table 1**) indicated that pAb 12239-1 efficiently captured its corresponding rProtein and detected the target antigen in extracts of *Cps*- and *Che*-infected leaves, but it did not react with the rProtein of 12239-2 or extracts of healthy leaves or leaves expressing *Volutella* Blight symptoms.

Table 1. Absorbance readings at 405 nm for DAS-ELISA using pAb 12239-1. Plate was coated with pAb and detected using horseradish peroxidase-conjugated pAb at 1:1000 dilution.

pAb	rProtein 12239-1	rProtein 12239-2	<i>Che</i>	<i>Cps</i>	Healthy leaves	Leaves shown <i>Volutella</i> blight
12239-1	2.273	0.137	1.129	1.306	0.378	0.224

For the DNA isothermal amplification approach, we designed seven primer pairs against two DNA targets (predicted genes P.25 and P.38) that are unique to *Cps* and *Che*. Preliminary specificity and sensitivity evaluations are underway using PCR.

1.2 Oregon nursery survey

Three of five nurseries scheduled to be surveyed in 2021 are complete. All three nurseries had boxwood blight. Data were collected on symptom presence, cultivar affected, and plant size and spacing. Preliminary results show that this year's drought and heat in Oregon have affected symptom expression. Symptoms were less common and less severe than in previous years when temperatures were more moderate and rainfall was more abundant. Leaf spots were mostly absent in 2021, with stem lesions and leaf drop occurring more frequently. Most symptoms developed during the rainy season in late 2020 and early 2021. Few new symptoms have developed since the onset of hot, dry weather in June 2021, unless the grower is watering extensively. This has made disease scouting more challenging this year. The most commonly infected cultivars include Winter Gem, Green Gem, Green Mountain, Green Velvet.

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

An experiment was conducted to evaluate symptom development on *B. microphylla* var. *japonica* 'Green Beauty' (representing resistant cultivars) and *B. sempervirens* cultivars (susceptible) inoculated outdoors in fall 2020, winter 2020, spring 2021, and summer 2021 in Oregon. Disease did not develop on most plants regardless of cultivar or season. Based on the lack of response, a modified growth chamber experiment is being implemented to explore infection at cooler temperatures on a resistant (Winter Gem) and susceptible (Green Velvet) boxwood cultivar at two temperatures at 75% relative humidity. Infection has occurred at both 15°C (average Oregon fall/spring temperature) and 25°C (optimum temperature for growth and infection). Data is being collected on disease incidence and the percent canopy affected by boxwood blight symptoms (leaf spots, leaf drop, and stem lesions).

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

These studies have been on hold due to the COVID-19 pandemic and restricted access to the lab facility.

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

We used detached leaves from *Buxus sempervirens* ‘Suffruticosa’, ‘Vardar Valley’, and ‘Justin Brouwers’, *B. harlandii* ‘Green Valley’, *B. microphylla* ‘Little Missy’ and NewGen Independence®, representing different levels of susceptibility to *Cps*. We collected similar-aged leaves from several plants of each cultivar, then pooled and randomized them. Five leaves were placed on moist filter paper in each Petri dish with the stomata-bearing lower surface facing up. We prepared inoculum of each *Calonectria* species and adjusted the concentration of each spore suspension to 10,000 conidia/mL. Each suspension was sprayed over open dishes containing leaves of the different cultivars. As a control, four dishes of each cultivar were sprayed with water. Dishes of each cultivar were incubated for 6 days at 12, 18, 24, and 30°C in growth chambers set at a 16-h photoperiod. After that, each dish was scanned using APS ASSESS 2.2 to measure total leaf area. Then the percent lesion area was determined for each leaf in the dish. After that, leaves were removed from dishes and put in 5 mL of sterile water in a 50-mL centrifuge tube. The centrifuge tube was vortexed for 30 sec, and 20-μL aliquots were placed into four cells of haemocytometer plates and conidia/mL calculated. From these measurements, the average lesion area is calculated for each cultivar at each temperature, and the number of spores per unit lesion area. This experiment has been run three times so far and data is being analyzed.

1.6 Bridging the blight knowledge gaps - field epidemiology

We took several approaches to the field epidemiology of boxwood blight. In collaboration with Dr. Thomas Brand in Germany, we have had full access to fourteen datasets of fungicide trials and five datasets of boxwood cultivar trials that he and his colleagues conducted from 2006 to 2020. We also have full access to all associated weather monitoring data. We have looked into the details on how each trial was conducted and how data were collected and visualized all datasets. We are currently assessing how weather may have impacted blight onset and progression of English boxwood starting with four data sets of fungicide trials with multiple blight assessments within each season. We also worked with Dr. Matthew Cromey and examined the historic records of boxwood blight samples submitted to the Royal Horticultural Society lab in the United Kingdom where this disease was first discovered in mid-1990s. Additionally, we worked with Dr. Len Coop and his associates – modeling the blight infection risk and establishment for both short and long-term prediction of this emerging invasive species.

We also networked with research and extension plant pathologists sharing interest in the blight epidemiology across the globe. We hosted a Zoom meeting on August 10, 2021 with the purpose of getting to know one another, briefing on recent and ongoing epidemiology research as well as future study plans, in order to brainstorm on future directions and foster collaborations. Nineteen scientists attended this introductory meeting and they are scheduled to meet again on October 25, 2021.

1.7 Identifying critical control points (CCPs) at production nurseries

We are working on a comprehensive national list of boxwood nursery growers of varying sizes. We are also working on the development of a survey questionnaire to determine growers’ boxwood production flow, baseline knowledge of boxwood blight disease, and knowledge and practice of boxwood blight best management strategies. The survey will also include questions

related with estimation of the current economic impact of boxwood blight disease and management techniques.

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

2.1 Evaluating antidesiccants for blight mitigation

Three best performing antidesiccant products (TransFilm, Vapor Gard, and Wilt Pruf) under controlled environments are being evaluated and compared to three fungicide standards (Daconil Weather Stik, Banner Maxx, and Concert II) under field conditions with an existing crop of Vardar Valley boxwood and natural inoculum in Western North Carolina. Also included in this trial are three combination treatments of a fungicide and TransFilm, and one nontreated control. For combination treatments, fungicides were applied one day before the application of TransFilm. Spring and fall treatments were applied to the foliage in Mid-April and late August 2021, respectively. Each antidesiccant and fungicide was applied at its labeled rate. Ten treatments each included six replicate plants, and treatments were arranged in a randomized complete block (RCB) design. Blight incidence is assessed monthly. The disease incidence remained low in this trial, so we have not seen any difference among the treatments yet as the peak disease season is yet to come in September to early November. This trial has been done in close collaboration with North Carolina Department of Agriculture and Consumer Services (NCDA&CS), North Carolina Cooperative Extension (NCCE), and the grower (**Figure 3**).



Figure 3. Dr. Olanike Omolehin working with Amanda Taylor of North Carolina Cooperative Extension, Dr. Hsien-Tzer (HT) Tseng, and Chad Taylor of NC Department Agriculture and Consumer Services (from left to right) evaluating selected antidesiccants for boxwood blight control in Western North Carolina

To expedite the field evaluation, another trial consisting of three antidesiccants, one fungicide standard (Concert II), one combination (Concert II and TransFilm), and one nontreated control was added to the project. This additional trial was established in Lowgap, NC with an existing crop of Justin Brouwers boxwood and natural inoculum. Severe blight has developed in this trial and TransFilm with one single spring treatment in Mid-April has reduced the disease severity. Almost no diseased leaves were seen on plants treated with Concert II every three weeks. Fall treatments were applied in late August. This trial has been conducted in close partnership with a private grower.

2.2 Further developing biocontrol agents (BCAs) into final products

Two field trials have been established in Western North Carolina to evaluate six selected BCAs, plus several of their combinations against one fungicide standard (Concert II) and a nontreated control, totaling twelve treatments. These treatments included seven replicate plants each and they were arranged using an RCB design within each trial. The first treatments were applied in late May and the second treatments done in late August. Blight incidence has been assessed monthly and remained low so far. Again, these trials have been performed in close collaboration with the NCDA&CS and NCCE as well as the growers.

Experiments were also conducted under controlled environments to determine the survival of one of the BCAs—*Burkholderia cepacia* strain SSG—and to identify the optimum culture media and age as well as surfactant formulations to improve the survival of BCAs on foliage and their performance in protecting boxwood from infection by the blight pathogen (**Figure 4**).



Figure 4. Dr. Olanike Omolehin examining how culture media and age may affect the antagonistic activities of selected biological control agents against the boxwood blight pathogen

Selected BCAs also were sent to Dr. Kurt Heungens at the Flanders Research Institute for Agriculture, Fisheries and Food in Belgium and they are being evaluated under local conditions. Both teams met twice via Microsoft Teams, sharing data and brainstorming on strategies to improve BCA survival on boxwood foliage and their performance against the blight disease.

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

We have propagated the small plants of susceptible cultivars needed to test SAR inducers and selected nutrients for their effect on boxwood blight. We have purchased plastic chambers that can be used to hold the plants at high relative humidity after inoculation. A preliminary trial in the fall of 2020 indicated that the inoculum level tested was too low for the moderately-susceptible boxwood cultivar used, so we will be increasing the inoculum 10-fold in our fall 2021 trials. No treatment effects were observed in our first runs because there were so few points of infection, even in the inoculated controls.

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

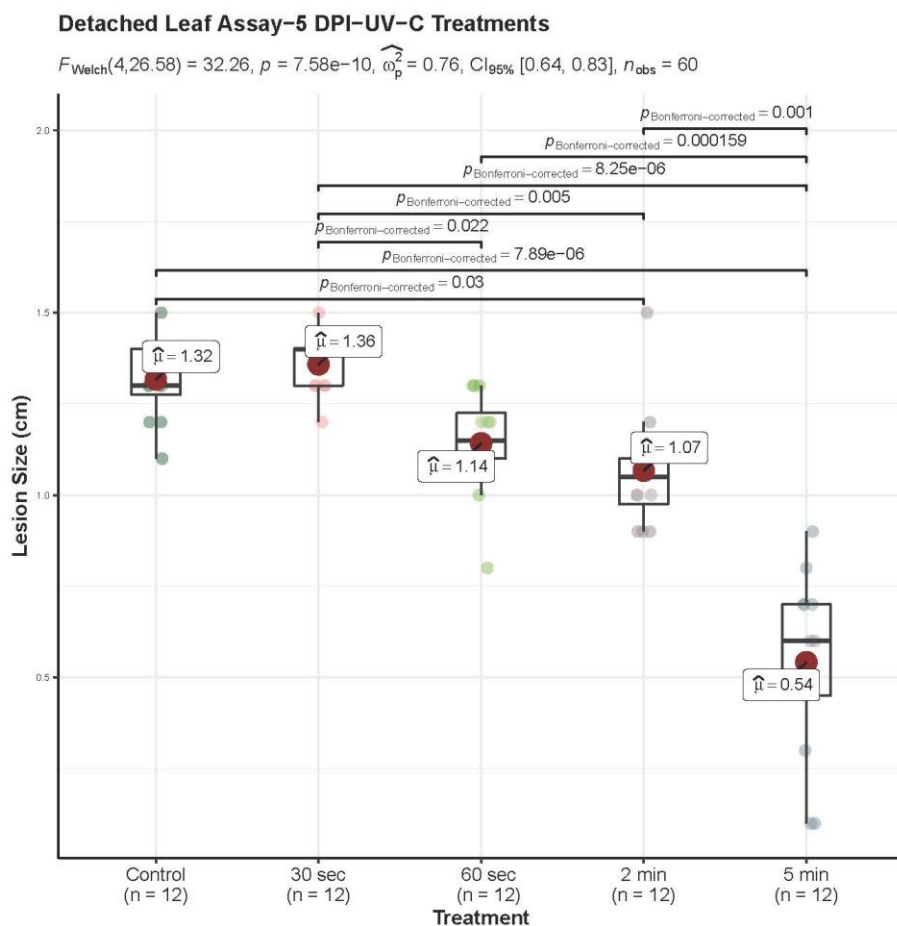


Figure 5. Boxplots showing mean lesion size from 12 replicate leaves of *B. sempervirens* ‘Suffruticosa’ after 5 days post inoculation (dpi) with *Cps* strain NCBB1 treated with UV-C light. Results from five levels of UV-C treatments are shown along with statistically significant differences between treatments ($P \leq 0.05$) shown with connecting lines using Welch’s analysis of variance (ANOVA) test.

Preliminary experiments have been conducted to assess disease-control strategy using low-dose UV-C irradiation (low dose/short duration). A detached leaf disc assay was used to evaluate the effectiveness of controlling boxwood blight and enhancement of natural defense compounds in the host plant. Initial testing used a highly susceptible cultivar, *B. sempervirens* ‘Suffruticosa’. Five treatment assays each containing 12 replicate leaves consisting of an untreated control, 30-sec, 1-min, 2-min, and 5-min exposure by UV-C lamps with an output of approximately 20 $\mu\text{W cm}^2$. Disease severity was determined by measuring lesion size across 5 days post-inoculation. Results showed marginally significant differences ($P \leq 0.05$) between all treatments except control vs 30-sec, control vs 60-sec, and 1-min vs 2-min treatments. A second round of treatment replicating the first experiment produced similar results as seen in **Figure 5** and **Figure 6**.

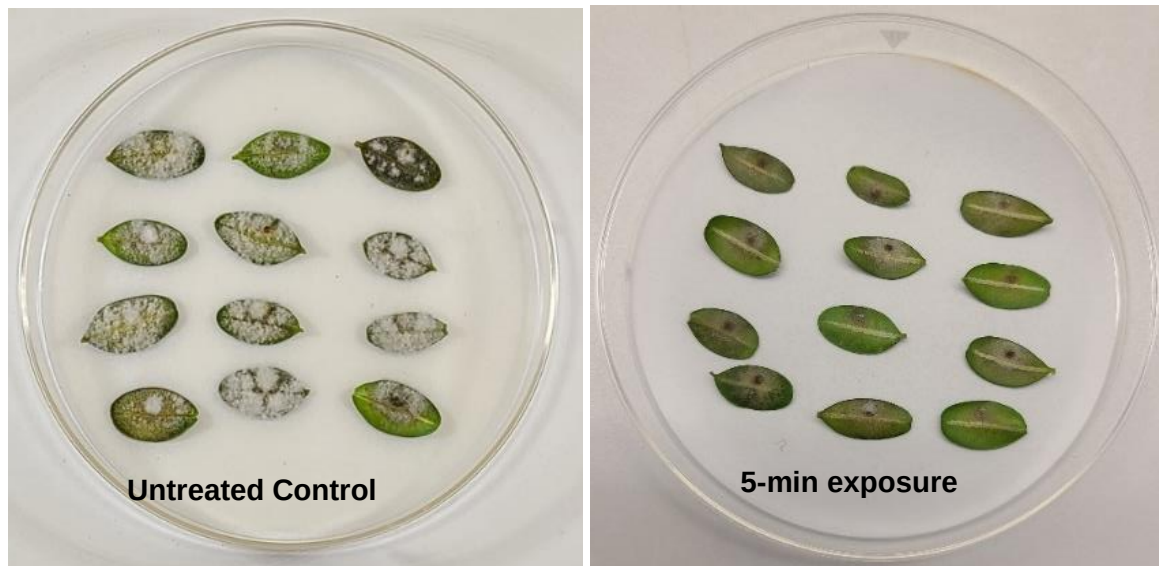


Figure 6. Inoculated detached leaves of *B. sempervirens* ‘Suffruticosa’ 5 days post inoculation with *Cps* strain NCBB1. Shown here are 12 replicate leaves used for an untreated control receiving no UV-C treatment and UV-C treatment with a 5-minute exposure.

Testing the effects of silicon (Si) treatment on boxwood blight incidence is underway. Two-year-old plants of two cultivars, *B. microphylla* ‘Little Missy’ and *B. sempervirens* ‘Suffruticosa’, are currently being treated and evaluated on the effect of continuous Si soil treatment. Treatments started on July 30, 2021, a time during which the plants are actively growing. We tested Si concentrations of 0, 2.5, 5, and 10 mg L^{-1} with YarVita Actisil (Bio Mineral N.V., Belgium), whose active compound is choline-stabilized orthosilicic acid. Treatments are being applied over an 8-week period and leaves and stems will be harvested and analyzed for Si content using variable pressure scanning electron microscopy with x-ray microanalysis. Treated plants will then be inoculated and evaluated for disease symptoms.

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

Understanding the epidemiological factors governing disease development is foundational for integrating recent innovations into boxwood blight best management practices (BMPs). We evaluated the effect of leaf wetness period and temperature on infection and sporulation of three boxwood cultivars under controlled conditions to develop predictive models for disease development. Specifically, we conducted detached leaf assays between 18 and 27 $^{\circ}\text{C}$, and

various leaf wetness periods (lwp), with the cultivars *B. sempervirens* ‘Suffruticosa’, *Buxus* × ‘Green Velvet’, and *B. microphylla* var. *japonica* ‘Winter Gem’. Detached leaves were inoculated with 200 conidia in suspension and disease incidence was recorded at 3 to 13 days post inoculation (dpi). Cultivar, lwp, temperature, and most of the interactions significantly influenced disease development. A minimum of 5 h of leaf wetness was required for any infection. Infection incidence increased most rapidly between 12 and 15 h and continued to increase to about 21 h of leaf wetness. Temperatures between 21 and 25°C were optimal for disease development. There was about a 7-day lag between appearance of lesions and maximal sporulation. The two less-susceptible cultivars had fewer lesions than ‘Suffruticosa’ under the same infection conditions; in addition, *Cps* sporulated on infected Winter Gem boxwood leaves later and at a smaller proportion. Response surfaces were developed for each cultivar to predict the infection percentage using the lwp and dpi.

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

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

Along with studying disease development in different cultivars, pictures of symptoms are being taken so differences in lesion appearance on different cultivars are documented and can be used in outreach bulletins.

Objective 3: To build resilience into boxwood production and plantings

3.1 Developing more resistant boxwood cultivars

To date, at least 70 sets of unique controlled pollinations have been performed, resulting in intra- and interspecific F₁ boxwood hybrids designed for breeding and genetic mapping purposes. These plants are being germinated and grown in polyhouses and will be planted out in nursery beds in years 3 and 4 of this project for further evaluation. Current hybrids and accessions are being evaluated for boxwood blight resistance using a detached leaf assay. These genotypes are expected to be genotyped using a genotyping-by-sequencing method in years 2, 3, and 4 of this project for trait mapping. In preparation for this, we have developed a new acetone-based DNA extraction method for woody plant taxa that works especially well for boxwood and reduces total extraction time by at least 2 hours, while producing yield quantities and quality similar to commercial kits. Additionally, a project to generate and annotate a boxwood reference genome is underway for the diploid cultivar *B. sempervirens* ‘Suffruticosa,’ using PacBio and Omni-C platforms. To-date 1.1 TB of PacBio Hi-Fi reads, 132 GB of RNA-Seq reads and 24 GB of Omni-C data have been generated. Initial genome assembly using the Hifiasm *de novo* assembler has produced a total assembly size of 1 Gb with 3,450 contigs and an N50=14.6 and L50=11. Repeat masking showed 65.5% of the total genome was masked, with 26.9% representing Class I and II TE repeats. Genome annotation results showed 32,326 total genes from *de novo* gene prediction and BUSCO analysis of predicted genes showed 80.4% complete single-copy BUSCOs. This is the first boxwood genome sequenced and will be an invaluable resource for breeding and genetic trait mapping. RNA-sequencing, gene prediction, and manual curation will continue as well as adding short-read Illumina data for gap filling and polishing the genome assembly.

3.2 Cataloging and manipulating boxwood microbiomes against *Cps*

Three studies are well underway aiming to better understand the boxwood phyllosphere microbiome (BPM). The first study focuses on the geographic location and climatic effects on the BPM. One nursery in Oregon, one in Virginia, and several boxwood cultivars representing different species were carefully selected for this study. Replicate samples have been taken from selected nurseries twice in May and August of 2021, respectively. Following initial processing, DNA has been extracted from all samples and amplicons of two genetic markers (ITS and 16S) are ready for sequencing with the next generation sequencing technology (Oxford Nanopore Technologies) to characterize both epiphytic and endophytic fungal and bacterial communities. This study is expected to continue until 2023.



Figure 7. Dr. Xiaoping Li taking plant sample from a research site in western North Carolina to assess how fungicide and antidesiccant treatment may affect the boxwood microbiome

The other two microbiome studies centered on fungicide and antidesiccant effects on the BPM (**Figure 7**). These studies were done in conjunction with the antidesiccant trials above (2.1). For fungicide impact, three chemistries were included: chlorothalonil (Daconil Weather Stik), propiconazole (Banner Maxx) and a combination of both (Concert II), plus a nontreated control. Samples were taken from boxwood plants immediately, 1 week, and 2 weeks after fungicide treatments. For the antidesiccant effect, three products were included: TransFilm, Vapor Gard, and Wilt Pruf, along with a nontreated control. The first samples were taken 1.5 and 3 months after antidesiccant treatments. DNA has been extracted from these samples and PCR products are ready for Nanopore sequencing. The second treatments were applied late August 2021 and boxwood samples are scheduled to be taken in Early October and late November. These two studies are expected to be completed by the end of 2021.

In addition, two English boxwood plantings were established on the research farm of Virginia Tech's Hampton Roads Agricultural Research and Extension Center in Virginia Beach, VA in July 2021. These plantings will be used to investigate how fertilization and irrigation regimes may affect the boxwood microbiome and plant health.

3.3 Facilitating adoption of more resistant boxwood cultivars

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

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

4.1 Developing baseline production model

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

4.2 Analyzing cost/benefit of changes in blight mitigation practice

Due to COVID, this objective has been delayed until year 2 of the project.

4.3 Partial budgeting: An economic engineering approach

This objective is not scheduled until year 2 of the project, therefore no progress is being reported on this objective.

4.4 Performing sensitivity tests

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

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

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

4.6 Assessing the overall project impact

This objective is not scheduled until year 3 of the project, but an initial analysis of the introduction and subsequent spread of boxwood blight since its detection in the U.S. has been conducted and published in the Journal of Environmental Horticulture. This provides a baseline level of boxwood blight to assess the progress of our various projects.

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

Delivering research and innovations to the end user has been an integral part of this project as detailed below. To best serve the horticulture industry, public and private gardeners, we networked with prominent scientists here in the U.S. and overseas, and partnered with stakeholders to establish an international boxwood blight research and education consortium – Boxwood Blight Insight Group (BBIG). We also designed a logo reflecting the project goal (sustainable boxwood production and gardening) and approach (focused science and application studies). Accordingly, we integrated this logo into our newsletter, PowerPoint presentation, Word, and PDF document templates for more effective and efficient technology transfer.

5.1 Translating research into products, protocols, and recommendations

BBIG Newsletter

The BBIG Team has developed a project newsletter to communicate the latest research and recommendations. This BBIG newsletter is published every three months. The inaugural issue was published in early December 2020, followed by three additional issues in March, June, and September of 2021, respectively.

Scouting Videos and BMPs

The Oregon and Tennessee teams developed three videos to help growers, landscapers, and other horticulturists to scout for and better manage boxwood blight at production and in the gardens and landscapes. The scouting video is in both English and Spanish to meet the needs of the diverse work force in the horticulture industry.

Bilingual Educational Resources

In addition to the scouting video, the Oregon team developed several extension publications on BMPs in both English and Spanish (**Figure 8**).

L. Santamaria

Tizón de boxwood
La enfermedad del tizón de boxwood
La caída de hojas de boxwood

Calonectria pseudonaviculata
También conocido como *Cylindrocladium*
pseudonaviculatum o *C. buxicola*

Photo: John S. Hamrick, Oregon State University

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Luisa Santamaria, patóloga de plantas para cultivos de vivero, profesor asistente y educadora bilingüe, North Willamette Research and Extension Center, Oregon State University

L. Santamaría

boxwood blight
box blight
blight disease of boxwood
boxwood leaf drop

Calonectria pseudonaviculata
Also called *Cylindrocladium*
pseudonaviculatum or *C. buxicola*

Photo: J. A. S. Smith, © Oregon State University

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Luisa Santamaria, Extension plant pathologist for nursery crops, assistant professor, and bilingual educator, North Willamette Research and Extension Center, Oregon State University



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Photo by Earl Pula, Oregon Department of Agriculture, used by permission. Visit www.oregon.gov/odag/soil/soil.htm

Other diseases in boxwood

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5.2 Delivering to end users

In addition to conventional means of technology transfer, we have developed and utilized several effective and efficient mechanisms and/or platforms for expedited and mass dissemination of research results from this project directly to end users.

Conventional Means

- Quarterly meetings with the project's Advisory Panel, all of whom are leaders in the nursery industry and public gardens, by direct presentations via Zoom.
- Two field demonstrations in Tennessee and Virginia.
- Three short courses and fifteen webinars on boxwood blight research at a number of virtual meetings of gardeners, nurserymen and arborists, as well as making a few in-person appearances over the past year.
- Five extension publications.

Expedited and Mass Dissemination

- Boxwood Blight Insight Group website (<https://www.boxwoodhealth.org>) was developed in collaboration with AmericanHort and the Horticultural Research Institute (HRI) and released to the public in November 2020 (**Figure 9**). In addition to developing a user-friendly interface, HRI is hosting this outreach website, including a knowledge center, for timely updates and continuing service to the horticulture industry and the public beyond the project period. This outreach website has been a powerful platform for communicating our project vision, mission, goals, and approach, as well as research updates to keep stakeholders informed, engaged, and excited about the project progress. Since its release, this website has had over 2,700 views. This website provides a platform for other research projects to share their results on boxwood blight and other invasive pests such as box tree moth.

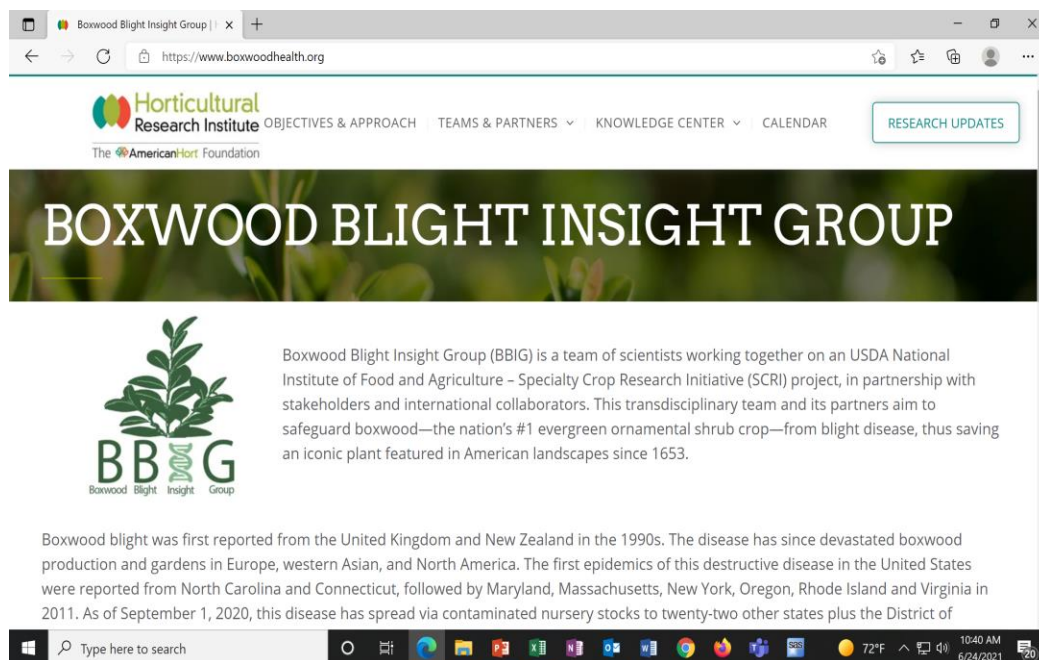


Figure 9. Screen shot of the Boxwood Blight Insight Group outreach website

- BBIG on Live Stage of the tHRive Knowledge Center at Cultivate'21 in Columbus, OH on July 11, 2021 (**Figure 10**). The Extension team leadership partnered with the AmericanHort/Horticultural Research Institute (HRI) leadership in planning this national event that provided the BBIG Team a platform to present and connect to various sectors of the horticulture industry. This session consisted of four presentations, three given by BBIG Project Directors (Drs. Fred Gouker, Chuanxue Hong, and James LaMondia) and one by a collaborator – Dr. Len Coop of Oregon State University.



Figure 10. BBIG partnered with AmericanHort and Horticultural Research Institute (HRI) who hosted a Live Stage session at Cultivate'21 in Columbus, OH on July 11, extending our connection to the industry while providing an outreach platform for a collaborating researcher – Dr. Len Coop of Oregon State University

- BBIG Google Group (bbig-g@vt.edu) was launched and used, along with other local and national networks, for dissemination of BBIG newsletters, research and extension publications, and boxwood health related news. A total of 10,391 boxwood enthusiasts were reached via the BBIG group alone during the first year.
- Outreach to the extension communities and large growers and landscapers through open access publications in Plant Health Progress.

Outreach to Researchers, Diagnosticians and Extension Personnel Outside of This Project

This was accomplished by:

- Organizing an Idea Café at the Annual Meeting of the APS – Plant Health 2021 Online on August 2, 2021. This was one of the well-attended breakout sessions with about 30 attendees.
- Publishing research results through 19 refereed journal articles.
- Communicating research data through 16 presentations at annual meetings of the APS and the Ecological Society of America.
- Communicating research through two invited lectures.

International Boxwood Seminar Series

The project team reached out to prominent boxwood scientists here in the U.S. and overseas, developed and added this seminar series to the project - bringing their expertise to the horticulture industry, master gardeners and consumers. The inaugural presentation was held on March 18, 2021 (**Figure 11**), followed by the second and third webinars on June 2 and August 25, 2021, respectively. These webinars target all sectors of the horticulture industry, public garden managers, consumers, boxwood research and extension communities, as well as regulatory personnel. The inaugural seminar attracted 359 attendees from the U.S. and a dozen foreign countries and received over 70 follow-up questions and discussion threads.



Figure 11. Dr. Thomas Brand, the Department Head of Ornamental Plant Cultivation, Tree Nurseries, Public Greenery at the Chamber of Agriculture of Lower Saxony, Germany, and the inaugural speaker of the International Boxwood Seminar Series, giving a presentation “Boxwood Blight – A 15-Year Love-Hate Relationship” on March 18, 2021

Citizen Science Projects

These projects were also added to meet the emerging needs.

- For pathogen monitoring at sites of infestation, we advised garden managers on experiment design, provided technical and material assistance, and helped analyze and interpret their data.
- For field trials evaluating boxwood collections for blight resistance, we helped growers analyze their research data, and advised on writing the results for publication.

- We also worked with a homeowner to rebuild her garden with NewGen® cultivars, plus sanitation, mulching, and science-directed fungicide protection after she lost over 300 well established boxwood to the blight.

5.3 Documenting project impacts

This project has resulted in many significant impacts on boxwood blight mitigation during the first 12 months.

Changes in Knowledge

- Our extension programs, including scouting and BMPs videos, the BBIG website at www.boxwoodhealth.org, and the International Boxwood Seminar Series, have enabled growers, retailers, landscapers, arborists, garden managers and other horticulturists, as well as regulatory personnel, diagnosticians, and extension specialists and agents better understand the blight disease, its diagnostic characters and current/latest mitigation technologies. Stakeholders are better able to prevent blight accidental introduction to their properties, identify and scout for blight hot spots, make informed decisions, and act in a timely manner to better manage and contain the disease at sites of contamination should it occur.
- Our research and innovation have advanced the science of boxwood blight biology, epidemiology, and diagnostic and mitigation technology. For example, preliminary evidence shows that spacing plants further apart reduces the chances for the pathogen to spread from an infected plant to nearby healthy plants. Furthermore, decreasing irrigation frequency reduces infection and disease severity. Thus, critical changes in the way nurseries grow boxwood (increased plant spacing and decreased irrigation) can reduce the chances for boxwood blight outbreaks and pathogen movement within the nursery. These advancements will continue to change blight diagnostics and improve disease mitigation in both the landscape and the nurseries, thereby benefiting the horticulture industry and consumers for years to come.

Changes in Practice and Condition

- Boxwood production has shifted from severely affected states to less affected states or regulated states as demonstrated by the Economic Team.
- Many growers have shifted their boxwood inventory to include less susceptible cultivars, a practice that will change the health of boxwood plantings nationally in the future.
- Many stakeholders have begun to take a systems approach to blight mitigation and boxwood crop health management while focusing on blight prevention and trying to contain and eradicate the disease at its earliest detection as exemplified by the public gardens we have worked with.
- Some of the stakeholders have learned through Citizen Science Projects and set up their own facilities and programs to monitor the blight pathogen in infested soil. While these efforts started at individual production facilities and gardens, they created conditions limiting pathogen spread, suppressive to pathogen survival, infection, and reproduction, consequently contributing to the blight mitigation and containment at local, regional, and national levels.

Other Impacts

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

- AmericanHort and HRI are hosting the project outreach website with a knowledge center and helped us develop a user-friendly interface. This invaluable partnership is projected to provide continual maintenance and service to the horticulture industry and consumers, even after this SCRI project ends. In addition to hosting our International Boxwood Seminar series, AmericanHort and HRI are playing key roles in planning, organization, publicizing, running, recording, posting recordings, and documenting attendance metrics.
- North Carolina Department of Agriculture and Consumer Services, North Carolina Cooperative Extension and local farmers all have played key roles in every important step of the ongoing field trials, including their hands-on efforts to identify naturally infested fields, evaluate antidesiccant and biocontrol agents, make scheduled applications of fungicide standards, and perform monthly blight assessments.
- In addition to sharing their expertise through the International Boxwood Seminar series, researchers in Belgium, Germany, and United Kingdom are collaborating on several important project components including biocontrol, field epidemiology. These collaborations accelerated understanding of the blight biology.
- We have also had extraordinarily fine support from our Advisory Panel members. In addition to providing feedback at our quarterly meetings, some individuals have shared additional time and talent. For example, Bennett Saunders and John Keller joined the BBIG Live Stage session at Cultivate'21; Lynn Batdorf presented one of the International Boxwood Webinars, and staff from Saunders Brothers nursery have worked directly with postdoc Dr. Xiaoping Li to collect boxwood samples for the microbiome studies.

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

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

Training for Project Team and Collaborators

- A training session on Microsoft Teams by Paige Ghra, Program Coordinator, Collaborative Computing Solutions at Virginia Tech, was organized and conducted on July 20, 2020 for Project Directors (PDs), associates, and Advisory Panel members in order to improve the internal communications for enhanced project productivity, quality and impacts.
- A seminar on how to study the plant microbiome by Xiaoping Li, postdoctoral microbiome scientist, for PDs and their associates, Advisory Panel members as well as some collaborators was presented via Zoom on February 12, 2021 as part of our efforts in team capacity-building.

Professional development opportunities for Postdocs and Students

- One postdoc has had experience as Project Manager, with extra responsibilities for handling communications via Teams, Zoom and email, assembling reports, and coordinating other postdocs in perfecting a poster on the BBIG research team's organization and objectives. Coaching is provided by the Project Leaders as needed.
- One postdoc – Applied Plant Pathologist, has transitioned into the role of coordinating field trials evaluating antidesiccants and biocontrol agents with NCDA&CS, NCCE and collaborating growers and become a point of contact for the collaboration with the Flanders Research Institute for Agriculture, Fisheries and Food in Belgium. PDs advised and supported as needed.
- One postdoc has coordinated boxwood sampling with the OSU/USDA ARS HCRL team in Corvallis, OR and collaborating growers in Virginia for the microbiome studies. This postdoctoral associate also directly worked with the growers taking samples late spring and summer. PDs helped connection with collaborating PDs and growers, advised on planning, and provided other support as needed.
- One postdoc – Epidemiologist, has become a point of contact person for collaboration with German scientists, re-analyzing their data of field trials evaluating fungicides, boxwood cultivars and other blight management strategies from the epidemiology perspective. This postdoctoral associate is transitioning into a role of coordinating the newly established International Working Group of Boxwood Blight Epidemiology, with PDs' support as needed.
- Two postdocs on the project have had the opportunity to coordinate multi-author efforts on two publications on boxwood diseases for the diagnostic community. PDs have provided advice and leadership as needed. The first of these publications, a diagnostic guide for boxwood blight disease, has been downloaded over 3,000 times, and was ranked as the second-most downloaded paper published in the journal for the year.
- Postdocs and graduate students presented at the quarterly Project Team meetings.
- Postdocs and graduate students were supported to attend the APS meetings and other professional meetings such as Phyllosphere Fortnight 2021 at [Phyllosphere Fortnight 2021](https://www.phyllospherefortnight.org/2021/) at [Phyllosphere Fortnight 2021 – PHYLLOSPHERE 2022 \(ucdavis.edu\)](https://www.phyllospherefortnight.org/2021/).

How have the results been disseminated to communities of interest?

We consider research result dissemination an integral part of this project. Please see Objective 5 for details.

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

We will make every effort to catch up on research components that were delayed due to the COVID-19 pandemic this past year, while striving to do even better for the other research and extension programming—where we have in some cases exceeded what we originally proposed, by adding the International Boxwood Seminar series and Citizen Science Projects.

CHANGES/PROBLEMS

Although the team has had many achievements in research and outreach over the first year of the grant, the ongoing pandemic of COVID-19 has caused unprecedented challenges to all the collaborating institutions. All USDA research labs and units have been in a federal government mandated gating phase, with only essential employees allowed to be onsite at a 25% facility capacity limit. While this has hampered laboratory-based research objectives, significant progress has been made in meeting objectives. PDs have collectively met and exceeded all extension goals. Eight of 13 research goals are at 100% completion or ahead of schedule, while the remaining five research goals are at or below 15% completion due to COVID-19-related restrictions on facility occupancy and/or travel, particularly at USDA locations. University programs have also faced reduced lab access and in some cases, requests from upper administration to cut back on planned activities and spending. Quarantine periods have been necessary on short notice when staff became ill with COVID-19. Our team has shown resilience under these circumstances and, as a result, the objectives all continue to appear to be achievable within the granting period.