



United States Department of Agriculture

National Institute of Food and Agriculture

Contents

Featured Research1
Featured Extension2
Extension Highlight4
Project Meeting Brief5
New Team Member5
Collaboration Corner6
Recent Publications7
Project Directors7
Advisory Panel7

FEATURED RESEARCH

Comparative Performance of Sanitizers in Managing Boxwood Blight and Volutella Blight by Ravi Bika and Fulya Baysal-Gurel

Boxwood blight caused by *Calonectria pseudonaviculata* and Volutella blight caused by *Pseudonectria foliicola* and *P. buxi* are a constant threat to boxwood production, landscape use and the cut boxwood greenery market. Both diseases cause significant economic loss to all parties (growers, retailers, and customers) in the horticultural chain. Boxwood blight symptoms appear as black, cloudy spots that lack a distinct edge, later transitioning into a tan spot with a graduated light brown to tan to yellow colored margin and leaf spots often enlarge to encompass entire leaves, ultimately resulting in severe defoliation. Volutella blight symptoms, in contrast, include light green, yellow, and red-bronze-colored leaves that become straw or yellow-tan in color, dieback of stem tissue distal of cankers, and salmon-pink colored fungal sporulation on twigs and the abaxial surface of leaves. Although Volutella blight typically does not kill boxwood plants as boxwood blight does, it severely affects the

ornamental characteristics of boxwood plants and greenery. These fungi can be easily dispersed in typical field conditions from infected to healthy plants through rain, irrigation water, contaminated tools (e.g., pruning and cutting shears), equipment and workers (contaminated boots, gloves, and clothing). The long-distance movement of these pathogens is from the transportation of infected plant materials, which may not show symptoms during harvest or at the time of shipment.

Sanitation is the major strategy for reducing inoculum density and limiting the spread of the pathogens. Sanitization of the cutting tools, equipment, clothes, boots, gloves as well as the plant material to be shipped is crucial for reducing the spread of boxwood blight and Volutella blight. Thus, the objective of this current study was to evaluate the efficacy of different commercially available sanitizers [quaternary ammonium compounds (QACs), peroxy, acid, alcohol, chlorine, cleaner] in reducing the plant-to-plant transfer of *C. pseudonaviculata* and *P. foliicola* on cutting tool blades, as well as

postharvest infection on boxwood cuttings. First, an in vitro study was conducted to identify the effective products and doses that showed complete or near complete inhibition of conidial germination of C. pseudonaviculata and P. foliicola. Then selected treatments from the in vitro study were tested for their ability to reduce the plant-to-plant transfer and manage postharvest infection on boxwood cuttings. For the plant-to-plant transfer study, we used Felco 19 shears for mechanical transfer of fungal conidia. The Felco 19 shears were attached to a hand pressurized reservoir so that solution was applied on blades while cutting through a spray nozzle mounted on shears. The Felco 19 shears were exposed to conidia by cutting through a 1 cmdiameter cotton roll dipped into fungal conidial suspension; the contaminated shears were used to prune the disease-free rooted boxwood cutting. Then, pruned boxwood plants were transplanted and incubated in room condition (21 °C, 60% RH) with 12 h of fluorescent light; data evaluation on disease severity was done every week for 1 month. In the dip application study of sanitizers, the harvested boxwood cuttings were inoculated with a conidial suspension of C. pseudonaviculata or P. foliicola and incubated for 3 days in a moisture chamber for sporulation. The boxwood cuttings were dipped for 10 s in sanitizer solution. Then, they were kept in a 50-mL centrifuge tube in room condition; boxwood blight and Volutella blight disease severities were assessed every 2 days for 1 week. A significant difference between treatments

RECENT DELIVERABLES March to May 2021

Research

- 4 Research articles
- 1 Conference proceedings article 1 Seminar

Extension & Outreach

- 2 Webinars/virtual meetings
- 2 Extension presentations
- 22 Samples diagnosed
- 53 Email/phone inquiries answered
- 1768 People reached with articles/news

Education

- 1 Postdoctoral associate
- 1 Graduate student

Publicity

1 Newsletter interview/report



was observed for reduction of boxwood blight or Volutella blight severity and AUDPC. The treatments **Simple Green D Pro 5** (2 fl oz/1 gal), **KleenGrow** (2 fl oz/1 gal), and **GreenShield** (13.7 qt/1 gal) were the most effective in reducing the plant-to-plant transfer of boxwood blight and Volutella blight when pruned with contaminated Felco 19 shears. In addition to the three effective treatments above, **Vinegar** (50% diluted), **KleenGrow** (1 fl oz/1 gal), **Clorox** (10%) and **Virkon** (2%) were effective in reducing postharvest boxwood blight whereas **Lysol all-purpose cleaner** (ready to use), **ethanol** (70%) and **Simple Green D Pro 3 plus** (2 fl oz/1 gal) were effective in reducing Volutella blight disease severity on boxwood cuttings.

In conclusion, we observed that QACs were the most effective treatments, when compared to representative of the peroxy, acid, alcohol, chlorine, and cleaner groups of sanitizers, in reducing the plant-to-plant transfer of *C. pseudonaviculata* and *P. foliicola* conidia and subsequent infection on boxwood cuttings. In future, the management of boxwood blight and Volutella blight will be improved as we gain a better understanding of pathogen biology and disease epidemiology. In the meantime, the findings reported in this study would effectively help to reduce the dispersal of these pathogens and should be integrated with other disease management strategies such as cultural practices, use of biorational products and fungicides.

Proteins Potentially Linked with Boxwood Blight Uncovered by Xiao Yang and Douglas Luster

Many fungi are known to secrete a group of unique proteins that are required for causing plant diseases. These proteins are called "effectors". The fungi causing boxwood blight, *Calonectria henricotiae* and *C. pseudonaviculata*, are no exception. Using mass spectrometry and bioinformatics analyses, the United States Department of Agriculture (USDA), Agricultural Research Service (ARS) research teams at Ft. Detrick and Beltsville, Maryland identified, for the first time, more than 120 putative effectors from the boxwood blight fungi. Based on this finding, we can further understand the boxwood blight disease on a molecular level. Interestingly, more than half of the identified effectors have never been found in any other pathogens. They may be the key to unlock the mystery: why boxwood blight is such a transmissive and aggressive disease. Furthermore, these effectors can potentially be utilized for screening purposes in downstream breeding programs of resistant boxwood cultivars and subsequently enhance scientist-stakeholder collaborations.

Outcomes of this research project have been published in the journal *Bioscience Reports*. The research article was made freely accessible online at <u>https://doi.org/10.1042/BSR20203544</u>.

FEATURED EXTENSION & OUTREACH

International Boxwood Seminar Series Launched by Xiao Yang and Margery Daughtrey



BBIG launched its **International Boxwood Seminar** series on March 18, 2021 with Dr. Thomas Brand as the inaugural speaker. Dr. Brand currently serves as Department Head of Ornamental Plant Cultivation, Tree Nurseries, Public Greenery at the Chamber of Agriculture of Lower Saxony, Germany. He has conducted numerous field trials evaluating fungicides and boxwood cultivars and other blight mitigation tools since 2004, when boxwood blight was first detected in his country.

In the first half of his presentation, Dr. Brand recalled the history of boxwood blight in Germany, which, in multiple ways, resembles our experience with this disease in the U.S. In the summer of 2004, a year after joining the Plant Protection Service of Lower Saxony, Dr. Brand documented Germany's first case of boxwood blight from a tree nursery. Although prompt

measures including plant removal and sanitation were taken, this disease, being novel at that time, re-occurred at the same nursery in the next year and unfortunately spread to many public and private gardens. Since then, Dr. Brand has been in a years-long fight against boxwood blight. He has conducted a multitude of research studies and trials and has provided consulting services to German boxwood growers. He described this experience dealing with boxwood blight as "a 15-year love-hate relationship": a plant pathologist's love for science combined with the hatred of seeing boxwood destroyed and stakeholders' economic loss. Based on scientific data and his experience, Dr. Brand pointed out that because no alternative plants can entirely replace boxwood, resistant varieties/cultivars, including many new boxwood breeds, are the long-term solution to control boxwood blight. By analyzing year-to-year results, he also suggested developing disease management

programs suited to the location and year, as environmental conditions are critical for the development of the boxwood blight disease. In the second part of his presentation, Dr. Brand reviewed the outcomes of his chemical trials and concluded that fungicides are important elements in Integrated Pest Management (IPM) programs, but their application can be restricted at places outside nurseries—for example, in private gardens and public landscaping sites. At the end of the presentation, Dr. Brand answered dozens of questions from diverse audience members.

More than 360 attended this inaugural seminar. The attendees included all sectors of the environmental horticulture industry, such as growers (both open field and greenhouse), retailers, landscapers (both outside and interior), arborists, industry suppliers and service providers, and chemical companies, plus public garden personnel, master gardeners, diagnosticians and extension agents, and government employees. Additionally, this seminar attracted attendees from Belgium, Canada, Czech Republic, France, Germany, Italy, New Zealand, Portugal, and the United Kingdom. This seminar including the Question and Answer session was recorded and the recording is posted in the Knowledge Center of the BBIG outreach website (www.boxwoodhealth.org/knowledge-center) for those who were unable to attend real-time.

Built upon the success of the inaugural session, four new seminars of the series have been announced with the first one set for June 2. These seminars will be held virtually via Zoom. They are free of charge although pre-registration is required. If you have not registered yet, it's time to do so. You do not want to miss these hard-to-find unique educational events. Click the "**FREE REGISTRATION**" links below to register TODAY.



Knowing and Growing Boxwood

June 2, 1:00 p.m. Eastern

Lynn Batdorf, Retired Curator of the National Boxwood Collection, U.S. National Arboretum

FREE REGISTRATION



Invasion, impact, and management of box tree moth in Europe

December 2, 11:00 a.m. Eastern Marc Kenis, Head Risk Analysis and Invasion Ecology @CABI

FREE REGISTRATION



Boxwood Breeding & Selection for Blight Resistance August 25, 11:00 a.m. Eastern

Katrijn Van Laere, Senior Researcher @Flanders Research Institute for Agriculture, Fisheries and Food-Plant Sciences Unit (ILVO)

FREE REGISTRATION



Progress in Boxwood Blight Management in the United Kingdom and New Zealand: 1990s to 2021 March 10, 2022, 3:00 p.m. Eastern

Matthew Cromey, Senior Plant Pathologist @Royal Horticultural Society, UK

FREE REGISTRATION

Outreach and Education on Boxwood Blight for the Spanish Speakers by Luisa Santamaria



In order to include the Spanish workforce at Oregon nurseries in our education and outreach efforts, a remote presentation on Boxwood blight was offered on March 2nd, 2021. This 1-hour presentation and Q&A was advertised and delivered through the Oregon Association of Nurseries (OAN) and supported by the Oregon Department of Agriculture (ODA) – Nursery & Christmas Tree Program. We had a small group of participants, but we were told at one place the presentation was displayed on a big screen; thus, additional workers benefitted.

One of the goals of both the Oregon State and BBIG Extension and Outreach teams is the development of multimedia material to facilitate educational activities. In February 2021, a video about boxwood blight scouting was completed by the

members of the team. This video presents key points to consider during scouting. The language of this original video is in English, and to extend its use, we translated it and recorded a voiceover in Spanish.

After completing the production of the video in Spanish, the next step was to use the videos in a training that could help all individuals interested in recognizing and scouting for boxwood blight, regardless of their primary language. Considering the limitations that the pandemic has posed on in-person training, my program developed a delivery format where participants can have a short introduction about the disease, biology of the boxwood blight pathogen, and the main diagnostic and scouting elements, followed by the scouting video and hands-on activity.

The target audience was nursery workers, nursery supervisors, landscape professionals, and potentially small groups at nurseries, such as 5-8 workers in charge of scouting or related activities. The focal learning outcomes were to increase awareness about symptoms of the disease and develop efficient scouting skills.

This initiative was supported and advertised by the ODA-Nursery Program through its listserv. The first pilot classes using this format were offered on April 12th in Spanish and May 17th in English. We are still in the process of evaluating the information from our pre- and post-survey questions that were designed to quantify how much participants learned throughout the workshop.

Structure of the educational event:

Format: Workshop presented remotely by Zoom, as a 3 hour-long class.

Requirement: Attendees had to have access to a phone or a computer with an internet connection.

Pre-workshop: We asked participants to identify an area for scouting at their nursery/place of work. We suggested an area with boxwood plants suitable for a scouting activity. If they were not at a nursery, they were free to select a location to practice their scouting skills. Also, we encouraged them to watch the scouting video before the workshop, if possible.

Hands-on activity: The time allocated for this activity was 45 minutes. During this time, participants had to take at least three photos at the place they identified for the activity: one broad photo of the scouting block, a second more detailed that shows a small area where plants look diseased or different from "normal" and a third one showing a close-up of a plant displaying possible disease symptoms. Participants had to send the pictures as soon they completed the task. There was a small break while we uploaded the photos into a presentation.



Discussion and Presentation: We assigned 25 minutes for this section. The slide show presenting the photos from the activity was a great resource to promote discussion and review the elements considered for scouting and disease symptoms. We did not specify names or places. We finished the workshop by reviewing BMPs to prevent boxwood blight, as well as presenting resources for further information. Questions from the participants were answered throughout the meeting.

EXTENSION HIGHLIGHT

The BBIG web site hosted by the Horticultural Research Institute of AmericanHort (launched Feb 2021) has been a great platform to keep stakeholders and the public informed, engaged and involved in this project. Among the most recent additions to the Knowledge Center are previous issues of BBIG Newsletters, images showing the blight pathogen affecting boxwood, pachysandras and sweet box, and a boxwood blight scouting video in both English and Spanish. Bookmark

www.hriresearch.org or www.boxwoodhealth.org for easy access and visit the site regularly to learn the latest research and innovations on boxwood blight mitigation.

Boxwood blight ID and management were presented during the 2021 Virtual Tree Steward Program held by Cornell Cooperative Extension of Westchester County, where Margery Daughtrey presented "Defying Disease" on March 17 to 37 people enrolled in the 5-session program.

R. Bika, W. Copes and F. Baysal-Gurel gave a presentation on the efficacy of sanitizers in reducing plant-to-plant transfer of *Calonectria pseudonaviculata* conidia on cutting tool blades at the 98th American Phytopathological Society (APS) Southern Division virtual meeting on Feb 15 to 19, 2021.

A live stage session is planned for Cultivate'21 in Columbus, OH to showcase BBIG and present the latest research and innovation on boxwood blight mitigation. Among invited speakers are Chuan Hong (Virginia Tech), Fred Gouker (USDA, ARS, Floral and Nursery Plants Research), Jim LaMondia (Connecticut Agricultural Experiment Station), and Len Coop (Oregon State University).

A recent article was published in the spring edition of the Friends of the National Arboretum's (FONA) quarterly newsletter <u>Arbor Friends</u>. The readership of the newsletters is aimed for plant enthusiasts and home gardeners. The article provides information about boxwood blight, boxwood breeding at the U.S. National Arboretum, and highlights the ongoing work of the BBIG project.

Diagnostic Character of Boxwood Blight by Margery Daughtrey

Often the best symptom to use to distinguish boxwood blight from other problems is short black streaks on the current season's shoots. The streaks generally do not girdle the stem – they resemble lines drawn by a black Sharpie marker. Along with the black streaks on shoots, there is often catastrophic leaf drop. Although some of the other fungal pathogens of boxwood, *Dothiorella candollei* (syn. *Macrophoma candollei*) and *Pseudonectria* spp. in particular, may cause some stem blackening, only boxwood blight caused by *Calonectria pseudonaviculata* is likely to cause leaf drop soon after infection.



PROJECT MEETING BRIEF

Project Directors, associates, and Advisory Panel members met on March 5, 2021 and discussed on project research progresses, BBIG website design and development, the launch of the International Boxwood Seminar series, and future project directions. **Dr. Charles Hall** presented a seminar entitled *Observations regarding the value of boxwood sales from 2009 to 2019*. **Dr. Chuan Hong** presented the current findings on antidesiccants as a physical barrier for blight mitigation.

In addition to the Extension/Outreach meetings reported above, three Project Leaders met monthly – March 16 and April 13, and they were joined by the new Project Manager on May 18, 2021, discussing project progress, planning project-wide activities, reviewing individual teams' quarterly progress reports, and preparing this issue of the BBIG Newsletter.

All Project Directors and their associates met on May 19, 2021, discussing the project progress and planning for the next quarter. **Ravi Bika**, a graduate student at the Baysal-Gurel lab, presented a seminar entitled "Efficacy of sanitizers in reducing plant-to-plant transfer and postharvest infection of boxwood blight and Volutella blight".

NEW MEMBERS AND PROMOTION



Ravi Bika is a master student at Tennessee State University, Otis L. Floyd Nursery Research Center, McMinnville, TN. He has completed his BS in agriculture (Horticulture) from Institute of Agriculture and Animal Science, Lamjung Campus, Tribhuvan University, Nepal in 2016 and joined the Baysal-Gurel lab in January 2019. His Master's research focuses on integration of sanitation, biorational products and fungicides for managing the postharvest Botrytis blight of ornamental cut flowers, boxwood blight and Volutella blight of boxwood and harvested greenery. He can be reached via email: ravibk002@gmail.com. Congratulations on his graduation and wish him well with his new adventure!

Bhawana Ghimire a first year PhD student (Plant Pathology) in the Baysal-Gurel Lab, has joined the BBIG team at Tennessee State University in McMinnville, TN. She is originally from Nepal and obtained her bachelor's degree in agricultural science (Plant Pathology) from Tribhuvan University, Nepal. Bhawana obtained her MS in Plant Pathology from the University of Georgia in 2019. She has research experience with stem canker of soybeans and different seed borne pathogens of wheat. As a part of her PhD projects, she will be working with the BBIG team aiming to develop a framework to identify critical control points and design boxwood blight best management practices in this project. Her approach incorporates appropriate steps and begins by surveying a large number of boxwood propagators and growers.



Survey results will inform the next segment, which includes visits to boxwood propagators and growers to create process flow diagrams, assessing the likelihood and potential risk of boxwood blight disease at a particular production point via operational risk management analysis, followed by 5-10 case studies with intensive sampling to identify critical control points. Customized mental modeling techniques will map findings from those segments to critical control points' specific training needs. She can be reached via email: bphimire@my.tnstate.edu.



Dr. Olanike Omolehin recently joined as a Postdoctoral Fellow (Applied Plant Pathologist) the BBIG family at Virginia Tech's Hampton Roads Agricultural Research and Extension Center in Virginia Beach, VA. A Nigerian by birth, she obtained both BS and MS degrees in Agronomy (Crop Science) from the University of Ibadan, Nigeria. Olanike recently graduated from the Louisiana State University with a Ph.D. in Plant Pathology. She has research experience on *striga* (witchweed) and ear rot disease in corn.

In her new role, Olanike is working with one of America's favorite ornamentals, boxwood. Specifically, she is investigating the performance of previously identified biocontrol agents and anti-desiccants for boxwood blight management under field conditions. She will also identify the modes of action of the best-performing biocontrol agents. She can be reached via email: <u>olanike@vt.edu</u>.

Dr. Xiao Yang recently took on a new role as the Project Manager while continuing to work as an ORISE postdoctoral research fellow developing rapid diagnostic assays for boxwood blight at the USDA, ARS – Luster Lab, Ft. Detrick, MD. He will coordinate communications and project reports among project directors, associates, collaborators, and advisory panel members, organize future project meetings, and help the extension team to translate research outcomes into outreach materials. He can be reached via email: <u>xiao.yang@usda.gov</u>.

COLLABORATION CORNER



Chad Taylor and Ginger Hemmings of North Carolina Department of Agriculture and Consumer Services (NCDA&CS) and Amanda Taylor of North Carolina Cooperative Extension helped set up a field trial evaluating three selected antidesiccants for their potential to protect boxwood crops from the blight pathogen in western North Carolina with the initial treatments in Mid-April. Chad/Ginger have since continued the fungicide standard treatments every 2 or 3 weeks as scheduled. Dr. Hsien Tzer (HT) Tseng and Chad did the May blight assessment as Olanike and Chuan had to cancel their field trip at the last minute due to the cyberattack to the Colonial Pipeline and consequent gasoline shortage.

A similar trial was also set up in another field of western North Carolina with history of boxwood blight, in collaboration with a grower. The collaborating grower helped prepare the field, planted, aves blighted in Mid May

and maintained the plots and recorded the percent leaves blighted in Mid-May.

The Biocontrol Team met with the **Belgium Collaborators** – Dr. Kurt Heungens and his student, Marie Froyen on April 28, 2021, sharing the latest data and brainstorming on what could have impacted the survival of selected biological control agents (BCAs) on boxwood foliage, how to dissect and look into this, and how to improve BCAs' survival and performance under field conditions.

RECENT PUBLICATIONS

Research Articles

Bika, R., Copes, W., **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. https://doi.org/10.1094/PDIS-03-21-0481-RE.

Castroagudín, V.L., Shishkoff, N., Stanley, O., Whitesell, R., Olson, T.N., **Crouch, J.A.** 2021. First report: coinfection of *Sarcococca hookeriana* (sweetbox) by *Coccinonectria pachysandricola* and *Calonectria pseudonaviculata* causes a foliar disease of sweetbox in Pennsylvania. Plant Disease. <u>https://doi.org/10.1094/PDIS-06-20-1198-PDN</u>.

Shishkoff, N., Miller, M.E., 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. <u>https://doi.org/10.24266/0738-2898-39.1.1</u>.

Yang, X., McMahon, M.B., Ramachandran, S.R., Garrett, W.M., LeBlanc, N., Crouch, J.A., Shishkoff, N., **Luster, D.G.** 2021. Comparative analysis of extracellular proteomes reveals putative effectors of the boxwood blight pathogens, *Calonectria henricotiae* and *C. pseudonaviculata*. Bioscience Reports. <u>https://doi.org/10.1042/BSR20203544</u>.

Peer-Reviewed Videos

Santamaria, L., Weiland, J.E. and Pscheidt, J.W. 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

Pscheidt, J.W., Weiland, J.E. and Santamaria, L. 2020. Scouting for Boxwood Blight. Corvallis, OR: Oregon State University. Video available at <u>https://www.youtube.com/watch?v=L-Yd8qCbb0A</u>

PROJECT DIRECTORS

Chuan Hong (Project Director), Virginia Tech, Virginia Beach, VA Margery Daughtrey (Extension Leader), Cornell University, Riverhead, NY Douglas Luster (Research Leader), USDA, ARS, Foreign Disease & Weed Science Research, Ft. Detrick, MD Charles Hall, Texas A&M University, College Station, TX Jerry Weiland, USDA, ARS, Horticultural Crop Research Lab, Corvallis, OR Fulya Baysal-Gurel, Tennessee State University, McMinnville, TN Fred Gouker, USDA, ARS, Floral & Nursery Plants Research, Beltsville, MD Ping Kong, Virginia Tech, Virginia Beach, VA Jo Anne Crouch, USDA, ARS, Mycology & Nematology Genetic Diversity and Biology Lab, Beltsville, MD James LaMondia, Connecticut Agricultural Experiment Station, Windsor, CT Jay Pscheidt, Oregon State University, Aurora, OR Nina Shishkoff, USDA, ARS, Foreign Disease & Weed Science Research, Ft. Detrick, MD Karen Snover-Clift, Cornell University, Ithaca, NY

ADVISORY PANEL & LIAISON TO AMERICANHORT

Jill Calabro (Chair), Sr Product Development Manager, Valent USA Corporation, Walnut Creek, CA Lynn Batdorf, Boxwood Cultivar Registration Authority, International Society for Horticultural Science Frank Collier, Owner, Pleasant Cove Nursery, Rock Island, TN Michael Gaines, President, CW Arborists, Ltd., Sagaponack, NY John Keller, Planning & Research Vice President, Monrovia – Grow Beautifully in CA, CT, GA, OR Bennett Saunders, General Manager, Saunders Genetics, LLC., Piney River, VA Richard Schnall, Vice President, Rosedale Nurseries, Inc., Hawthorne, NY Casey Sclar, Executive Director, American Public Gardens Association, Kennett Square, PA Mark Sellew, Owner, Prides Corner Farms, Lebanon, CT Martha Sleeper, Plant Health Manager, Hines Growers, Forest Grove, OR Jennifer Gray (Liaison to AmericanHort), Administrator, Horticultural Research Institute, Washington, DC

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