2023 Oregon Department of Agriculture Nursery Research Proposal
Evaluating the ability of the boxwood blight pathogen to cause asymptomatic infections

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Background and significance

Boxwood is the top selling evergreen shrub with $141 million annual sales in the United States of which Oregon is the top producer supplying 19% ($27 million) of total sales. Unfortunately, boxwood production has been severely threatened since the emergence of boxwood blight in Oregon in 2011. Symptoms of boxwood blight start as leaf spots and stem lesions, and lead to severe defoliation when conditions are conducive such as under high humidity. However, in Oregon where the summers are hot and dry, symptoms can be very mild and easily overlooked. This observation led some Oregon growers to wonder whether the boxwood blight pathogen may be capable of causing asymptomatic (or latent) infections during the summer that initially appear healthy, but can later develop disease when conditions become favorable, such as when the fall rains arrive or when the plants are shipped to more humid regions. The possibility of asymptomatic boxwood blight infections has also been raised in several State Department of Agriculture and University publications. Nevertheless, there have not been any studies that prove asymptomatic infection occurs. Therefore, our goal is to determine whether the boxwood blight pathogen is capable of causing asymptomatic infections. Information obtained from this study will allow Oregon growers and inspectors to devise practical management and inspection strategies to minimize the spread of boxwood blight through nursery trade.

Our objectives are to:

1. Determine whether symptomatic boxwood plants can harbor the boxwood blight pathogen in asymptomatic tissue.
2. Evaluate the ability of the boxwood blight pathogen to cause asymptomatic infections under different environmental conditions and on different cultivars.
3. Educate growers, Oregon Department of Agriculture inspectors, and Oregon State University extension personnel on these findings through nursery visits, stakeholder meetings (Oregon Boxwood Health Workshop), Boxwood Blight Insight Group meetings, and trade magazine articles (Digger).

Benefit to the Oregon nursery industry

Oregon has been perceived as a source for boxwood blight infected plants within the country. This could be due to infected plants being unknowingly shipped from Oregon because asymptomatic infections occur or because mild symptoms were overlooked. If asymptomatic infections do occur, it can have serious consequences for boxwood trade because visual inspection of plants will not be sufficient. On the other hand, if asymptomatic infections do not occur, Oregon growers can be more confident that plants are disease-free after careful visual inspection. Thus, the Oregon nursery industry would greatly benefit from knowing whether the boxwood blight pathogen causes asymptomatic infections because it would allow Oregon growers and inspectors to devise practical strategies that ensure disease-free plants for trade.

Methods

Objective 1: Determine whether plants with boxwood blight symptoms can harbor the pathogen in asymptomatic tissue.

Nurseries will be visited to sample tissues from plants displaying boxwood blight symptoms. From each plant, 4 to 6 branches that have both a symptomatic section (with leaf spots and stem lesions) and asymptomatic section (no visible symptoms) will be collected. Asymptomatic tissues will be separated from symptomatic tissues and then both sets of tissues will be analyzed separately using a DNA-based assay developed in our lab that detects the presence of the boxwood blight pathogen, *Calonectria pseudonaviculata*. To differentiate between presence of the pathogen on the surface of the plant versus an internal asymptomatic infection, half of the sampled asymptomatic tissues will be surface sterilized prior to analysis. If plants are able to harbor the pathogen within apparently healthy asymptomatic tissue, we expect to detect *C. pseudonaviculata* DNA from the portions of the branch that were surface sterilized.

Objective 2: Evaluate the ability of the boxwood blight pathogen to cause asymptomatic infections under different environmental conditions and on different cultivars.

Liners of two *Buxus* cultivars that are reported to differ in boxwood blight susceptibility (e.g. Common boxwood for susceptible and Winter Gem for tolerant) will be spray inoculated with *C. pseudonaviculata* spores. Following an initial infection period, the plants will be placed in growth chambers under two humidity levels (40% and 80% relative humidity) at each of two temperatures (15 and 25 °C). One week post-inoculation, the plants will be observed for symptoms. Five weeks post-inoculation, asymptomatic tissues will be collected from each plant and analyzed using a DNA-based assay that detects the presence of *C. pseudonaviculata*. If any symptomatic tissue is present on plants, it will be analyzed as a positive control. To differentiate between the presence of the pathogen on the surface of the plant versus an internal asymptomatic infection, half of the collected asymptomatic tissue will be surface sterilized prior to analysis. This experiment will be performed twice. If *C. pseudonaviculata* is able to cause asymptomatic infections under certain humidity, temperature, or host conditions, we expect to detect *C. pseudonaviculata* DNA from asymptomatic tissues that were surface sterilized.

Timeline for 2023

	Objective 1 activity	**Objective 2 activity**	**Objective 3 activity**
January – March		Set up and analyze trial 1	
April – June	Sample nurseries	Set up and analyze trial 2	
July – September	Analyze samples		Present at meetings
October – December			Publish results

Budget summary

Travel to field sites and meetings	$1,000
Molecular biology supplies	$4,000
Boxwood plants	$1,000
Salary + benefits (0.2 OSU postdoctoral researcher)	$15,648
Total	**$21,648**