Does Composting Eradicate the Pathogen Responsible for Boxwood Blight? An Outline of Future Investigations©

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BOXWOOD BLIGHT: AN OVERVIEW

Boxwoods (*Buxus* spp.) have been a staple ornamental in both Europe and the United States for hundreds of years (Bir et al., 1997; Henricot and Culham, 2002; Varela et al., 2009). Controversy exists surrounding the current naming of the pathogen responsible for boxwood blight. This stems from the pathogen being isolated and proposed as a new species independently by two different lab groups in 2002. The first of these reports (Crous et al., 2002), documented a new species of fungus infecting boxwoods in New Zealand and described it as *Cylindrocladium pseudonaviculatum*. Shortly thereafter, Henricot and Culham (2002), published a paper documenting their findings and named the fungus *Cylindrocladium buxicola*. Although the teleomorph has yet to be observed, the name *Calonectria pseudonaviculata* has been proposed for the sexual stage by Lombard et al. (2010). However, within the research community *Calonectria pseudonaviculata* is becoming the preferred name, and will be used in this paper.

Due to the polycyclic nature of the disease, boxwood blight poses a significant threat to the boxwood industry. The adhesive nature of C. *pseudonaviculata*'s spores also contribute to the rapid spread and infection of new hosts (Henricot, 2006). Infested tools and clothing, if not properly sanitized, can vector pathogen propagules inadvertently to healthy plants and non-threatened areas. The life cycle for the pathogen is rather straightforward. Germination takes place approximately 3 h after inoculation, with penetration occurring approximately 5 h post-inoculation under ideal weather conditions (Henricot, 2006). Penetration occurs directly through the cuticle, or through a stoma. The presence of an appressorium has not been reported for this pathogen. Once the fungus enters the host, the mycelium grows intercellularly within the mesophyll; the fungus re-emerges through the stomata 2 to 3 days after initial infection. After 1 week, conidiophores can be observed on the abaxial leaf surface (Henricot, 2006). The presence of microsclerotia, which represents a method of survival during adverse environmental conditions, has also been noted (Henricot, 2006; Ivors et al., 2012).

The disease and symptom progression of boxwood blight is as follows. Circular lesions appear initially on the leaf, forming concentric rings which appear due to the outward growing of the fungus (Akilli et al., 2012; Cech et al., 2010; Crepel et al., 2003; Elmhirst and Auxier, 2013; Gorgiladze et al., 2011; Henricot and Culham, 2002; Henricot, 2006; Ivors et al., 2012; LaMondia et al., 2012; Mirabolfathy et al., 2013; Saurat et al., 2012; Varela et al., 2009). Over time, the lesions expand, eventually coalescing and leading to leaf death. Symptoms are not limited to the leaves. Large black cankers and streaks appear on the stems, eventually leading to total defoliation and plant death (Akilli et al., 2012; Cech et al., 2010; Crepel et al., 2003; Elmhirst and Auxier, 2013; Gorgiladze et al., 2011; Henricot and Culham, 2002; Henricot, 2006; Ivors et al., 2012; LaMondia et al., 2012; Mirabolfathy et al., 2013; Saurat et al., 2012; Varela et al., 2009).

The host range of *C. pseudonaviculata* is not fully understood; however, in vitro experiments have yet to uncover an immune species of *Buxus*. *Buxus balearica* appears to be most resistant to the pathogen. This putative resistance is attributed to its thick leaves, leading to the postulate that the pathogen experiences difficulties penetrating the leaf. Unfortunately, *B. sempervirens* represents one of the most popular boxwood speicies, and shows the most susceptibility towards the pathogen (Henricot, 2006; Henricot et al., 2008). Other member species of ornamental importance in the *Buxaceae* family include *Sarcococca* sp. and *Pachysandra* sp., both of which have been evaluated for susceptibility. *Sarcococca* has illustrated some susceptibility to the pathogen, but not to

the same extent as in *Buxus* (Henricot, 2006, 2008). However, *Pachysandra terminalis* (LaMonida et al., 2012) and *P. procumbens* (LaMondia and Li, 2013) have been confirmed as susceptible.

Severe damage and losses have occurred due to the rapid rate of boxwood blight spread in the United States. Ten thousand plants were confirmed to have boxwood blight in North Carolina alone, with the amount of infected plants found in Connecticut being 15-fold higher. Within two nurseries, 150,000 infected boxwood plants were found (Ivors et al., 2012). The estimated monetary loss in Connecticut alone amounts to $3,000,000 (LaMondia, 2014). Boxwood blight is a major concern for the nursery industry, as the boxwood market is valued at $103 million annually. Fourteen states have confirmed cases of boxwood blight in the USA (Fig. 1) as well as Quebec, Ontario, and British Columbia in Canada, as of December 2014.

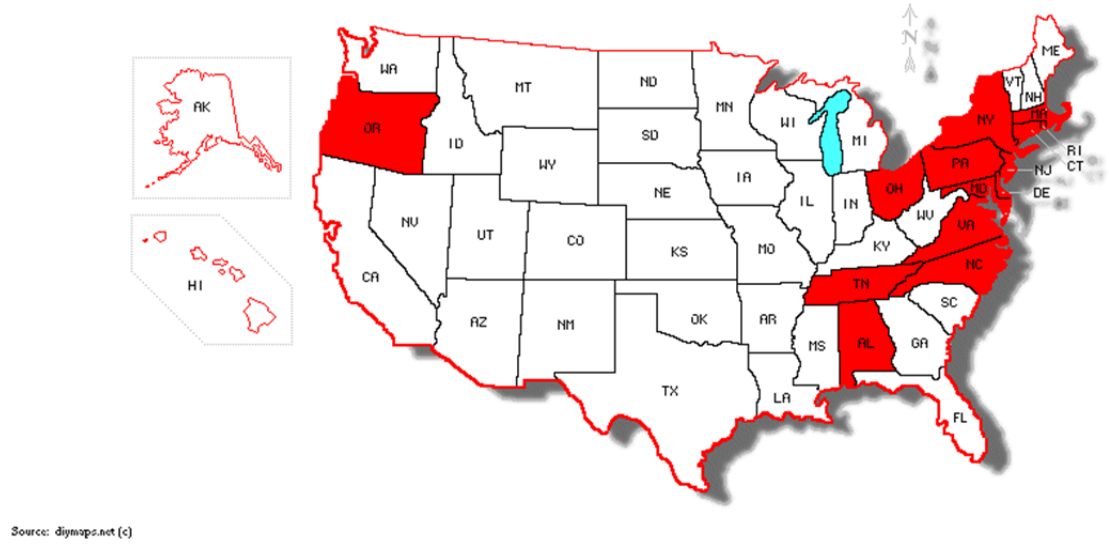

Fig. 1. Incidence of confirmed boxwood blight cases in the United States. Fourteen total states have reported the disease, mainly on the East Coast. Map as of December 2014.

COMPOSTING AS A METHOD FOR CONTROL

Composting is a complex process involving multiple physical and biological factors. Generally, composting involves microbial decomposition processes that transform heterogeneous organic waste to a homogenous soil-like material. These decomposition processes produce heat, leading to internal temperatures that vastly exceed ambient (Hassen et al., 2001). Overall the composting process can be divided into three separate phases: mesophilic, thermophilic, and cooling (Hassen et al., 2001; Hoitink et al., 1997).

Temperature appears to be the key factor involved with pathogen eradication by composting (Fayolle et al., 2006; Harnik et al., 2004; Hassen et al., 2001; Hoitink et al., 1997; Noble and Roberts, 2004; Noble et al., 2009). Heat can be an effective killer, even when not with in the composting system. Harnik et al. (2004) reported that chlamydospores of the pathogen *Phytophthora ramorum* were killed in 3 min when exposed to temperatures of 53°C. Indirect evidence of pathogen eradication was reported by Hassen et al. (2001), when they observed that fungal populations declined during the thermophilic stage of the composting process, indicating that many fungi cannot tolerate the high temperatures. Generally, many fungi can be eliminated under composting conditions at 52°C for 7 consecutive days (Hoitink et al., 1976; Noble and Roberts, 2004; Noble et al., 2009). However, not all fungi are eradicated under these conditions. Windrow composting produces a temperature cross sectional profile, with uneven

heating, due to air flow and insulation properties of the substrate. Therefore, windrows must be turned on a regular basis to ensure that all material is exposed to high temperatures (Hoitink et al., 1997).

Aeration is another factor that influences pathogen eradication. Fayolle et al. (2006) noted that under aerated conditions, pathogen eradication was successful in compost; however, no aeration led to incomplete pathogen eradication. When compost is not aerated or turned properly, the system can become anaerobic, which results in lower temperatures in the compost pile (Fayolle et al., 2006).

High moisture leads to eradication temperatures that are lower than in drier composts (Noble et al., 2004). Fayolle et al. (2006) demonstrated that *Plasmodium brassicae* eradication was not as efficient in drier composts as compared to composts that had higher moisture contents. There was one exception, however. The level of moisture in wood-chip-compost did not influence eradication by heating (Fayolle et al., 2006).

RATIONAL/PLAN OF ACTION

Pathogen presence in compost is an important issue. To alleviate landfill pressure, many green wastes are being diverted to composting operations (Fayolle et al., 2006). However, many states have regulations limiting landfilling of organic wastes. Burning is another method utilized to dispose of organic waste. However, bans on open burning are also increasing (Noble et al., 2009). As a result, yard waste from many locations currently ends up in the compost stream. Such organic waste may include infected boxwood materials. If composting is not able to eradicate the pathogen, then reapplication of the finished compost as mulch near healthy boxwoods could lead to disease outbreak. However, the safety of compost must be ensured, and protocols must be established to ensure pathogen elimination (Fayolle et al., 2006; Noble et al., 2009). Both environmental and plant pathological perspectives must be accounted for, in order to prevent the introduction and spread of pathogens from non-infested to healthy areas (Noble et al., 2009).

Calonetria pseudonaviculata produces microsclerotia which are extremely resistant to extreme environmental conditions, and function as survival structures. It is unknown whether the temperatures and environmental conditions within a compost pile are adequate to destroy microsclerotia. However, if composting is shown to destroy the pathogen, then composting could be employed as an environmentally friendly control option. If the pathogen survives temperatures routinely reached in commercial composting operations, then compost being used for mulches or as soil amendments could be a potential source of inoculum, further contributing to spread of the pathogen.

A small bioreactor system has been constructed within the Mushroom Research Center at The Pennsylvania State University (Fig. 2). This system includes three independently controlled incubators, each of which holds three reactor vessels. Each reactor vessel has a diameter of 15.2 cm and a height of 31.5 cm. The benefit of a small bioreactor system is that precise temperature and oxygen controls can be incorporated, eliminating variability found in a windrow or aerated composting systems. Flow regulators control the volume of air that enters the system, and each incubator can be programmed to maintain a different temperature regime. Another benefit is the ability to monitor multiple metrics in the system. Each reactor vessel is connected to a data collection system that gives real-time readings, as well as allowing data storage on a computer. Oxygen and carbon dioxide meters, as well as boric acid traps for ammonia analyses, allow for measurement of exhaust gas from each vessel. Each vessel also has a probe for temperature monitoring.

Overall, this bioreactor design allows performance of carefully controlled experiments to examine specific questions related to pathogen eradication. We plan to first investigate the effect of compost temperature and time on pathogen survival, as this is likely the most important factor. Other options can then be explored, such as comparison of different starting materials to determine if their content plays a role in pathogen eradication. For example, a low C:N ration would mean that more ammonia would be produced, which might help with pathogen eradication. In addition, the effects

of other microbes could be investigated to determine if different microbe populations influence pathogen eradication.

Fig. 2. Three bioreactors inside of a high-temperature incubator. The system consists of three incubators, allowing for the simultaneous operation of nine bioreactors. Humidified air enters the reactor at the bottom, and exits the top. The reactors hold approx. 4 L and are constructed with 15.2 cm diameter PVC.

It is our goal to utilize this system to evaluate the survival of *C. pseudonaviculata* during the composting process. As this new pathogen presents a significant threat to the nursery industry, it is extremely important to recognize and identify any and all pathways by which the pathogen can spread. Results from these experiments can hopefully be used as a tool in developing integrated pest management plans to minimize the spread of boxwood blight.

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