A Performance Comparison of Bareroot and Containerized *Pinus taeda* L. Seedlings as Affected by Ophiostomatoid Fungi

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Abstract

The objective of this study was to evaluate responses of containerized and bareroot seedlings from the same *Pinus taeda* L. families to ophiostomatoid fungi, *Leptographium trerebrantis* and *Grosmannia huntii*. Seedlings from four families were artificially inoculated with *L. terebrantis* and *G. huntii*. After 8 weeks, tissue necrosis and occlusion caused by the fungi were measured. Seedlings from both *P. taeda* stockypes showed similar susceptibility to fungi, suggesting both seedling stocktypes can be used to screen the susceptibility of *P. taeda* families against tested ophiostomatoid fungi. This paper was presented at the Joint Annual Meeting of the Southern Forest Nursery Association and the Northeast Forest and Conservation Nursery Association (Pensacola, FL, July 17–19, 2018).

Introduction

Pinus taeda L. (loblolly pine) is one of the most commercially valuable timber species in the southern United States. *Pinus taeda* plantations contribute a considerable portion of the economy of this region by providing marketable forest products, habitat for wildlife, recreational areas, and others (Poudel et al. 2017, Schultz 1997). However, root-infecting fungi, including bark beetle-vectored ophiostomatoid fungi, are frequently associated with root infection and the decline of this species (Eckhardt et al. 2007).

Leptographium terebrantis and *Grosmannia huntii* are the most pathogenic ophiostomatoid fungi associated with *Pinus taeda* decline (Matusick et al. 2010; Singh et al. 2014). Root-feeding bark beetles act as vectors in introducing these fungi into the roots of pine trees during their feeding activity (Jankowiak and Bilański 2013). Inoculation of these fungi and subsequent host defense responses result in tissue necrosis and occlusions of xylem and phloem tissues, respectively (Devkota et al. 2018b). Thus, the fungal infection, together with activated host defense responses, disturbs plant water transport and results in tree decline.

Bareroot and containerized seedlings are the two major stocktypes used in forest restoration programs. Bareroot seedlings are grown in soil beds in an open field with the removal of soil during harvest. Containerized seedlings are grown in containers containing artificial media under a shelter or controlled greenhouse environment with root and growing medium maintained together from harvest to outplanting (Grossnickle and El-Kassaby 2016). The lifting of bareroot seedlings in nurseries in the southeastern United States involves a number of operational procedures that might affect the root viability (Starkey and Enebak 2013). In contrast, root damage is minimal in containerized seedling. The use of a greenhouse and artificial growing medium may, however, increase susceptibility of containerized seedlings to biotic diseases, compared with bareroot seedlings (Grossnickle and El-Kassaby 2016).

Few studies have been conducted to screen the susceptibility and resistance of different *Pinus taeda* families to *Leptographium* and *Grosmannia* spp. (Devkota et al. 2018a, Devkota and Eckhardt 2019). These studies examined either bareroot or containerized seedlings, but never compared the relative performance of both seedling stocktypes from the same family. For example, Singh et al. (2014) studied bareroot and container-grown seedlings from different families in the screening in the year 2011 and 2012, respectively. Variation in seedling stocktype used in

individual trials have made it difficult to compare family performance between trials.

Thus, there is a need to compare variations in susceptibility and resistance of containerized and bareroot seedlings from the same family of *Pinus taeda* to ophiostomatoid fungi. The objective of our study was to utilize the established method of fungal inoculation for evaluation of bareroot and containerized seedlings from the same four *P. taeda* families to *Leptographium terebrantis* and *Grosmannia huntii*.

Methodology

An artificial seedling inoculation study was conducted in 2014. This study was a subset of a larger experiment conducted to screen the tolerance of various *Pinus taeda* families to *Leptographium terebrantis* and *Grosmannia huntii*. Bareroot and containerized seedlings from four half-sib *Pinus taeda* families were studied. Each family was assigned a random name (i.e., L109, L81, L38, and L09). The genetic distinction between these families and the original names are not disclosed to maintain confidentiality.

Seeds from all families were collected and sown within a single forest company nursery (Elberta, AL) in March 2013. Bareroot seedlings from all families were grown in an operational nursery bed and containerized seedlings were grown in 600 cm^3 containers. Seedlings were lifted from nursery beds and containers in early January 2014 and transported to the research facility at Auburn University, Auburn AL.

To minimize individual seedling variation, seedlings with 30 ± 0.5 cm average height and 4.5 ± 0.1 mm root-collar diameter (RCD) were chosen for the inoculation experiment. A total of 128 seedlings were chosen from each seedling stocktype. Seedlings were transplanted into plastic pots (16.19-cm diameter and 18.41-cm height) with peat-based potting medium (ProMix BX®, Premier Tech, Quebec, Canada) and grown in an outdoor growing area. The study design was a randomized complete block with six blocks (figure 1). Soil water was regularly monitored and the pots were watered to meet the volumetric content of each pot (V/V: 0.28).

Two months after transplanting, seedling mortality, RCD, and height of seedlings were measured. Then, seven randomly selected seedlings from each family/stocktype combination per treatment in each block were inoculated with one of three treatments. Single isolates of *Leptographium terebrantis* and *Grosmannia huntii* were used as the two fungal treatments and sterile agar plugs were used as the control treatments. The

Figure 1. Randomized blocks with bareroot and containerized seedlings transplanted in an outdoor research facility of Auburn University at Auburn, AL. (Photo by Pratima Devkota, 2014)

fungal isolates were originally from the roots of declining *Pinus* stands from the southern United States, as described by Eckhardt et al. (2007). The fungal isolates were cultured in 2 percent malt extract agar (MEA) plates for 14 days prior to the inoculation experiment.

To perform the inoculation (figure 2), a 1-cm vertical flap of bark was cut with a sterile razor blade in the seedling stem 2 cm above the soil line (Devkota and Eckhardt 2018). Then, a 3-mm agar plug with actively growing fungi (fungus side down) was inoculated in the wound. To prevent the desiccation of the agar medium, the inoculation point was covered with a moist cotton ball and wrapped with Paraflim®.

Seedling mortality, RCD, and seedling height were evaluated 8 weeks after inoculation. Then the individual seedlings were clipped above the soil line and placed in a bucket filled with 0.25 g Fast-Green dye (FastGreen

Figure 2. Artificial inoculation of fungal mycelial plug in the stem wound. (Photo by Pratima Devkota, 2014)

Figure 3. Dark necrotic lesions in *Pinus taeda* seedling stems inoculated with *Leptographium terebrantis*. (Photo by Pratima Devkota, 2014)

FCF; Sigma Chemical Co., St. Louis, MO) mixed in 1 L of deionized distilled water. To allow the dye to translocate throughout the stem, seedlings were left upright in the stain mix for 48 hours. After 48 hours, the bark around the inoculation point was carefully scraped until the necrotic tissue was observed and lesion and occlusion length were measured with a digital caliper. The vertical length of the dark dead necrotic tissue (figure 3) and the occluded tissue not taking up the dye was regarded as lesion and occlusion length, respectively. Two 2-mm sections of the stem tissue around the lesion was plated on MEA containing 800 mg L^{-1} of cycloheximide and 200 mg L^{-1} of streptomycin sulfate medium to confirm re-isolation of the inoculated fungus.

Seedling height growth was calculated by subtracting seedling height before inoculation from height during harvest. Similarly, seedling diameter growth was calculated by subtracting seedling RCD before inoculation from RCD during harvest. Data were analyzed using a general linear model (GLM) in SAS statistical software (SAS Institute, 9.4 versions, Cary, NC). Assumptions of normality and equal variance were satisfied. Pair-wise comparisons between the stocktypes were performed using the Post Hoc Tukey's test at $\alpha = 0.05$.

Results

Seedling mortality 2 months after transplanting and prior to the fungal inoculation differed significantly between stocktypes (5 and 30 percent of bareroot and container seedlings, respectively). There was, however, no further seedling mortality 8 weeks after the fungal inoculation. Dark-brown, necrotic lesions and vascular occlusions were observed in the inoculated seedling stems. *Leptographium terebrantis* and *Grosmannia huntii* were re-isolated from 90 and 92 percent of the inoculated seedlings, respectively.

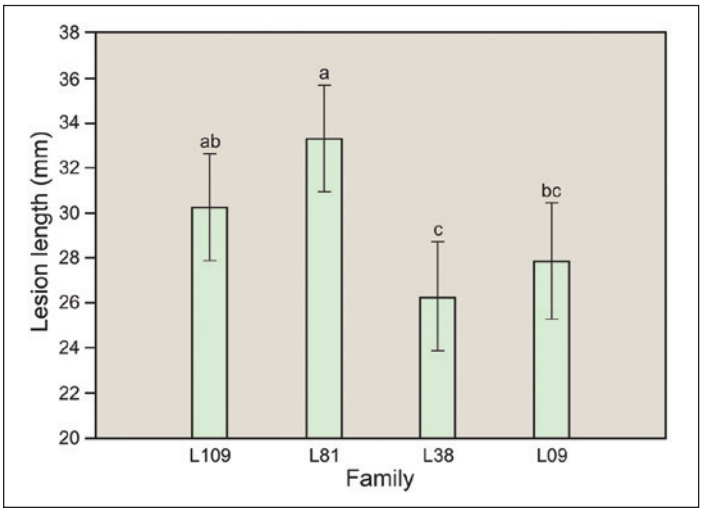

Figure 4. Lesion length caused by *Leptographium terebrantis* and *Grosmannia huntii* in seedlings from the four different *Pinus taeda* families. Different letters indicate Tukey's Honest Significant Differences among different families at $\alpha = 0.05$.

At first, the model was fitted to the data with three treatments (two fungi and one control). Tukey's multiple comparison test revealed that the lesion and occlusion length in the control seedlings did not occur beyond the inoculation point. Also, when compared to the fungal treatments, the lesion and occlusion length in seedlings receiving control treatments were significantly shorter. Thus, the model was fitted again with the two fungal treatments and without control (Devkota et al. 2018a).

The lesion length caused by two fungi varied among the four *Pinus taeda* families ($F_{(3,498)} = 5.8339$, $P = 0.0064$) (figure 4). *Grosmannia huntii* caused a relatively longer lesion than *Leptographium terebrantis* ($F_{(1,498)} = 21.085$, $P < 0.0001$) (figure 5). Overall, the lesions caused by the two inoculum treatments did not differ significantly between bareroot and containerized seedlings ($F_{(1,498)} = 1.0964$, $P = 0.29556$) or within each family ($F_{(3, 498)} = 1.2358$, $P = 0.29604$). Also, there was no significant three-way interaction among family, fungi, and stocktype. There was no variation in lesion length of bareroot and container-grown seedlings from the same family to *L. terebrantis* and *G. huntii* ($F_{(3,498)} = 0.178.08$, $P = 0.91125$).

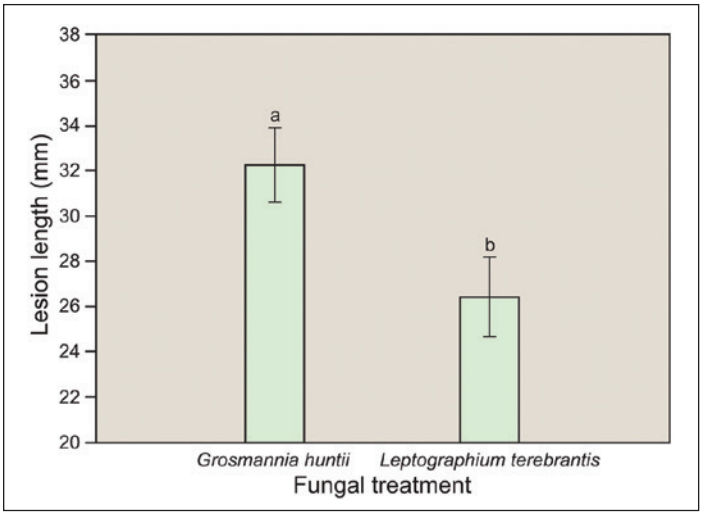

Figure 5. Lesion length caused by *Grosmannia huntii* and *Leptographium terebrantis* in *Pinus taeda* seedlings. Different letters indicate Tukey's Honest Significant Differences between two fungal treatments at $\alpha = 0.05$.

Overall, the occlusion length was significantly different among the four families ($F_{(3,477)} = 6.0584$, $P = 0.00047$; figure 6). There was, however, family and fungal interaction ($F_{(7,477)} = 17.384$, $P = 0.00004$). *Grosmannia huntii* caused significantly longer occlusion lengths compared with *Leptographium terebrantis*. Overall occlusion length did not differ significantly between the two seedling stocktypes within each family ($F_{(3,477)} = 0.06565$, $P = 0.97805$). Seedlings in the family L81 and L38 had the longest and shortest occlusion lengths, respectively.

Diameter growth differed significantly among the *Pinus taeda* families ($P < 0.00001$). Family L109 and L38 had the highest and lowest RCD increment, respectively. Both bareroot and containerized seedlings from family L09 had the highest RCD increment as compared to seedlings from other families. Diameter growth was significantly higher in containerized seedlings compared with bareroot seedlings ($P = 0.00432$; figure 7a).

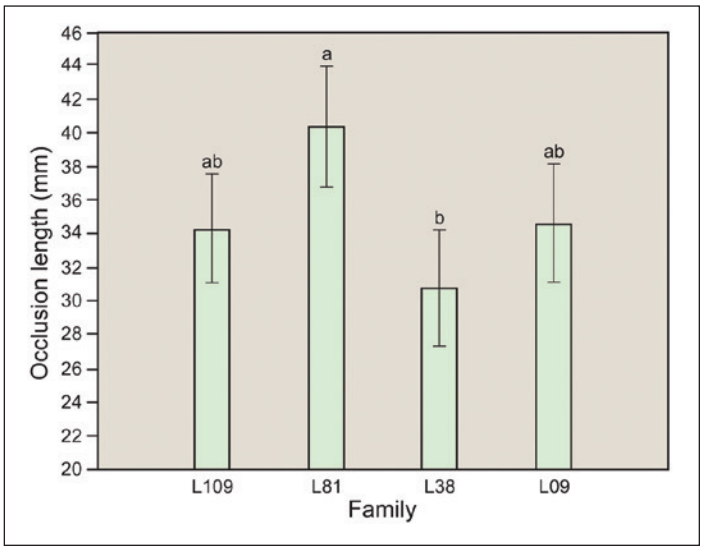

Figure 6. Occlusion length caused by *Grosmannia huntii* and *Leptographium terebrantis* in bareroot and containerized *Pinus taeda* seedlings. Different letters indicate Tukey's Honest Significant Differences among four families at $\alpha = 0.05$.

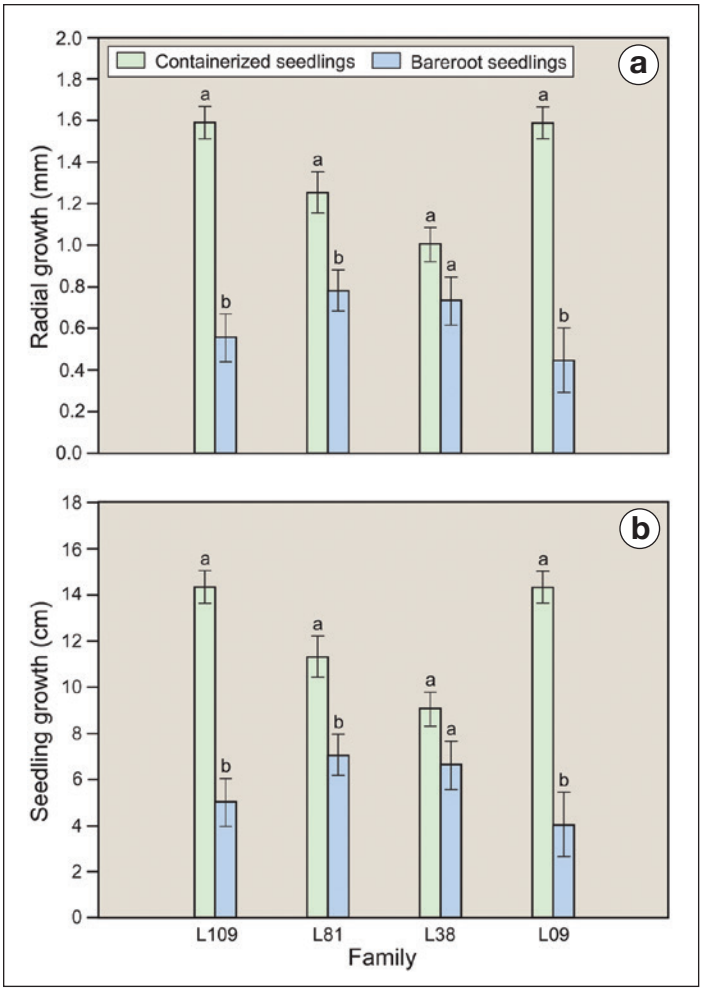

Figure 7. (a) Diameter and (b) height growth of bareroot and containerized seedlings from various *Pinus taeda* families. Different letters indicate Tukey's Honest Significant Differences between two seedling stocktypes at $\alpha = 0.05$.

Overall, seedling height growth differed significantly among the four families. Family L109 had the most growth, whereas L38 had the least growth. Height growth of seedlings inoculated with fungi was not significantly different from the control seedlings ($P = 0.85114$). Containerized seedlings for three of the four families had more growth compared with the bareroot seedlings ($P < 0.00001$; figure 7b).

Discussion

Our results show that intraspecific variation in tolerance of *Pinus taeda* to ophiostomatoid fungi is independent of seedling stocktype. Both seedling stocktypes from the same family responded similarly to *Leptographium terebrantis* and *Grosmannia huntii* (in terms of lesion and occlusion length). Therefore, either containerized or bareroot seedlings from each family may be utilized in screening *P. taeda* families to these fungi in the future screening studies. Our results suggest that this variation in susceptibility of *Pinus taeda* families to these fungi observed in different trials of Singh et al. (2014) may not be attributed to the stocktype differences but may be due to genotype x environment interaction. Containerized seedlings had more height and diameter growth than bareroot seedlings and less mortality in the 2 weeks after transplanting. Therefore, containerized seedlings may be a better choice for susceptibility screening though bareroot seedlings can also be used to accommodate necessary sample sizes.

Development of necrotic lesions and occlusions in the seedling root collar area 8 weeks following fungal inoculation serves as a reliable estimate of the host susceptibility (Matusick et al. 2010). The sizes of lesions and occlusions determine the susceptibility and tolerance of conifer hosts to the ophiostomatoid fungal pathogen. The fungal inoculation in *Pinus* spp. induces production of ethylene, which further regulates monoterpene production and influences lesion and occlusion formation (Devkota et al. 2018c, Paine et al. 1997, Popp et al. 1995). Accumulation of a high level of monoterpene causes heightened host response and longer lesion and occlusion. The trees with larger necrotic lesions have a greater decline in phloem non-structural carbohydrates and sapwood lipids. Larger lesions and occlusions cause disruption of conductive xylem tissue, decline in the radial growth, and tree mortality (Joseph et al. 1998, Krokene et al. 2008, Oliva et al. 2014).

Our study has some limitations. Early seedling performance in the weeks following infection may not necessarily be a predictor of longer term seedling performance on an outplanting site. Therefore, future studies should include field evaluations of both stocktypes. In addition, our conclusions were based only on four families of *Pinus taeda*. Therefore, future studies should focus on other *P. taeda* families as well.

Family genetics, but not the seedling stocktype, of *P. taeda* appears to be an important factor in disease development and plant tolerance to ophiostomatoid fungi. Thus, both containerized and bareroot seedling stocktypes can be used interchangeably in screening studies such as the one described in this article. Deployment of these *P. taeda* families in the field, however, should consider factors such as soil

management, drought, and various other biotic and abiotic stressors associated with ophiostomatoid fungal-vectoring beetle attacks.

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