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Research Article

The Impact of Drought and Vascular-Inhabiting Pathogen Invasion in *Pinus taeda* Health

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The complex interaction of various biotic and abiotic factors may put the overall stand health of *Pinus* spp. at risk. A study was designed to determine the combined impact of drought and vascular-inhabiting fungi (*Leptographium terebrantis* and *Grosmannia huntii*) in *Pinus taeda*. Seedlings from two *P. taeda* families were planted and watering treatments, (i) normal watering, (ii) moderate drought, and (iii) severe drought, were applied. One month following the initiation of watering treatments, seedling stems were artificially inoculated with *L. terebrantis* and *G. huntii*. Drought and fungal interaction significantly affected lesion length/seedling height, occlusion length/seedling height, and seedling fine root biomass. *Leptographium terebrantis* was more pathogenic under moderate and severe drought than normal watering condition, whereas the pathogenicity of *G. huntii* remains unaltered. The susceptibility of the families to vascular-inhabiting fungi remained the same under different watering treatments. Drought and specific vascular-inhabiting fungi may negatively impact *P. taeda* stand health.

1. Introduction

Adverse climatic conditions like drought have been shown to be responsible for a number of forest health problems around the world [1, 2]. Recent incidents of tree decline and mortality have been related to increased mean annual temperatures and decreased mean annual rainfall in European forests [3] and increased droughts in southwestern [4] and southeastern US [5, 6]. Drought events are expected to become more common in the future as provided by IPCC 2013 [7] resulting in drought-induced forest mortality [8]. Despite the adverse effects of drought on forest functions, mechanisms underlying forest health decline and mortality are not understood [9].

The impact of drought on forest health is a function of host tree resistance and pathogen performance. Drought influences the production of specific chemicals in conifers rendering the trees more susceptible to pathogens and insect attacks [10, 11]. For example, bark beetle infestation in drought-weakened *Pinus* forests may occur many years after the end of the climatological drought [12]. Beetle-vectored, vascular-inhabiting pathogens can also have a devastating effect on drought-stressed trees [13].

The vascular-inhabiting fungal pathogens are considered to be the dominant factors in the final phase of the drought-induced tree and stand mortality [13]. Vascular wilt pathogens such as *Ceratocystis* Ellis & Halst., *Leptographium,* and *Grosmannia* Goid. species thrive in the xylem of *Pinus* spp. [14, 15]. Host, *Pinus* spp., defends against these fungi by producing resins that clog the plant vascular conducting tissues [16]. A tremendous amount of carbon is required in defense which results in the scarcity of carbon required for the plant growth and functioning. In addition, clogging of plant xylem disturbs plant water transport, resulting in hydraulic failure leading to tree mortality [13].

Many pathologists have had a false dichotomy of drought vs. biotic attack [17]. Many studies have focused primarily

on individual factors: (i) drought and its subsequent effect on plant physiology [18, 19] or (ii) Biotic agents and its subsequent impact tree health [15, 16]. However, the evidence for the mechanisms suggested by these individual factors is inconclusive and a more integrated approach focusing on relations between drought and biotic agents on tree growth and functioning is needed.

Recently, a few studies have focused on the interaction of drought and vascular-inhabiting fungi [20, 21]. However, these studies deployed both drought and fungal treatment at the same time, despite the fact that these vascular-inhabiting fungi come into play only after the predisposition of trees to a drought event [20, 21]. Thus, a closer examination of the impact of *Leptographium terebrantis* and *Grosmannia huntii* on *P. taeda* trees predisposed to drought is needed. Therefore, the objectives of the study were (i) to determine whether the pathogenicity of *L. terebrantis* and *G. huntii* in *P. taeda* alters under different soil moisture conditions and (ii) to determine whether the susceptibility of *P. taeda* families to *L. terebrantis* and *G. huntii* alters under different soil moisture conditions. An experiment was conducted to address these objectives, in which seedlings from two *P. taeda* families were grown under different watering treatments followed by fungal inoculation. The extent of necrotic and occluded vascular tissues and plant growth parameters was used as a measure of fungal pathogenicity and seedling family susceptibility.

2. Materials and Methods

2.1. Experimental Location. The experiment was conducted in the research facility of the Southern Forest Nursery Management Cooperative Auburn, AL, USA. The facility contained an open outdoor pavilion with 12 raised wooden boxes (120 cm long, 100 cm wide, and 120 cm deep) filled with pure sand. Plastic transparent roof covered the pavilion to exclude any ambient rainfall.

2.2. Seedling Planting. One-year-old, bare-root seedlings from two commercially grown *P. taeda* families were used for inoculation. To establish those seedlings, seeds were sown in February 2014 and seedlings were lifted from the nursery in February 2015. Based on previous findings by Singh et al. [15], one seedling family used was considered "susceptible" (S) and one family was considered "tolerant" (T) to vascular-inhabiting ophiostomatoid fungi. In February 2015, 630 seedlings (35 per family in each box) were planted in 9 wooden boxes and watered to field capacity for 4 weeks until watering treatments were initiated.

2.3. Watering Treatment. Three watering treatments, (i) normal watering, (ii) moderate drought, and (iii) severe drought, were deployed to 3 boxes (3 replicates/treatment) in March 2015. The watering treatments were determined based on the volumetric water content of the pure sand. The wet weight and dry weight (72 h at 105°C) of the soil were determined, and the volumetric water content (V) of the soil sample was determined by using the following formula:

$$V = \frac{Wmass - Dmass}{Pw\ Vs} \quad (1)$$

where *Wmass* was the mass of the soil before drying and *Dmass* was the mass of the soil after drying, *Pw* is the density of water (1000 kg m^{-3}), and *Vs* is the total volume of the soil sample (sum of air, water, and soil). The volumetric water content for the field capacity (FC) was 0.32 $m^3\ m^{-3}$. The watering treatments were as follows: (i) 75 % of FC (normal watering i.e., 0.28 $m^3\ m^{-3}$), (ii) 50 % of FC (moderate drought, i.e., 0.18 $m^3\ m^{-3}$), and (iii) 25 % of FC (severe drought, i.e., 0.11 $m^3\ m^{-3}$). Soil water content was constantly monitored in each box throughout the experiment using an external moisture probe (SM150T mit HH150, Delta-T Devices, Ltd. Giesbeek, The Netherlands), and irrigation was programmed as required to meet volumetric water content of each box.

2.4. Inoculation Treatment. One month into the three watering treatments (April 2015), artificial stem inoculations were conducted as described by Nevill et al. [22], Singh et al. [15], and Chieppa et al. [21] using wound + inoculum method. Five inoculation treatments applied were as follows: *L. terebrantis* (LOB-R-00-805/ATCC accession no. MYA-3316), *G. huntii* (LLP-R-02/ATCC accession no. MYA-3311), wound, wound + media, and no wound. To perform the inoculation, 13 mm bark (<1.5 mm depth) of seedling at the stem, ~ 3 cm above soil line was cut vertically with a sterile razor blade. The single prepunched plug of agar (3 mm) with actively growing fungal mycelium was placed (fungus-side-towards wound) in the wound in each seedling. Sterile agar plug was put in the wound in case of wound + media inoculation. A sterile cut was made for wound control. No wound was made in seedling receiving no wound treatment. Wounds on the stems were then wrapped with sterile cotton balls moistened with deionized water to prevent desiccation of Malt Extract Agar (MEA) and wrapped with Parafilm[5] to avoid contamination. Seven seedlings per family within a box received each inoculation treatment. These fungal isolates have been used in previous artificial inoculation studies [15, 16, 21]. The fungal isolates were maintained at 4°C in MEA before use and were placed on 2 % MEA plate, 14 days prior to the inoculation experiment.

2.5. Preharvesting Measurements

2.5.1. Growth and Size Measurement. Height and Root-Collar Diameter (RCD) measurements were collected from each seedling prior to water treatments (March 2015), stem inoculation (April 2015), and seedling harvesting (September 2015). The number of new buds developed was counted on individual seedlings prior to watering (March 2015) and prior to seedling harvesting (September 2015).

2.6. Postharvest Measurement

2.6.1. Inoculation Response. In September 2015, four seedlings from each treatment were cut at the stem above the soil level (September 2015) and placed vertically in the mixture of stain (FastGreen FCF; Sigma Chemical Co., St. Louis, MO, USA) 0.25 g L^{-1} for 72 h. After staining, the bark near inoculation point was scraped to the xylem with the lesion

Table 1: Generalized linear mixed models utilized for each of the response variables.

Y	Model
LL/HT	$\beta 0 + \beta 1F + \beta 2M + \beta 3T + \beta 4F * T + \beta 5M * T + \epsilon$
OL/HT	$\beta 0 + \beta 1B + \beta 2F + \beta 3M + \beta 4T + \beta 5F * T + \beta 6M * T + \epsilon$
NB	$\beta 0 + \beta 1B + \beta 2F + \beta 3M + \beta 4T + \beta 5F * T + \beta 6M * T + \epsilon$
HTI	$\beta 0 + \beta 1B + \beta 2F + \beta 3M + \beta 4T + \beta 5F * M + \beta 6F * T + \epsilon$
Ny	$\beta 0 + \beta 1B + \beta 2F + \beta 3M + \beta 4T + \beta 5F * T + \epsilon$
Sy	$\beta 0 + \beta 1B + \beta 2F + \beta 3M + \beta 4T + \beta 5F * M + \epsilon$
CRY	$\beta 0 + \beta 1B + \beta 2F + \beta 3M + \epsilon$
FRY	$\beta 0 + \beta 1B + \beta 2F + \beta 3M + \beta 4T + \beta 5F * M + \beta 6M * T + \epsilon$

Note. Y: response variable, LL/HT: lesion length/seedling height, OL/HT: occlusion length/seedling height, NB: new bud-break, HTI: increase in seedling height, Ny: needle dry biomass, Sy: stem dry biomass, CRY: coarse root dry biomass, FRY: fine root dry biomass, and Ny/Fry: needle/Fine root dry biomass. $\beta 0$ is the intercept, F is the family effect, T is the fungal effect, M is the moisture effect, $F*M$ is the family fungal interaction, $F*T$ is the interaction of fungal treatment and moisture, B is the random effect of the box, and ϵ is the residual error.

and occlusion length and width measured. The necrotic bark and phloem were measured as the lesion. The xylem that did not allow the stain to pass through was measured as the occlusion. Ratios of lesion length\seedling height and occlusion length\seedling height were calculated. Two pieces (~ 3 mm) of stem tissue surrounding the lesion were cut and plated on MEA with cycloheximide at 800 mg L^{-1} and streptomycin sulfate at 200 mg L^{-1} to confirm fungal reisolation. Stem sections of control seedlings were also plated to confirm no contamination.

2.6.2. Seedling Biomass. Three remaining seedlings from each treatment combination per box were used for dry biomass measurements. With each seedling separated into needles (N), stem (S), coarse root (CR, roots ≤ 2 mm), and fine root (FR, roots < 2 mm), biomass was let to dry at 75°C for 72 h and then weighed.

2.7. Statistical Analysis. The experimental design was a randomized control block design. The generalized linear mixed models were used to analyze the response variables. The most parsimonious model for each of the response variables was selected by Akaike Information Criterion (AIC). The model with lowest AICc score and high percentage weight of the total weight of the models considered was selected as the best model for each response variable and is presented in Table 1. The response variables were lesion length and seedling height ratio, occlusion length and seedling height ratio, seedling height change, new bud-break, needle, stem, coarse root, and fine root dry biomass. Multiple comparisons were performed by using post hoc Tukey (Honest Significant Difference) procedures at $\alpha = 0.05$. All the assumptions of normality and homogeneity of the variance were inspected. Lesion width, stem dry biomass, needle dry biomass, and fine root dry biomass were log transformed. All the statistical analysis was conducted using SAS (Version 9.4, SAS Institute, Inc., Cary, NC, USA).

3. Results

Dark brown necrotic tissues were observed at the inoculation point in all the inoculated seedlings. Lesions on seedlings with the control inoculations were significantly smaller than lesions from fungal inoculations, indicating that the fungi, not the wound, caused the lesion. Likewise, lesions in the wound and wound + media did not extend beyond the inoculation zone. The reisolation success of *G. huntii* and *L. terebrantis* was 89 % and 92 %, respectively, indicating successful fungal inoculation.

Lesion length/seedling height ratio was significantly affected by family, watering treatment, inoculation, family x inoculation, and watering treatment x inoculation (Table 2). Family S (susceptible family) had higher lesion length/seedling height ratio than that compared to family T (susceptible family) (Table 3). The seedlings under moderate and severe drought had significantly higher lesion length/seedling height ratio as compared to that under normal watering treatment (Table 4). Seedlings inoculated with *Leptographium terebrantis* had significantly higher lesion length/seedling height ratio than that compared to seedlings inoculated with *G. huntii* (Table 5). *Leptographium terebrantis* resulted in significantly longer lesion than *G. huntii* within both tolerant and susceptible family (Table 6). The lesion caused by *L. terebrantis* was significantly longer in the susceptible family than the tolerant family. The seedlings under moderate drought challenged with *L. terebrantis* had highest lesion length/seedling height ratio followed by severe drought (Table 7). However, the pathogenicity of *G. huntii* remained unaltered under different watering treatment (Table 7). *Leptographium terebrantis* resulted in the higher lesion length/height ratio in seedlings under moderate and severe drought as compared to that under normal watering.

Occlusion length/seedling height ratio was affected by family, inoculation, family x inoculation, and watering treatment x inoculation (Table 2). This ratio was significantly higher in seedlings from the susceptible family as compared to that of tolerant family (Table 3). Within each watering treatment, *L. terebrantis* caused significantly higher occlusion length/seedling height ratio than *G. huntii* and control inoculations (Table 7). This ratio was significantly higher in seedlings inoculated with *L. terebrantis* than *G. huntii*, indicating the high virulence of *L. terebrantis*.

Overall seedling height growth was significantly affected by family, inoculation, family x watering treatment, and family x inoculation (Table 2). The growth of seedlings from the tolerant family was significantly higher than that compared to seedlings from the susceptible family (Table 3). The overall seedling height growth did not differ between the three watering treatments (Table 4). The seedlings inoculated with *L. terebrantis* had significantly less growth than that compared to wound + media control (Table 5). The growth of seedlings from the tolerant family was significantly lower in severe drought than that compared to normal watering and moderate drought conditions whereas it did not alter under different watering treatments in the seedlings from the susceptible family. The growth of the seedlings varied significantly between the tolerant and susceptible

Table 2: Type three fixed effects of lesion and occlusion length by seedling height ratio, height increase, new bud-break, and dry biomass of various plant parts.

Variable	Effect	Num DF	Den DF	F value	Pr > F
LL/HT	Fam	1	408	47.02	<.0001
	Mos	2	408	6.18	0.0023
	Trt	3	408	138.94	<.0001
	Fam∗Trt	3	408	6.43	0.0003
	Mos∗Trt	6	408	6.86	<.0001
OL/HT	Mos	2	418	1.16	0.3159
	Fam	1	418	64.74	<.0001
	Trt	3	418	312.91	<.0001
	Fam∗Trt	3	418	11.07	<.0001
	Mos∗Trt	6	418	6.4	<.0001
HTI	Fam	1	613	70.58	<.0001
	Mos	2	613	0.9	<0.0001
	Trt	4	613	3.32	0.0105
	Fam∗Mos	2	613	3.11	0.0455
	Fam∗Trt	4	613	2.25	0.0625
Bud-break	Fam	1	559	8.21	0.0043
	Mos	2	559	1.16	0.3132
	Trt	4	559	6.22	<.0001
	Fam∗Trt	4	559	3.19	0.0132
	Mos∗Trt	8	559	1.71	0.0930
Ny	Fam	1	405	10.82	0.0011
	Mos	2	405	0.93	0.395
	Trt	4	405	4.73	0.001
	Fam∗Trt	4	405	3.41	0.0093
Sy	Fam	1	405	107.48	<.0001
	Mos	2	405	2.87	0.0578
	Trt	4	405	8.05	<.0001
	Fam∗Mos	2	405	1.06	0.3482
CRY	Fam	1	397	46.32	<.0001
FRY	Fam	1	397	37.41	<.0001
	Mos	2	397	2.16	0.1161
	Trt	4	397	6.30	<.0001
	Fam∗Mos	2	397	7.13	0.0009
	Mos∗Trt	8	397	3.18	0.0017

Note. LL/HT: lesion length/height, OL/HT: occlusion length/seedling height, HTI: height-increase, Ny: needle dry biomass, Sy: stem dry biomass, CRY: coarse root dry biomass, and FRY: fine root dry biomass, Fam: family, Trt: fungal treatment, and Mos: moisture treatment.

family under all watering treatment (Table 8). Bud-break was significantly affected by family, inoculation, and their interaction (Table 2). The tolerant family had significantly higher bud-break than that compared to susceptible family. There was no significant variation in the number of bud-breaks in *L. terebrantis*, and *G. huntii* treated seedlings from the susceptible and tolerant families (Table 6).

Family, watering treatments, inoculation, and family x inoculation impacted dry needle biomass (Ny) (Table 2). The needle dry biomass of the seedlings from the tolerant family was significantly higher than the susceptible family (Table 3). *Leptographium terebrantis* and *G. huntii* inoculated seedlings from the tolerant family had significantly higher Ny than control inoculated seedlings (Table 6). Stem biomass was significantly different among family, watering treatment, and inoculation treatment (Table 2). However, none of the interactions were significant. Seedlings from the tolerant family had significantly higher stem biomass when compared to that of the susceptible family (Table 3). The seedlings inoculated with *G. huntii* had significantly lower stem dry biomass than that compared to no wound and wound control (Table 5).

Table 3: Least square means and standard error of various response variables in susceptible and tolerant family.

Variables	Tolerant family LS mean ± SE	Susceptible family LS mean ± SE
LL/HT	0.44 ±0.02a	0.61 ±0.02b
OL/HT	0.50 ±0.03a	0.68 ±0.03b
HTI (cm)	22.37 ±1.19a	16.70 ±1.20b
Bud-break	2.22 ±0.17a	1.81 ±0.17b
Ny (g)	5.71 ±0.42a	5.05 ±0.42b
Sy (g)	5.00 ±0.22a	3.61 ±0.22b
CRY (g)	1.43 ±0.09a	1.00 ±0.09b
FRY (g)	1.22 ±0.10a	0.92 ±0.10b

Note. SE: standard error, LL/Ht: lesion length/seedling height, OL/HT: occlusion length/seedling height, Ny: needle dry biomass, Sy: stem dry biomass, CRY: coarse root dry biomass, and FRY: fine root dry biomass. Different letters indicate Tukey pair-wise differences between two families within each variable at $\alpha = 0.05$.

Fine root dry matter biomass (FRY) was significantly affected by family, watering treatment, inoculation, family x watering treatment, and watering treatment x inoculation (Table 2). Overall, the seedlings from the tolerant family had significantly higher FRY than that compared to the susceptible family (Table 3). Seedlings inoculated with *L. terebrantis* and *G. huntii* had significantly lower FRY than that compared to no wound and wound control seedlings (Table 5). However, FRY of seedlings inoculated with *L. terebrantis* was significantly less in seedlings under severe drought as compared those under the moderate drought and normal watering treatment. Fine root biomass of seedlings inoculated with *G. huntii* under severe drought was lower than under normal watering treatment (Table 7). Similarly, FRY of the two families did not alter under different watering conditions (Table 8). The susceptible family had significantly lower FRY than that compared to the tolerant family under normal watering treatment. Coarse root dry matter biomass (CRY) was significantly different between the two families. However, none of the interactions were significant (Table 2). Seedlings from the tolerant family had significantly higher CRY than that compared to the susceptible family (Table 3).

4. Discussion

The pathogenicity of *L. terebrantis* (in terms of lesion length\height, occlusion length\height) increased under

Table 4: Least square means and standard error of various response variables under three different watering conditions.

Variables	Normal watering LS Mean± SE	Moderate drought LS Mean ± SE	Severe drought LS Mean ± SE
LL/HT	0.44 ±0.03a	0.56 ±0.03b	0.57 ±0.03b
OL/HT	0.54 ±0.04a	0.63 ±0.04a	0.61 ±0.04a
HTI (cm)	20.57 ±1.99a	20.66 ±1.99a	17.36 ±1.98b
Bud-break	2.27 ±0.26a	2.07 ±0.26a	1.71 ±0.26a
Ny (g)	5.56 ±0.71a	6.00 ±0.71a	4.59 ±0.71a
Sy (g)	4.72 ±0.36a	4.56 ±0.36a	3.64 ±0.36a
FRY (g)	1.29 ±0.16a	1.09 ±0.16a	0.82 ±0.16a

Note: SE: standard error, LL/HT: lesion length/seedling height, OL/HT: occlusion length/seedling height, HTI: height growth, Ny: needle dry biomass, Sy: stem dry biomass, and FRY: fine root dry biomass. Different letters indicate Tukey pair-wise differences among watering treatments within each variable at $\alpha = 0.05$.

Table 5: Least square means and standard error of various response variables in seedlings treated with different inoculations.

Variables	GH LS Mean ± SE	LT LS Mean ± SE	W LS Mean ± SE	WM LS Mean ± SE	NW LS Mean ± SE
LL/HT	0.57 ±0.03a	0.94 ±0.03b	0.30 ±0.03c	0.29 ±0.03c	-
OL/HT	0.66 ±0.03a	1.15 ±0.03b	0.26 ±0.03c	0.28 ±0.03c	-
HTI	18.60 ±1.33ab	17.58 ±1.33b	20.43 ±1.33ab	20.59 ±1.33a	20.48 ±1.33ab
BB	1.69 ±0.21a	1.60 ±0.21a	2.09 ±0.21ab	2.01 ±0.21a	2.68 ±0.22b
Ny	5.11 ±0.46ab	4.64 ±0.45b	5.74 ±0.46a	5.56 ±0.46a	5.84 ±0.45a
Sy	3.77 ±0.24a	4.12 ±0.24ab	4.59 ±0.25bc	4.23 ±0.24ab	4.81 ±0.24c
FRY	0.96 ±0.10a	0.94 ±0.10a	1.20 ±0.10bc	1.01 ±0.10ab	1.23 ±0.10c

Note. SE: standard error, LL/HT: lesion length/seedling height, OL/HT: occlusion length/seedling height, HTI: height growth, BB: new bud-break, Ny: needle dry biomass, Sy: stem dry biomass, FRY: fine root dry biomass, GH: *Grosmannia huntii*, LT: *Leptographium terebrantis*, W: wound, WM: wound + media, NW: no wound, and NW -: not applicable. Different letters indicate Tukey pair-wise differences among different inoculations within each variable at $\alpha = 0.05$.

moderate and severe drought conditions when inoculated in *P. taeda* seedlings. However, pathogenicity of *G. huntii* remained unaltered under all watering treatments. In general, the presence of the *Leptographium* spp. in *Pinus* spp. and the decline in tree health has been linked to many abiotic factors including moisture stress [23, 24]. Salle et al. (2008) [25] reported that *L. yunnanense* caused a longer lesion in moderately water-stressed *P. yunnanensis*(Franch.). In contrast, Christiansen and Glosli [26] reported that phloem has damage and blue staining due to *Ceratocystis polonica* (Siem.). C. Moreau was greater in the well-watered *Picea abies* [L.] Karst (Norway spruce) trees than in the water-stressed *P. abies* trees. This difference in result between their study and present study might be due to the variation in the host-specific response to water stress [27] and ophiostomatoid fungi [28].

The moderate and severe drought had an impact on seedlings inoculated with vascular-inhabiting fungi, *L. terebrantis,* but not with *G. huntii*. Lesion length should be considered as a function of seedling height [21]. The ratio of lesion length and seedling height and occlusion length and seedling height was greatest in seedlings inoculated with *L. terebrantis* under drought treatment as compared to normal watering treatment. Previous studies by Matusick et al. [20] and Chieppa et al. [21] did not find any evidence of variation in lesion size formation in *Pinus* seedlings inoculated with vascular-inhabiting fungi under different soil moisture levels. Similar results to our study were also observed, for example, for *P. resinosa* and *Populus nigra* L. x *P. maximowiczii* Rupr. (hybrid poplar) grown under drought stress. Under drought stress, the susceptibility of *P. resinosa* to *Sphaeropsis sapina* (Fr.) Dyko & B. Sutton was greatly increased (Blodgett et al. [29]). *Septoria musiva* Peck caused the increased size of canker in hybrid poplar grown under drought stress [30]. Trees with larger lesion size are the result of the greater utilization of nonstructural carbohydrates (NSC) by the trees in defense [31]. Thus, trees with larger sizes of lesion have a greater reduction in resource required for plant growth and functioning [31]. Furthermore, the spread of the fungus in the sapwood may result in enhanced vulnerability to cavitation and reduction in xylem water potential [32].

There was no significant three-way interaction between family, watering treatment, and inoculation suggesting that susceptibility of *P. taeda* families to fungal and control inoculation remain unaltered under different watering treatments. Overall, the tolerant family grew better than the susceptible family. The sizes of the lesion, occlusion, seedling growth, and biomass were all higher in the tolerant family than that compared to the susceptible family. Both the present study and few additional studies by Chieppa et al. [21] and Chieppa et al. [33] indicate that the families chosen for tolerance to ophiostomatoid fungi have more growth potential in terms of seedling volume change and height increment. Taken together, the previous studies and present study together

Table 6: Least square means and standard errors of number of various response variables in tolerant and susceptible family receiving various inoculations.

Variable	Inoculation	Tolerant family No. of bud-break ± SE	Susceptible family No. of bud-break ± SE
LL/HT	*G. huntii*	0.46 ±0.04c	0.68 ±0.04c
	L. terebrantis	0.77 ±0.04b	1.11 ±0.04a
	Wound	0.27 ±0.04c	0.33 ±0.04c
	Wound + media	0.26 ±0.04c	0.33 ±0.04c
OL/HT	*G. huntii*	0.56 ±0.03a	0.77 ±0.03a
	L. terebrantis	0.96 ±0.04b	1.35 ±0.03c
	Wound	0.24 ±0.03d	0.29 ±0.04d
	Wound + media	0.25 ±0.03d	0.32 ±0.04d
HTI (mm)	*G. huntii*	20.87 ±1.13a	16.42 ±1.15b
	L. terebrantis	19.64 ±1.13a	15.40 ±1.13b
	No wound	24.96 ±1.16c	16.16 ±1.13b
	Wound	24.37 ±1.22c	16.75 ±1.17b
	Wound + media	22.23 ±1.12ac	18.85 ±1.13ab
BB	*G. huntii*	1.56 ±0.26c	1.83 ±0.27bc
	L. terebrantis	1.67 ±0.28bc	1.53 ±0.27bc
	No wound	3.20 ±0.28a	2.16 ±0.28abc
	Wound	2.62 ±0.27ab	1.57 ±0.27c
	Wound + media	2.10 ±0.26b	1.93 ±0.26bc
Ny(g)	*G. huntii*	4.93 ±0.51cd	5.30 ±0.51abcd
	L. terebrantis	4.52 ±0.50d	4.75 ±0.50cd
	No wound	6.52 ±0.51a	5.17 ±0.50ab
	Wound	6.43 ±0.51ab	5.05 ±0.52abcd
	Wound + media	6.13 ±0.51abc	4.99 ±0.51bcd

Note. SE: standard error, LL/HT: lesion length/seedling height, OL/HT: occlusion length/seedling height, HTI: height increase, BB: new bud-break, and Ny: needle dry biomass. Different letters indicate Tukey pair-wise differences between all watering treatment and inoculations within each variable at $\alpha = 0.05$.

Table 7: Least square means and standard errors of three response variables caused by various inoculations under three different watering treatments.

W	I	LL/HT ± SE (mm)	OL/HT ± SE (mm)	Fry ± SE (g)
N	GH	0.49 ±0.03b	0.62 ±0.06d	1.28 ±0.18ab
N	LT	0.65 ±0.04b	0.93 ±0.06bc	1.13 ±0.19ab
N	NW	-	-	1.27 ±0.18ab
N	W	0.28 ±0.03c	0.28 ±0.06e	1.69 ±0.19a
N	WM	0.30 ±0.04c	0.30 ±0.06e	1.10 ±0.18ab
MD	GH	0.57 ±0.03b	0.68 ±0.06cd	0.90 ±0.18ab
MD	LT	1.08 ±0.03a	1.32 ±0.06a	1.04 ±0.18ab
MD	NW	-	-	1.45 ±0.18ab
MD	W	0.27 ±0.03c	0.25 ±0.06e	1.02 ±0.18ab
MD	WM	0.26 ±0.04c	0.25 ±0.06e	1.06 ±0.18ab
SD	GH	0.56 ±0.03b	0.69 ±0.06cd	0.71 ±0.18bc
SD	LT	1.03 ±0.03a	1.19 ±0.06ab	0.65 ±0.18c
SD	NW	-	-	0.97 ±01.8ab
SD	W	0.30 ±0.03c	0.26 ±0.06e	0.92 ±0.18ab
SD	WM	0.29 ±0.03c	0.30 ±0.06e	0.86 ±0.18ab

Note. SE: standard error, W: watering treatment, I: inoculation, N: normal watering, MD: medium drought, SD: severe drought. LL/HT: lesion length/seedling height, OL/HT: occlusion length/seedling height, FRY: fine root dry biomass, GH: *Grosmannia huntii*, LT: *Leptographium terebrantis*, W: wound, and WM: wound + media. Different letters indicate Tukey pair-wise differences between all inoculations and watering treatments within each variable at $\alpha = 0.05$.

Table 8: Least square means and standard errors of various response variables of seedlings from two different families receiving three different watering treatments.

Variable	Family	Normal watering Mean ± SE (cm)	Moderate drought Mean ± SE (cm)	Severe drought Mean ± SE (cm)
HTI	Tolerant	23.99 ±2.07a	24.09 ±2.07a	19.01 ±2.07b
	Susceptible	17.16 ±2.08b	17.24 ±2.07b	15.7 ±2.06b
FRY	Tolerant	1.57 ±0.17a	1.15 ±0.16ab	0.94 ±0.17ab
	Susceptible	1.01 ±0.17ab	1.04 ±0.17ab	0.71 ±0.17b
Sy	Tolerant	5.70 ±0.18a	5.05 ±0.17a	4.14 ±0.17ab
	Susceptible	3.92±0.18b	3.79 ±0.17b	2.97 ±0.17b

Note. SE: standard error, HTI: height increase, FRY: fine root dry biomass, and Sy: stem dry biomass. Different letters indicate Tukey pair-wise differences between all family and watering treatment conditions within each variable at $\alpha = 0.05$.

show some support for the higher growth potential of tolerant families. Future studies should be conducted to understand the anatomical and chemical factors governing increased disease tolerance and higher growth potential in those families.

Vascular-inhabiting fungi (*L. terebrantis*) is likely to enhance tree health decline directly through increased investment in occlusion and lesion. Localized damage and blockage in the vascular conducting tissue was observed in inoculated *P. taeda* seedlings. The spread of the fungal mycelium into the sapwood might have caused damage to the tracheid walls [34]. Such damage can further result in cavitation and embolism [35]. The xylem blockage can be irreversible due to resin deposition and tyloses formation [36]. Under severe drought, complete xylem blockage due to occlusion in some of the seedlings inoculated with *L. terebrantis* was observed. In such seedlings, the development of the new tissues on the opposite side of the fungal inoculation would have helped in the survival of the plant. However, the growth of the tissues around the fungi inoculated side was completely halted, and the fine root biomass was reduced. It could be an adaptive trait of *P. taeda* that would allow the plant to be decoupled from drought as well as pathogen stress. Moreover, the growth of such seedlings was halted suggesting a potential tradeoff between this adaptive trait and plant growth. Massive inoculation of the fungi might lead to a more detrimental impact on the *P. taeda* seedlings [35]. Unlike Croisé et al. [37], we only performed single-point inoculation. Future studies should be focused on studying the impact of multiple-point fungal inoculations on *P. taeda* under drought.

The family considered as tolerant to ophiostomatoid fungi exhibited higher growth rates and more bud-production under all watering treatments, implying that the fungi tolerant family tend to have higher growth rate. Under severe drought conditions, seedlings exhibited greatly reduced plant height growth as compared to that under normal watering and moderate drought. The responsiveness of *Pinus* species height (by limited growth) to drought conditions is now well documented in the literature. Reduced soil moisture has been reported to cause the reduction in growth [38, 39] and the degree of reduction is linked to the location of seed source [40]. The drought and the fungi did not interact together to inhibit plant growth during the study period. The family which is tolerant to ophiostomatoid fungi has higher growth rates under all watering treatments, implying that the family tolerant to the fungi tends to have higher growth rate.

The tolerant family tended to have high needle, stem, coarse root, and fine root dry biomass compared to the susceptible family in general. The seedlings from the tolerant family have been previously reported to show higher biomass than that from the susceptible family [21]. The overall seedling biomass did not alter under different watering treatments. Seedlings from tolerant family inoculated with both fungi had significantly lower needle dry biomass than that compared to the control seedlings from the same family. *Leptographium terebrantis* inoculated seedlings had significantly less fine root biomass under severe drought treatment compared to the normal watering treatment. The fine root biomass was progressively declining from normal watering to severe drought in seedlings inoculated with both fungi. However, no specific significant pattern can be concluded for *G. huntii*. It is likely that the significant shifts in the seedling biomass were not observed as the experimental period was short (20 weeks). Therefore, the results of the present study should be carefully considered. In general, the present study suggests that invasion of specific vascular-inhabiting fungi can be a critical factor for fine root growth during severe drought. The allocation of carbohydrates from needles to roots may have been partially blocked by the fungal invasion [13], resulting in decreased root growth. With the decreasing root growth, the plant has less access to the soil available water [41, 42]. Plant survival decreases as needle-to-fine-root ratio reaches a certain threshold. Above that threshold, evaporative surface (needles) increases as compared to the absorbing root surface [43].

Future studies should be focused on longer-term monitoring of the fungal inoculated *P. taeda* seedlings under projected climate change scenarios. The damage on an ecological scale might be higher than what we observed in our controlled study as we know that the mass attack of the beetles occurs in trees prestressed with drought in the natural scenario. Thus, mass inoculation of the fungi in the stressed mature *P. taeda* trees could provide a better understanding of host-microbe and environment interactions.

5. Conclusion

Drought and specific vascular-inhabiting fungi may negatively impact *P. taeda* stand health. The pathogenicity of *L. terebrantis* in *P. taeda* alters under different soil watering treatments. However, no specific pattern was observed for *G. huntii*. The necrotic lesion and vascular occlusion caused by *L. terebrantis* increased under increasing drought in *P. taeda* seedlings. The susceptibility of *P. taeda* families to *L. terebrantis* and *G. huntii* did not alter under different soil watering treatments. Infection by specific vascular-inhabiting fungi is likely to influence tree health through increased investment in occlusion and reduction of plant growth. Families selected for tolerance to ophiostomatoid fungi are consistently tolerant to fungi and have the ability to grow better than the susceptible family.

Disclosure

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Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Pratima Devkota conducted the experiment, analyzed data, and generated tables and graphs. Lori G. Eckhardt and Scott A. Enebak provided idea and guidance for the research. All authors contributed to the writing of the manuscript.

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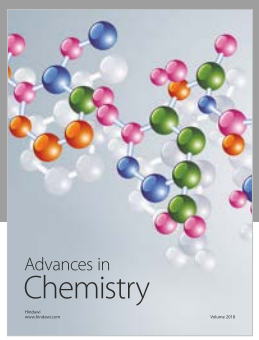

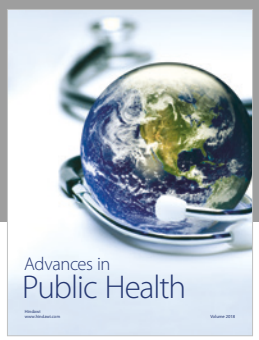

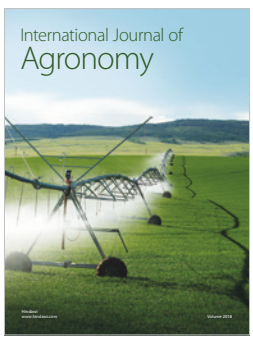

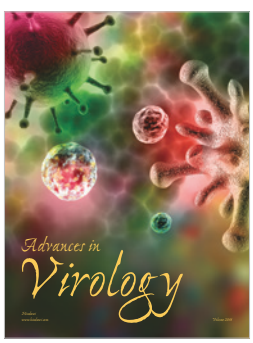

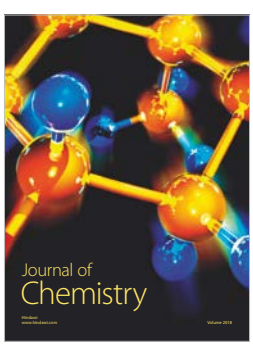

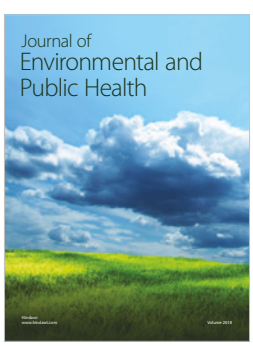

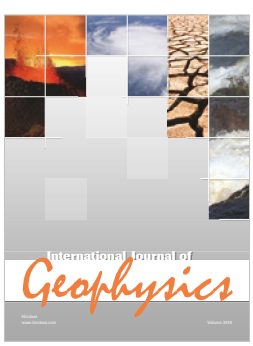

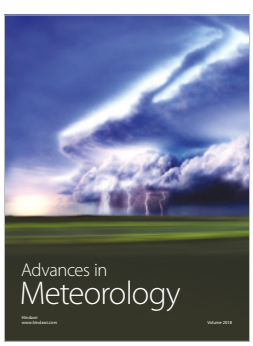

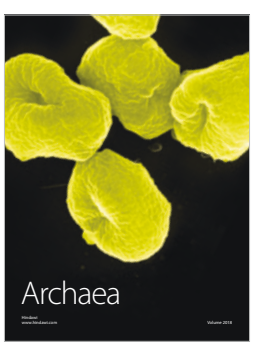

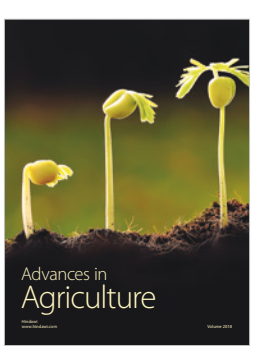

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