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SHORT COMMUNICATION

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Variation in pathogenicity of different *Leptographium terebrantis* isolates to *Pinus taeda* L.

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Abstract

Variation in the pathogenicity of 42 isolates of *Leptographium terebrantis* to *Pinus taeda* was examined. Stems of 1-year-old *P. taeda* seedlings were artificially inoculated with the fungal isolates. Eight weeks following inoculation, *L. terebrantis* isolates caused dark necrotic lesion and sapwood occlusion in the seedling stems. The fungal isolates varied in their ability to cause lesion and tissue occlusion. Lesions caused by fungal isolates were, however, significantly longer than the control. Results suggest that different isolates of *L. terebrantis* may not put the health of *P. taeda* at equal risk.

KEYWORDS
lesion, occlusion, pathogenicity

1 | INTRODUCTION

Leptographium species are distributed worldwide with the most species as inhabitants of conifers (Jacobs & Wingfield, 2001). These fungi are more common in the southern United States where conifer are the major species. *Leptographium procerum* (W.B. Kendr.), M.J. Wingf, and *L. terebrantis* S.J. Barras and T.J. Perry, are among the root-feeding bark beetle-vectored *Leptographium* species, frequently isolated from roots of declining *Pinus taeda* L. in the southern United States (Eckhardt, Jones, & Klepzig, 2004). *Leptographium terebrantis* is arguably the most important *Leptographium* species, as it has shown to be pathogenic to various *Pinus* hosts in a number of previous studies (Eckhardt et al., 2004; Wingfield, 1986).

Leptographium terebrantis causes lesions and occlusion in the periderm and vascular tissues, respectively, in the stems and roots of *Pinus* species (Repe, Bojović, & Jurc, 2015). It may play an important role in the death of the tree, directly by blocking water transport (Paine, Raffa, & Harrington, 1997) and indirectly by stimulating the tree defence mechanisms that may exhaust the host (Lieutier, Yart, & Salle, 2009). It enters the host through an artificially created wound and spreads in the vascular tissue. Hosts, on the other hand, defend themselves with the production of resin response. The fungal virulence and the pathogenicity are usually determined by measuring the visual host symptoms such as the length of the dark necrotic lesion and occluded sapwood (Matusick, Somers, & Eckhardt, 2012). Pathogenicity tests using *L. terebrantis* have been carried out in various *Pinus* hosts. However, intra-species variation in pathogenicity of fungi is lacking in the literature. The high frequency of *L. terebrantis* isolation from *Pinus* forests (Eckhardt, Weber, Menard, Jones, & Hess, 2007) shows that the fungus may have the potential to become a devastating pathogen in the future. It is, however, still unknown whether all the isolates have equal potential to impact the health of the tree? Therefore, the objective of the study was to determine the variation in pathogenicity of 42 different *L. terebrantis* isolates to *P. taeda*.

2 | MATERIALS AND METHODS

A total of 820 1-year-old bare-root *P. taeda* seedlings from the single-family were re-planted in one-gallon pots filled with ProMix BX® (Premier Tech, Quebec, and Canada) peat-based potting media in January 2015. The seedlings were allowed to grow under natural conditions in an outdoor research facility. Stems of seedlings were artificially inoculated with mycelial agar plugs of fungal isolates taken from the leading edge of 14-day-old Malt Extract Agar plate. Nineteen seedlings were assigned for each of 42 different *L. terebrantis* isolate. These *L. terebrantis* isolates were isolated from the roots of declining *Pinus* stands from various locations in Alabama, USA, by Eckhardt et al. (2007) as the isolation method described by Otrosina,

TABLE 1 Means and standard errors of lesion length and occlusion length caused by 42 different *Leptographium terebrantis* isolates

Isolate	Lesion length (mm)	Occlusion length (mm)
I-03-3150	19.28 ± 0.95 cdef	23.55 ± 1.29 bcdef
I-03-2040	18.67 ± 0.95 cdef	23.68 ± 1.29 bcdef
I-03-2069	19.47 ± 0.95 bcdef	23.00 ± 1.29 bcdef
I-03-3125	18.57 ± 0.95 cdef	20.97 ± 1.29 def
I-03-2072	20.48 ± 0.95 bcdef	22.68 ± 1.29 bcdef
I-03-1848	21.93 ± 0.95 bcde	26.06 ± 1.29 bcdef
I-03-2122	18.05 ± 0.95 cdef	19.92 ± 1.29 fg
I-03-2077	18.78 ± 0.95 cdef	21.36 ± 1.29 defg
I-03-3107	19.76 ± 0.95 bcdef	22.17 ± 1.29 bcdef
I-03-2013	20.40 ± 0.95 bcdef	25.67 ± 1.29 bcdef
I-03-1770	21.10 ± 0.95 bcdef	25.10 ± 1.29 bcdef
I-03-2031	21.64 ± 0.95 bcdef	25.64 ± 1.29 bcdef
I-03-3196	19.62 ± 0.95 bcdef	24.02 ± 1.29 bcdef
I-03-1953	21.68 ± 0.95 bcde	26.20 ± 1.29 bcdef
I-03-1772	20.24 ± 0.95 bcdef	25.47 ± 1.29 bcdef
I-03-2123	19.40 ± 0.95 bcdef	25.22 ± 1.29 bcdef
I-03-2030	21.15 ± 0.95 bcdef	26.65 ± 1.29 bcdef
I-03-2098	21.06 ± 0.95 bcdef	26.61 ± 1.29 bcdef
I-03-2099	20.13 ± 0.95 bcdef	23.85 ± 1.29 bcdef
I-03-3179	18.13 ± 0.95 cdef	22.33 ± 1.29 bcdef
I-03-1973	18.92 ± 0.95 cdef	20.75 ± 1.29 defg
I-03-2163	18.81 ± 0.95 cdef	23.43 ± 1.29 bcdef
I-03-2026	22.83 ± 0.95 bcde	27.55 ± 1.29 bcd
I-03-1911	19.91 ± 0.95 bcdef	21.70 ± 1.29 bcdef
I-03-1995	17.10 ± 0.95 cdef	20.39 ± 1.29 efg
I-03-2133	24.58 ± 0.95 b	29.98 ± 1.29 ab
I-03-3123	20.12 ± 0.95 bcdef	23.93 ± 1.29 bcdef
R-03-3122	20.43 ± 0.95 bcdef	23.56 ± 1.29 bcdef
R-03-2088	19.26 ± 0.95 bcdef	23.48 ± 1.29 bcdef
R-03-3134	21.62 ± 0.95 bcde	27.23 ± 1.29 bcd
R-03-1687	19.86 ± 0.95 bcdef	24.86 ± 1.29 bcdef
R-03-3134	22.48 ± 0.95 bcde	25.33 ± 1.29 bcdef
R-03-3111	18.55 ± 0.95 cdef	21.30 ± 1.29 cdefg
R-03-1593	23.65 ± 0.95 bc	26.07 ± 1.29 bcdef
R-03-1622	22.07 ± 0.95 bcde	25.91 ± 1.29 bcdef
R-00-407	18.16 ± 0.95 cdef	22.21 ± 1.29 bcdef
R-00-366	20.72 ± 0.95 bcdef	25.67 ± 1.29 bcdef
R-00-87-ss205	19.04 ± 0.95 cdef	23.33 ± 1.29 bcdef
R-00-805	33.47 ± 0.95 a	38.83 ± 1.29 a
R-00-44-ss6	23.24 ± 0.95 bcd	28.53 ± 1.29 bc
I-03-1731	20.45 ± 0.95 bcdef	20.81 ± 1.29 def
Clem	21.49 ± 0.95 bcdef	26.89 ± 1.29 bcde
Control	14.93 ± 0.95 g	16.92 ± 1.29

Bannwart, and Roncadori (1999). Seedling stem inoculations were performed as described by Devkota, Enebak, and Eckhardt (2018). Control seedlings were inoculated with a sterile agar plug without fungus. After 8 weeks, seedlings were cut above the soil line placed in the distilled water that contained the Fast Green stain (Fast Green FCF; Sigma Chemical Co., St. Louis, MO, USA) mixed in a ratio of 0.25 g/L^{-1} (Devkota, Nadel, & Eckhardt, 2018; Devkota, Enebak et al., 2018). Seedlings were left in the stain mix for 48 hr to allow the dye to translocate throughout the stem. The bark around the inoculation point was carefully scraped, and the dark necrotic tissue was measured as the lesion. The vertical portion of the seedling stem that did not allow the capillary movement of dye was recorded as occlusion length. Two, 2-mm sections of the stem tissue around the lesion were plated on selective media (MEA containing 800 mg/L of cycloheximide and 200 mg/L of streptomycin sulphate) media to confirm re-isolation of the inoculated fungus. Data were analysed using a general linear model (GLM) in SAS statistical software (SAS Institute, 9.4 versions, Cary, NC). Pairwise comparisons were undertaken using the post hoc Tukey's test on the inoculation treatments at $\alpha = 0.05$.

3 | RESULTS

All of the 42 *L. terebrantis* isolates resulted in lesions and occlusions in all the inoculated seedlings. *Leptographium terebrantis* isolates were re-isolated from the 95% of the inoculated seedlings. Lesion length caused by different isolates of *L. terebrantis* varied significantly ($F_{(42, 773)} = 7.62$, $p = <0.0001$). Similarly, different isolates of *L. terebrantis* caused significantly different occlusion length in seedlings stems ($F_{(42,773)} = 6.49$, $p = <0.0001$).

Thirty-nine and forty isolates among 42 isolates of *L. terebrantis* caused significantly longer lesion length and occlusion length than control, respectively. Isolate R-00-805 caused the highest mean lesion length and the occlusion in the inoculated seedlings than that compared to the control and other *L. terebrantis* isolates (Table 1). Also, isolates I-03-2133, R-03-011, R-03-3134 and R-00-44-ss6 showed higher pathogenicity among others (Table 1). Similarly, isolates I-03-2122, I-03-3179, I-03-1995 and I-03-17 showed a lower level of pathogenicity among others (Table 1).

4 | DISCUSSION

The pathogenicity of *L. terebrantis* to *P. taeda* varied among the fungal isolates. These results suggest some of the isolates may be highly pathogenic whereas others may be weakly pathogenic to *P. taeda* host. Thus, all isolates of *L. terebrantis* may not put the health of *P. taeda* at equal risk. The results are similar to those reported previously for would inoculations of *Pinus ponderosa* Laws (Parmeter, Slaughter, Chen, Wood, & Stubbs, 1989) with two different isolates of *L. terebrantis*. Our findings are also supported by Lieutier, Yart, Ye, Sauvard, and Gallois (2004) that reported

variations in the virulence of *L. wingfieldii* Morelet. in *P. sylvestris*. In contrast, Wingfield (1986) did not find any significant difference in the virulence of different *L. procerum* isolates in *P. strobus* seedlings. These variations in results may have been due to the difference in fungal and host tree species.

Mortality of *P. taeda* seedlings was not observed during this study as had been observed in other pathogenicity studies (Harrington & Cobb, 1983; Parmeter et al., 1989). Pathogenicity and virulence of fungal inoculants to *Pinus* species are usually assessed based on lesion length and area caused by the fungus (Devkota, 2017; Wingfield, 1986). The *L. terebrantis* isolate R-00-805 caused the longest lesion and occlusion. The longer lesion denotes the higher pathogenicity of some fungi as compared to the other fungal isolates. With the same level of defence by *P. taeda*, relatively more virulent fungal isolates were able to cause varying lengths of necrotic lesions and blockage of the water-conducting vascular tissue. Intra-species variation in pathogens should be considered while generalizing the pathogenicity of *L. terebrantis* to *Pinus* hosts. Also, further studies should focus on the factors governing the variation in pathogenicity among the isolates.

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