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Effect of *Leptographium terebrantis* inoculation and drought on oleoresin and soluble phenolic levels in plantation *Pinus taeda*

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ABSTRACT

The influence of *Leptographium terebrantis* stem infection on constitutive and induced oleoresin and soluble phenolics in terminal shoots was assessed in a *Pinus taeda* plantation using three inoculum densities. The pathogen causes wilt disease by invading and colonizing root xylem tissues. We observed three levels of *P. taeda* defense in response to *L. terebrantis* during a two-year period interrupted by drought. Normal carbon allocation to growth was maintained and constitutive oleoresin production was observed at low and medium inoculum densities, with oleoresin induction observed among the medium inoculum density trees. At the high inoculum level, oleoresin flow occurred at a level similar to the medium inoculum density trees and carbon allocation to growth was diminished. Terminal shoot phenolic concentration was unaffected by the pathogen. Our findings validate the view that simultaneous occurrence of wilt disease and drought reduces constitutive and induced defenses by interfering with carbon fixation and allocation to growth.

1. Introduction

Pinus taeda (loblolly pine) is the most extensively planted forest tree species in North America (McKeand et al., 2021) and is the primary commercial tree species grown in the southern United States. This is attributed to productivity gains achieved by application of genetic improvement and intensive silviculture across this species' diverse range of habitats (Schultz, 1997). Over 1.4 million hectares of loblolly pine are planted in the southeastern United States annually (Haase et al., 2021; Enebak et al., 2022).

Nonetheless, loblolly pine is susceptible to diseases and pest infestations which adversely affect its physiological function and growth. Several fungi such as *Grosmannia alacris, Leptographium terebrantis*, *L. serpens*, and *L. procereum* have frequently been isolated from woody roots of declining loblolly pine trees in the southeastern U.S.A and have been shown to be pathogenic (Eckhardt et al., 2004; Matusick et al., 2013, 2016). These fungi are vectored and deposited into woody roots by root-feeding bark beetles such as *Hylastes salebrosus* and *Hylastes tenuis*, and the weevil species *Hylobius pales* and *Pachylobius picivorus* (Barras and Perry, 1971; Klepzig et al., 1991; Eckhardt et al., 2004). The fungi grow into the xylem tissue and interfere with sapwood hydraulic conductivity (Mensah et al., 2020).

In spite of the susceptibility of planted tree species to insect pests and pathogens, loblolly pine has several structural defense barriers and chemical mechanisms that offer protection against such attacks (Paine et al., 1987; Seybold et al., 2006; Metsämuuronen and Sirén, 2019; Turner et al., 2019). These defense barriers are both mechanical and chemical in nature and act sequentially and collectively to ward off attack by invading insect pests and pathogens (Nebeker et al., 1992; Ruel et al., 1998; Franceschi et al., 2005). Mechanically, the bark is the first line of defense which is strengthened in Pinaceae by intracellular accumulation of the solid chemical compound calcium oxalate ($C_2H_2CaO_5$). Calcium oxalate crystals embedded in the phloem provide extra support to the bark (Hudgins et al., 2003; Franceschi and Nakata, 2005). Although the crystals have no effect on pathogen spread, they deter bark-boring and chewing insects from entering the tree (Hudgins et al., 2003). According to Runyon et al. (2022), constitutive defenses are baseline defenses present in pines prior to attack whereas induced defenses are activated or increased after an attack or stress event. When mechanical barriers are breached, defense chemicals are activated. The

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quantity and quality of induced secondary metabolites differ based on existing stressors affecting physiological and biochemical processes of the host (Burke and Carroll, 2016; Yang et al., 2018). Changes in the concentration of these metabolites following pathogen invasion may provide an indication of host susceptibility to disease.

For pines, synthesis of secondary metabolites with known antimicrobial properties such as terpenes, phenolics, and alkaloids may be activated by pathogen invasion (Franceschi et al., 2005). These compounds exert defense pressure against invading insect pests, pathogens, and pest-pathogen complexes (Lewinsohn et al., 1991; Lieutier, 1993). Among secondary metabolites, oleoresins derived from terpenes, synthesized and stored in resin ducts, are the major defense compound in pines (Ruel et al., 1998; Turner et al., 2019). Once resin cells are disrupted, oleoresins are released which prevents the advancement of or kills the invader (Franceschi et al., 2005). Ultimately, wounds created by the invader are sealed when volatile components of oleoresin evaporate, leaving behind the viscous non-volatile crystalline component of oleoresins.

Oleoresins are a complex mixture of monoterpenes, diterpenes, and sesquiterpenes (Keeling and Bohlmann, 2006), and monoterpenes have been shown to inhibit fungal growth and reproductive development. For instance, Raffa and Smalley (1995) found that mature *Pinus resinosa* and *P. banksiana* responded to inoculation with the bark beetle-associated fungi, *Ophiostoma ips*, *O. nigrocarpa*, and *L. terebrantis*. They found increasing monoterpene concentrations at the inoculation site, thereby, inhibiting fungal growth. Similarly, Klepzig et al. (1995) demonstrated the ability of 25-year-old *P. resinosa* trees to respond to *L. terebrantis* inoculation by producing resinous lesions in the phloem.

Phenolic compounds are the most studied plant secondary metabolite and they have a wide range of biological roles (Beckman, 2000). These compounds exhibit antimicrobial and antioxidant properties which help plants evade pathogenic infections and protect plant tissues from toxic reactive oxygen species (Maddox et al., 2010; Daayf et al., 2012; Kumar et al., 2020). Synthesized by chloroplasts and plastids, and compartmentalized in vacuoles, the release of phenolics is usually triggered by wounding, or infestation by a pathogen or insect pest (Wink, 1997).

Among the vast array of plant phenolics, stilbenes, flavonoids, and hydroxycinnamic acid have demonstrated antifungal properties (Viiri et al., 2001; Witzell and Martín, 2008). Among conifers, stilbenes and flavonoids have been extensively studied (Lieutier et al., 1996; Bois and Lieutier, 1997; Hammerbacher et al., 2013). For instance, stilbenes such as pinosylvin and pinosylvin monomethyl ether and flavonoids like catechin have been found to accumulate in the infection zone of *Ophiostoma brunneo-ciliatum* in *Pinus sylvestris* to inhibit pathogen spread (Lieutier et al., 1996; Bois and Lieutier, 1997).

This study was undertaken to assess the induction of defensive chemical compounds in plantation loblolly pine following artificial inoculation with the bark beetle-vectored fungus, *L. terebrantis*. Specifically, we evaluated the induction of oleoresins in the stem and soluble phenolic compounds in terminal shoots of mature loblolly pine trees using three inoculum densities of *L. terebrantis.* We tested the hypothesis that higher pathogen inoculum density will induce a significant production of oleoresins and phenolic compounds in the inoculated trees. Additionally, we undertook an *in vitro* study to assess the effect of catechol on radial colony growth of *L. terebrantis*, *L. procereum*, and *Grosmannia alacris* which are known pathogens of *Pinus* species (Matusick et al., 2013, 2016). We further hypothesized that amending malt extract agar (MEA) media with catechol will suppress the radial growth of *L. terebrantis*, *L. procereum*, and *Grosmannia alacris in vitro*. Catechol was chosen as the test phenolic compound because it has been isolated from leaf and needle litter of several deciduous and coniferous tree species (Topal et al., 2006). The compound has been shown to have antifungal effects on *Colletotrichum circinans* (Farkas and Kiraaly, 1962), *Fusarium oxysporum* and *Penicillium italicum* (Kocaçalışkan et al., 2006). Catechol also has an antimicrobial effect on bacteria such as *Pseudomonas putida*, *Pseudomonas pyocyanea*, and *Corynebacterium xerosis* (Kocaçalışkan et al., 2006).

2. Materials and methods

2.1. Study site and experimental design

The study was located in a loblolly pine plantation near Eufaula, Alabama, U.S.A. in Barbour County (32°1′13.10"N, 85°12′31.76"W) as described by Mensah et al. (2022b). Mean annual precipitation and air temperature during the 30-year period between 1989 and 2019 at the site were 1407.8 mm and 22.4 °C, respectively (NOAA, 2024). Loblolly pine seedlings from an open-pollinated seed source were used to establish the plantation at a 1.2 m × 3.0 m spacing in 2003. The soil is a complex of Annemaine and Wahee fine sandy loams. Both series consist of fine sandy loam surface soil. Annemaine, the predominant soil series, is moderately well-drained and Wahee is poorly drained due to its clay–loam subsoil (Trayvick, 2005; Ditzler et al., 2017). Fifteen treatment plots containing two rows of 20 planted trees were established at age 13 years in December 2015. At the time of plot establishment in December 2015, mean and standard error values of measurement tree DBH, total tree height, and stemwood volume were 16.9 ± 0.5 cm, 14.0 ± 0.2 m, and 0.32 ± 0.02 m^3, respectively.

Five inoculation treatments were randomly assigned to three treatment plots in a completely random experimental design. Within each treatment plot, five trees were randomly chosen as measurement trees from among the dominant and codominant trees that were not forked. Measurement trees were outfitted with manual dendrometer bands (D1 UMS GmbH, Munich, Germany) installed at breast height (DBH, 1.37 m).

It is noteworthy that a 27-month period of normal rainfall between December 2016 and February 2019 was followed by moderate to severe drought between March and September 2019. Mean weekly Palmer Drought Severity Indices (PDSI) between March and September 2019 decreased from −0.2 to −3.0, indicating that drought severity increased from mild to severe during this period**.** This drought period overlapped our post-treatment oleoresin measurements in June 2019 (Mensah et al., 2022b).

2.2. Fungal isolate and inoculation method

The fungal isolate *Leptographium terebrantis* (LOB-R-00-805) used for the study was originally isolated from woody roots of declining *P. taeda* trees at the U.S.D.A. Forest Service Talladega National Forest, Oakmulgee Ranger District, AL, U.S.A. (Eckhardt et al., 2007) and cultured on sterile toothpicks as described by Devkota et al. (2019). Previous studies found this fungal isolate to be the most virulent among 42 *L. terebrantis* isolates (Devkota and Eckhardt, 2018).

Inoculation treatments were applied to measurement trees in March 2017 by inserting toothpicks containing *L. terebrantis* inoculum (mycelium and spores) into bored holes in the lower stem within 5 min of drilling (Devkota et al., 2019). Treatments were no inoculation or wounding (control), no inoculation but sterile toothpick wounding (wound), and three levels of increasing fungal inoculum density, low, medium, and high. Inoculum densities were selected based on earlier studies that established the relationship between number of *L. terebrantis* toothpick inoculum points, occluded radial area of the stem (Devkota et al., 2019), and stem hydraulic conductivity of loblolly pine (Mensah et al., 2020). Trees treated with the low, medium, or high inoculum densities received one inoculated toothpick per 10.0, 2.4, or 1.2 cm over the bark circumference, respectively. As a result, trees treated with the low, medium, or high inoculum density received 5 to 8, 20 to 28, or 40 to 58 *L. terebrantis*-colonized toothpicks, respectively. Wound-treated trees received sterile toothpicks at the high inoculum density level (Mensah et al., 2022a).

2.3. Resin sampling

Prior to treatment application in March 2017, 30 dominant or codominant trees within the loblolly pine stand but outside the 15 treatment plots were sampled for oleoresin production. Non-measurement trees were used to reduce the risk of attracting bark beetles to measurement trees. The pre-treatment resin yield of the measurement trees was estimated by a subset of these non-measurement trees. The DBH of each of these trees was quantified with a diameter tape and expressed in cm and tree heights were measured with a TruPulse 200 Rangefinder-Hypsometer (Laser Technology Inc., Centennial, CO, USA), and expressed in m. Stem volumes (V) were calculated by the equation d^2h with d and h as DBH and total height, respectively, expressed in m.

The north and south side of each tree was prepared for oleoresin collection by using a 1.9 cm diameter arch punch (C.S. Osbourne & Co., Harrison, NJ, U.S.A.), to remove the bark and phloem, exposing a 2.8 cm^2 area of the outer ring of sapwood. A plastic connector attached to a 10 mL plastic tube was screwed into each hole to collect resin from the exposed sapwood (Fig. 2a). The tubes were removed 24 h after attachment and transported to the laboratory in an ice chest containing ice. Mean oleoresin fresh weight from the north and south sides of a 2.8 cm^2 area of exposed sapwood per tree was determined and expressed as g/cm^2/day.

Among the 30 non-measurement trees, an effort was made to identify vigorous individuals with light and soil resources representative of desirable stand conditions. Trees that were potentially light-limited were identified by co-occurrence of stem volumes (V) less than mean V minus one standard deviation of V and total tree heights (TH) less than mean TH minus one standard deviation of TH. This excluded the five smallest trees from the data set. Likewise, trees with V greater than mean V plus one standard deviation of V were removed from the data set. This excluded the three largest trees with an average V that was 18% larger than mean V ($n = 30$). One additional tree was removed from the data set because of its codominant crown position and apparent loss of crown vigor. A polynomial equation to predict resin fresh weight by V was developed with data from the remaining 20 non-measurement trees, $y = 39.4\ V^2 - 42.7\ V + 12.5$ where y is resin fresh weight expressed as g/cm^2/day and V is stem volume expressed as m^3 ($P = 0.0107$). Measurement tree resin fresh weights at the time of inoculation were estimated by this equation (Fig. 1).

2.4. Branch tissue sampling and phenolic assay

In June 2017, one shoot was shot with a rifle from the upper one-third of the crown of 60 dominant or codominant non-measurement trees from across the stand. For each shoot, foliage was removed, buds of main and lateral branches were excised, and terminal 10 cm sections from the base of the bud of the main and lateral branches were cut. Terminal shoot sections were pooled by tree in paper bags, transported to the laboratory in an ice chest containing dry ice, and freeze-dried for 24 h to equilibrium. The dried samples were ground in a Wiley mill to pass through a 0.5 mm mesh screen. Sample collection and processing were repeated two years later (post-drought period), in June 2019 with two randomly chosen measurement trees per plot (30 trees).

Ground samples (50 mg) were extracted three times with 1.0 mL of 70% acetone in 1.5 mL micro-centrifuge tubes. Extracts were mixed for 30 min at 25 °C and centrifuged at 16,000 g for 2 min. Supernatants were pooled by sample and diluted to a 6.5 ml volume with 70% acetone. Total soluble phenolic concentration was determined by the Folin-Ciocalteu method as described by Booker et al. (1996). A 40 μL aliquot of diluted extract was mixed with 475 μL of 0.25 N Folin-Ciocalteu reagent (Sigma Chemical Co., St. Louis, MO, U.S.A.), in a 1.5 mL micro-centrifuge tube and incubated at room temperature for 3 min. Subsequently, 475 μL of 0.6 M Na_2CO_3 was added to each tube, mixtures were agitated for 5 s, and then incubated at room temperature in darkness for 45 min. Absorbance was measured at 760 nm with a Beckman-Coulter DU-800 spectrophotometer. Catechin (Sigma Aldrich Corp., Saint Louis, MO, U.S.A.), was used to prepare a standard curve and total soluble phenolic concentration of each sample was expressed as micrograms catechin equivalent per mg dry mass.

2.5. In vitro study

In this experiment, malt extract agar medium (MEA, BD Bacto ™ Malt Extract, BD Biosciences, San Jose, CA, U.S.A.), as described by Devkota et al. (2019) was amended with catechol at 0, 10, 20, 50 and 100 mg/L. Subsequently, 15 ml each of the five media was poured into Petri dishes (100 mm diameter x 15 mm deep) and replicated five times. Prior to media pouring, two perpendicular lines were drawn on the

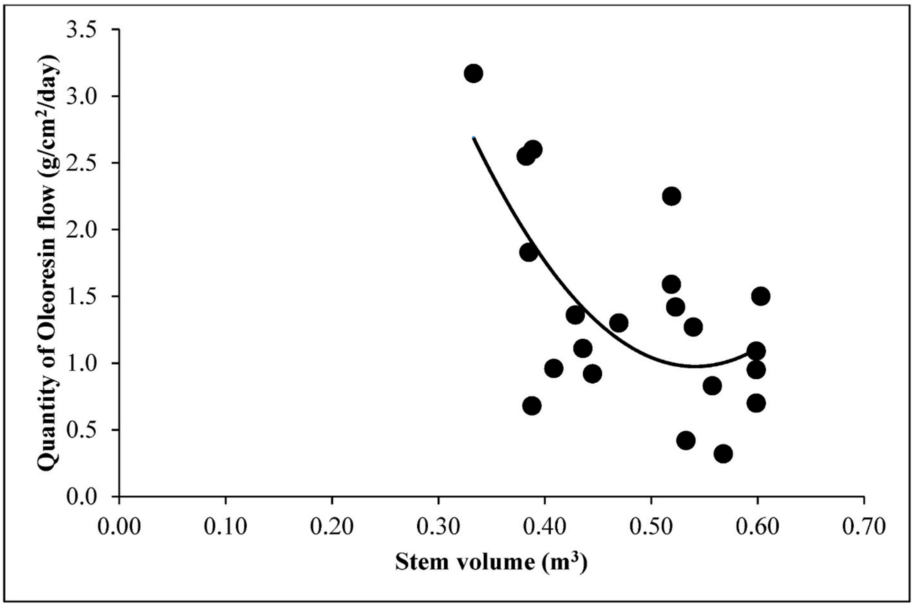

Fig. 1. Regression relationship ($y = 39.4\ V^2 - 42.7\ V + 12.5$, $p = 0.0107$) between stem volume (V) and oleoresin fresh weight (y) among 21 non-measurement trees. This relationship estimates the resin flow by fresh weight, among measurement trees before inoculation in March 2017.

Fig. 2. (a) Oleoresin sampling from the north and south sides of a non-measurement loblolly pine tree prior to stem inoculation with *Leptographium terebrantis*; (b) Appearance of oleoresin exudates after 5 months of inoculation of high inoculum density (c) crystals of oleoresin superior to the phloem of a loblolly pine tree at study conclusion, two years after stem inoculation with the high inoculum density.

bottom of the Petri dishes and at their intersection, the medium was inoculated with a 5 mm disc of actively growing pure culture of *L. procereum*, *L. terebrantis,* or *Grosmannia alacris*. The Petri dishes were incubated at 23 °C in the dark for 8 days. Radial growth of each fungal colony was measured with a ruler along the four previously marked lines and mean radial growth by colony was determined.

2.6. Statistical analysis

Estimates of pre-treatment and actual post-treatment tree oleoresin exudate fresh weight and total soluble phenolic concentration averaged among measurement trees by plot and mean radial growth of fungal colonies over an 8-day period were analyzed by one-way analysis of variance (SAS Inc., Cary, NC, U.S.A.). Prior to analyses, each dependent variable was checked for normality and homogeneity of variance using Shapiro-Wilk and Levene's tests, respectively. Main and interaction treatment effects were considered significant at $p \leq 0.05$ and were further evaluated by a pair-wise comparison among means using the post-hoc Tukey honest significant difference (HSD) test for multiple comparisons at a significance level of 0.05. The relationship between tree V and oleoresin exudate fresh weight was analyzed by linear regression (SAS Inc., Cary, NC, U.S.A.). The F-statistics and mean differences were considered significant at a significance level of 0.05.

3. Results

Prior to *L. terebrantis* inoculation treatment in March 2017, oleoresin flow among the subset of 21 non-measurement trees averaged 1.37 ±

0.76 (mean ± standard error) g/cm^2/day. Pre-treatment measurement tree oleoresin flow rates estimated by non-measurement tree regression (Fig. 1) did not differ significantly by treatment and averaged 1.69 ± 0.16 (mean ± standard error) g/cm^2/day. By five months after inoculation with *L. terebrantis,* oleoresin exudation was visibly apparent, particularly among the high inoculation treatment trees (Fig. 2b). At the end of the 34-month treatment period, examination of the inoculation area under the bark revealed crystals of oleoresin in the phloem of the high inoculation treatment trees (Fig. 2c) which was absent in the low, medium, wound, and control inoculation treatment trees.

Post-treatment oleoresin flow among the measurement trees was significantly ($p = 0.0007$) affected by inoculation treatment. Oleoresin flow rate was significantly higher in the medium and high inoculation treatments compared to the control treatment. The mean oleoresin flow rate of the medium and high inoculum density treatments was approximately 50% greater than that of the control treatment. Average oleoresin flow rate of the wound and low inoculum density treatments was 43.1% lower than the mean oleoresin flow rate of the medium and high inoculum density treatments (Fig. 3). Transverse sections of the inoculated trees showed that centrally located sapwood in the high inoculum density trees was heavily soaked with oleoresin, the wound treatment trees had a minor amount of oleoresin around the sterile toothpicks, and the transverse stem section appearance of other treatments was intermediate between these extremes (Fig. 4).

Total soluble phenolic concentration of the woody portion of upper-crown terminal shoots of 60 non-measurement trees sampled prior to treatment application averaged 43.1 ± 1.9 (mean ± SE) ug catechin equivalent/mg dry tissue. Two years after inoculation treatment, total soluble phenolic concentration of upper-crown woody shoots was not significantly different among inoculation treatments with an average total soluble phenolic concentration of 42.5 ± 2.3 (mean ± SE) ug catechin equivalent/mg dry tissue (Fig. 5).

The *in vitro* growth of the pathogens on MEA medium amended with catechol demonstrated the growth of *L. terebrantis, L. procereum,* and *G. alacris* occurred on the media until a threshold of catechol concentration was reached. This threshold was 50 mg/L of catechol for both *L. terebrantis* and *L. procereum* at which radial colony growth was significantly suppressed (Fig. 6). However, significant colony suppression occurred at 100 mg/L for *G. alacris* relative to that on the unamended media.

4. Discussion

We assessed induction of oleoresin and soluble phenolics in loblolly pine trees in response to stem inoculation with the bark beetle-vectored fungus *Leptographium terebrantis*. The pathogen caused induction of oleoresins at medium and high inoculum levels but only constitutive oleoresin was observed at the low inoculum level. Terminal shoot phenolic concentration was unaffected by stem inoculation with *L. terebrantis* irrespective of the pathogen inoculum level. Hence, we partly accept the hypothesis that higher pathogen inoculum levels induce a significant oleoresins response but we failed to accept that higher pathogen inoculum levels induce phenolic synthesis in the terminal shoots of inoculated trees.

Oleoresin and soluble phenolic responses to the pathogen perhaps differed by the occurrence of drought. Although drought was not accommodated in our experimental design, these observations implicate drought effects on available carbon as a contributing factor in pine defense against *L. terebrantis* at our study site. When soil resources are adequate, vigorous stemwood growth and high oleoresin yield co-occur (Lombardero et al., 2000; Hood and Sala, 2015). With the onset of one or more factors that restrict growth but not carbon fixation, photosynthate is distributed away from growth toward non-structural compounds such as secondary metabolites (Loomis, 1932; Lorio et al., 1986). Because secondary metabolites serve as constitutive defense chemical precursors, this carbon allocation shift has the potential to heighten resistance to insect attack and disease infestation (Lorio Jr, 1986; Lombardero et al., 2000). As limiting factors advance such that photosynthesis is hindered, the production of secondary metabolites decreases and tree vulnerability to these threats may increase (Rissanen et al., 2022; Thompson et al., 2024).

It is noteworthy that the drought period overlapped our post-treatment oleoresin measurements in June 2019 (Mensah et al., 2022b). Among control trees, oleoresin flow in June 2019 was low relative to that produced before drought (in March 2017). This comparison suggests that constitutive oleoresin production is dynamic and potentially influenced by localized drought. Similar oleoresin responses to available water have been observed in loblolly pine (Mason, 1971; Lorio Jr, 1986; Blanche et al., 1992).

Past research documents a positive correlation between annual patterns of oleoresin flow and seasonal air temperature (Blanche et al., 1992; Warren et al., 1999). In the present study pre-treatment oleoresin

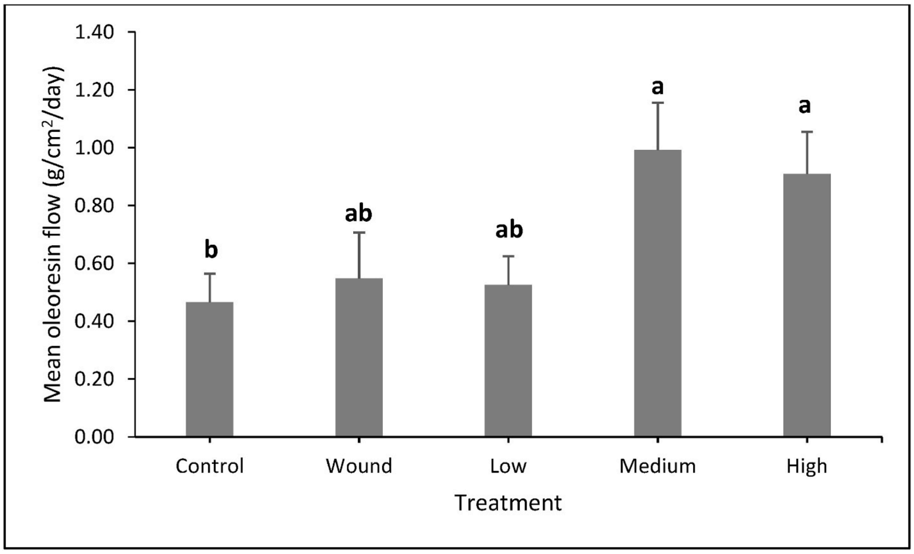

Fig. 3. Oleoresin flow from the 2.8 cm^2 area of the outer ring of sapwood by *Pinus taeda* trees two years after stem inoculation with *Leptographium terebrantis*. Means associated with different lower-case letters are significantly different at a significance level of 0.05 by the Tukey HSD test for multiple comparisons. Bars represent one standard error of the mean.

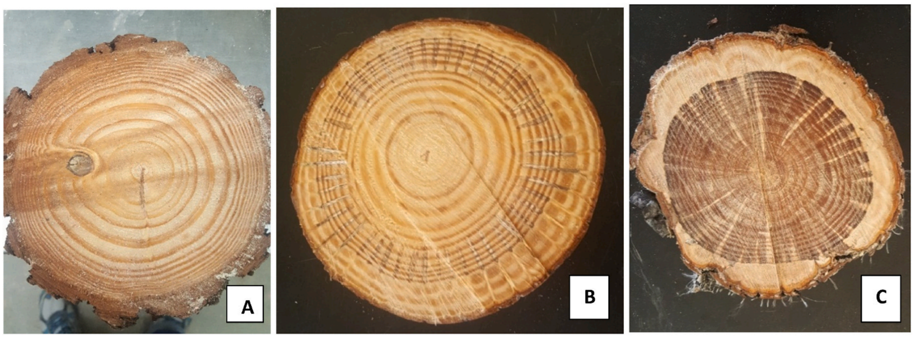

Fig. 4. Transverse sections of the stem of loblolly pine showing (a) the sapwood of a non-inoculated tree, (b) minor oleoresin deposition around sterile toothpicks of a wound treatment tree but without centrally located oleoresin, and (c) centrally located oleoresin-soaked sapwood surrounded by post-inoculation, new sapwood of a high inoculation treatment tree.

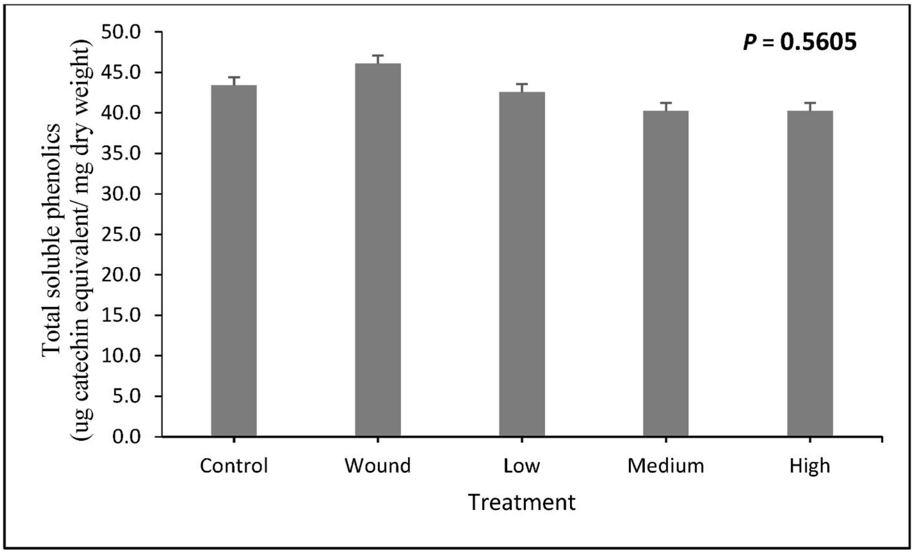

Fig. 5. Quantity of total soluble phenolics produced among terminal woody shoots in the upper crown of *Pinus taeda* two years after stem inoculation with *Leptographium terebrantis.* Bars represent one standard error of the mean.

flow was measured when daily average air temperature was 14.5 °C (58 °F), and that measured among control trees in June 2019 was 12.7 °C (55 °F). Although these air temperatures and oleoresin flow values are positively correlated, the magnitude of difference between air temperatures is small relative to that between oleoresin flow values. Therefore, it is likely that available water had a greater influence than air temperature on constitutive oleoresin production during our study.

Despite negative drought effects on constitutive oleoresin flow, we observed induced oleoresin exudation in stems of trees inoculated with *L. terebrantis.* Specifically, oleoresin yield was similar among the control trees, the wounded trees, and those receiving the low inoculum density. Absence of an induced oleoresin response to stem infection at the low inoculum density suggests that at this treatment level, loblolly pine exhibited *L. terebrantis* tolerance. In contrast, the mean oleoresin flow of medium and high inoculum density trees was approximately 50% greater than that of the control trees (Fig. 3). It appears that oleoresin production was induced when the pathogen spread was beyond a threshold tolerated by trees receiving the low inoculation treatment. Furthermore, elevated oleoresin exudation may have contributed to the significant growth loss observed among the high inoculum density trees (Mensah et al., 2022b).

Several studies report high oleoresin production after induction by wounding or pathogenic infection (Klepzig et al., 1995, 2005; Lombardero et al., 2000; Knebel et al., 2008; Hood and Sala, 2015). Knebel et al. (2008) found that inoculating 6- and 12-year-old loblolly pines with one of two densities of *Ophiostoma minus* increased stem oleoresin flow relative to wounding alone. Nonetheless, resin yield was uniform regardless of the *O. minus* inoculum density applied to tree stems. In contrast, we observed three thresholds of pathogenesis across inoculum densities. First, among the low inoculum density trees, pathogen impacts were limited to constitutive defense but not induced oleoresin production. The second threshold of *L. terebrantis* infection occurred at the medium inoculum density level with the action of both constitutive and induced oleoresin that again sustained enough stem hydraulic function for normal tree physiology and growth. The high treatment level yielded a final threshold of pathogenesis with the action of both constitutive and induced oleoresin but the loss of tree vigor.

Additionally, the high treatment trees exhibited phloem that appeared resin-soaked in the inoculation zone with deposition of the non-volatile components of oleoresin as crystals in the outer phloem (Fig. 2b and c), similar to losses in phloem function and carbon allocation to the root system caused by stem girdling (Högberg et al., 2001; Rainer-Lethaus and Oberhuber, 2018; Clausing et al., 2021). Thus obstruction of significant phloem function by oleoresin crystals may have not only decreased new root growth (Mensah et al., 2022b), but contributed to widespread root system deterioration (Rainer-Lethaus and Oberhuber, 2018; Clausing et al., 2021), and tree mortality (Mensah et al., 2021).

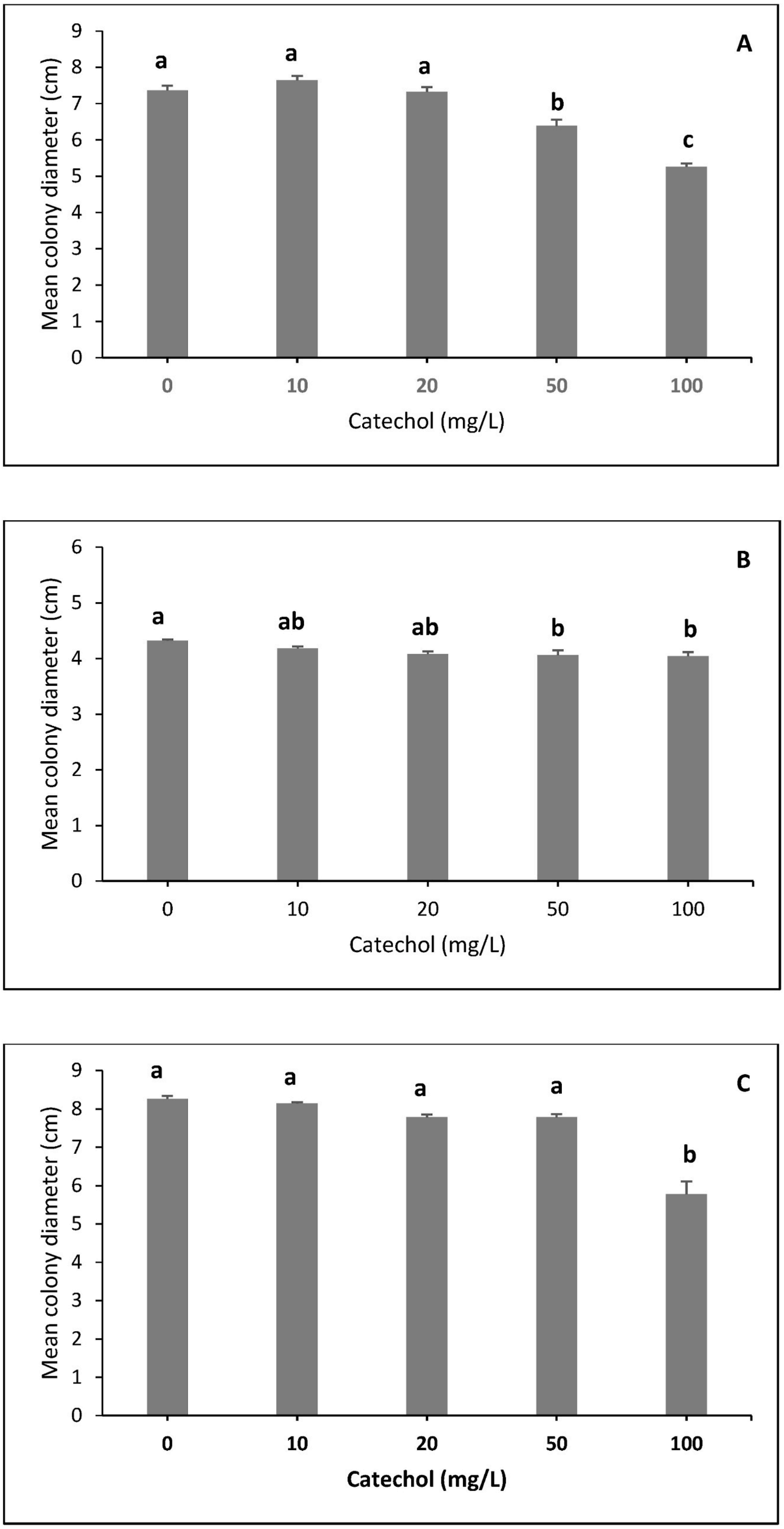

Fig. 6. Mean radial colony growth of bark beetle-associated fungi growing on MEA medium amended with catechol: (a) *Leptographium terebrantis,* (b) *Leptographium procereum*, (c) *Grosmannia alacris*. Means associated with different lower-case letters are significantly different at a significance level of 0.05 by the Tukey HSD test for multiple comparisons. Bars represent one standard error of the mean.

Unlike stem oleoresin exudation, woody shoot soluble phenolic concentration was not significantly affected by stem inoculation with *L. terebrantis*. This suggests that the defense response to stem infection with the pathogen was localized and not systemic in our study. Similarly, Klepzig et al. (1995) reported absence of a systemic phenolic defense response in the stem phloem of *P. resinosa* trees with roots infested by the root pathogens, *L. terebrantis* and *L. procerum*.

Pre- and post-treatments total phenolic concentrations values were 23% less than that reported by Sayer et al. (2016) for 10-year-old loblolly pine in January. The total phenolic values observed in our study were determined in the first half of the growing season while those reported by Sayer et al. (2016) were measured during the dormant season when a surplus of current photosynthate exists in terminal shoots (Chung and Barnes, 1980). In our study, modest terminal shoot total phenolic concentrations may be attributed to a short-lived period in which carbon allocation to defense was reduced in support of carbon allocation to new shoot and fascicle growth.

Catechol-amended MEA medium suppressed the growth of *L. terebrantis, L. procereum,* and *G. alacris* with greater effects on *L. terebrantis* and *L. procereum* compared with *G. alacris*. Previous studies have also found antifungal effects of catechol on fungi such as *Fusarium oxysporum* and *Penicillium italicum* (Kocaçalışkan et al., 2006), and *Colletotrichum circinans* (Farkas and Kiraaly, 1962). We did not quantify the soluble phenolic compound response to infection near the site of stem inoculation. However, it is possible that induction of soluble phenolic compounds in the localized area of stem inoculation with *L. terebrantis* occurred at the same time as induced oleoresin flow, potentially acting as an antimicrobial pesticide to suppress fungal spread.

5. Conclusion

We observed the constitutive and induced nature of chemical defense by oleoresin flow in response to stem infection by *L. terebrantis.* As stem infestation with the pathogen increased, whole-tree carbon dynamics demonstrated three responses indicative of either the success or failure of disease tolerance. At the low inoculum density, constitutive oleoresin flow, and at the medium inoculum density, both constitutive and induced oleoresin flow allowed sufficient sapwood function for adequate defense and normal tree growth. Under the high inoculation treatment, a loss of sapwood function caused by the combined effects of the pathogen and perhaps drought led to inadequate oleoresin to prevent disease spread. These results indicate fixed carbon and its allocation to oleoresin may have been key to the outcome of pine decline at our study site. Furthermore, observation of growing season drought effects on constitutive oleoresin production suggests its effectiveness could be at risk where soil and climate interact to create or worsen water deficits. In settings prone to pine decline, stand density and thinning regimes benefit resource availability and whole-tree carbon dynamics for sustained tree vigor (Mason, 1971; Eckhardt et al., 2010; Coyle et al., 2015). As such, our findings demonstrate the need for research on stand density management regimes that maintain whole-tree carbon balance and oleoresin defense where *L. terebranitis* root disease and drought are likely. Additionally future studies should consider assessing the total phenolic concentration near the infection zone of the inoculated trees.

CRediT authorship contribution statement

John K. Mensah: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Mary Anne S. Sayer:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Formal analysis, Conceptualization. **Ryan L. Nadel:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Lori G. Eckhardt:** Writing – review & editing, Validation, Supervision, Software, Resources, Project administration, Funding acquisition, Conceptualization.

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Declaration of competing interest

Authors declare no conflict of interest.

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