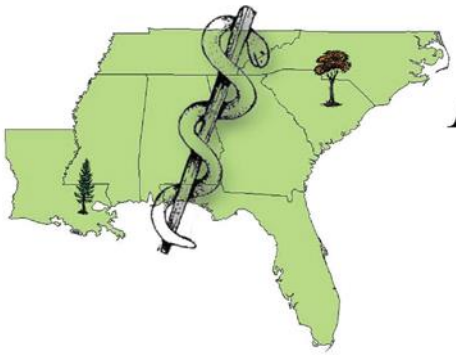

AUBURN UNIVERSITY
FOREST HEALTH
COOPERATIVE

REASEARCH REPORT 22-03

ALLELOCHEMICALS PRODUCTION FROM LOBLOLLY SEEDLINGS INOCULATED WITH OPHIOSTOMATOID FUNGI

by

Sylvester Menanyih and Lor Eckhardt

ABSTRACT

Root-feeding bark beetles are considered pests of *Pinus taeda* in the southeastern United States. The bark beetles and their associated ophiostomatoid fungi contribute to decline disease in pine trees. Trees produce monoterpenes as a defense mechanism when they are infected by insect pests and pathogens. *Pinus taeda* seedlings were inoculated with four ophiostomatoid fungi: *Grosmannia alacris, Grosmannia huntii, Leptographium terebrantis,* and *Leptographium procerum.* Monoterpenes were extracted and analyzed with GC-MS after 4, 8 and 12 weeks after inoculation. Fourteen monoterpenes were identified and quantified: α-pinene, camphene, β-pinene, limonene, myrcene, terpineol, p-cymene, bornyl acetate, ocimene, γ-terpinene, *trans*-verbenol, 3-carene, camphene, *cis*-verbenol, and borneol. Higher quantities of compounds were produced in week 8 when compared to weeks 4 and 12. Monoterpenes produced can be used as attractant or deterrent either individually or synergistically.

4.1. INTRODUCTION

The root-feeding bark beetles are considered pest of loblolly pine tree in the southeastern United States (Eckhardt, 2007). The insect bores into the roots of the host tree allowing its associated fungi to colonize the phloem and overwhelm the tree's defense system. Root-feeding bark beetles and their fungal associates are biotic factors associated with pine decline (Eckhardt et al., 2007). Ophiostomatoid fungi are carried either in the mycangia or on the exoskeleton of the bark beetle and enhances the decline (Bridges and Moser, 1986; Six, 2003). The role played by ophiostomatoid fungi during bark beetle colonization can be complicated. The fungi can have antagonistic effect on the beetle's larval stage (Barras, 1970) or be involved in the success of the beetle in the early stages of the attack due to the fungi's rapid growth and phloem colonization that compromises host defenses (Klepzig et al., 2005; Lieutier et al., 2009).

When bark beetles and their associated fungi rapidly invade a pine tree, the attackers trigger a defense system. A major chemical defense system of conifers is the production of oleoresin which consists primarily of a complex mixture of different volatile monoterpenes, non-volatile diterpenoids, and sesquiterpenes (Keeling and Bohlmann, 2006; Kolosova and Bohlmann, 2012; Zulak and Bohlmann, 2010). Constitutive and induced defense responses are crucial for the survival of pine trees after infection (Karban and Baldwin, 1997). Conifers continuously produce baseline amounts of some monoterpenes that form constitutive defenses to repel, kill or contain invaders such as pathogens or insects (Bonello et al., 2006). They may be the first line of defense to inhibit the initial growth of pathogenic fungi that infect conifer trees (Bridges 1987; Michelozzi et al., 1995; Lombardero et al., 2006). Monoterpenes can also form inductive defenses when trees synthesize or up-regulating compounds in response to specific stressors (Evenesen et al., 2000). In addition to phenolics, monoterpenes are also important chemical

defense of conifers (Franceschi et al., 2005). The majority of monoterpenes produced are secondary compounds, that is, they are mainly required for communication and tree defense but not for growth (Gershenzon and Dudareva, 2007). In addition, these monoterpene-based defenses play an important role in constraining fungi from transitioning from endemic to epidemic population densities (Boone et al., 2011). Apart from defense, conifer terpenes have several ecological functions. Conifer terpenes can function as insect-attracting odors in conifers (Nordlander, 1991), or as building blocks for *in vivo* synthesis of pheromones by wood-boring beetles (Martin et al., 2003).

The present study was conducted to test the hypothesis that, comparing *P. taeda* monoterpene responses to infection by different fungi (*G. alacris, G. huntii, L. terebrantis,* and *L. procerum*). To test this hypothesis, seedlings were inoculated with *G. alacris, G. huntii, L. terebrantis,* and *L. procerum,* monoterpenes were extracted, and were qualitatively and quantitively assessed.

4.2. MATERIALS AND METHODS

4.2.1 Potting and inoculation of seedlings

A total of 225 1-year-old bare-root *P. taeda* seedlings from a single genetic family were grown in one-gallon pots filled with ProMix BX® peat-based potting media. The seedlings were allowed to grow under natural conditions in an open field of the College of Forestry and Wildlife Sciences, Auburn University, located in Auburn, Alabama. Seedlings' stems were artificially inoculated with mycelial agar plugs of fungal isolates taken from the leading edge of 14-day-old malt extract agar plate. Forty-five seedlings were assigned for each of four different pathogens (*L. terebrantis, L. procerum, G. alacris* and *G. huntii*). Control seedlings were inoculated with a sterile agar plug without fungus. Inoculations were made by making a small (1 cm) vertical slit in the stem was made with a sterile razor blade extending into the vascular tissues, followed by

placing a 3 mm diameter plug of colonized MEA in the wound. Inoculation points were covered with sterile moist cotton balls to prevent desiccation of the fungal media and then wrapped with parafilm to prevent further contamination.

Figure 4.1. Seedlings inoculated with *G. alacris, G. huntii, L. terebrantis,* and *L. procerum.*

4.2.2. Terpene extraction

Seedlings were selected at three different time points (four, eight- and twelve-weeks post-inoculation) for terpene extraction. Fifteen seedlings were randomly selected from each treatment during each time point. Same was done for control seedlings. For terpene extraction, inoculation point, or stem of seedlings were destructively extracted and ground into powder in liquid nitrogen. Lesion length, width and depth were measured before seedlings were destructive sampled. Grinding was done to prevent damage to gas chromatography (GC) columns and equipment. Samples were freeze dried at -40ºC. One hundred milligram of ground sample was weighed and extracted with dichloromethane together with 0.004% of tridecane as the internal standard. The mixture was vortexed for 30 sec, sonicated for 10 min, and centrifuged (at 30,000

rpm) for 15 minutes. Extracts were transferred to glass vials for GC analysis. One microliter of each extract was injected from GC vials into an Agilent 7890A/5062C gas chromatograph-mass spectrometer (GC-MS, 7890A/5975C, Agilent Tech., Santa Clara, CA, USA) with a DB-5MS UI column 30 m x 0.25 mm ID x 0.25 μm film. Helium was used as a carrier gas flowing at 1 mL min^{-1} with initial temperature of 50ºC for 2 minutes, increased to 60ºC and held for 1 minute. The temperature was increased again from 20ºC to 250ºC and held for 1 minute. The standards used for quantification were α-pinene, β-pinene, 3-carene, myrcene, limonene, p-cymene, camphor, 4-allyanisole, borneol, γ-terpinene, α-terpinene, pulegone, terpineol, ocimene, terpinolene, bornyl acetate, and camphene.

4.2.3. Data analysis

The lesion area was calculated to standardize the concentration of analyte as the amount of compound (ng/mm^2). Data were analyzed using STATA (version 14) statistical software. A multivariate analysis of variance (MANOVA) was conducted to determine if there were differences between the monoterpenes produced on fungal and time treatments. Data were first checked for normality and equal variance using Wilks' lambda. Pair-wise comparisons were undertaken using the Tukey Honest Significant Difference (HSD) on the four fungal treatments and their interaction with time at $\alpha = 0.05$. Graphs were created in Microsoft Excel.

4.3. RESULTS

A multivariate analysis of variance (MANOVA) was conducted to determine whether the different compounds identified and quantified by the GC analysis differed based on time and fungal group as well as the interaction between time and fungal treatments. From the analysis, significant differences were observed between fungi, time, and their interactions at ($p<0.001$), ($p<0.001$) and ($p<0.001$) respectively (Table 4.1).

Fourteen monoterpenes were identified and quantified after GC analysis. In decreasing abundance from species, the compounds are α-pinene, camphene, β-pinene, limonene, myrcene, terpineol, p-cymene, bornyl acetate, ocimene, γ-terpinene, trans verbenol, 3-carene, camphene, *cis* verbenol, and borneol (Fig 4.2). Nine compounds (α-pinene, β-pinene, limonene, p-cymene, camphene, bornyl acetate, γ-terpinene, terpineol and trans verbenol) were produced by *G. alacris. Grosmannia huntii* produced 8 compounds: α-pinene, β-pinene, limonene, p-cymene, camphene, bornyl acetate, terpineol and myrcene. Ten compounds were detected in *L. procerum* (α-pinene, β-pinene, 3-carene, myrcene, limonene, p-cymene, camphene, borneol, ocimene and bornyl acetate). α-pinene, β-pinene, 3-carene, myrcene, limonene, p-cymene, camphene, γ-terpinene, terpineol, ocimene, and *cis* verbenol were detected from seedlings inoculated with *L. terebrantis.* Increased quantity of compounds was observed in all the experiments after inoculation when compared with control, though the quantities differed across compounds. Six compounds were visible in all fungal treatments (α-pinene, β-pinene, camphene, limonene, bornyl acetate, and p-cymene). Statistical significances were observed in α-pinene, limonene, bornyl acetate, and p-cymene. However, β-pinene (p=0.1162) and camphene (p=1.72) were not statistically different. Terpineol was only present in *G. alacris, G. huntii* and *L. terebrantis* showed significant difference at (p= 0.0259). Ocimene ($p<0.001$) and 3-carene (p=0.0036) were compounds produced in only *L. procerum* and *L. terebrantis.* No significant difference (p=0.0605) was seen in the production of myrcene. Myrcene was only present in *G. huntii, L. procerum* and *L. terebrantis.* However, *trans*-verbenol, borneol and *cis*-verbenol were only present in *G. alacris, L. procerum* and *L. terebrantis* (Table 4.2).

The quantity of compounds produced differed across time. From the analysis, the results showed that higher quantities of compounds were produced in week 8 when compared to weeks 4 and 12

(figure 4.3). Quantitative analysis showed that the production of compounds was induced due to fungal inoculation. All fungal treatments had significantly higher quantities of the measured compounds when compared with the control group. The most abundant compounds in all treatments were α-pinene, β-pinene, camphene, and limonene. A-pinene, β-pinene and limonene showed statistical significance at (p=0.0414), (p<0.001) and (p<0.0026), respectively. Camphene, however, did not show significant (p=0.2025) increase over time although absolute values increased gradually. Most compounds concentration in control treatments were low often below detection limits. Myrcene was an exception as it was not detected during the fourth weeks but was seen in week 8 and marginally increased in week 12. There was no significant difference (p= 0.1619) of myrcene across time. Also, γ-terpinene and *trans*-verbenol was not produced in week 4 but was detected abundantly in week 8 but decreased in week 12. γ-terpinene and *trans*-verbenol differed significantly at (p=0.0161) and (p=0.0025) respectively. There was statistical significance seen in the concentration of compounds p-cymene, terpineol, bornyl acetate and ocimene, and their *p*-values were (0.0147), (0.0226), (<0.001) and (0.01) respectively. Terpineol, p-cymene, bornyl acetate and ocimene compounds were produced during all the time points. *Cis*-verbenol was only produced in weeks 4 and 12 and there was a statistical difference (p=0.0414) between the production of *cis* verbenol in weeks 4 and 12. Production of 3-carene was only detected in week 12 (Table 4.3).

Significant differences were observed in compounds produced for time and fungal interactions except compounds camphene and myrcene. *Grosmannia alacris* and *L. procerum* produced in week 8 had relative higher interactions with all fungi at different periods and were statistically significant (Table 4.4).

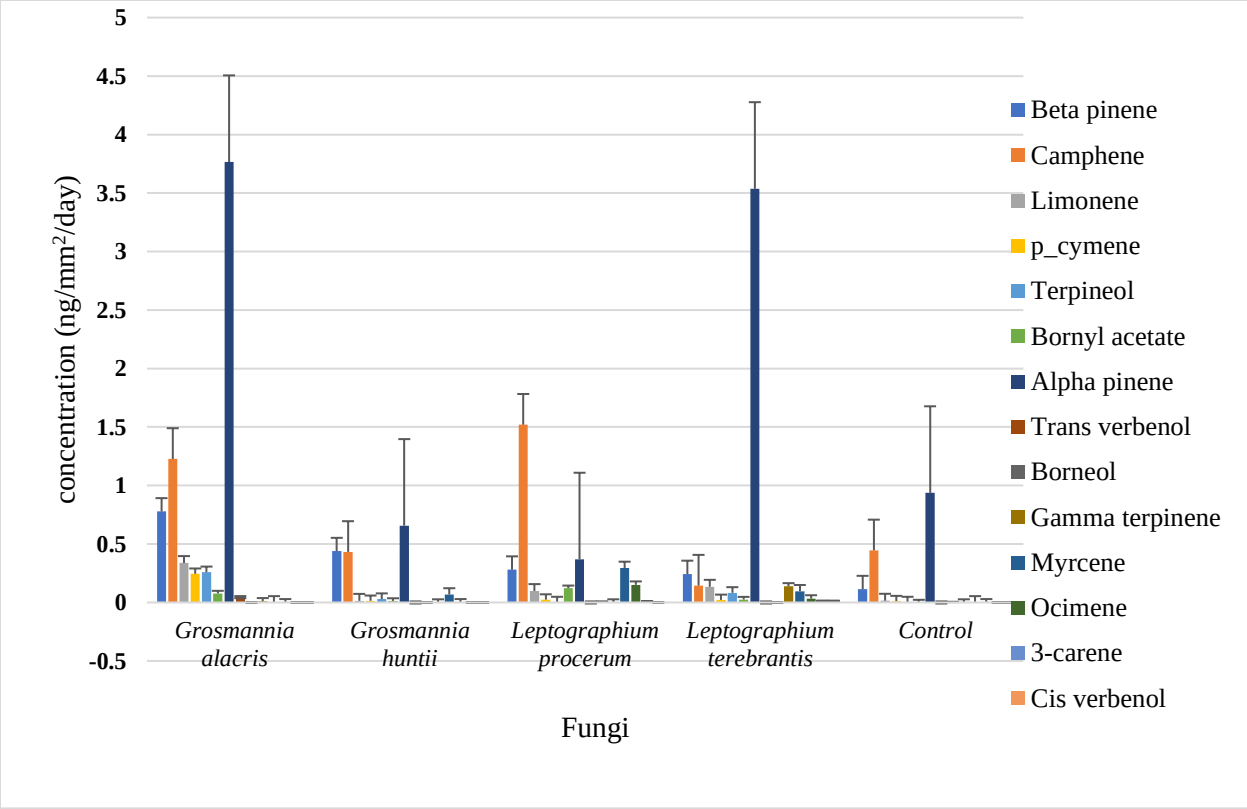

Figure 4.2. Graph of compounds detected in seedlings inoculated with *G. alacris*, *G. huntii*, *L. terebrantis*, and *L. procerum.*

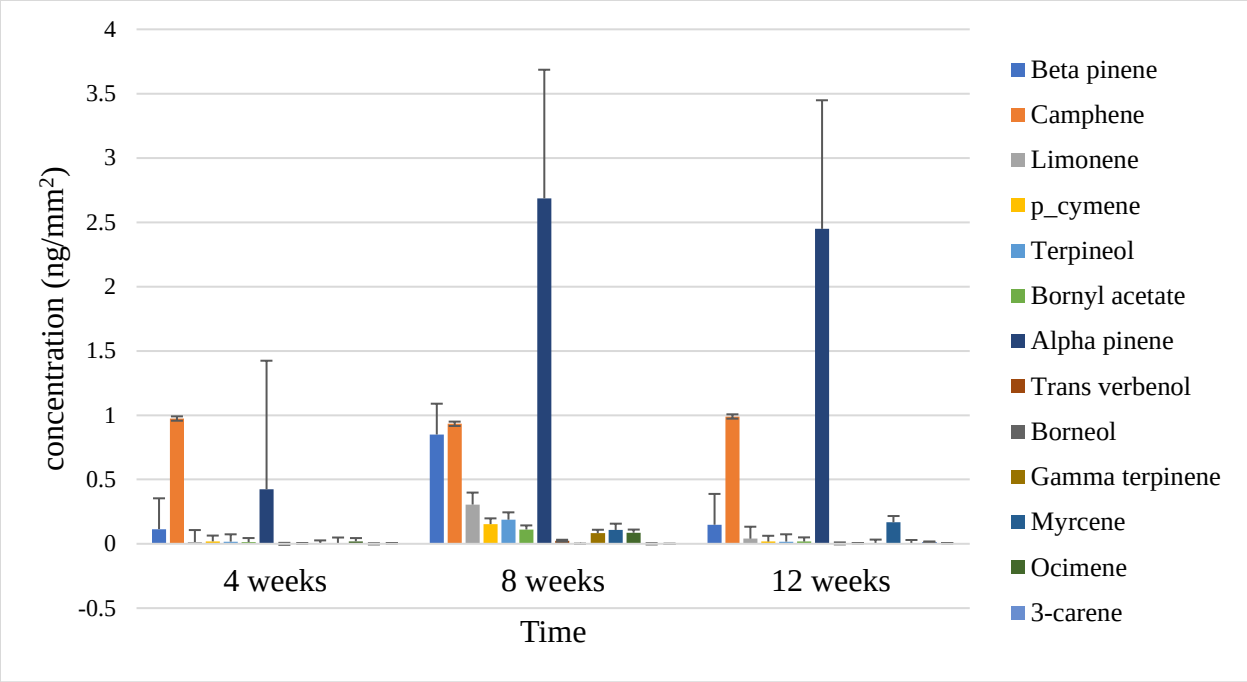

Figure 4.3. Graph of compounds detected at time points.

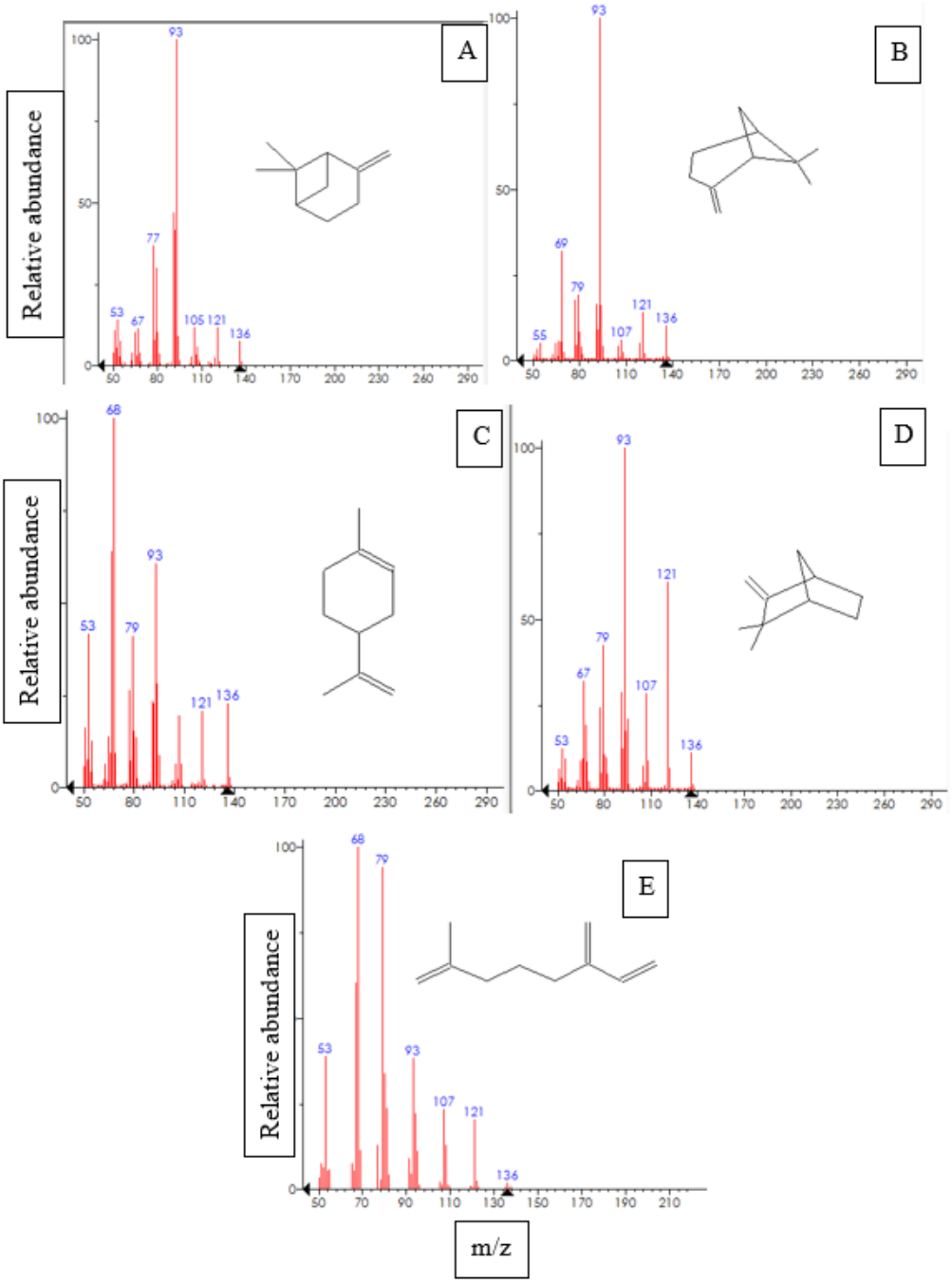

Figure 4.4. Spectral diagrams of abundant monoterpenes α-pinene (A), β-pinene (B), limonene (C), Camphene (D) and myrcene (E).

Table 4.1. Summary of descriptive statistics of compounds based on fungi treatments, time and as well as their interactions.

Variable	df	F	P-value
Fungi	14	4.92	<0.001
Time	2	3.2	<0.001
Interaction	14	4.92	<0.001

Table 4.2. Mean and standard error concentrations (ng/mm^2/day) of compounds detected from seedlings inoculated with *G. alacris*, *G. huntii*, *L. terebrantis*, and *L. procerum.* Compounds not detected during headspace collection are indicated with "ND".

Volatile emission (ng/mm^2)	**Fungal treatments**				**df**	**F**	**P-value**
	Grosmannia alacris	*Grosmannia huntii*	*Leptographium procerum*	*Leptographium terebrantis*			
β-pinene	0.78±0.35	0.44±0.19	0.28±0.08	0.24±0.07	4	1.88	0.1162
Camphene	1.23±0.73	0.43±0.23	1.52±0.83	0.14±0.05	4	1.72	0.21
Limonene	0.34±0.18	0.01±0.005	0.1±0.03	0.13±0.04	4	2.44	0.0494
p_cymene	0.25±0.1	0.01±0.004	0.02±0.009	.022±0.005	4	4.71	<0.001
Terpineol	0.26±0.14	0.03±0.006	ND	0.08±0.02	3	2.85	0.0259
Bornyl acetate	0.08±0.03	0.01±0.004	0.12±0.04	0.03±0.005	4	4.84	<0.001
α-pinene	3.77±1.67	2.69±0.18	2.45±0.13	3.54± 0.94	4	3.6	0.0079
Trans-verbenol	0.05±0.01	ND	ND	ND	-	-	-
Borneol	ND	ND	0.007±0.002	ND	-	-	-
γ-terpinene	0.01±0.004	ND	ND	0.14± 0.06	2	4.65	<0.001
Myrcene	ND	0.07±0.06	0.29±0.16	0.1±0.04	3	2.31	0.0605
Ocimene	ND	ND	0.15±0.05	0.03±0.02	2	7.29	<0.001
3-carene	ND	ND	0.01±0.004	0.01±0.006	2	4.09	0.0036
Cis-verbenol	ND	ND	ND	0.01±0.003	-	-	-

Table 4.3. Mean and standard error concentrations (ng/mm^2) of compounds detected from volatiles of seedlings at periods 4, 8 and 12 weeks. Compounds not detected during headspace collection are indicated with "ND".

Volatile emission (ng/mm^2)	**Time (weeks)**			**df**	**F**	**P-value**
	4	8	12			
β-pinene	0.11±0.03	0.85±0.29	0.15±0.03	2	8.98	<0.001
Camphene	0.09±0.04	0.93±0.55	0.99±0.64	2	1.61	0.2025
Limonene	0.01±0.01	0.31±0.14	0.04±0.01	2	6.21	0.0026
p_cymene	0.02±0.01	0.15±0.08	0.02±0.003	2	4.35	0.0147
Terpineol	0.02±0.01	0.19±0.11	0.02±0.003	2	3.89	0.0226
Bornyl acetate	0.01±0.003	0.11±0.04	0.02±0.004	2	9.7	<0.001
α-pinene	0.42±0.16	2.69±1.21	2.45±0.82	2	3.25	0.0414
Trans-verbenol	ND	0.024±0.01	0.003±0.002	1	10.65	<0.001
Borneol	ND	ND	0.004±0.002	-	-	-
γ-terpinene	ND	0.08± 0.05	0.006±0.004	1	4.25	0.0161
Myrcene	ND	0.11±0.04	0.11±0.13	1	1.84	0.1619
Ocimene	0.02±0.01	.086±0.04	0.005±0.002	2	4.75	0.01
3-carene	ND	ND	0.01±0.005	-	-	-
Cis-verbenol	0.004±0.001	ND	0.004±0.002	1	3.25	0.0414

Table 4.4. Significant compounds detected across fungi and time interactions.

Compound	Interaction of fungi and time	t-stat	P-value
β-pinene	8 weeks Ga vs 4 weeks Ga	4.96	0.000
	8 weeks Ga vs 4 weeks Gh	5.24	0.000
	8 weeks Ga vs 4 weeks Lp	4.96	0.000
	8 weeks Ga vs 4 weeks Lt	4.75	0.000
	8 weeks Ga vs 8 weeks Lp	4.07	0.007
	8 weeks Ga vs 8 weeks Lt	4.27	0.003
	8 weeks Ga vs 12 weeks Gh	5.18	0.000
	8 weeks Ga vs 12 weeks Lp	4.71	0.001
	8 weeks Ga vs 12 weeks Lt	5.02	0.000
p_cymene	8 weeks Ga vs 4 weeks Ga	6.65	0.000
	8 weeks Ga vs 4 weeks Gh	6.9	0.000
	8 weeks Ga vs 4 weeks Lp	6.95	0.000
	8 weeks Ga vs 4 weeks Lt	6.53	0.000
	8 weeks Ga vs 8 weeks Gh	7.00	0.000
	8 weeks Ga vs 8 weeks Lp	6.46	0.000
	8 weeks Ga vs 8 weeks Lt	7.00	0.000
	8 weeks Ga vs 12 weeks Ga	6.89	0.000
	8 weeks Ga vs 12 weeks Gh	6.9	0.000
	8 weeks Ga vs 12 weeks Lp	6.68	0.000
	8 weeks Ga vs 12 weeks Lt	6.85	0.000
Bornyl acetate	8 weeks Ga vs 4 weeks Gh	4.5	0.001
	8 weeks Ga vs 4 weeks Lp	4.76	0.000
	8 weeks Ga vs 8 weeks Lt	4.76	0.006
	8 weeks Ga vs 12 weeks Ga	4.76	0.000
	8 weeks Ga vs 12 weeks Gh	4.1	0.006
	8 weeks Ga vs 12 weeks Lp	4.41	0.002
	8 weeks Ga vs 12 weeks Lt	3.687	0.025
	8 weeks Lp vs 4 weeks Ga	7.51	0.000
	8 weeks Lp vs 12 weeks Lt	7.43	0.000
	8 weeks Ga vs 12 weeks Lp	6.68	0.000
	8 weeks Ga vs 12 weeks Lt	6.85	0.000
	8 weeks Lp vs 8 weeks Gh	8.09	0.000
	8 weeks Lp vs 8 weeks Lt	8.09	0.000
	8 weeks Lp vs 12 weeks Ga	8.09	0.000
	8 weeks Lp vs 12 weeks Gh	7.43	0.000

	8 weeks Lp vs 12 weeks Lp	7.74	0.000
	8 weeks Lp vs 12 weeks Lt	7.01	0.000

4.4. DISCUSSION

Fungal infections increase the production of monoterpenes in conifers, a set of primary defense compounds against bark beetles (Munro et al., 2020; Cale et al., 2017; Boone et al., 2011; Erbilgin et al., 2017a, b). Monoterpenes are generally toxic to insects and may increase bark beetle mortality during host tree colonization (Gershenzon and Dudareva, 2007). Monoterpenes can also inhibit the growth of fungal pathogens, for example blue stain fungi vectored by bark beetles (Novak et al., 2014). Bark beetle associated fungi when inoculated into Norway spruce (*Picea abies* (L.) Karst) increased drastically terpene concentration. The monoterpene increase inhibited the colonization by the spruce bark beetle (*Ips typographus* Linnaeus) in a dose-dependent manner (Zhao et al., 2011). Same pattern was observed for this study when *Grosmannia alacris, G. huntii, Leptographium procerum,* and *L. terebrantis* were inoculated into *Pinus taeda.* The observation suggests that loblolly pine can activate appropriate defenses when infected.

In the southeastern United States, emission of monoterpenes is dominated by α-pinene, β-pinene, camphene, limonene and myrcene (Geron et al., 2000; Klepzig et al., 1995). The same compounds are also known to be dominant compounds of pines though terpineol, p-cymene, bornyl acetate, ocimene, γ-terpinene, *trans*-verbenol, 3-carene, camphene, *cis*-verbenol, and borneol are produced by pine species (Chiu et al., 2017; Jost et al., 2008). This study supports previous findings that the compounds α-pinene, β-pinene, camphene, limonene and myrcene are the abundant monoterpenes produced after fungal infections in pine. Several studies have demonstrated that 3-carene was produced in increased quantities when lodgepole and pines were

inoculated with *Grosmannia clavigera* (Lusebrink et al., 2011; Sadof and Grant, 1997), however, our study showed otherwise. Only small quantities of 3-carene were detected when loblolly pine seedlings were inoculated with the four ophiostomatoid fungi used for the studies. Production of the compound is only seen in seedlings inoculated with *L. procerum* and *L. terebrantis*. Some monoterpenes such as β-pinene, 3-carene and limonene have antifungal properties (Himejima et al., 1992). In the southern United States coniferous system, the growth and germination of bark beetle- associated fungi are inhibited by monoterpenes and other compounds in the resin of host pine (Klepzig et al., 1996; Eckhardt et al., 2009). Limonene is known to inhibit bark beetle attack (Cale et al., 2017). α-pinene is a precursor to aggregation pheromone *trans*-verbenol is toxic to some bark beetles. *Trans*-verbenol can be converted into verbenone by some ophiostomatoid fungi contributing to the anti-aggregation chemicals emitted by bark beetle when it colonizes trees (Cale et el., 2019). *Trans*-verbenol is known to be the primary compound of mountain pine beetle aggregation pheromone (Borden et al., 2008). Additionally, both α-pinene and β-pinene have been identified as attractants or attractant synergists for many beetles infesting conifers, serving as host susceptibility to the insects (Hofstetter et al., 2008; Miller and Rabaglia, 2009). Also, α-pinene and β-pinene reduce fungal growth (Eckhardt et al., 2009). Certain bark beetles (e.g., *Dendroctonus frontalis*) exploits myrcene synergistically to produce its aggregation pheromone (*exo*-brevicomin and frontalin) to mass attack pine trees (Miller and Lindgren, 2000; Boone et al., 2008; Borden et al., 2008). Although when myrcene and limonene are combined with α- and β-pinene, they become directly toxic to some adult bark beetles in loblolly pine (Raffa and Berryman, 1987). Studies have shown that, myrcene can also inhibit the growth of fungi associated with bark beetles (Hofstetter et al., 2005). Induction of myrcene and β-pinene may also promote beetle aggregation (Cale et al., 2017; Clark et al., 2012). Limonene is

a monoterpene identified as a resistance factor against many bark beetles by acting as a toxicant and oviposition deterrent (Sadof and Grant, 1997), and reduces fungal growth (Eckhardt et al., 2009). There is strong evidence that 3-carene also synergizes with some bark beetles' aggregation pheromone response (Borden et al., 2008). 3-carene showed strongly attractive to mountain pine beetle during an olfactory test (Conn, 1981). This compound sometimes functions as an indicator of a weakened tree thus supporting aggregation of beetles on susceptible hosts (Lusebrink et al., 2011). In adult *Dendroctonus valens,* 3-carene is a major volatile attractant in flight response (Erbilgin et al., 2007). *Cis* verbenol has been identified as an attractant to some bark beetles, and isolated from female *Dendroctonus* species. It is an attractive component in the pheromone blend of mountain pine beetle as well as several *Ips* spp. (Sullivan, 2005; Cognato, 2011). γ-terpinene is also known to inhibit the growth of fungal pathogens, as well as playing an active role in constitutive and inducive defenses against the bark beetles (Faccoli et al., 2005; Seybold et al., 2006). Additionally, γ-terpinene plays an indirect role to attract or repel beetles or their enemies (Villari et al., 2012).

Generally, pine trees respond to fungal infection by producing terpenes within 3 – 14 days and the rise is drastic (Litvak and Monson, 1998; Raffa and Smalley, 1995). Subsequently, monoterpenes level off or decline after reaching an initial peak (Raffa and Smalley, 1995). Similarly, monoterpenes were recorded at week 4, increased significantly at week 8 and declined during week 12. Faldt et al., (2006) reported that, high quantities of α-pinene, β-pinene and limonene were observed 17 weeks after fungal inoculation. Consistent with our experiment, previous studies conducted showed α-pinene, camphene, β-pinene, limonene, myrcene and 3-carene were compounds produced 1 – 4 weeks after inoculation with *L. terebrantis* and *Ophiostoma minus* indicating a strong induced response against fungal attack (Klepzig et al.,

1995; Arango-Velez et al., 2018; Delorme and Lieutier, 1990). Significant increase in monoterpenes were observed during the 9th week when *Pinus strobus* Linnaeus was inoculated with blue stain fungi.

The four ophiostomatoid fungi: *G. alacris, G. huntii, L. procerum,* and *L. terebrantis* we studied are pathogenic to loblolly pines (Eckhardt et al., 2004; Pan et al., 2018). *Grosmannia alacris* previously *L. serpens* has been described as the most virulent compared to *G. huntii, L. procerum,* and *L. terebrantis* (Eckhardt et al., 2004; Matusick et al., 2010). This can be attributed to why *G. alacris* had significant interactions with other fungi used for the studies. Although *L. procerum* is considered a weak pathogen (Eckhardt et al., 2008), it had considerable interactions across fungi and time.

4.5. CONCLUSION

Pine trees produce monoterpenes as defense mechanism when they are infected by bark beetles or pathogens. After several weeks of fungi inoculation, seedlings produce a significant number of monoterpenes. In this study, the monoterpenes produced by seedlings can be used to manage both bark beetle and its fungal symbiont. A field experiment should be conducted to determine whether compounds can be used either as an attractant or repellent or as precursor.