

## Allelopathic potential of *Sassafras albidum* and *Pinus taeda* essential oils

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### ABSTRACT

The bark essential oil of *Sassafras albidum* was obtained by hydrodistillation and analyzed by GC-MS. The major components were  $\alpha$ -pinene (38-62%), camphene (3-5%),  $\beta$ -pinene (10-13%), 1,8-cineole (7-10%), camphor (2-5%) and  $\alpha$ -terpineol (4-12%). *Pinus taeda* leaf, bark and wood essential oils were obtained and analyzed by GC-MS.  $\alpha$ -Pinene dominated in all three *P. taeda* essential oils (50%, 44% and 67%, respectively), however,  $\beta$ -pinene was also abundant (22%, 8% and 27%, respectively). *P. taeda* bark oil also had large amounts of myrcene (18%) and  $\beta$ -phellandrene (17%). *S. albidum* bark oil and *P. taeda* leaf oil, along with the essential oil components, [ $\alpha$ -pinene, camphene,  $\beta$ -pinene, limonene, 1,8-cineole, camphor and  $\alpha$ -terpineol] were screened for phytotoxic effects on lettuce (*Lactuca sativa*) and perennial ryegrass (*Lolium perenne*). *S. albidum* bark oil inhibited the seed germination of *L. sativa* ( $IC_{50}$  = 1834  $\mu$ g/mL) and *L. perenne* ( $IC_{50}$  = 1848  $\mu$ g/mL), while, camphor ( $IC_{50}$  = 3.1 mM) and  $\alpha$ -terpineol ( $IC_{50}$  = 2.9 mM) inhibited the germination of *L. sativa*; and  $\beta$ -pinene ( $IC_{50}$  = 6.4 mM) and limonene ( $IC_{50}$  = 8.8 mM) inhibited *L. perenne* seed germination. Both *S. albidum* bark oil and *P. taeda* leaf oil, as well as all of the major essential oil components tested, but especially  $\alpha$ -terpineol, significantly inhibited the radicle elongation of *L. sativa*. Similarly, both essential oils and all of the components except camphene significantly inhibited the radicle elongation in *L. perenne*. The phytotoxic effects of *S. albidum* bark oil and *L. perenne* leaf oil are consistent with their hypothetical allelopathy.

**Key words:** Camphor, cineole, essential oil composition, *Lactuca sativa*, limonene, *Lolium perenne*, pinene, *Pinus taeda*, *Sassafras albidum*, terpineol.

### INTRODUCTION

Numerous essential oils to exhibits the allelopathic activity e.g. *Artemisia* (7,13), *Chenopodium* (20), *Cinnamomum* (11), *Eucalyptus* (51), *Juniperus* (49), *Mentha* (6), *Minthostachys* (4), *Pictacia* (9), *Ruta* (12) and *Tagetes* (37). *Sassafras albidum* (Nutt.) Nees had previously shown allelopathic effects in old-field succession (15) and it has been hypothesized that allelopathic effects may be responsible for the negative host association of poison ivy, [*Toxicodendron radicans* (L.) Kunth.] vines with *S. albidum* (44).

Many herbaceous and woody plants interfere with loblolly pine (*Pinus taeda* L.) seedling growth (16,18,30,42,45). Conversely, allelopathic substances from *Pinus* species inhibit the germination and growth of understory plants (5,14,23,24,28,29,31,39). We have

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observed that grasses do not grow well under stands of loblolly pine (personal observations). Additionally, Spanish moss, *Tillandsia usneoides* L., has shown a negative host preference for *Pinus* species (10,36). To probe the potential allelopathic effects of volatile components in *Sassafras albidum* bark and *Pinus taeda*, we have obtained the essential oils from these plants, determined the chemical compositions and screened the essential oils and major components for phytotoxic activity against two model plants lettuce [*Lactuca sativa* L. (47)] and perennial ryegrass [*Lolium perenne* L. (8)].

## MATERIALS AND METHODS

### I. Essential Oils

The stem bark of *S. albidum* was collected on February 18, 2008, from four different mature trees growing on the campus of University of Alabama, Huntsville (34° 43.34' N, 86° 38.30' W, 185 m elevation). The fresh bark samples were chopped and hydrodistilled using a Likens-Nickerson apparatus for 4 h with continuous extraction with dichloromethane to give the essential oils [#1: 51.40 g bark → 0.789 g oil (1.54%); #2: 50.52 g bark → 0.540 g oil (1.07%); #3: 50.12 g bark → 1.069 g oil (2.13%); #4: 51.43 g bark → 1.178 g oil (2.29%)]. *P. taeda* leaves, bark and wood were collected on April 7, 2010, from a mature tree growing on the campus of University of Alabama, Huntsville. The fresh *P. taeda* tissue samples were chopped and hydrodistilled as above using a Likens-Nickerson apparatus to give the essential oils [needles: 200.0 g → 12.753 g oil (6.38%); bark: 46.74 g → 2.733 g oil (5.85%); wood: 77.49 g → 7.602 g oil (9.81%)].

### II. Gas Chromatographic – Mass Spectral Analyses

A gas chromatographic-mass spectral analysis was performed on each of the essential oils using an Agilent 6890 GC with Agilent 5973 mass selective detector (EIMS, electron energy = 70 eV, scan range = 45-400 amu and scan rate = 3.99 scans/s) and a fused silica capillary column (HP 5 ms, 30 m × 0.25 mm) coated with 5% phenyl-polymethylsiloxane (0.25 mm phase thickness). The carrier gas was helium with a flow rate of 1 mL/min and the injection temperature was 200°C. The oven temperature was programmed to initially hold for 10 minutes at 40°C, then ramp to 200°C at 3°C/min and finally to 220°C at 2°C/min. The interface temperature was 280°C. A 1% w/v solution of each sample in dichloromethane was prepared and 1 µL was injected using a splitless injection technique. Identification of the oil components was based on their retention indices determined by reference to a homologous series of *n*-alkanes and by comparison of their mass spectral fragmentation patterns with those reported in the literature (3) and stored on the MS library [NIST database (G1036A revision D.01.00)/ChemStation data system].

### III. Allelopathy Assays

An allelopathic bioassay based on lettuce (*Lactuca sativa*) and perennial ryegrass (*Lolium perenne*) germination and subsequent radicle and hypocotyl growth was done to examine the effects of the essential oil of *S. albidum* and *P. taeda* and the major essential oil components. Pure compounds were purchased from Sigma-Aldrich. Stock solutions of essential oils (2.0 g/L essential oil and 1.0 g/L Tween-80 in water) and the pure

compounds (10 mM compound and 1.0 g/L Tween-80 in water) were used. Two-fold serial dilutions of stock test solutions were prepared to give test concentrations of 2000, 1000 and 500 µg/mL (essential oils) and 10, 5 and 2.5 mM (pure compounds). The control was 1.0 g/L aqueous Tween-80. Seeds were placed in 6-well test plates (10 seeds per well) each well lined with two layers of Whatman No. 1 filter paper moistened with test solution. The test plates were sealed with Parafilm. The test plates were incubated at room temperature in dark for 5 days. After 5 days, the number of germinated seeds was determined and the root (radicle) and shoot (hypocotyl) lengths were measured. The allelopathic assay results are summarized in Table 3.

**Statistical analysis:** Calculations were carried out using Excel. Student's *t*-test (50) was used to compare radicle and hypocotyl test means with controls. Seed germination  $IC_{50}$  values were determined using the Reed-Muench method (34).

## RESULTS AND DISCUSSION

### *Sassafras albidum* bark essential oil

The hydrodistillation of bark of *S. albidum* produced pale yellow essential oils in yields ranging from 1.07% to 2.29%. The main components (Table 1) in all four essential oils were monoterpenoids, which included  $\alpha$ -pinene (38-62%),  $\beta$ -pinene (10-12%), 1,8-cineole (7-10%),  $\alpha$ -terpineol (4-12%), camphene (3-5%), camphor (2-5%) and limonene (2-3%). This was in contrast to the leaf oil compositions of *S. albidum*, which are dominated by geranial (11-27%) and neral (10-18%) (21), or the root bark essential oils, composed largely of safrole (80-85%) (22,38,46).

### *Pinus taeda* essential oils

The leaves (needles), stem bark and wood of *P. taeda* were hydrodistilled to give 6.38%, 5.85% and 9.81% yields of colourless essential oils, respectively. The leaf oil was composed predominantly of  $\alpha$ - and  $\beta$ -pinenes (50.2% and 21.8%, respectively, Table 2). The wood oil was also dominated by  $\alpha$ - and  $\beta$ -pinenes (67.2% and 27.2%, respectively), while the bark essential oil, in addition to  $\alpha$ -pinene (44.3%) and  $\beta$ -pinene (7.8%), also contained a large concentration of myrcene (17.8%) and  $\beta$ -phellandrene (16.8%). Both  $\alpha$ - and  $\beta$ -pinene have been reported to be abundant components of a number of *Pinus* leaf oils (27,33) and these two compounds are the abundant monoterpenes emitted from *P. taeda* pine branches (17). *P. taeda* oleoresin from north Alabama is generally comprised of high  $\alpha$ - and  $\beta$ -pinenes, myrcene and  $\beta$ -phellandrene, but low limonene (43), consistent with the bark essential oil composition observed in this present study.

### Allelopathic activity of essential oils and components

*S. albidum* bark oil at concentration of 2000 µg/mL inhibited the germination of both lettuce (50% germination) and rye grass (57% inhibition). The major components of *S. albidum* bark oil that showed allelopathic activity, based on inhibition of *L. sativa* seed germination, were camphor and  $\alpha$ -terpineol, with  $IC_{50}$  values of 3.1 and 2.9 mM, respectively (see Fig. 2). The compounds that inhibited the *L. perenne* germination were  $\alpha$ -pinene,  $\beta$ -pinene and limonene ( $IC_{50}$  = 4.3, 6.4 and 8.8 mM, respectively).

Table 1. Chemical compositions of *Sassafras albidum* bark essential oils

RI <sup>a</sup>	Compound	Composition (%)			
		#1	#2	#3	#4
941	$\alpha$ -Pinene	61.5	49.3	37.9	51.3
952	Camphene	5.1	2.9	3.1	4.1
977	$\beta$ -Pinene	10.0	13.0	11.2	12.2
994	Myrcene	0.2	0.1	0.3	0.1
1025	<i>p</i> -Cymene	0.3	0.2	0.3	0.2
1029	Limonene	3.2	2.2	1.7	3.4
1033	1,8-Cineole	10.0	8.7	7.8	7.3
1093	Terpinolene	Tr <sup>b</sup>	Tr	0.3	Tr
1108	Unidentified	0.9	0.1	0.3	0.5
1118	Fenchol	Tr	0.2	0.4	0.1
1142	<i>Trans</i> -Sabinol	Tr	1.1	2.0	0.9
1148	Camphor	1.7	4.2	4.6	2.9
1165	Pinocarvone	ND	0.2	0.5	0.2
1167	Borneol	0.6	1.1	1.7	1.4
1178	Terpinen-4-ol	Tr	0.4	0.8	0.4
1190	$\alpha$ -Terpineol	4.2	9.4	11.6	8.6
1193	Myrtenol	Tr	1.3	1.4	1.0
1201	Verbenone	Tr	0.5	1.4	0.5
1459	( <i>E</i> )- $\beta$ -Farnesene	ND	ND	0.1	ND
1477	<i>Trans</i> -Cadinane-1(6),4-diene	Tr	Tr	0.1	Tr
1501	$\alpha$ -Muurolene	Tr	0.1	0.3	0.1
1510	$\beta$ -Bisabolene	ND	Tr	0.2	Tr
1513	$\gamma$ -Cadinene	0.2	0.4	0.8	0.4
1522	<i>Trans</i> -Calamenene	0.3	0.1	0.2	Tr
1523	$\delta$ -Cadinene	0.3	0.8	1.3	0.7
1541	$\alpha$ -Calacorene	ND <sup>c</sup>	Tr	0.1	Tr
1624	1- <i>epi</i> -Cubenol	ND	Tr	0.2	Tr
1639	$\tau$ -Cadinol	0.7	1.5	3.9	1.4
1644	Torreyol	Tr	Tr	0.8	0.2
1652	$\alpha$ -Cadinol	0.9	2.2	5.0	2.1

<sup>a</sup>RI = Retention Index determined with respect to a homologous series of normal alkanes, <sup>b</sup>TR = Trace (< 0.1%), <sup>c</sup>ND = not detected.

In addition to inhibiting the germination, *S. albidum* bark oil significantly reduced the radicle growth in *L. sativa*, but was less effective to inhibit the hypocotyl elongation. The most effective compound in inhibiting the *L. sativa* radicle and hypocotyl growth was  $\alpha$ -terpineol, but limonene and camphor were also effective. Although not effective in germination inhibition, camphene and  $\beta$ -pinene did inhibit *L. sativa* radicle elongation and limonene inhibited both the radicle and hypocotyl growth. *S. albidum* bark oil significantly inhibited both *L. perenne* radicle and hypocotyl growth, with  $\alpha$ -pinene,  $\beta$ -pinene, limonene, camphor and  $\alpha$ -terpineol causing significant growth reduction.

*P. taeda* leaf essential oil, although not especially active in terms of inhibition of seed germination, did show growth inhibitory activity, notably on ryegrass (*L. perenne*).

Table 2. Chemical compositions of *Pinus taeda* essential oils

RI <sup>a</sup>	Compound	Composition (%)		
		Leaf	Bark	Wood
934	Tricyclene	0.1	0.2	0.1
945	$\alpha$ -Pinene	50.2	44.3	67.2
956	Camphene	0.4	0.5	0.7
979	$\beta$ -Pinene	21.8	7.8	27.2
993	Myrcene	0.5	17.8	0.7
1003	$\alpha$ -Phellandrene	Tr <sup>b</sup>	0.3	ND <sup>c</sup>
1012	$\delta$ -3-Carene	Tr	ND	ND
1018	$\alpha$ -Terpinene	Tr	ND	ND
1024	<i>p</i> -Cymene	Tr	TR	ND
1028	$\beta$ -Phellandrene	1.7	16.8	1.9
1040	( <i>Z</i> )- $\beta$ -Ocimene	Tr	ND	ND
1050	( <i>E</i> )- $\beta$ -Ocimene	Tr	ND	ND
1059	$\gamma$ -Terpinene	Tr	ND	ND
1086	Terpinolene	0.2	0.1	0.1
1098	Linalool	0.1	0.2	ND
1112	Phenylethyl alcohol	0.1	ND	ND
1138	Camphor	ND	Tr	ND
1162	Borneol	Tr	Tr	ND
1171	(6 <i>Z</i> )-Nonenol	0.1	ND	ND
1174	Terpinen-4-ol	Tr	Tr	ND
1189	$\alpha$ -Terpineol	0.8	0.3	0.3
1197	Methyl chavicol (= Estragole)	ND	ND	0.2
1207	Decanal	0.1	ND	ND
1232	Thymol methyl ether	ND	0.1	ND
1241	Carvacrol methyl ether	ND	Tr	ND
1257	2-Phenylethyl acetate	0.1	ND	ND
1273	1-Decanol	Tr	ND	ND
1286	Bornyl acetate	0.1	ND	ND
1293	(2 <i>E</i> ,4 <i>Z</i> )-Decadienal	Tr	ND	ND
1312	Carvacrol	ND	Tr	ND
1316	(2 <i>E</i> ,4 <i>E</i> )-Decadienal	0.1	ND	ND
1335	$\delta$ -Elemene	0.1	ND	ND
1348	$\alpha$ -Terpinyl acetate	0.1	ND	ND
1372	$\alpha$ -Copaene	0.1	ND	ND
1390	$\beta$ -Elemene	0.7	ND	ND
1393	Phenyl ethyl isobutanoate	0.1	ND	ND
1403	Methyl eugenol	0.1	0.2	0.2
1408	Dodecanal	0.2	ND	ND
1417	$\alpha$ - <i>cis</i> -Bergamotene	ND	0.1	ND
1420	( <i>E</i> )-Caryophyllene	3.1	2.5	0.1
1428	$\beta$ -Copaene	Tr	ND	ND
1436	$\alpha$ - <i>trans</i> -Bergamotene	0.1	0.3	ND
1438	Aromadendrene	0.1	ND	ND
1443	2-Phenylethyl butanoate	0.3	ND	ND
1446	6,9-Guaiadiene	Tr	ND	ND
1453	$\alpha$ -Humulene	0.6	0.5	ND
1458	Sesquisabinene	ND	Tr	ND
1461	<i>cis</i> -Cadina-1(6),4-diene	0.1	ND	ND
1465	Ethyl cinnamate	Tr	ND	ND

RI <sup>a</sup>	Compound	Composition (%)		
		Leaf	Bark	Wood
1474	<i>trans</i> -Cadina-1(6),4-diene	0.1	ND	ND
1477	$\gamma$ -Muurolene	0.3	ND	ND
1482	Germacrene D	1.2	ND	ND
1485	$\beta$ -Selinene	0.1	ND	ND
1490	<i>trans</i> -Muurolo-4(14),5-diene	0.1	ND	ND
1501	Bicyclogermacrene	4.7	ND	ND
1503	$\alpha$ -Muurolene	0.3	ND	ND
1507	Germacrene A	0.2	ND	ND
1515	$\gamma$ -Cadinene	0.7	ND	ND
1528	$\delta$ -Cadinene	4.0	ND	ND
1533	<i>trans</i> -Cadina-1,4-diene	0.1	ND	ND
1537	$\alpha$ -Cadinene	0.2	ND	ND
1541	$\alpha$ -Calacorene	Tr	ND	ND
1563	( <i>E</i> )-Nerolidol	0.2	ND	ND
1575	Germacrene D-4-ol	0.2	ND	ND
1577	Spathulenol	0.1	ND	ND
1582	Caryophyllene oxide	0.1	Tr	ND
1592	Globulol	Tr	ND	ND
1616	1,10-di- <i>epi</i> -Cubenol	Tr	ND	ND
1628	1- <i>epi</i> -Cubenol	0.1	ND	ND
1631	$\gamma$ -Eudesmol	Tr	ND	ND
1641	$\tau$ -Cadinol	1.5	ND	ND
1641	Phenylethyl hexanoate	1.3	ND	Tr
1646	Torreyol (= $\alpha$ -Muurolool)	0.2	ND	ND
1653	$\alpha$ -Cadinol	2.1	ND	ND
1714	(2 <i>E</i> ,6 <i>Z</i> )-Farnesol	Tr	ND	ND
1737	Phenylethyl heptanoate	Tr	ND	ND
1812	Hexadecanal	Tr	ND	ND
1844	Phenylethyl octanoate	0.1	ND	ND
1879	1-Hexadecanol	Tr	Tr	ND
1931	<i>ent</i> -Rosa-5,15-diene	Tr	Tr	Tr
1942	Pimaradiene	Tr	Tr	0.1
1957	( <i>Z,Z</i> )-Geranyl linalool	ND	1.1	ND
1989	Manool oxide	Tr	0.4	0.1
1990	( <i>E,Z</i> )-Geranyl linalool	ND	0.4	ND
2012	13- <i>epi</i> -Manool oxide	Tr	0.6	Tr
2033	Abieta-8,12-diene	Tr	ND	ND
2081	Abietadiene	Tr	0.1	ND
2148	Abienol	ND	3.0	0.1
2147	Abieta-8(14),13(15)-diene	Tr	ND	ND
2161	Unidentified diterpenoid	ND	0.4	0.7
2222	3,5-Dimethoxystilbene	ND	Tr	ND
2228	Unidentified diterpenoid	ND	0.6	ND
2299	4- <i>epi</i> -Abietal	ND	0.7	ND
2307	Abietal	ND	ND	Tr
2309	Methyl levopimarate	Tr	ND	ND
2340	Methyl dehydroabietate	Tr	0.1	Tr
2382	Methyl abietate	Tr	0.2	ND
2439	Methyl neoabietate	Tr	0.2	Tr
2499	Methyl 7,13,15-abietaTrienoate	ND	0.1	ND

<sup>a</sup>RI = Retention Index determined with respect to a homologous series of normal alkanes, <sup>b</sup> Tr = Trace (< 0.1%), <sup>c</sup>ND = not detected.

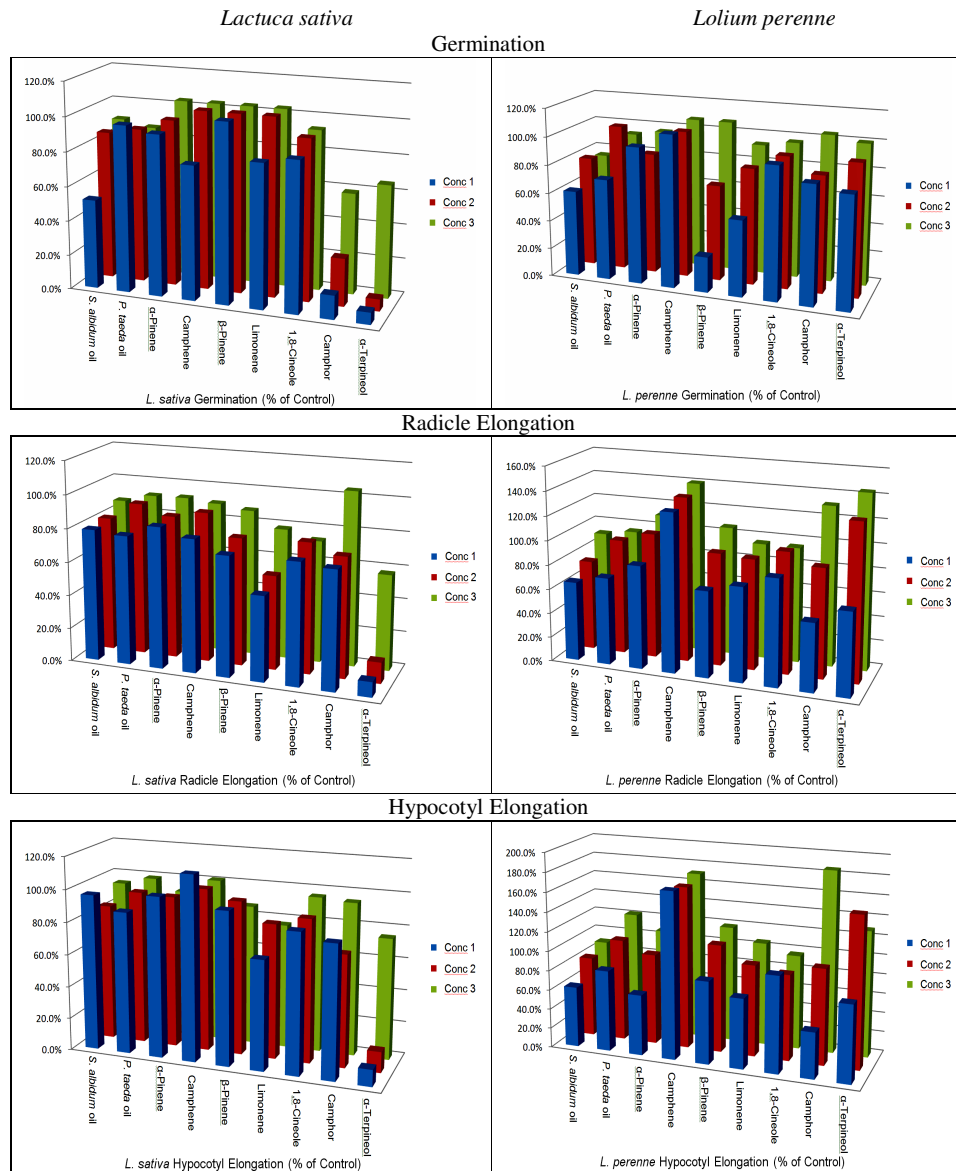


Figure 2. Inhibitory effects of essential oils of *S. albidum*, *P. taeda*, and components on germination and seedling growth of lettuce and rye grass.

Table 3. Inhibitory effects of *Sassafras albidum* and *Pinus taeda* essential oils and essential oil components on seed germination and seedling growth of *Lactuca sativa* and *Lolium perenne*

Sample	<i>Lactuca sativa</i>				<i>Lolium perenne</i>			
	Concentration	Germination (%)	Radicle length (mm)	Hypocotyl length (mm)	Germination (%)	Radicle length (mm)	Hypocotyl length (mm)	Hypocotyl length (mm)
Control <sup>a</sup>	---	96.7	27.7(6.7)	19.4(3.5)	93.3	21.4(6.8)	13.9(4.8)	13.9(4.8)
<i>Sassafras albidum</i> bark essential oil	2000 µg/mL	50.0	21.9(7.1) <sup>b</sup>	18.7(4.3)	56.7	13.9(6.2)	8.6(4.5)	8.6(4.5)
	1000 µg/mL	83.3	22.4(8.4)	16.4(5.5)	73.3	15.9(4.7)	11.4(4.5)	11.4(4.5)
	500 µg/mL	86.7	24.1(8.1)	18.3(3.3)	70.0	19.4(8.2)	12.4(5.9)	12.4(5.9)
<i>Pinus taeda</i> leaf essential oil	2000 µg/mL	93.3	21.4(7.7)	17.0(3.8)	66.7	15.3(8.6)	11.5(8.9)	11.5(8.9)
	1000 µg/mL	86.7	25.3(7.0)	18.4(4.3)	96.7	20.3(6.7)	14.5(4.4)	14.5(4.4)
	500 µg/mL	83.3	25.4(10.2)	19.2(4.7)	86.7	20.3(9.1)	17.0(5.5)	17.0(5.5)
$\alpha$ -Pinene	10 mM	90.0	23.4(7.9)	19.2(3.9)	90.0	18.1(7.1)	8.6(6.5)	8.6(6.5)
	5 mM	93.3	23.7(8.6)	18.2(4.5)	80.0	22.0(8.3)	12.9(4.5)	12.9(4.5)
	2.5 mM	100.0	25.5(7.8)	17.9(3.9)	90.0	23.8(7.6)	15.0(4.9)	15.0(4.9)
Camphene	10 mM	75.0	22.0(8.6)	22.1(7.2)	100.0	27.8(11.4)	23.6(9.8)	23.6(9.8)
	5 mM	100.0	24.8(8.2)	19.5(4.3)	96.8	28.9(11.0)	23.0(10.4)	23.0(10.4)
	2.5 mM	100.0	25.0(9.1)	19.6(4.9)	100.0	30.0(8.4)	23.8(8.0)	23.8(8.0)
$\beta$ -Pine ne	10 mM	100.0	19.9(7.8)	18.3(4.5)	23.3	15.1(6.9)	11.7(5.2)	11.7(5.2)
	5 mM	100.0	21.2(8.8)	18.4(4.7)	63.3	19.8(10.3)	15.3(9.6)	15.3(9.6)
	2.5 mM	100.0	24.3(9.2)	16.8(4.5)	100.0	22.7(8.4)	16.5(8.2)	16.5(8.2)
Limonene	10 mM	80.0	14.1(5.9)	13.1(5.4)	50.0	16.5(9.5)	9.9(5.8)	9.9(5.8)
	5 mM	100.0	15.6(5.8)	16.1(5.1)	76.7	19.4(8.3)	13.0(6.9)	13.0(6.9)
	2.5 mM	100.0	21.7(6.4)	14.9(4.5)	86.7	20.4(12.5)	14.7(8.2)	14.7(8.2)
1,8-Cineole	10 mM	83.3	20.1(7.1)	16.7(4.7)	86.7	18.6(6.4)	13.6(5.1)	13.6(5.1)
	5 mM	90.0	21.6(7.3)	17.1(5.7)	86.7	21.3(6.9)	12.2(3.9)	12.2(3.9)
	2.5 mM	90.0	20.3(6.3)	18.6(3.9)	90.0	20.3(7.3)	13.4(5.3)	13.4(5.3)
Camphor	10 mM	13.3	19.5(3.9)	15.8(8.3)	77.4	11.9(7.1)	6.5(5.2)	6.5(5.2)
	5 mM	26.7	19.9(7.8)	13.4(6.4)	76.7	19.2(7.5)	13.6(6.1)	13.6(6.1)
	2.5 mM	56.7	28.9(7.3)	18.3(7.9)	97.0	28.1(10.3)	25.8(6.9)	25.8(6.9)
$\alpha$ -terpineol	10 mM	6.7	2.5(0.7)	2.0(0.0)	73.3	14.5(9.7)	10.9(3.9)	10.9(3.9)
	5 mM	6.7	3.5(0.7)	2.5(0.7)	86.7	27.5(8.1)	21.4(4.5)	21.4(4.5)
	2.5 mM	63.3	15.9(8.0)	14.5(5.5)	93.3	30.8(12.2)	17.9(4.0)	17.9(4.0)

<sup>a</sup>Values in bold are significantly different ( $P < 0.05$ ) than controls; standard deviations are in parentheses., <sup>b</sup>Control = 0.1% aqueous Tween-80.

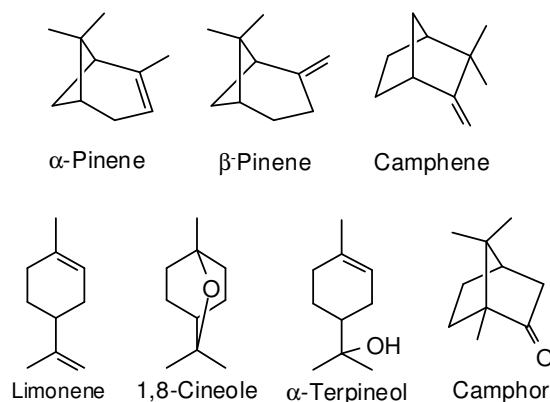


Figure 1. Chemical structures of essential oil components discussed in this manuscript.

Both  $\alpha$ -pinene (2,12,41) and  $\beta$ -pinene (9), limonene (9,19,37), 1,8-cineole (26,35,40), camphor (1,7,32) and  $\alpha$ -terpineol (24,46), have previously been shown to exhibit allelopathic activity.

Both *Sassafras albidum* bark essential oil and *Pinus taeda* leaf essential oil have shown phytotoxic effects against two model plant species, lettuce and perennial ryegrass. The allelopathic effects of *S. albidum* bark essential oil and the major components are consistent with the negative host preference of poison ivy (*Toxicodendron radicans*), an adventitious-root-climbing vine, for this tree. The growth inhibitory activities of major components of *P. taeda*,  $\alpha$ -pinene and  $\beta$ -pinene, are consistent with the allelopathic activity of leaf oil and may account for the observed sparse growth of understory herbaceous plants under *P. taeda*, as well as the negative host association of Spanish moss (*Tillandsia usneoides*) for *Pinus* species.

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