**Essential oil, insect, and microbe relationship with *Juniperus osteosperma* trees killed by wildfire**

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**ABSTRACT**

[Abstract: will be written later]

**KEY WORDS**: *Juniperus osteosperma*, aromatic profile, trunk, wildfire, insect, fungi, bacteria. [Please add additional key words as you see fit]

Pinyon-juniper forests are estimated to cover over 18 million hectares of the Intermountain West (Miller and Tausch 2001). Three aromatic tree species are common to the pinyon-juniper forests in Utah, *Juniperus osteosperma* (Utah juniper), *Pinus edulis* (pinyon pine), and at certain elevations and growing conditions, *J. scopulorum* (Rocky Mountain juniper) (Cronquist et al. 1972; Welsh 1993). Utah juniper is the most common tree in Utah (Cronquist et al. 1972). The trunk wood of all three species has been found to contain volatile oil (Poulson et al. 2020; Poulson et al. 2021; Wilson et al. 2019).

The aromatic profile for Utah junipertrunk essential oil was found to be prominent in α-pinene (59.4%), δ-3-carene (4.4%), cis-thujopsene (11.0%), and cedrol (3.0%) (Wilson et al. 2019). The aromatic profile for pinyon pine trunk essential oil was found to be prominent in α-pinene (50.3%), δ-3-carene (7.3%), ethyl octanoate (2.9%), longifolene (6.7%), and germacrene D (5.8%) (Poulson et al. 2020). The aromatic profile for Rocky Mountain juniper trunk essential oil, was found to be prominent in α-pinene (20.5%), cis-thujopsene (34.2%), and cedrol (18.9%) (Poulson et al. 2021). A primary difference between Utah juniper and Rocky Mountain juniper trunk is found in the purple-red heartwood that is unique to Rocky Mountain juniper(Cronquist et al. 1972; Poulson et al. 2021; Welsh 1993)*.*

Pinyon-juniper forests are often casualties of forest fires which result in the deaths of many trees (Gruell 1999). It has long been known that the decomposition of dead trees is an intricate process involving both abiotic and biotic factors (Shigo 1979). Examples of biotic decomposers include saprophytic fungi and bacteria (Janusz et al. 2017; Kubartová et al. 2009; Song et al. 2017) and insects through both indirect, acting as a means of introducing microorganisms, and direct means (Jacobsen et al. 2018; Krivosheina 2016; Ulyshen et al. 2016).

Previous work (Clark et al. 1990) has shown both antimicrobial and antifungal properties of volatile oil extracted from Utah juniper heartwood and bark/sapwood. *Juniperus recurva*, which is native to Nepal, was shown to contain two volatile compounds, cis-thujopsene and cedr-8-en-13-ol, that demonstrate insecticidal properties against mosquitos (Oda et al. 1977). Both compounds have been shown to be present in the trunk wood of Utah juniper (Wilson et al. 2019). Additionally, Utah juniper heartwood demonstrated termiticidal activity (Adams et al. 1988). Utah juniper’s durability, as well as abundance, lend to its popular use as fence posts (Cronquist et al. 1972); which use has also been attributed to the volatile compounds acting as preserving agents in the wood (Adams 2014).

It was hypothesized that essential oil is retained in dead Utah juniper trees and that these volatile compounds would help slow the process of decomposition. Utilizing wildfire data between 1998-2018, the present study aimed to investigate whether essential oils remain in dead Utah juniper trunks following wildfires, to characterize any changes in the essential oil composition following tree death, and to examine the relationship between biotic decomposition influencers of the dead trees, namely fungi, bacteria, and insects.

[Please add additional content throughout introduction as you see fit]

**MATERIALS AND METHODS**

*Juniperus osteosperma* burnt trunks were collected from sixteen locations, one tree per site, throughout the state of Utah (Figure 1). Historical fire dates were obtained from the Bureau of Land Management’s records (reference needed). Study areas were carefully searched and burnt *J. osteosperma* were selected and cut with a chainsaw. The burnt trunk is defined as a 1 m, charred section (starting 0.25 m above ground) including heartwood, sapwood, cambium, and any remaining bark. Due to the lack of leaf and reproductive tissue, conventional voucher samples were unable to be obtained. However, the identity of specimens was determined through means of volatile compound analysis and, in the case of Rocky Mountain juniper, the color of the heartwood (Cronquist et al. 1972; Poulson et al. 2020; Poulson et al. 2021; Wilson et al. 2019; Welsh 1993).

Samples of burnt trunk (n=16) of *J. osteosperma* were cut and processed as follows: 30 cm section for steam distillation and analytical analysis, 30 cm section for entomology analysis, 30 cm section for mycology and bacterial analysis, and 10 cm section for moisture analysis (Figure 2). All sections were stored in a sealed bag at ambient temperature and out of direct sunlight.

Laboratory scale distillation was as follows: 3 L of water added to the bottom of a 12 L distillation chamber (Albrigi Luigi S.R.L., Italy), plant material chipped and accurately weighed before being added to the distillation chamber, distillation for 4 hours by direct steam, essential oil separated by a cooled condenser and Florentine flask. Essential oil samples were filtered and stored in a sealed amber glass bottle until analysis.

Essential oils were analyzed, and volatile compounds identified by GC-MS using an Agilent 7890B GC/5977B MSD and J&W DB-5, 0.25 mm x 60 m, 0.25 μm film thickness, fused silica capillary column. Operating conditions: 0.1 μL of neat sample, 150:1 split ratio, initial oven temperature of 40 °C with an initial hold time of 5 minutes, oven ramp rate of 4.5 °C per minute to 310 °C with a hold time of 5 minutes. The electron ionization energy was 70 eV, scan range 35–650 amu, scan rate 2.4 scans per second, source temperature 230 °C, and quadrupole temperature 150 °C. Volatile compounds were identified using the Adams volatile oil library (Adams 2007, pdf at www.juniperus.org) using Chemstation library search in conjunction with retention indices. Note that in some samples widdrol/cedrol elute as single peaks, but their amounts are determined by the ratio of masses 68 and 79 (limonene), 77 and 93 (β-phellandrene), 151 (widdrol), and 150 (cedrol). Volatile compounds were quantified and are reported as a relative area percent by GC-FID using an Agilent 7890B and J&W DB-5, 0.25 mm x 60 m, 0.25 μm film thickness, fused silica capillary column. Operating conditions: 0.1 μL of sample (5% soln. for essential oils, 1% for reference compounds in hexane), 25:1 split ratio, initial oven temperature at 40 °C with an initial hold time of 2 minutes, oven ramp rate of 3.0 °C per minute to 250 °C with a hold time of 3 minutes. For quantification, compounds were identified using retention indices coupled with retention time data of reference compounds.

The percent yield was calculated as the ratio of mass of processed plant material immediately before distillation to the mass of essential oil produced, multiplied by 100.

[Entomology method]

[Microbial method]

[Water Content method]

[Statistical method(s)] Spearman’s correlation was used to evaluate the relationship between variables over a range of time.

**RESULTS AND DISCUSSION**

It should be noted that the overall health and condition of each tree, prior to being burnt, is unknown.

The aromatic profiles of each trunk are detailed in Table 1. Interestingly, while α-pinene and δ-3-carene were prominent compounds in Utah juniper trunk essential oil from living trees (Wilson et al. 2019), neither greatly contributed to the profile of burnt Utah juniper trees (α-pinene: from nd to 3.9%; δ-3-carene: nd). It is possible that these lighter fractions are readily lost to the atmosphere or that they are primarily contained in the outer layers of the trunk, which were burnt. Prominent compounds (defined as an average area %, from all samples, >5) include α-cedrene (10.4%), cis-thujopsene (19.8%), widdrol (7.6%), cedrol (20.1%), and cedr-8-en-13-ol (5.7%). Based on the date when these trees were burnt, older trunks display an increasing relative abundance of more volatile fractions (α-cedrene, cis-thujopsene, widdrol) (Figure 3) and decreasing relative abundance of less volatile fractions (cedrol, cedr-8-en-13-ol) (Figure 4).

The raw material mass and essential oil yield are detailed in Table 2. Not surprisingly, older trees displayed a trend of containing less water and, as a result, a higher content (relative to the weight of the raw material) of essential oil (Figure 5). There is a moderate positive correlation (0.5054) between essential oil and water content in brunt trunks.

[Results and discussion: please add additional content as you see fit]

Sequence data has been deposited in the Sequence Read Archive under accession PRJNA730829.

**CONCLUSIONS**

[Conclusion: will be written later]

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Table 1. Aromatic profile of *J. osteosperma* essential oil from the dead trunk (n=16). Compound not detected are denoted as not detected (nd) and values less than 0.1% as traces (t). Unidentified compounds less than 1.0% are not included. KI is the Kovat’s Index using a linear calculation on DB-5 column (Adams 2007). Relative area percent is determined by GC-FID. Essential oil samples were analyzed in triplicate to ensure reproducibility (SD <1 for all compounds).

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Site ID:** | **CC1** | **CC2** | **M1** | **F1** | **CC3** | **CC4** | **SL2** | **SL3** | **SL4** | **SL6** | **SL7** | **SL8** | **SL9** | **CC5** | **SL11** | **SL12** |
|  | **Year of Fire:** | **1998** | **1999** | **2000** | **2001** | **2004** | **2005** | **2007** | **2009** | **2009** | **2011** | **2012** | **2013** | **2014** | **2016** | **2017** | **2018** |
| **KI** | **Compound** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 932 | α-pinene | 0.1 | nd | nd | nd | nd | 3.9 | t | t | 0.5 | t | nd | nd | 0.8 | 0.3 | 2.0 | 1.0 |
| 953 | thuja-2,4(10)-diene | nd | nd | nd | nd | nd | 0.8 | nd | nd | t | nd | nd | nd | nd | t | 0.2 | nd |
| 1020 | p-cymene | nd | 0.2 | 2.9 | 0.5 | 1.7 | 1.2 | 0.6 | 0.3 | 1.0 | 0.6 | 0.2 | t | 0.3 | 1.4 | 0.5 | 0.5 |
| 1186 | α-terpineol | 0.3 | t | 3.2 | 1.8 | 5.3 | 1.5 | 0.6 | 0.3 | 1.5 | 3.5 | 0.3 | t | 1.1 | 1.2 | 0.5 | 2.8 |
| 1194 | myrtenol | t | t | t | 0.1 | 0.7 | 0.4 | t | 0.1 | 0.3 | 0.1 | 0.1 | t | 2.7 | 0.1 | 0.2 | 0.7 |
| 1195 | myrtenal | t | t | t | 0.1 | 0.4 | 1.1 | t | 0.2 | 0.3 | 0.3 | 0.2 | t | 1.3 | 0.4 | 0.2 | 0.1 |
| **1 1225** | Unknown 1 | nd | nd | nd | nd | nd | t | t | nd | nd | nd | nd | nd | nd | 2.0 | nd | nd |
| 1241 | carvacrol methyl ether | 0.2 | t | 0.9 | 0.4 | 0.3 | 0.7 | t | 0.3 | 0.1 | 0.3 | 0.2 | nd | 1.0 | 1.1 | 0.4 | 0.5 |
| 1249 | piperitone | nd | nd | t | t | 0.2 | nd | nd | 1.0 | nd | nd | nd | nd | 1.3 | t | nd | t |
| 1387 | α-duprezianene | 0.7 | 1.1 | 1.0 | 0.7 | 0.7 | 1.2 | 0.9 | 1.0 | 0.6 | 0.5 | 0.5 | 1.2 | 0.8 | 1.2 | 0.9 | 0.3 |
| 1410 | α-cedrene | 14.3 | 16.7 | 3.6 | 2.4 | 5.6 | 18.1 | 7.2 | 34.1 | 8.1 | 8.0 | 11.4 | 7.8 | 7.1 | 11.7 | 8.0 | 2.1 |
| 1419 | β-cedrene | 2.9 | 3.3 | 0.9 | 0.6 | 2.3 | 4.1 | 1.9 | 7.2 | 2.4 | 2.5 | 1.8 | 0.7 | 1.9 | 2.2 | 2.0 | 0.9 |
| 1421 | β-duprezianene | 0.4 | 0.8 | 0.8 | 0.6 | 0.3 | 0.8 | 0.8 | 0.7 | 0.4 | 0.4 | 0.4 | 2.1 | 0.6 | 0.8 | 0.8 | 0.4 |
| 1429 | cis-thujopsene | 8.7 | 16.1 | 30.7 | 25.8 | 26.4 | 15.2 | 30.2 | 15.8 | 13.9 | 27.2 | 12.0 | 13.8 | 22.4 | 12.0 | 20.7 | 26.0 |
| 1449 | α-himachalene | t | 0.6 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.3 | nd | 0.2 | 0.1 | t | 0.3 | 0.4 | 0.3 | 0.2 |
| 1469 | β-acoradiene | 0.4 | 0.7 | 0.8 | 0.5 | 0.6 | 0.9 | 0.7 | 0.7 | 0.1 | 0.3 | 0.5 | 0.6 | 0.5 | 0.9 | 0.4 | 0.3 |
| 1474 | 10-epi-β-acoradiene | 0.3 | 0.7 | 0.6 | 0.4 | 0.9 | 0.9 | 0.8 | 0.7 | 0.1 | 0.4 | 0.4 | 0.8 | 0.5 | 0.6 | 0.6 | 0.3 |
| 1476 | β-chamigrene | 0.5 | 0.9 | 1.5 | 1.0 | 0.7 | 1.4 | 0.8 | 1.5 | 0.3 | 0.4 | 1.1 | 0.8 | 0.8 | 1.6 | 0.9 | 0.3 |
| 1498 | pseudowiddrene | 0.9 | 2.3 | 1.9 | 1.1 | 1.2 | 2.2 | 1.1 | 2.3 | 0.6 | 0.5 | 1.6 | 1.6 | 1.3 | 3.7 | 1.5 | 0.2 |
| 1504 | cuparene | 1.6 | 2.3 | 3.5 | 2.8 | 2.1 | 2.5 | 2.4 | 2.5 | 1.6 | 1.4 | 2.9 | 1.9 | 2.0 | 3.2 | 1.9 | 1.5 |
| 1541 | 8,14-cedranoxide | t | 0.5 | 0.9 | 0.9 | 0.3 | 0.3 | t | t | nd | nd | 2.5 | 1.4 | 0.8 | 1.9 | 0.4 | 0.8 |
| 1589 | allo-cedrol | 0.3 | 0.4 | 0.4 | 0.5 | 0.4 | 0.2 | 0.6 | t | 0.6 | 0.5 | t | 0.7 | 0.5 | t | 0.3 | 1.2 |
| 1595 | cis-dihydro-mayurone | 0.5 | 1.4 | 0.6 | 0.6 | 0.4 | 0.6 | 0.4 | t | 0.4 | t | 0.5 | 1.9 | 0.3 | 0.4 | 0.2 | 0.3 |
| 1599 | widdrol | 7.5 | 7.1 | 10.1 | 10.3 | 6.0 | 7.9 | 7.0 | 5.5 | 4.3 | 2.6 | 10.0 | 15.5 | 7.4 | 11.8 | 6.4 | 1.4 |
| 1600 | cedrol | 37.3 | 17.8 | 5.8 | 7.4 | 15.1 | 14.6 | 18.4 | 15.9 | 36.6 | 38.1 | 15.2 | 20.5 | 20.6 | 8.6 | 15.0 | 35.2 |
| 1607 | β-biotone | 0.3 | 0.4 | t | 0.3 | 0.3 | t | 0.3 | t | 0.6 | nd | 0.3 | 0.6 | 0.5 | 0.3 | 0.3 | 0.2 |
| 1630 | γ-eudesmol | 0.5 | 1.1 | 2.0 | 3.4 | 0.4 | 0.4 | 0.3 | 0.2 | 1.2 | 0.2 | 0.7 | 0.8 | 0.2 | 2.8 | 2.4 | nd |
| 1632 | α-acorenol | 0.8 | 0.4 | 1.2 | 1.1 | 1.4 | 0.9 | 1.6 | 0.4 | 1.2 | 1.5 | 0.4 | 2.6 | 1.1 | nd | 0.7 | 3.1 |
| 1636 | β-acorenol | 0.4 | 0.5 | 0.5 | 0.7 | 0.6 | 0.4 | 0.7 | t | 0.3 | 0.5 | 0.3 | 0.9 | 0.6 | 0.3 | 0.5 | 0.8 |
| 1639 | 1,7-diepi-alpha-cedrenal | 1.3 | 0.9 | 1.4 | 1.7 | 0.8 | 0.8 | 0.9 | 0.7 | 1.0 | 0.4 | 1.6 | 1.6 | 0.8 | 1.1 | 0.5 | 0.5 |
| 1649 | β-eudesmol | 1.0 | 1.1 | 4.2 | 6.2 | 0.9 | 1.0 | 1.9 | 1.0 | 3.9 | 1.0 | 0.9 | 3.2 | 0.7 | 1.9 | 3.1 | 1.5 |
| 1652 | α-eudesmol | 0.6 | 0.7 | 2.2 | 3.1 | 0.5 | 0.6 | 0.6 | t | 2.0 | 0.5 | 0.5 | 2.0 | 0.4 | 1.6 | 1.9 | 0.6 |
| **1 1681** | Unknown 2 | 0.8 | 0.8 | 0.7 | 1.1 | 0.7 | 0.5 | 0.8 | 0.4 | 0.4 | 0.5 | 0.9 | 1.2 | 0.9 | 0.8 | 0.9 | 0.9 |
| 1688 | cedr-8-en-13-ol | 4.9 | 5.7 | 1.5 | 2.2 | 7.8 | 5.0 | 9.2 | 3.0 | 6.1 | 0.8 | 13.0 | 3.3 | 6.5 | 8.8 | 11.4 | 1.8 |
| **1 1714** | cedr-8-en-15-ol | 3.3 | 2.9 | 2.1 | 3.4 | 2.2 | 2.1 | 2.9 | 1.6 | 2.8 | 1.7 | 3.9 | 2.3 | 3.1 | 3.1 | 3.7 | 2.7 |
| 1709 | mayurone | 0.4 | 0.5 | 0.8 | 0.8 | 0.4 | 0.4 | t | t | 0.5 | t | 0.5 | 0.6 | t | 0.3 | nd | 0.1 |
| 1708 | thujopsenal | 0.7 | 0.5 | 0.6 | 0.9 | 0.5 | 0.4 | 0.4 | 0.4 | t | 0.7 | 0.5 | 0.5 | 0.5 | 0.7 | 0.5 | 0.5 |
| **1 1725** | Unknown 3 | 1.2 | 1.5 | 0.7 | 1.4 | 2.1 | 0.9 | 2.4 | 0.5 | 1.4 | 1.0 | 1.1 | 1.0 | 2.0 | 0.9 | 2.4 | 1.8 |
| **1 1739** | 4a,8,8-Trimethyloctahydrocyclopropa[d]  naphthalen-2(3H)-one | 1.1 | 2.6 | 1.6 | 1.4 | 0.9 | 1.3 | 0.9 | 0.7 | 0.5 | 0.7 | 1.6 | 2.2 | 0.7 | 1.3 | 0.5 | 0.2 |
| **1 1746** | 3,3,4-Trimethyl-4-(4-methylphenyl) cyclopentanol | 0.4 | 0.3 | 0.3 | 0.9 | 0.4 | 0.2 | 0.4 | t | t | t | 0.9 | nd | 0.4 | 0.5 | 0.4 | 0.3 |
| 1788 | 8-cedren-13-ol acetate | nd | nd | nd | nd | nd | nd | nd | nd | nd | 0.4 | 0.4 | nd | nd | nd | nd | nd |
| 1806 | nootkatone | t | t | 1.0 | 0.4 | t | t | t | t | nd | nd | nd | t | nd | nd | nd | 0.2 |
| 1889 | 8S,14-cedranediol | t | 0.2 | 0.6 | 0.7 | 0.4 | 0.3 | 0.3 | t | nd | nd | 1.4 | 0.7 | 1.0 | 1.2 | 0.6 | 0.9 |
|  | column total: | 94.6 | 93.0 | 91.9 | 88.6 | 92.3 | 96.1 | 98.4 | 99.3 | 95.6 | 97.7 | 90.8 | 94.8 | 95.7 | 93.1 | 94.1 | 93.1 |

1KI was calculated using alkane standards.

Table 2. Mass distilled and essential oil (EO) yield from a single *J. osteosperma* tree (the number was limited to one as per permit restrictions). Tree was cut 0.25 m above ground; all measurements and calculations are reflective of above ground portions.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **sample identification** | **year of fire** | **mass distilled (g)** | **yield EO (g)** | **yield EO (%)** |
| **CC1** | 1998 | 900.2 | 1.7 | 0.2 |
| **CC2** | 1999 | 813.5 | 3.8 | 0.5 |
| **M1** | 2000 | 1050.5 | 4.4 | 0.4 |
| **F1** | 2001 | 832.7 | 2.3 | 0.3 |
| **CC3** | 2004 | 936.1 | 7.6 | 0.8 |
| **CC4** | 2005 | 1416.7 | 5.2 | 0.4 |
| **SL2** | 2007 | 1205.7 | 5.6 | 0.5 |
| **SL3** | 2008 | 1426.1 | 11.0 | 0.8 |
| **SL4** | 2009 | 1030.7 | 4.4 | 0.4 |
| **SL6** | 2011 | 984.6 | 9.4 | 1.0 |
| **SL7** | 2012 | 844.1 | 4.5 | 0.5 |
| **SL8** | 2013 | 933.6 | 0.7 | 0.1 |
| **SL9** | 2014 | 1127.5 | 4.7 | 0.4 |
| **CC5** | 2016 | 1109.9 | 3.7 | 0.3 |
| **SL11** | 2017 | 1641.5 | 4.8 | 0.3 |
| **SL12** | 2018 | 1196.2 | 2.2 | 0.2 |

Figure 1. Map showing collection sites of *J. osteosperma* plant material. Samples were collected in sequence, 1-10. The following are sample collection dates and elevation: [will be added later]

Figure 2. Illustrated portions of *J. osteosperma* trunk used for essential oil extraction and analytical analysis, entomology and mycology studies, and moisture content. [will be added later]

Figure 3. [will be added later]

Figure 4. [will be added later]

Figure 5. [will be added later]