

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

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In the state of Utah (USA), pinyon-juniper forests are common and are primarily composed of three essential oil-bearing tree species: *Juniperus osteosperma* (Utah juniper), *J. scopulorum* (Rocky Mountain juniper), and *Pinus edulis* (pinyon pine). Previous work has shown antimicrobial properties of volatile oil extracted from Utah juniper heartwood and bark/sapwood (Clark, 1990). Pinyon-juniper forests are often destroyed by wildfire, and it was hypothesized that essential oils are retained within dead trees and that their antimicrobial properties would slow the rate of decomposition.

METHODS

To determine if burnt, dead trees could be identified through their essential oils alone, live trees of *J. osteosperma*, *J. scopulorum*, and *P. edulis* were harvested, processed, distilled, and the resulting oils analyzed by GC-FID and GC-MS. Utilizing wildfire data from 1998-2018, sixteen samples of dead Utah juniper trunks were harvested. Since conventional voucher samples could not be created from burnt plant material, chemical profiling of steam distilled plant material was used to distinguish species. The trunk was separated into four different sections (Figure 1) to characterize fungal and bacterial species colonizing the dead trees, to catalogue insect activity, and for essential oil distillation.



Figure 1

Essential Oils of Pinyon-Juniper Forests

J. osteosperma trunk essential oil was high in α -pinene, δ -3-carene, cis-thujopsene, and cedrol (Wilson, 2019). *J. scopulorum* trunk essential oil was high in α -pinene, (E)-caryophyllene, widdrol, cedrol, and allo-aromadendrene epoxide (Poulson, 2021), and can also be distinguished by its unique purple-red heartwood. *P. edulis* trunk essential oil was high in α -pinene, δ -3-carene, ethyl octanoate, longifolene, and germacrene D (Poulson, 2020). The trunk essential oil of dead Utah juniper had a similar composition to living Utah juniper, except the ratios of monoterpenes to sesquiterpenes differed. Live Utah juniper trunk contains nearly 60% α -pinene (Wilson, 2019). Dead Utah juniper essential oil showed a great deal of variation but was highest in α -cedrene (2.1% - 34.1%), cis-thujopsene (8.7% - 30.7%), widdrol (1.4% - 15.5%), and cedrol (5.8% - 38.1%), and low in all monoterpenes (Table 1).

Insect and Microbial Activity

Insect exit holes from flatheaded/metallic woodboring beetles (Buprestidae) were present in 75% of trunk samples, ranging from 1 to 35 exit holes per sample. Five living flatheaded borer larvae were recovered (2013, 1 larva; 2014, 4 larvae). There was no apparent trend in number based on year since fire, suggesting the essential oil is not a deterrent to insect activity. Microbial results showed 1258 bacterial and 326 fungal taxa. Years since burn did not have an apparent effect on fungal diversity or composition, but it did influence bacterial diversity and community composition (Figure 2). However, the combination of α -acorenal and β -acorenal was a statistically significant and negative predictor of fungal diversity [GLM; $P = 0.0171$]. No predictable effect of these factors on discrete microbial taxa was found.

Figure 2

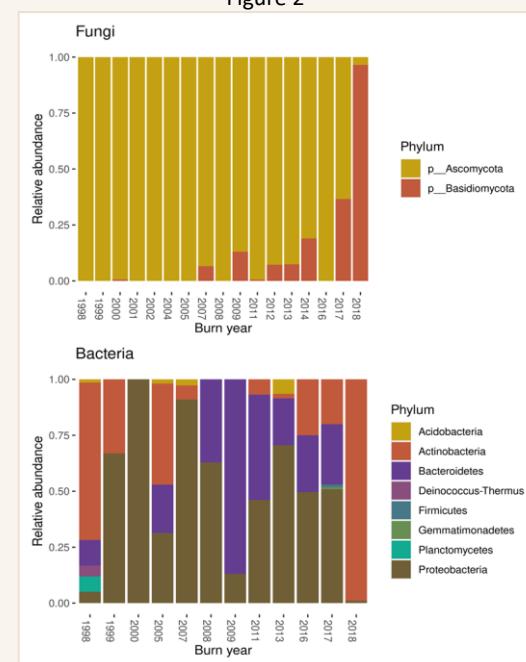


Table 1. Aromatic profile of *J. osteosperma* essential oil from burnt trunks (n = 16).

Compounds not detected are denoted as not detected (nd) and values less than 0.1% as trace (t). KI is the Kovat's Index using a linear calculation on DB-5 column (Adams, 2007).¹ KI was calculated using alkane standards.

| KI | Compound | Year of Fire: | | | | | | | | | | | | | | | |
|------|--|---------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| | | 1998 | 1999 | 2000 | 2001 | 2004 | 2005 | 2007 | 2009 | 2009 | 2011 | 2012 | 2013 | 2014 | 2016 | 2017 | 2018 |
| 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 | myrtanal | 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 |
| 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 -acorendiene | 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 -acorendiene | 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 -acorenal | 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 -acorenal | 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- α -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 | 0.9 | 3.2 | 1.0 | 0.9 | 3.2 | 0.7 | 1.9 | 3.1 |
| 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 | 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 |
| 1681 | | | | | | | | | | | | | | | | | |
| 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 | 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 |
| 1714 | | | | | | | | | | | | | | | | | |
| 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 | t | 0.7 | 0.5 | 0.5 | 0.5 | 0.5 | 0.7 | 0.5 | 0.5 |
| 1 | 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 |
| 1725 | | | | | | | | | | | | | | | | | |
| 1 | 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 |
| 1739 | 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 |
| 1746 | 8-cedren-13-ol acetate | nd | nd | nd | nd | nd | nd | nd | nd | nd | 0.4 | 0.4 | nd | nd | nd | nd | nd |
| 1788 | nootkatone | t | t | 1.0 | 0.4 | t | t | t | t | nd | nd | nd | t | nd | nd | nd | 0.2 |
| 1806 | | | | | | | | | | | | | | | | | |
| 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 |

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CONCLUSION

Insects from the Buprestidae family predominated trunk samples, and numerous bacterial (1258) and fungal (326) taxa were found, though no predictive effects influencing tree decomposition were observed when compared with essential oils. However, years since burn did appear to influence bacterial diversity and community composition, and the combination of α - and β -acorenal in the essential oil was a significant predictor of fungal diversity, suggesting a potential synergy. While there were no clear trends in essential oil composition or yield from year to year, results show that essential oils can be retained in dead trees for over twenty years. Complete research is expected to be published in *Phytologia* later this year.

