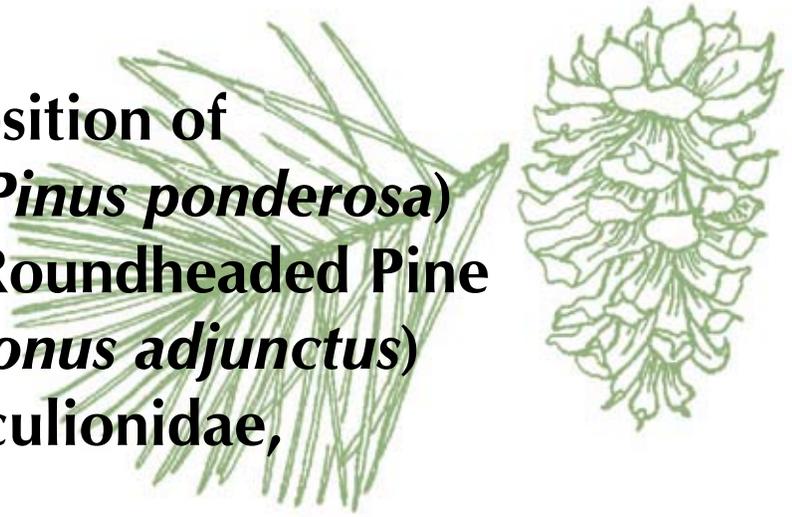


# The Resin Composition of Ponderosa Pine (*Pinus ponderosa*) Attacked by the Roundheaded Pine Beetle (*Dendroctonus adjunctus*) (Coleoptera: Curculionidae, Scolytinae)



Melissa J. Fischer, Kristen M. Waring, Richard W. Hofstetter, and Thomas E. Kolb, *School of Forestry, Northern Arizona University, Flagstaff, AZ*

**Abstract**—*Dendroctonus adjunctus* is an aggressive bark beetle species that attacks several species of pine throughout its range from southern Utah and Colorado south to Guatemala. A current outbreak of *D. adjunctus* provided a unique opportunity to study the relationship between this beetle and pine resin chemistry in northern Arizona. We compared the resin composition of trees that had been attacked by *D. adjunctus* compared with unattacked trees and found significant differences in the composition of the monoterpenes  $\alpha$ -pinene,  $\beta$ -pinene, myrcene and limonene between attacked and unattacked trees. Attacked trees contained significantly higher percentages of  $\alpha$ -pinene, myrcene, and limonene, but lower levels of  $\beta$ -pinene when compared to unattacked trees. Although it is unknown whether *D. adjunctus* prefers or is repelled by trees with specific monoterpene content, our results suggest that *D. adjunctus* may use specific chemical cues in host tree selection.

## Introduction

The roundheaded pine beetle, *Dendroctonus adjunctus* (Coleoptera: Curculionidae, Scolytinae), is an aggressive bark beetle (Negrón 1997, Negrón and others 2000) that attacks several species of pine throughout its range from southern Utah and Colorado south to Guatemala (Chansler 1967). *Dendroctonus adjunctus* has periodic outbreaks that cause extensive tree mortality in the southwestern United States (Negrón 1997, Negrón and others 2000). Outbreaks have been associated with dense stand conditions and drought (Negrón 1997, Negrón and others 2000),

---

In: Olberding, Susan D., and Moore, Margaret M., tech coords. 2008. Fort Valley Experimental Forest—A Century of Research 1908-2008. Proceedings RMRS-P-53CD. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 408 p.

which is similar to other *Dendroctonus* species (Fettig et al. 2007). Host selection by bark beetles for specific trees within these dense stands remains unclear (Wood 1982). Multiple hypotheses have been presented to describe the mechanisms driving bark beetle host selection. These include: 1) locating damaged or diseased trees by the volatile chemicals they emit (Byers 1995, Hofstetter et al. 2008, Wood 1982); 2) attraction or repulsion caused by monoterpenes released by trees (Byers 1995, El-Sayed and Byers 2000, Fettig et al. 2007, Hofstetter et al. 2008, Wood 1982); 3) attraction to aggregation pheromones released by beetles of the same species or to volatiles produced by competing insect species during colonization (Byers 1995, Hofstetter et al. 2008, Wood 1982); and 4) bark beetles choose trees for attack randomly (Byers 1995, Wood 1982). Finally, stand conditions may affect host selection on a larger scale; for example, microclimate and tree vigor vary with stand density and may partially determine which stands will be attacked (Fettig et al. 2007, Miller and Keen 1960, Wood 1982).

The resin of conifers contains monoterpenes that have been found to both attract and repel bark beetles (Byers 1995, El-Sayed and Byers 2000, Fettig et al. 2007, Hofstetter et al. 2008, Smith 1966, Sturgeon 1979). Some common monoterpenes found within resin include  $\alpha$ -pinene,  $\beta$ -pinene, and limonene (Latta et al. 2000). Which monoterpenes repel or attract bark beetles is still uncertain, even among the most well-studied bark beetle species (El-Sayed and Byers 2000). Studies on host monoterpene and bark beetle interactions have been inconclusive. For example, lodgepole pines with high levels of limonene were readily attacked and killed by *D. ponderosae* (Byers 1995); conversely, ponderosa pines with high levels of limonene were not attacked by *D. brevicomis* (Sturgeon 1979).

The overall goal of this project is to gain a better understanding of host selection behavior of bark beetles. We use *D. adjunctus*—ponderosa pine as a model system to investigate:

1. If attacked trees exhibit different size, resin composition, growth rate, crown characteristics, or phloem thickness than unattacked trees;
2. If beetles cause changes in tree resin composition following attack, and
3. If surrounding forest stand density is correlated with beetle attacks in northern Arizona.

In this paper, we test the hypothesis that attacked trees exhibit a different resin composition, specifically monoterpenes, than unattacked trees.

## Materials and Methods

### Study Area

The study was located near Flagstaff, Arizona, approximately 2.14 km (1.33 miles) north of the Flagstaff Nordic Center. Twenty-five pairs of “attacked” and “unattacked” ponderosa pine trees (50 trees total) were selected in 2007. Paired trees were similar in diameter at breast height (dbh) (table 1) and location, with the greatest distance being 5.52m (18.11ft) apart. Numerous pitch tubes and the presence of frass were used to identify successfully attacked trees (Smith 1966, Chansler 1967, Nebeker 1993); bark was removed and galleries inspected on a sub-sample

of trees to confirm beetle identification. Trees selected as unattacked had no pitch tubes and/or frass. Attacks by the western pine beetle (*D. brevicomis*) were also present on site. We differentiated between trees attacked solely by *D. adjunctus* and *D. brevicomis* by the size of the pitch tubes on the bole of attacked trees (*D. brevicomis* attacked trees have smaller pitch tubes) and location of pitch tubes on the bole (pitch tubes caused by *D. brevicomis* tend to be higher on the bole than those caused by *D. adjunctus*). We did not select trees that had signs of only *D. brevicomis* attack.

During September 2007, the bark and phloem of each tree was punched at breast height (1.37m) using a number fifteen 2-cm (0.79 inches) metal punch and a glass vial inserted to collect resin. The glass vials were then removed from the trees and moved to cold storage prior to composition analysis. Resin collection corresponded to *D. adjunctus* fall flight; trees that were chosen as pairs were in the process of being attacked when resin was collected. Resin was analyzed for total monoterpene content and composition using gas chromatography by the Analytical Chemical Laboratory at Northern Arizona University. Because some of the trees had no flowing resin at the time of collection and others did not produce enough resin to be analyzed, only resin from 13 of the attacked trees and 17 of the unattacked trees were used for this analysis. Due to the fact that we did not have resin for all trees, the resin data was not analyzed as matched pairs, but was instead pooled by attacked or non-attacked. Data were not normally distributed so statistical comparisons were made using the Kruskal-Wallis test.

## Results

No significant difference was found between the diameters of the paired trees (Table 1).

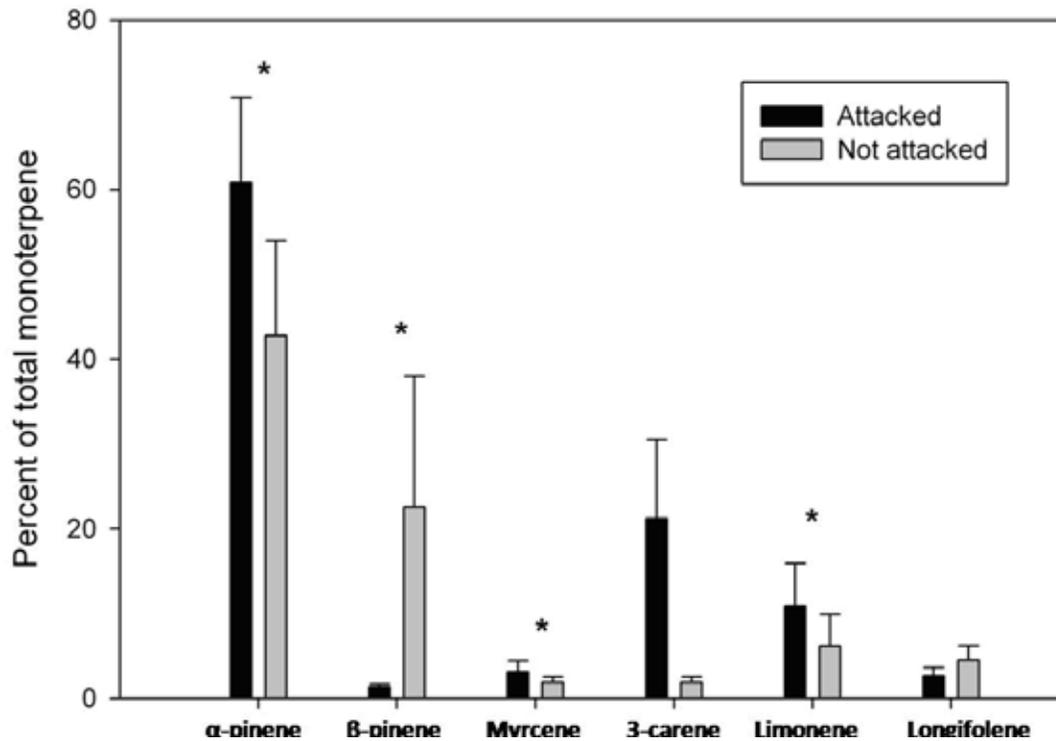
Percentages of  $\alpha$ -pinene,  $\beta$ -pinene, myrcene and limonene were significantly different between attacked and unattacked trees (Table 2, Kruskal-Wallis test,  $p < 0.05$ ), while 3-carene and longifolene were not significantly different between tree pairs (Table 2, Kruskal-Wallis test,  $p > 0.05$ ).

**Table 1.** Matched pairs t-test comparing the diameters of the ponderosa pine trees paired as attacked and unattacked.

	Mean DBH	Mean Difference	SEM	p-value
<b>Attacked</b>	29.6 cm (11.65 inches)	1.12 cm (0.44 inches)	0.9392	0.2447
<b>Non-attacked</b>	30.72 cm (12.09 inches)			

**Table 2.** Results of Kruskal-Wallis tests comparing percentage monoterpene content between *D. adjunctus* attacked and unattacked ponderosa pine trees in northern Arizona.

	$\alpha$ -pinene	$\beta$ -pinene	Myrcene	3-carene	Limonene	Longifolene
Chi-Square	5.591	4.828	4.646	0.036	4.206	1.738
df	1	1	1	1	1	1
Asymp. Sig.	0.018	0.028	0.031	0.850	0.040	0.187



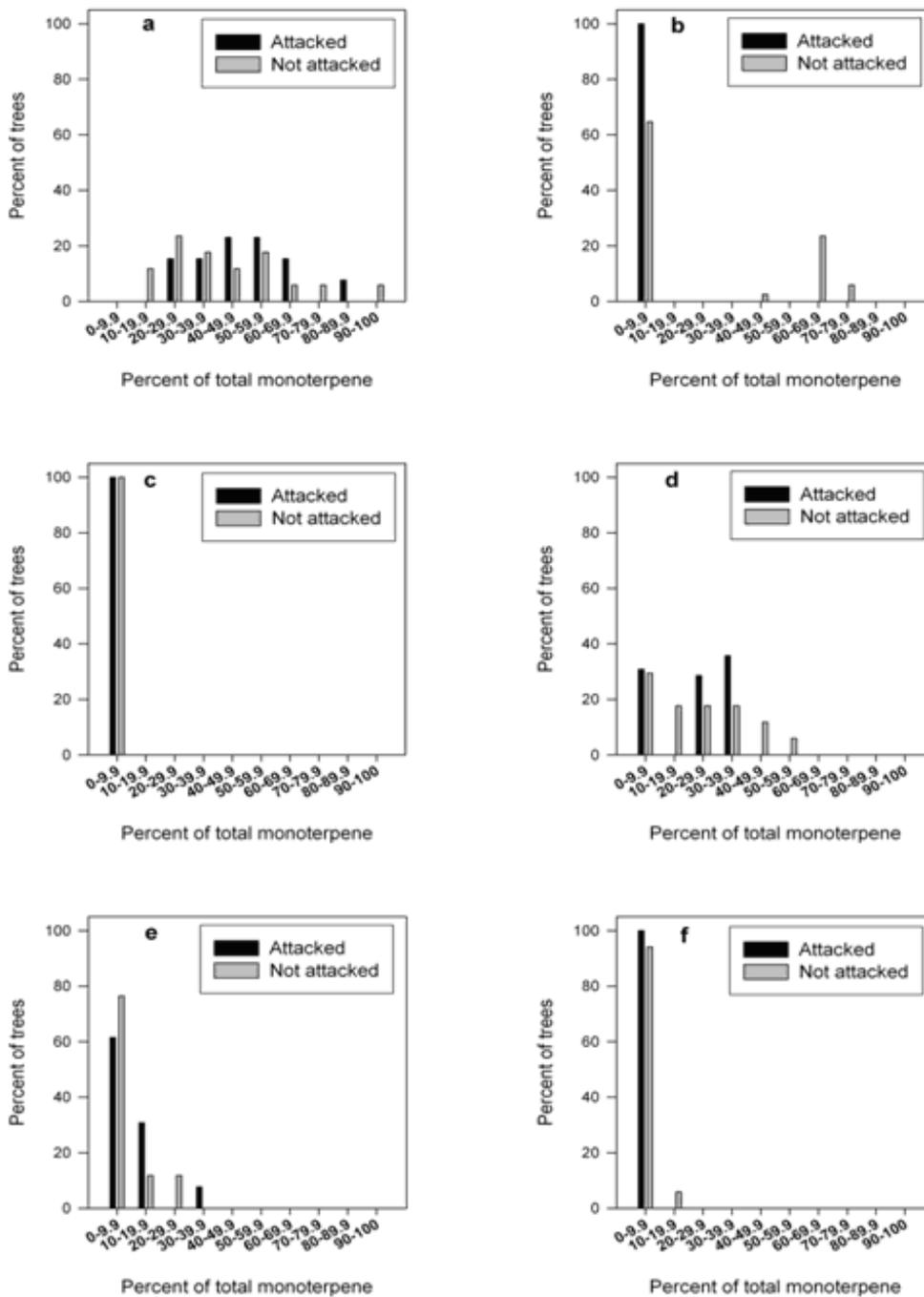
**Figure 1.** Average percent of individual monoterpenes in the total monoterpene content of *D. adjunctus* attacked and unattacked ponderosa pine trees in northern Arizona. Error bars represent 95 percent confidence intervals and asterisks signify significant differences ( $p < 0.05$ ; see text for details).

Attacked trees contained significantly higher percentages of  $\alpha$ -pinene, myrcene, and limonene, but lower levels of  $\beta$ -pinene when compared to unattacked trees (Figure 1).

Although  $\beta$ -pinene showed a significant difference between attacked and unattacked trees, high levels of  $\beta$ -pinene were not found in all of the unattacked trees (Figure 2b). Amount of individual monoterpenes as a percentage of the total monoterpene content varied between attacked and unattacked trees for most analyzed monoterpenes (Figure 2).

## Discussion

Our study demonstrated that there are significant differences in monoterpene composition between bark beetle attacked and non-attacked trees. Contrary to past studies on other bark beetle species that found high levels of myrcene (Byers 1995) and limonene (Sturgeon 1979) in unattacked trees, half of our unattacked trees showed high levels of  $\beta$ -pinene. Differences between the monoterpene composition of unattacked trees in our study compared with previous studies (Smith 1966, Byers 1995, Sturgeon 1979) may be explained by geographic variation (Byers 1995, Hofstetter et al. 2008). Monoterpenes vary widely between geographic regions and among trees within local populations of ponderosa pine (Smith 1966, Latta et al. 2000, Thoss and Byers 2006, Hofstetter et al. 2008). Thus, those monoterpenes shown to be important in host selection for a bark beetle species in one geographic region might not be attractive to populations of the same species in another region (Byers 1995, Hofstetter et al. 2008).



**Figure 2.** Individual monoterpene content as a percent of total monoterpene found in *D. adjunctus* attacked and unattacked ponderosa pine trees in northern Arizona: a.  $\alpha$ -pinene; b.  $\beta$ -pinene; c. myrcene; d. 3-carene; e. limonene and f. longifolene.

Genetic differences among beetles from different geographic regions may reflect the variation in the monoterpene composition of their host as well (Byers 1995, Hofstetter et al. 2008). Bark beetle populations may be adapted to monoterpene ratios specific to geographic region (Byers 1995, Hofstetter et al. 2008). As a result, total monoterpene composition or certain ratios may be more important in determining host repellency than individual monoterpenes such as limonene (Byers 1995).

Attraction of *D. adjunctus* to host tree compounds, including monoterpenes, has not been previously studied (Byers 1995). Therefore, whether *D. adjunctus* prefers or is repelled by trees with specific monoterpene composition is not known. Our results suggest that *D. adjunctus*, or at least this particular local population of *D. adjunctus*, may be attracted to or repelled from certain host tree compounds. Additional research has yet to be completed, including a baiting study to ensure that the resin composition of the trees analyzed was not induced by *D. adjunctus* attack.

## Acknowledgments

This research was supported, in part, by McIntire-Stennis appropriations to NAU and the State of Arizona. Resin analyses were paid for by U.S.D.A. Cooperative Agreement 06-CA-11330129-046 (to R.W.H) and completed by the Analytical Chemical Laboratory at Northern Arizona University. Barbara Bentz, Joel McMillin and José Negrón provided beneficial reviews of the draft manuscript.

## References

- Byers, John A. 1995. Host chemistry affecting colonization in bark beetles. In: Carde, T.; Bell, William J. Chemical ecology of insects 2. New York: Chapman & Hall: 154-213.
- Chansler, John F. 1967. Biology and life history of *Dendroctonus adjunctus* (Coleoptera: Scolytidae). *Annals of the Entomological Society of America*. 60: 760-767.
- Christiansen, Erik; Waring, Richard H.; Berryman, Alan A. 1987. Resistance of conifers to bark beetle attack: Searching for general relationships. *Forest Ecology and Management*. 22(1-2): 89-106.
- El-Sayed, Ashraf M.; Byers, John A. 2000. Inhibitory effect of monoterpenes on response of *Pityogenes bidentatus* to aggregation pheromone released by piezoelectric sprayer for precision release of semiochemicals. *Journal of Chemical Ecology*. 26: 8.
- Fettig, Christopher J.; Klepzig, Kier D.; Billings, Ronald F.; Munson, A. Steven; Nebeker, T. Evan; Negrón, José F.; Nowak, John T. 2007. The effectiveness of vegetation management practices for prevention and control of bark beetle infestations in coniferous forests of the western and southern United States. *Forest Ecology and Management*. 238: 24-53.
- Hofstetter R. W.; Chen, Z.; Gaylord, M. L.; McMillin, J. D.; Wagner, M. R. 2008. Synergistic effects of  $\alpha$ -pinene and exo-brevicomin on pine bark beetles and associated insects of Arizona. *Journal of Applied Entomology*. 132: 387-397.
- Miller, J. M.; Keen, F. P. 1960. Biology and control of the western pine beetle. A summary of the first fifty years of research. Washington D.C.: U.S. Department of Agriculture; 381 p.
- Nebeker, T. E.; Hodges, J. D.; Blanche, C. A. 1993. Host response to bark beetle and pathogen colonization. In: Scholwalter, T. D.; Filip, G. M., eds. Beetle-pathogen interactions in conifer forests. New York: Academic Press: 157-169.

- Negrón, José. 1997. Estimating probabilities of infestation and extent of damage by the roundheaded pine beetle in ponderosa pine in the Sacramento Mountains, New Mexico. *Canadian Journal of Forest Research*. 27: 1936-1945.
- Negrón, José; Wilson, Jill L.; Anhold, John A. 2000. Stand conditions associated with roundheaded pine beetle (Coleoptera: Scolytidae) infestations in Arizona and Utah. *Environmental Entomology*. 29(1): 20-7.
- Smith, R. H. 1966. The monoterpene composition of ponderosa pine xylem resin and of *D. brevicomis* pitch tubes. *Forest Science*. 12: 1.
- Smith, R. H. 1975. Formula for describing the effect of insect and host tree factors on resistance to western pine beetle attack. *Journal of Economic Entomology*. 68: 841-844.
- Sturgeon, Kareen B. 1979. Monoterpene variation in ponderosa pine xylem resin related to western pine beetle predation. *Evolution*. 33: 803-814.
- Thoss, Vera; Byers, John A. 2006. Monoterpene chemodiversity of ponderosa pine in relation to herbivory and bark beetle colonization. *Chemoecology*. 16: 51-58.
- Wood, D. L. 1982. The role of pheromones, kairomones, and allomones in the host selection and colonization behavior of bark beetles. *Annual Review of Entomology*. 27: 411-446.

---

The content of this paper reflects the views of the author(s), who are responsible for the facts and accuracy of the information presented herein.