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CHANGES IN HEARTWOOD CHEMISTRY OF DEAD YELLOW-CEDAR TREES THAT REMAIN STANDING FOR 80 YEARS OR MORE IN SOUTHEAST ALASKA

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Abstract—We measured the concentrations of extractable bioactive compounds in heartwood of live yellow-cedar (*Chamaecyparis nootkatensis*) trees and five classes of standing snags (1–5, averaging 4, 14, 26, 51, and 81 years-since-death, respectively) to determine how the concentrations changed in the slowly deteriorating snags. Three individuals from each of these six condition classes were sampled at four sites spanning a 260-km distance across southeast Alaska, and the influence of geographic location on heartwood chemistry was evaluated. Cores of heartwood were collected at breast height and cut into consecutive 5-cm segments starting at the pith. Each segment was extracted with ethyl acetate and analyzed by gas chromatography. Concentrations of carvacrol, nootkatene, nootkatol, nootkatone, nootkatin, and total extractives (a sum of 16 compounds) for the inner (0–5 cm from pith), middle (5–10 cm from pith), and surface (outer 1.1–6.0 cm of heartwood) segments from each core were compared within each tree condition class and within segments across condition classes. Heartwood of class 1 and 2 snags had the same chemical composition as live trees. The first concentration changes begin to appear in class 3 snags, which coincides with greater heartwood exposure to the external environment as decaying sapwood sloughs away, after losing the protective outer bark. Within core segments, the concen-

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trations of all compounds, except nootkatene, decrease between snag classes 2 and 5, resulting in the heartwood of class 5 snags having the lowest quantities of bioactive compounds, although not different from the amounts in class 4 snags. This decline in chemical defense is consistent with heartwood of class 5 snags being less decay-resistant than heartwood of live trees, as observed by others. The unique heartwood chemistry of yellow cedar and the slow way it is altered after death allow dead trees to remain standing for up to a century with a profound impact on the ecology of forests in southeast Alaska where these trees are in decline.

Key Words—*Chamaecyparis nootkatensis*, snags, decay resistance, chemical defense, carvacrol, nootkatin, antifungal compounds.

INTRODUCTION

Yellow cedar, *Chamaecyparis nootkatensis* (D. Don) Spach, is a socially, ecologically, and economically valuable tree in southeast Alaska. Its wood is highly desired in foreign markets, especially Japan. Around 1880, yellow cedar growing near open bogs, or on semibog sites with poor drainage, began to die, initiating a forest decline that continues to this day (Hennon et al., 1990a). Circumstantial evidence suggests that this decline was triggered by climatic warming (Hennon and Shaw, 1994), with minimal involvement of biotic agents (Hennon, 1990; Hennon et al., 1990b,c,d). Interactions of site characteristics with maritime-continental climate patterns are associated with tree mortality (Hennon and Shaw, 1997) that has left standing dead trees (snags) on more than 200,000 ha widely spread across the landscape. Yellow-cedar trees can remain standing for up to a century after their death because of the heartwood's natural durability and superior strength. As a consequence of this slow deterioration and the progressive nature of the decline, standing snags accumulate on affected sites and can average about 65% of the yellow-cedar basal area (Hennon et al., 1990c). Although live trees are commercially valuable, snags have been used primarily for firewood. With snags scattered over large distances and with limited road access, their commercial salvage is not economically justified unless their wood properties are comparable with those of live trees.

In an attempt to develop products with greater value, investigators compare the various heartwood characteristics of snags with those of live yellow-cedar trees. Snags can be visually sorted into six classes with increasing mean time-since-death of 4, 14, 26, 51, and 81 years, respectively (class 6 not dated; Hennon et al., 1990c) by evaluating their retention of foliage, twigs, and branches. By class 6, the heartwood is decaying and the bole is broken, often close to the ground. Mechanical properties of snag heartwood do not change much through class 5 (McDonald et al., 1997; Green et al., 2002), but there is a modest reduction in recoverable wood and grade from snags in classes 4 and 5,

compared with live trees and younger snags (Hennon et al., 2000). Decay resistance of heartwood from class 5 snags is adequate for products used above ground, but may be less durable than the heartwood from live trees and younger snags when in contact with soil (DeGroot et al., 2000).

Heartwood durability and decay resistance in yellow cedar have been attributed to the tropolone, nootkatin, that inhibits growth of various wood decay fungi (Rennerfelt and Nacht, 1955). Reducing its concentration in the heartwood decreases the wood's resistance to decay (Smith, 1970; Smith and Cserjesi, 1970). Carvacrol is another major component in yellow-cedar heartwood with antifungal properties (Voda et al., 2003). Because the heartwood of class 5 snags is less resistant to decay than heartwood from live trees and younger snags, it is likely to have lower concentrations of one or more of these bioactive compounds. The objective of this study was to measure the concentrations of carvacrol, nootkatin, and other extractable constituents in heartwood of live yellow-cedar trees and the five classes of snags to determine how they changed over the course of 80 years or more, in the slowly deteriorating, standing dead trees. Live trees and snags were sampled at each of four sites spanning a 260-km distance across southeast Alaska to evaluate whether geographic location had an influence on heartwood chemistry.

METHODS AND MATERIALS

Study Sites. Live and dead yellow-cedar trees were sampled at four locations in southeast Alaska (Figure 1): Goose Cove, Baranof Island (57°30′35N, 135°30′48W); Halibut Point near Sitka, Baranof Island (57°05′26N, 135°22′43W); Nemo Point, Wrangell Island (56°16′58N, 132°19′38W); and Sal Creek, Prince of Wales Island (55°48′41N, 132°32′13W). Each of these locations represents forests typical of yellow-cedar decline.

Tree and Snag Selection. Three dead trees from each of the five snag classes and three live trees were selected for sampling at each of the four study sites, yielding a total of 72. Snag classes were identified by their retention of dead foliage, twigs, and secondary or primary branches as described by Hennon et al. (1990c). Diameters of each bole were measured at breast height (dbh at 1.4 m) on the uphill side. All trees and snags sampled at a site were in close proximity to one another; most were separated by less than 100 m and none more than 200 m apart. They were sampled as encountered and discarded as unacceptable if decay prevented the removal of a continuous heartwood core to the pith.

Heartwood Samples. Two cores of heartwood extending to the center of the live tree or snag were removed with an increment borer (5.0 mm i.d.) on opposite sides of the bole at breast height. The boundary between sapwood and

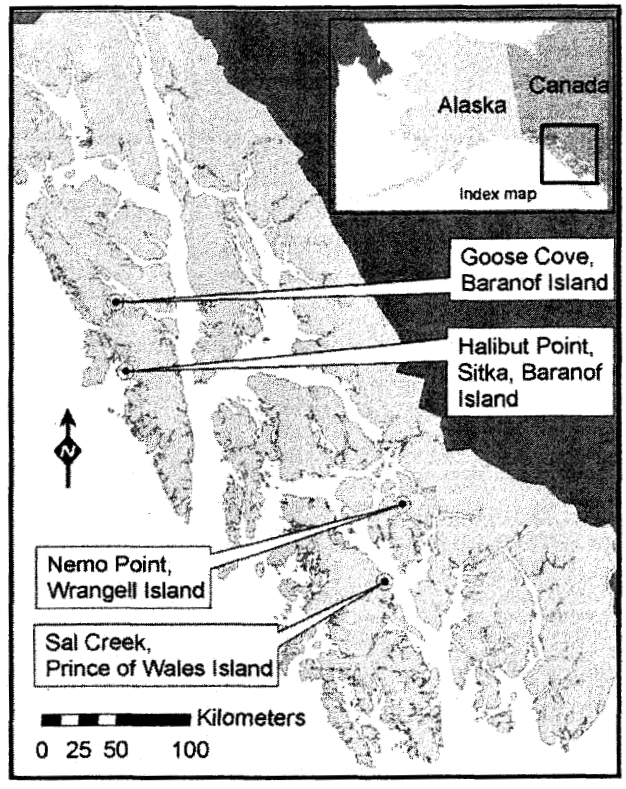


FIG. 1. A map showing the four sampling sites of yellow cedar in southeast Alaska.

heartwood was marked on cores from live trees and snags from classes 1 to 3 to assist the removal of sapwood in the laboratory. The pale yellow heartwood of yellow cedar is distinguishable from the white sapwood of live trees and black-stained or brown decayed sapwood of dead trees. Residual sapwood on class 4 snags was minimal (mean of 2–3 mm; Hennon et al., 2000) and readily detectable without marking. There was no sapwood remaining on class 5 snags. Each core was sealed in a plastic straw with tape (two straws for longer cores), placed inside a sealed plastic bag, and stored on ice in the field. Upon returning to the laboratory, cores were frozen at −36°C until analyzed.

Solvent Extraction. One increment core from each live tree or snag was removed from the freezer in random order and processed for extraction in a cold room at 7 ± 1°C. Cores were cut into consecutive 5.0-cm segments starting at the pith and progressing outward. The outermost heartwood segment was cut at the sapwood boundary (sapwood discarded) or terminated at the bole surface for

some class 4 and all class 5 snags. The two innermost segments from 0 to 5 and 5 to 10 cm from the pith are referred to as the inner and middle segments, respectively. The outermost or newest heartwood is referred to as the surface segment because it included the outer layer of heartwood left exposed after the sapwood had weathered away in class 4 and 5 snags. This surface segment varied in length. When greater than 1.0 cm, it was processed and extracted separately, but when 1.0 cm or less, it was combined with the previous segment yielding lengths ranging from just over 1.0 cm (11 surface segments between 1.0 and 1.9 cm) up to 6.0 cm. This resulted in seven small diameter individuals (one live tree, one class 1, two class 3, and three class 4 snags) having only two segments (inner and surface).

Each segment was sliced into disks 0.5–1.0 mm in thickness with a razor blade. The bulk of each sample was transferred to a preweighed vial (15-ml vol.) sealed with a Teflon®-lined screw cap for extraction. A small subsample was sealed in a preweighed 4-ml vial to measure the tissue water content. Both vials were adjusted to room temperature before recording tissue fresh weight. Four milliliters of ethyl acetate (Fisher Scientific, HPLC grade) were added to vials containing 3- to 6-cm length segments, whereas vials containing 2- to 3- or 1- to 2-cm segments were extracted with 2.0 and 1.0 ml of solvent, respectively. They were allowed to soak at room temperature (22 ± 1°C) for 7 d in the dark. Each extract was transferred by pipette into a 4-ml sealed vial containing anhydrous sodium sulfate (Mallinckrodt reagent grade, 0.68, 0.34, and 0.17 g/ 4-, 2-, and 1-ml solvent, respectively) to remove excess water. These were stored in a dark cold room until further processed for analysis. Just prior to analysis, 900 µl of room temperature extract were transferred into a 1.5-ml autosampler vial followed by 100 µl of ethyl acetate containing *R*-(+)-limonene (Aldrich, 99.7% purity) as the internal standard. Subsamples for water content measurements were dried at 102°C for 16 hr, then cooled to room temperature in a desiccator box before weighing.

Chromatography. Extracts were analyzed by gas chromatography using a Hewlett Packard 5890 Series II gas chromatograph (GC) equipped with a 6890 autosampler, flame ionization detector, and J&W Scientific DB-5 column (30 m × 0.25 mm, 0.25-µm film thickness). Helium was the carrier gas set at 1.0 ml/min flow through the column at the initial 100°C column temperature, with a split ratio of 1:10. The column oven was held for 1.0 min at 100°C, then increased to 150°C at 5°C/min, and then to 220°C at 3°C/min with no final temperature hold. Two microliters of extract were injected. Compound concentrations were calculated from a six-level standard curve of carvacrol (Sigma, 99%), with a limonene (Aldrich, 99.7%) internal standard at the same concentration as added to the extracts. All compound concentrations were calculated using the same response factor as carvacrol and normalized per gram dry weight using tissue water content measurements. Preliminary analysis indicated

that the response factors for carvacrol and nootkatin differed by less than 5%. Compounds were identified by GC–mass spectrometry using the same GC with an HP 5970 mass selective detector and a Phenomenex ZB-5 column (equivalent to the DB-5), with the oven conditions as above. Compounds were identified by comparison of their spectra with those in the spectral library or spectra obtained from authentic samples analyzed with this instrument.

Statistical Analyses. This sampling scheme is considered a balanced generalized randomized block because in all four blocks (sites), each of the six tree condition classes (live trees plus five snag classes) or treatments was independently received by three experimental units (trees or snags; Steele et al., 1997). This design allowed us to test whether any block (site) × tree condition class interactions were apparent. Each tree was repeatedly measured, once in each of the three heartwood core segments. Pearson's correlation coefficients among concentration estimates for the heartwood compounds provided information regarding the independence or correlation among these responses.

To assess whether there were systematic differences in dbh among snag age classes, the following one-way ANOVA model was fit to the data using SAS v8.2 PROC Mixed,

$$Y_{ijk} = m + \beta_i + a_j + \alpha_{ij} + \lambda_{ijk}$$

where Y_{ijk} is the dbh of the kth tree in the jth tree condition class in the ith block; m is the overall mean dbh for all trees; β_i is the random effect of block i ($i = 1, 2, \ldots, 4$); a_j is the effect of the jth tree condition class (j = live tree, snag class $1, \ldots, 5$); α_{ij} is the random error term that represents variability among sets of k trees ($k = 1, 2, 3$) from the same tree condition class among the blocks and is the error term used to test for condition class effect, $\alpha_{ij} \sim N(0, \sigma_\alpha^2)$; and λ_{ijk} is the random error term that represents variability among the k trees of the same tree condition class within the blocks, $\lambda_{ijk} \sim N(0, \sigma_\lambda^2)$, and is the error term used to test for site × tree condition class interaction.

The above model was expanded to model the concentration responses for the compounds described below using SAS v8.2 PROC Mixed (SAS Institute Inc., 1999),

$$Y_{ijkl} = m + \beta_i + a_j + \alpha_{ij} + \lambda_{ijk} + c_l + ac_{jl} + \varepsilon_{ijkl}$$

where Y_{ijkl} is the concentration of the compound of interest in the lth core segment of the kth tree in the jth tree condition class in the ith block; m, β_i, a_j, α_{ij}, and λ_{ijk} are defined as above; c_l is the effect of the lth core segment (l = inner, middle, surface); ac_{jl} is the interaction effect of the jth level of tree condition class and the lth core segment; and ε_{ijkl} is the random error term that

represents variability among core segments and is the error term used to test for effects of core segment and tree condition class by core segment interaction,

$$\varepsilon_{ijk} \sim \text{MVN}\left(\begin{pmatrix}0\\0\\0\end{pmatrix}, \begin{pmatrix}\sigma_{11}^2 & \sigma_{12}\sigma_{13} & \\ \sigma_{12} & \sigma_{22}^2 & \sigma_{23} \\ \sigma_{13} & \sigma_{23} & \sigma_{33}^2\end{pmatrix}\right)$$

and represents the covariance among core segments within a tree.

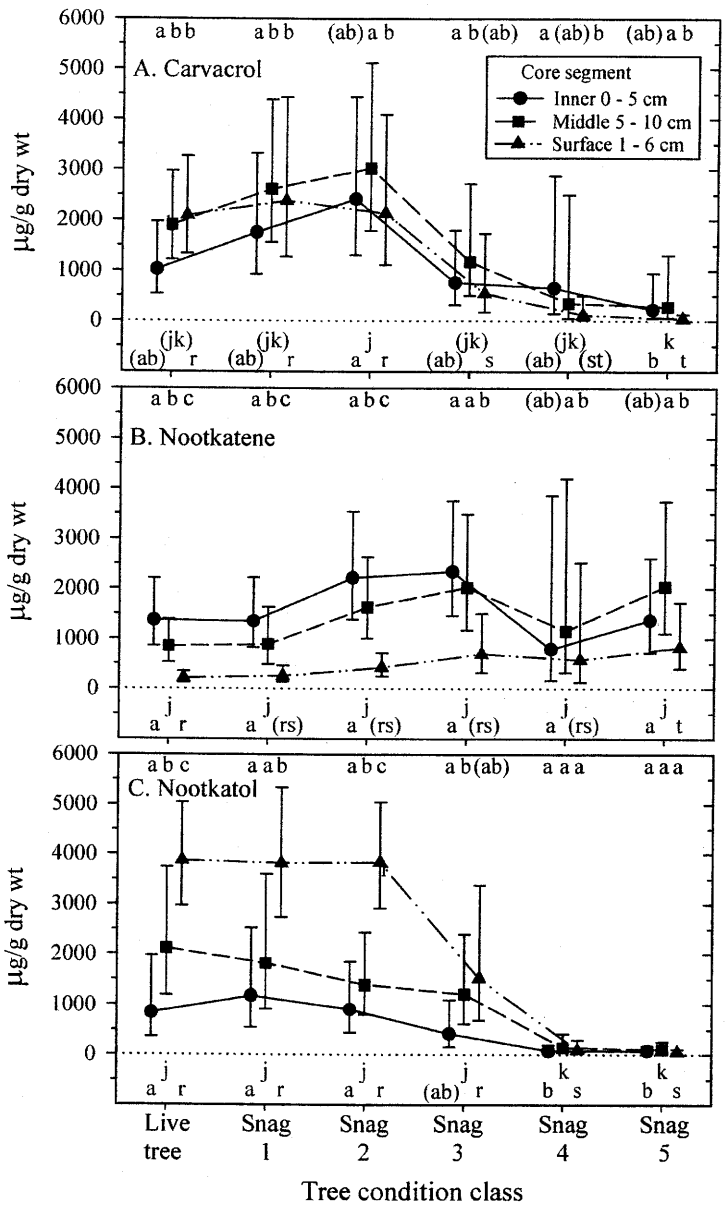
A heterogeneous variance model was fit allowing different estimates of the values of this covariance matrix for each tree condition class. The appropriate within-tree covariance structure was selected using the minimum Akaike's information criterion (AIC). All individual compounds had a fully unstructured covariance [UN(3) model] with unequal variance among core segments and unequal covariances between segments. The total extractives had a much simpler within-tree covariance model [TOEP(1) model] with equal variance among core segments and covariance among segments estimated to be 0. Each compound response was also modeled with dbh as a covariate.

Scatter plots of the initial data for each compound revealed a few samples with excessively high values (5–10 times the mean) that were removed as outliers. Homogeneity of variances and normality were evaluated prior to analysis by examining plots of the residuals (observed vs. predicted) and normal probability plots. All five individual compounds (but not the total) required natural logarithm transformation to meet these assumptions. For carvacrol, nootkatene, and nootkatol (all with interactions between core segments and tree condition class), the differences among means (on the log scale) of core segments within tree condition classes, and differences among tree condition classes within segments, were determined with pairwise comparisons using Bonferroni adjusted confidence intervals (CIs). For nootkatone and nootkatin (on the log scale), and the total extractives (all without interactions between core segments and tree condition classes), the differences among means of the tree condition classes were determined by pairwise comparisons using Tukey 95% CIs. Transformed means and their 95% confidence limits were back-transformed to medians for presentation.

An F statistic, treating block as a fixed effect, was calculated to assess effectiveness of blocking and site-specific differences in average response for all response variables, including dbh.

RESULTS

Sixteen compounds were selected for quantitation in the ethyl acetate extracts of yellow-cedar heartwood. Based on the results from the GC-MS analysis, including spectral comparisons with some authentic samples recently


A. Carvacrol
B. Nootkatene
C. Nootkatol
Core segment
Inner 0 - 5 cm
Middle 5 - 10 cm
Surface 1 - 6 cm
µg/g dry wt
6000
5000
4000
3000
2000
1000
0
Live tree
Snag 1
Snag 2
Snag 3
Snag 4
Snag 5
Tree condition class

isolated by Xioung (2000) or Khasawneh (2003), four compounds were identified as monoterpenes (3-carene, 4-terpineol, methyl carvacrol, and carvacrol), eight were sesquiterpenes (valencene, nootkatene, δ-cadinene, epi-nootkatol, nootkatol, valencene-13-ol, nootkatone, and valencene-11,12-diol), one was a tropolone (nootkatin), and three were unknown. Carvacrol, nootkatene, nootkatol, nootkatone, and nootkatin were selected for individual analysis (Figures 2 and 3) because of their biological activities, abundance, or chemical reactivity. A total extractives concentration using the combination of all 16 compounds was also analyzed (Figure 4). It is important to note that concentrations of the five individual compounds analyzed and the total extractives were related with one another as indicated by Pearson's correlation coefficients all greater than 0.62, except for nootkatene, whose coefficients with other compounds never exceeded 0.28. Differences in carvacrol, nootkatene, or nootkatol concentrations among core segments (Figure 2) were dependent on the tree condition class (significant interactions; Table 1), whereas concentrations of nootkatone and nootkatin (Figure 3) were dependent only on the tree condition class, with no differences among core segments within any of the condition classes (no interaction; Table 1).

Within live trees and class 1 snags, carvacrol concentrations in the inner core segments were significantly less than in the middle and surface segments (Figure 2A). This pattern began to reverse in class 2 snags resulting in surface core segments having lower concentrations than either the inner or middle segments, in all older snag classes. Within the surface core segments, carvacrol was significantly lower in class 5 snags than in live trees and all younger snags and significantly lower in class 4 than in live trees or class 1 and 2 snags. Concentrations of carvacrol within inner and middle core segments were significantly higher in class 2 snags than those in class 5.

Nootkatene concentrations within the middle and inner core segments were not different among any of the tree condition classes (Figure 2B). Quantities within the surface segments increased progressively starting in class 1, and by class 5, the concentrations were significantly higher than in live trees, with no other differences among classes. Within each tree condition class, the surface

FIG. 2. Median concentrations (±95% CIs) for the individual compounds carvacrol (A), nootkatene (B), and nootkatol (C) extracted with ethyl acetate from yellow-cedar heartwood of live trees and snags. Magnitudes of the CIs are indicative of the tree-to-tree variation about medians and are not accurate measures for identifying significant differences between medians. Within each tree condition class, the medians of core segments with similar letters above their symbols at the top of each graph are not significantly different. Within each core segment, the medians among tree condition classes with similar letters below their symbols at the bottom of each graph are not significantly different. In both instances, $\alpha = 0.05$.

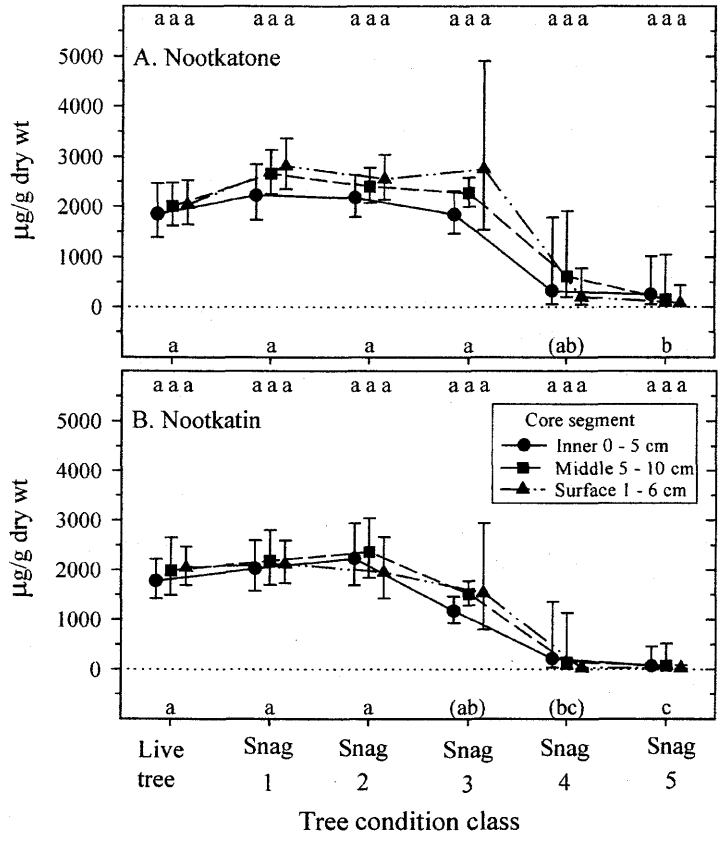


FIG. 3. Median concentrations (±95% CIs) for the individual compounds nootkatone (A) and nootkatin (B). The CIs and letters are the same as in Figure 2.

core segments contained significantly less nootkatene than the middle segments, and the inner segments in live trees and snags from classes 1 to 3. For live trees and class 1 or 2 snags, the inner core segments had significantly more nootkatene than the middle segments, with no differences between them in class 3–5 snags.

Nootkatol levels within live trees were highest in the surface core segments, followed by the middle and inner segments, with all differences being significant (Figure 2C). These relationships remained until class 3 when quantities generally declined, especially in the surface segments (but not significantly from levels in class 2). By class 4, nootkatol concentrations were the same in all core segments and significantly lower than in live trees and all previous snag classes. There were no differences in concentrations within segments or among segments for snags in classes 4 and 5.

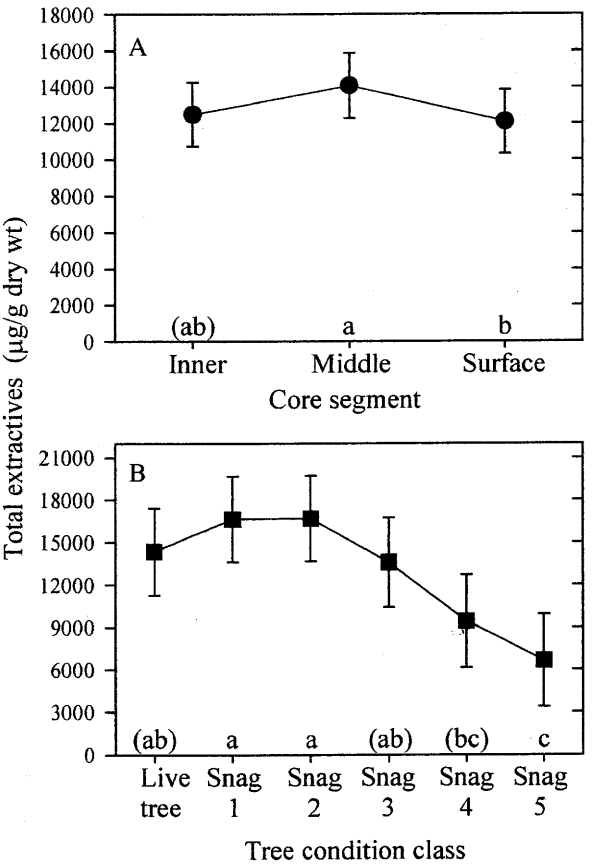


FIG. 4. Mean total extractives (±95% CIs) for 16 compounds removed with ethyl acetate from heartwood of live trees and snags of yellow cedar collected across southeast Alaska shown separately for core segments (A) and tree condition classes (B) because their interaction was not significant (Table 1). Means with the same letters at the bottom of the graph are not significantly different; $\alpha = 0.05$.

Nootkatone concentrations within all tree condition classes exhibited no significant differences among core segments (Figure 3A). Concentrations within segments remained unchanged from live trees to class 3 snags, followed by a nonsignificant decline in class 4. Class 5 snags had significantly less nootkatone than any of the more recent snags, except class 4.

Nootkatin exhibited a response similar to nootkatone, with no differences for core segments within any of the condition classes (Figure 3B). Within segments, the concentrations began to drop in class 3 rather than class 4.

TABLE 1. ANOVA RESULTS FOR EACH INDIVIDUAL COMPOUND AND THE TOTAL EXTRACTIVES FROM YELLOW-CEDAR HEARTWOOD

		Carvacrol		Nootkatene		Nootkatol		Nootkatone		Nootkatin		Total	
Factor in model	*df*[a]	*F*	*P*-value	*F*	*P*-value	*F*	*P*-value	*F*	*P*-value	*F*	*P*-value	*F*	*P*-value
Tree condition class (Tcc)	5, 15	6.51	0.002	2.30	0.097	22.58	<0.001	5.55	0.004	9.26	<0.001	8.75	<0.001
Core segment (Cs)	2, 173[b]	8.28	<0.001	103.64	<0.001	19.43	<0.001	1.41	0.246	2.87	0.059	4.68	0.011
Tcc × Cs	10, 173[b]	4.76	<0.001	4.32	<0.001	2.53	0.007	1.12	0.352	1.41	0.180	1.60	0.115

[a] Degrees of freedom for the numerator, denominator.
[b] Due to some missing values and removal of outliers, the denominator *df* is 172 for nootkatol, 171 for nootkatone and nootkatin, and 113 for the total extractives, which is much lower than for the individual compounds because of the different covariance structure used in the analysis.

Nootkatin concentrations in class 5 snags were significantly lower than all but class 4, and those in class 4 were lower than in live trees and class 1 or 2 snags.

Concentrations of the total extractives varied among core segments (Figure 4A), and among tree condition classes (Figure 4B), but without an interaction between them (Table 1). Middle core segments had significantly higher concentrations than the surface segments (Figure 4A). The class 4 snags had significantly lower amounts than class 1 and 2 snags, and the quantities in class 5 snags were lower than live trees, and all snag classes, except class 4 (Figure 4B).

The F statistic analysis showed that there were no differences among sites (all P values > 0.09) for the median concentrations of any compound or dbh. The analysis of variance (ANOVA) of compound concentrations with dbh as a covariate did not significantly reduce the variance, and the results were essentially unchanged from the ANOVA without dbh as a covariate. There was some evidence ($F_{5,15} = 2.57$, $P = 0.072$) that class 5 snags were smaller in diameter (averaging 25.6–33.2 cm dbh) than live trees or any of the other snag classes (averaging 31.2–41.3 cm dbh), with no differences among any of the latter.

DISCUSSION

Changes in compound concentrations in heartwood of yellow-cedar snags are associated with major physical changes of the boles as these standing dead trees deteriorate. Class 1 snags have intact outer bark and stained sapwood, whereas class 2 snags have sloughing bark and decaying sapwood. These changes, however, have minimal impact on heartwood chemistry, as the concentrations of most compounds in these snags remained the same as in live trees (Figures 2, 3, and 4B). By snag class 3, the outer bark is gone, the decaying sapwood is starting to slough away, and the heartwood surface begins to experience direct exposure to the external environment. This stage, some 25 years after tree death, marks the first substantial changes in heartwood chemistry. Because the outer heartwood is first exposed in class 3 snags, greater rates of chemical change might be expected to occur in the surface core segments compared with the middle and inner segments. This was observed, but only for nootkatol (Figure 2C). Tree to tree variation (as indicated by the larger confidence intervals) within the surface segments was greater in class 3 snags than in live trees or most younger snags for all individual compounds, except carvacrol. This probably reflects the variation in rates of sapwood decay and sloughing among class 3 snags, which results in different levels and durations of heartwood exposure to the environment. By snag class 4, all of the bark and most of the sapwood are gone from the bole, with checks or cracks penetrating about 3 cm deep into the heartwood (Hennon et al., 2002). Concentration changes that begin to appear in class 3 snags continue into class 4. Class 5 snags have no bark or sapwood

remaining and have deeper heartwood cracks (4 cm or greater) than in class 4. Heartwood of class 5 snags typically has lower concentrations of each compound (excluding nootkatene; Figure 2B) and total extractives than all younger snag classes, except class 4. Geographic location of the live trees and snags had minimal influence on their heartwood chemistry, probably because environmental parameters among the sites sampled in southeast Alaska were similar.

Potential mechanisms contributing to the concentration changes of heartwood components in aging yellow-cedar snags (Figures 2 Figures 3 Figures 4) are volatilization to the atmosphere, leaching, structural changes from reactions such as dehydration or oxidation, and possibly polymerization. As the heartwood surface becomes exposed to the elements, all of the compounds could be lost to some degree by volatilization, similar to the emissions of monoterpenes from the boles of lodgepole pine, *Pinus contorta murrayana* (Grev. and Balf.) Engelm. (Rhoades, 1990). Carvacrol is the most volatile of the individual compounds, and was likely impacted more by this mechanism than the other compounds, and partially responsible for the lower quantities measured in surface cores of class 4 and 5 snags with cracked and exposed heartwood. The forests of southeast Alaska are humid and wet (150- to 500-cm annual precipitation; Harris et al., 1974), so temperature and humidity both may function as the drivers of heartwood emissions to the atmosphere (Schade et al., 1999). Although these heartwood compounds all have limited water solubility, they could be gradually leached from the outermost layers of tissues exposed for many years to heavy rainfall.

Structural changes could be responsible for the decline of nootkatol, as a simple and facile dehydration reaction will yield nootkatene. Evidence supporting this rearrangement occurs in the heartwood of live trees, where the concentrations (transformed on the natural log scale) of these two compounds exhibit a nonlinear, inverse relationship (cubic polynomial; $R^2 = 0.567$). In addition, shifts in their relative concentrations appear to be associated with heartwood age. For example, surface core segments represent the youngest heartwood and contain 18.7 times more nootkatol than nootkatene (Figure 2B and C). In older, middle core heartwood, the concentrations of nootkatol are lower and the concentrations of nootkatene are higher than in surface core segments, resulting in nootkatol being only 2.5 times greater than nootkatene. The oldest heartwood from inner core segments of live trees contains the lowest nootkatol and highest nootkatene among the three segments, making the nootkatene 1.6 times more abundant than nootkatol. Loss of nootkatol from the surface cores is accelerated as the heartwood becomes more exposed between snag classes 2 and 4. Whereas nootkatene concentrations do increase in the surface core segments between these snag classes, the amount accumulated represents only a fraction of the nootkatol lost during the same period. Thus, nootkatol in exposed heartwood may be also changing to products other than

nootkatene (e.g., oxidation to nootkatone) or volatilizing to the atmosphere. Alternatively, nootkatene could be the initial rearrangement product of nootkatol, but is probably a transient intermediate because of the reactive conjugated double-bond system that is easily oxidized, thus minimizing the accumulation of nootkatene.

Longevity of yellow-cedar trees and the long persistence of standing snags in southeast Alaska are attributed to compounds in the heartwood functioning as a defense against disease and insects, with resistance to decay credited primarily to nootkatin, shown to have fungicidal activity at relatively low concentrations. This chemical defense also contributes to the durability of yellow-cedar wood used as lumber or other building materials (Rennerfelt and Nacht, 1955). Removal of nootkatin by the growth of a black-stain fungus, an organism that invades the wood of live trees and degrades this compound, dramatically decreased the resistance of the stained wood to decay fungi (Smith, 1970; Smith and Cserjesi, 1970). But other compounds also contribute to yellow-cedar durability. The antifungal properties of carvacrol were reported by Anderson in 1961 (cited in Barton, 1976), and it was one of the most active growth inhibitors of 22 compounds tested against the wood-decaying white rot, *Trametes versicolor*, and brown rot, *Coniophora puteana* (Voda et al., 2003). It also inhibits mycelial growth of various species of plant pathogens in the genus *Fusarium* (Thompson, 1996). Carvacrol is bactericidal toward a variety of human pathogens commonly found on meats, vegetables, rice, and dairy products (Ultee et al., 1999; Knowles and Roller, 2001; Friedman et al., 2002; Burt, 2004). Chamic acid and isochamic acid are two other monoterpenes that may contribute to decay resistance of yellow-cedar heartwood, but were not detected in our analysis because of their polarity. They have only 1/10th or less the activity of nootkatin (Rennerfelt and Nacht, 1955).

Extracts of yellow-cedar heartwood and some of the individual compounds are also biologically active toward a variety of invertebrates. Nootkatone repels and inhibits tunneling of Formosan termites, *Coptotermes formasanus* Shiraki (Maistrello et al., 2001; Zhu et al., 2001). Yellow-cedar fiber used in producing wall paneling was not eaten by eastern subterranean termites, *Reticulitermes flavipes* (Kollar), in free choice tests with five other panel wood products and pine sapwood as alternative choices (Kard and Mallette, 1997). However, in no-choice tests, the yellow-cedar fiber was severely damaged. Essential oil from yellow-cedar heartwood, nootkatone, carvacrol, valencene-13-ol, and nootkatol all have insecticidal and/or acaricidal activity toward various agricultural, stored products, or medicinal arthropod pests (Panella et al., 1997; Ahn et al., 1998; Khasawneh, 2003). Carvacrol also has nematicidal properties (Oka et al., 2000).

Loss of the antifungal compounds as dead trees deteriorate, as shown here, is most likely responsible for the reduced decay resistance of heartwood from class 5 yellow-cedar snags. In laboratory tests against the brown-rot fungus,

Gloeophyllum trabeum Pers. Ex. Fr., heartwood from class 5 snags lost significantly more weight (56.3%) from fungal decay than heartwood from live trees (21%) or class 3 snags (12%, not significantly different from live trees), when collected from individuals with a large diameter (425–535 mm or 17–21 in.) (DeGroot et al., 2000). A similar response was observed for smaller diameter snags (300–400 mm or 12–16 in.), but the average weight loss for class 5 heartwood (29.2%) was not significantly greater than heartwood from similarly sized live trees (15.9%). In a separate study of decay resistance, heartwood was placed in contact with soil for up to 4 years. As in the laboratory study described above, the wood from snag class 5 had significantly greater weight loss (i.e., decay) than wood from class 3 snags and live trees, which did not differ (Hennon, unpublished data). Furthermore, DeGroot et al. (2000) observed substantial tree-to-tree variation in decay resistance during laboratory bioassays, which probably results, in part, from the large tree-to-tree variation in concentrations of the antifungal compounds, as observed here. Because the composition and concentrations of chemicals in yellow-cedar heartwood protect it from fungal decay, it might be feasible to evaluate and rank the level of resistance for live trees or snags by quantifying the concentrations of nootkatin, carvacrol, and possibly other compounds. Development of an accurate predictive model for this purpose would require further testing of heartwood samples for decay resistance in conjunction with a quantification of their chemical constituents, as undertaken here.

Collectively, the studies of standing dead yellow-cedar trees suggest that changes in heartwood chemistry precede other major changes in wood properties. As demonstrated in this study, the heartwood constituents remain largely unchanged from live trees until snag class 3, about 25 years after tree death. Initiation of chemical changes corresponds with the class of snags experiencing increased heartwood exposure to external environmental parameters that can expedite these changes. Concentrations of most compounds, including those with antifungal activity, began to decline in class 3 snags. This gradual reduction in chemical defense continues as the snags age and subsequently leads to a decline in heartwood decay resistance. Whereas heartwood of class 5 snags is less decay-resistant than heartwood of live trees (DeGroot et al., 2000; Hennon, unpublished data), the strength properties of heartwood at the class 5 stage, some 80 years after death, remain unchanged from live trees (McDonald et al., 1997; Green et al., 2002). The unique heartwood chemistry and the slow way in which it is altered after tree death have profound ecological and economic implications. Dead yellow-cedar trees across southeast Alaska remain standing as snags for up to a century, and because the heartwood stays strong, hard, and undecayed, they probably offer little in the way of habitat for cavity-nesting animals. This limited deterioration and surprising persistence of wood properties also offer opportunities for recovering valuable wood products.

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