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A SIMPLE QUANTITATIVE METHOD FOR THE DETERMINATION
OF TERPENOID IN DOUGLAS-FIR SAPWOOD

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A novel analytical method requiring only a simple solvent extraction was developed for the quantitation of mono- and sesquiterpenoids in sapwood. Capillary gas chromatography with mass selective detection was used to identify and quantify terpenoids present in Douglas-fir (*Pseudo menziesii*) sapwood. Secondary metabolites such as terpenoids are considered to behave as feeding deterrents. This method was employed to analyze sapwood collected from Douglas-fir trees to assess the terpenoid content relative to the damage inflicted by Black bear (*Ursus americanus*). Bears often utilize tree sapwood as a source of forage in the spring when other sources of food are limited. Bear damage to trees causes mortality, delayed growth, and decay which results in significant economic losses and retards reforestation efforts.

Sapwood samples were collected from two 40 x 10 cm sites on mature trees, immediately frozen in liquid nitrogen in the field and maintained frozen. The samples were homogenized in plastic freezer bags with a rubber mallet. Three to four gram frozen sapwood subsamples were horizontally shaken in 50-mL screw-cap tubes for 10 minutes with 10.0 mL of ethyl acetate. Following centrifugation, 1 μ L aliquots of each extract were injected directly into the GC/MS and quantified versus external standards. A 5%-phenyl:95%-poly(dimethylsiloxane) gas capillary column (J&W Scientific DB-5) was used.

Composite sapwood samples were also subjected to lyophilization in order to eliminate the majority of the volatile terpenoids. The dried material was then rehydrated to yield "blank" sapwood and fortified to assess terpenoid recovery. Recovery of 23 different terpenoids generally exceeded 80% with good precision (approximately 15%).

To evaluate the sample handling procedures and demonstrate recovery from real samples, ten samples ranging from 50 to 80 grams were fortified in the field with 998 μ g of endoborneol. Excellent borneol recovery (89% \pm 10%) was observed.