

Chemical and nutritional status of dwarf mistletoe, Armillaria root rot, and Comandra blister rust infected trees which may influence tree susceptibility to bark beetle attack

T.E. Nebeker, R.F. Schmitz, R.A. Tisdale, and K.R. Hobson

Abstract: The terpenoid and phenyl propanoid content of xylem resin as well as phloem nitrogen and carbohydrate levels of lodgepole pine trees infected with Armillaria root disease, Comandra blister rust, and dwarf mistletoe and check (asymptomatic) trees were determined. Comparisons were made to determine if differences existed that might influence their susceptibility to bark beetle attack. These variables were also contrasted with respect to aspect (north and south). Five volatiles (tricyclene, α -pinene, camphene, γ -terpinene, and bornyl acetate) were significantly higher in trees with one or more diseases than in check trees. Four volatiles (myrcene, camphor, 4-allylanisole, and γ -terpineol) were significantly lower in diseased trees. Camphene was the only resin constituent found to differ with respect to aspect, with a higher concentration on the north aspect of check trees. There were no significant differences in carbohydrate or nitrogen content with respect to aspect. The check trees were found to have significantly higher starch, total nitrogen, and free amino-N contents than diseased trees. Trees infected with Comandra blister rust were found to contain lower levels of reducing and nonreducing sugars than the other diseased trees and the check trees. Changes in terpenoids and phenyl propanoids in trees susceptible to mountain pine beetle attack suggest a biochemical basis for host selection.

Key words: mountain pine beetle, lodgepole pine, Comandra blister rust, dwarf mistletoe, Armillaria root disease, resin chemistry, susceptibility.

Résumé : Les auteurs ont déterminé les teneurs en terpénoïdes et en phényles propanoïdes dans la résine du xylème ainsi que les teneurs en azote et en glucides du phloème chez des pins lodgepoles infectés par l'armillaire dans leurs racines ou portant la rouille du comandra, ou du gui nain, y inclus des arbres témoins (asymptomatiques). Les comparaisons ont été effectuées dans le but de vérifier s'il y a des différences qui pourraient influencer leur susceptibilité aux attaques par les insectes corticoles. Ces variables ont été également observées en fonction de l'exposition (nord et sud). Cinq substances volatiles (tricyclène, α -pinène, camphène, γ -terpinène et acétate de bornyle) sont significativement plus élevés dans les arbres qui portent une ou plusieurs des maladies, comparativement aux arbres témoins. Quatre substances volatiles (myrcène, camphre, 4-allylanisole et γ -terpinéol) sont significativement moins abondantes chez les arbres malades. Le camphène est le seul constituant de la résine qui diffère selon l'exposition, la teneur étant plus élevée du côté nord que du côté sud, chez les arbres témoins. Il n'y a pas de différences significatives dans les teneurs en glucides et en azote selon l'exposition. On observe des teneurs significativement plus grandes en amidon, en azote total et en azote d'acides aminés libres chez les arbres témoins, comparativement à ceux qui sont malades. Les arbres atteints de la rouille à comandra ont des teneurs moindres en sucres réducteurs et non-réducteurs que les arbres atteints d'autres maladies ou que les témoins. Les modifications en terpénoïdes et en phényles propanoïdes, chez les arbres susceptibles aux insectes corticoles, suggèrent qu'une base biochimique serait impliquée dans la sélection des hôtes.

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T.E. Nebeker and R.A. Tisdale. Department of Entomology and Plant Pathology, Mississippi State University, Mississippi State, MS 39762-9775, U.S.A.

R.F. Schmitz. Intermountain Forest and Range Experiment Station, Ogden Forestry Sciences Lab, 507 25th Street, Ogden, UT 84401, U.S.A.

K.R. Hobson. Department of Entomology, 345 Russell Labs, University of Wisconsin, Madison, WI 53706, U.S.A.

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Introduction

A considerable research effort has been expended on bark beetles that attack conifers. These research efforts have added immensely to our understanding of the bark beetles and their hosts. From a review of the literature (Blanche et al. 1983), hypotheses (Berryman 1972; Shrimpton 1978; Hodges et al. 1985) have been developed that suggest the importance of the secretory system of pine as a constitutive defense against bark beetle attack. The mechanisms controlling susceptibility to bark beetle attack are then associated, in part, with the host's resin system. It is essential that we understand the host's repertoire of chemical defenses and their relative abundances, which are contingent upon the genotypic and phenotypic vigor of the hosts, especially during endemic periods (Nebeker et al. 1984).

Shrimpton (1978) has described the resin duct system of lodgepole pine (LPP) *Pinus contorta* Dougl. var. *latifolia* Engelm. and the production of secondary resin associated with wounding. The overall process of resin duct formation and secondary resin formation increases to about age 50 but declines after culmination of mean annual stem volume growth (Shrimpton 1973a). Measures of the resin content of lodgepole pine in Utah at the time of mountain pine beetle flight revealed β -phellandrene was most abundant, followed by Δ -3-carene + myrcene + β -pinene group and α -pinene, respectively (Cole et al. 1981). Analysis of these data to establish possible links between tree characteristics and phloem constituents suggested that the monoterpene concentrations in the phloem would tend to increase with tree vigor and tree size up to diameters of about 33 cm. In contrast, Raffa and Berryman (1982) reported no relationship in LPP between resistance to mountain pine beetle (MPB), *Dendroctonus ponderosae* Hopkins, and the daily rate of resin flow, rate of resin crystallization, monoterpene content, monoterpene composition, or current growth rate. They state the major difference between trees which survived or died during exposure to naturally occurring high MPB populations was the extent of their active response to fungal invasion. This wound response is general in nature, quantitatively variable, metabolically active, rapid, and localized. It appears to form the major line of defense to MPB and its associated fungi, and to be related to the general vigor of the tree.

Monoterpenes are primary elements of resin, and intensive research has suggested an attractiveness of these compounds to bark beetles. Myrcene enhances the response of the female western pine beetle (WPB), *Dendroctonus brevicomis* Leconte, to host trees (Bedard et al. 1969; Silverstein 1970). α -Pinene has been observed to enhance the attractiveness of host trees to the southern pine beetle (SPB), *Dendroctonus frontalis* Zimmermann (Renwick and Vité 1969). Myrcene and α -pinene have also been documented as beetle pheromone precursors (Brand et al. 1975, 1976; Hughes 1974, 1975). Limonene, on the other hand, has been observed to be toxic to the SPB (Coyne and Lott 1976; Hodges et al.

1979) and WPB (Smith 1965, 1966, 1975; Sturgeon 1979). Intraspecific variation in monoterpene concentration and composition has also been observed by Rockwood (1973), Blight and McDonald (1964), Roberts (1970), and Smith (1964, 1977). Also, monoterpene composition may change as trees mature (Squillace 1976). Significantly higher amounts of myrcene and α -pinene were observed in older age-classes of loblolly pine (Schmitt et al. 1988), which may partially explain why SPB generally attacks the older age-classes.

Observations made during a 2-year study revealed that 36% of the initial *Dendroctonus* attacks on LPP occurred on the north aspect, 25% on the west, 21% on the east, and 18% on the south (Rasmussen 1974). Rasmussen also reported that Shepard (1965) found significant differences in total number of attacks per square foot among aspects; north aspects received the largest number, south aspects the smallest, and east and west aspects an intermediate number. Reid (1963) also found attack density greatest on the north aspect.

Tkacz and Schmitz (1986) found a significant and consistent association between infection by the root pathogen *Armillaria mellea* (Vahl. ex Fr.) Kummer, sensu lato, and the incidence of infestation by low population (endemic) levels of MPB. This is the first documentation of the association in LPP that may be an important factor affecting the dynamics of endemic level populations of the beetle. Rasmussen (1987) investigated the selection by mountain pine beetle of lodgepole pine infected with dwarf mistletoe (DMT) (*Arceuthobium americanum* Nutt. ex Engelm.) and Comandra blister rust (CBR) (*Cronartium comandrae* Pk.). He points out there was some evidence that MPB chose to attack trees with heavier infections of CBR. However, due to the high incidence of DMT in the areas examined, comparisons of beetle–DMT interactions were difficult. There are very few studies that report on associations of bark beetle populations and tree diseases, especially during endemic periods.

Nutritional status of trees is an important component of resistance to beetles as well as microorganisms. Lodgepole pine with lower nitrogen levels have been shown to be more susceptible to MPB attack than trees with sufficient levels of nitrogen (Waring and Pitman 1983). It has also been shown that carbohydrates are essential for the formation of the hypersensitive response (Christiansen and Ericsson 1986; Cook and Hain 1986).

It is critical that we continue to build upon our understanding of host–insect–microorganism interactions, with particular attention to potential hosts of bark beetle populations during endemic periods. Our interest here is comparing the resin composition and nutrient status in LPP with respect to aspect and disease status. Hence, our objective was to determine if the aromatic volatiles of LPP xylem resin, phloem nitrogen, and carbohydrate levels of trees infected with *A. mellea* (AM), DMT, and CBR differ from that present in apparently healthy (CK), asymptomatic trees.

Materials and methods

Study area

The area selected for this study was located on the north slope of the Uinta Mountains in northeastern Utah at an elevation of 2600 m. The area was between the Hayden Fork and Stillwater Fork of the Bear River, some 4 km south of the Bear River Ranger Station, which is approximately 48 km south of Evanston, Wyoming. The area consisted of primarily LPP with scattered quaking aspen (*Populus tremuloides* Michx.). Lodgepole pine selected for study had an average diameter of 24 cm, height of 20 m, and age of 95 years. Within the area the diseases of interest were also present.

Trees selected for sampling were separated into four groups (after Nebeker et al. 1995): (i) CK, (ii) infected with DMT, (iii) infected with CBR, and (iv) infected with AM. The six-class DMT rating system devised by Hawksworth (1977) was used to determine the degree of infection. CK trees were free of CBR and AM but did have light infections (rating of 3 or less) of DMT. Only trees with an infection rating of 6 were used in the DMT group. The eight-class rating system devised by Brown (1977) was used to classify the severity of infection by CBR. All degrees of infection (mostly classes 2–4) were used in the CBR group. Presence of AM was determined by examining the lower bole for evidence of basal bleeding. Upon completion of the study those trees suspected of AM infection were examined by digging at least three roots. Trees were considered infected if mycelial fans, rhizomorphs, and (or) resinosis were present. Those not exhibiting these symptoms were excluded from the data set for analysis. Experimental areas were selected so that all four categories of test trees were within the same area. In total 78 trees were selected, 21 DMT, 20 CBR, 18 AM, and 19 CK.

Resin composition

Resin from LPP was collected as described by Nebeker et al. (1995). As soon as approximately 1 mL of resin had accumulated in the pipette it was collected and placed in a 1-dram vial, evacuated with nitrogen, and placed on dry ice. Upon returning to the laboratory, the samples were placed in freezers ($\sim 0^{\circ}\text{C}$) until the monoterpene analysis was conducted. Resin samples were obtained with respect to aspect and disease status (DMT, CBR, or AM) along with samples from disease-free (asymptomatic) trees used as controls (CK).

The monoterpenes, sesquiterpenes, and oxygenated monoterpenes were analyzed as outlined by Blanche et al. (1985) using a 5880A Hewlett Packard gas chromatograph equipped with a flame ionization detector. A 2.44 m \times 2 mm (inside diameter) glass column was packed with 10% Carbowax 20M on 80/100 mesh acid-washed Chromosorb W. Samples were run under the following conditions: N_2 flow rate = 20 mL/min; H_2 flow rate = 40 mL/min; air flow rate = 250 mL/min; detector temperature = 200°C ; injection port temperature = 225°C ; oven temperature was programmed with initial temperature of 60°C for 5 min, increasing at the rate of 5°C per minute to the final temperature of 130°C and remained constant at that temperature for 12 min. Identities of the compounds were verified co-chromatographically using authentic terpenes. Quantities of terpenes are expressed

in milligrams per 100 mg of oleoresin using terpinene-4-ol as the internal standard.

Nutrient analysis

After resin flow and collection an area at breast height, on each aspect (north and south), of the outer bark was removed with a draw knife, exposing the primary phloem. A sample of the primary phloem was removed, placed in aluminum foil, labeled, and then placed on dry ice in a cooler. The samples were approximately 20 cm^2 . Samples were returned to the laboratory and placed in freezers until the laboratory analysis could be conducted. Prior to analysis the samples were removed from the freezer and ground in liquid nitrogen using mortar and pestle and lyophilized. The lyophilized samples were stored in desiccators at -16°C until chemically analyzed.

Total nitrogen was determined by digesting the sample in 20% sulfuric acid and heating until completely charred, then clearing it with 30% reagent-grade hydrogen peroxide. The cleared solution was neutralized with 4 M KOH prior to standard nesslerization. Absorbance was read at 490 nm. Quantification was based on a standard curve developed using ammonium sulfate.

Determination of total sugar (reducing and nonreducing sugars) was based on the Somogyi–Nelson procedure (Hodge and Hofreiter 1962). This procedure was slightly modified in that polyvinylpyrrolidone (PVPP) was used to clean the extract. Boiling 80% ethanol was used to extract the sugars from 50-mg samples. The extract was filtered with white ribbon filter paper No. 589 (Schleicher and Schuell, Inc., Keene, N.H.). The residue was recovered and saved for starch analysis. The filtrate was evaporated to near dryness, resuspended in 10 mL distilled water, stirred in 200 mg PVPP, further filtered with blue ribbon filter paper No. 589, and made to volume. An aliquot was assayed colorimetrically at 500 nm. Nonreducing sugar was determined through invertase digestion from a portion of the filtrate used for reducing sugar determination and then assayed as described above. Starch was analyzed from the residue saved after ethanol extraction. The residue was hydrolyzed by boiling in 0.1 M sulfuric acid for 1 h, filtered, and then assayed colorimetrically for reducing sugars. Details of the procedure are found in Smith et al. (1964). Accuracy of the procedure was checked using the phenol sulfuric acid method of Dubois et al. (1956).

The amino nitrogen fraction was determined from a portion of the reducing sugar extract (Rosen 1957). After color reaction with ninhydrin, the solution was diluted with isopropyl alcohol – water (1:1 v/v) and colorimetrically read at 570 nm. Quantification was based on a standard curve developed using aspartic acid.

Analysis

The data were analyzed using SPSS V4.0 procedures (SPSS Inc. 1990) for ANOVA and multiple range tests (LSD procedure). Level of significance was set at $\alpha = 0.05$. An arc-sine transformation was performed on all percentage data.

Results

The basic descriptors of the trees are contained in Table 1. There were no significant differences in the ages of the trees

Table 1. Summary of lodgepole pine components with respect to aspect and associated diseases on the north slope of the Uinta Mountains, south of Evanston, Wyoming, 1989.

Component	DMT	CBR	AM	CK
Age (years)	100.3a	95.2a	97.6a	95.0a
DBH (cm)	24.4a	22.4b	23.9ab	24.6a
Height (m)	17.6b	15.9c	17.6b	19.0a
Radial growth (mm/last 5 years)	3.4ab	2.9b	3.6ab	4.7a
Radial growth (mm/last 10 years)	6.1b	5.7b	6.3b	8.8a
Resin flow				
North (N) aspect (mL/2 h)	0.60a	0.46ab	0.33ab	0.20b
South (S) aspect (mL/2 h)	0.46ab	0.42ab	0.59a	0.11b
North aspect (mL/24 h)	2.37a	1.75a	1.58a	1.28a
South aspect (mL/24 h)	1.89a	1.69a	1.96a	0.96b
N+S (mL/2 h)	1.05a	0.82ab	1.02ab	0.31b
N+S (mL/24 h)	4.26a	3.24ab	3.79ab	2.24b
Tricyclene (mg/100 mg)				
North aspect	0.017a	0.013a	0.019a	0.007b
South aspect	0.016a	0.010bc	0.015ab	0.004c
α -Pinene (mg/100 mg)				
North aspect	0.729a	0.695a	0.592a	0.683a
South aspect	0.763a	0.671ab	0.709ab	0.527b
β -Pinene (mg/100 mg)				
North aspect	2.32a	2.59a	2.31a	0.57b
South aspect	2.25a	2.59a	1.93a	1.78a
Camphene (mg/100 mg)				
North aspect	0.142a	0.132a	0.124a	0.127a*
South aspect	0.145a	0.121ab	0.139a	0.093b*
Sabinene (mg/100 mg)				
North aspect	0.375a	0.350a	0.516a	0.386a
South aspect	0.636a	0.349a	0.466a	0.329a
Δ -3-Carene (mg/100 mg)				
North aspect	4.64a	5.31a	5.68a	4.80a
South aspect	4.29a	6.27a	6.53a	4.68a
Myrcene (mg/100 mg)				
North aspect	0.975ab	0.907b	0.948ab	1.480a
South aspect	0.982a	0.980a	1.020a	1.070a
α -Terpinene (mg/100 mg)				
North aspect	0.001b	0.010b	0.040a	0.011b
South aspect	0.012a	0.027a	0.016a	0.022a
β -Phellandrene (mg/100 mg)				
North aspect	17.26a	17.21a	17.01a	16.58a
South aspect	17.69a	15.32ab	16.14ab	14.46b
γ -Terpinene (mg/100 mg)				
North aspect	0.084b	0.110b	0.232a	0.078b
South aspect	0.083ab	0.113a	0.101a	0.050b
<i>p</i> -Cymene (mg/100 mg)				
North aspect	0.011a	0.015a	0.009a	0.008a
South aspect	0.013a	0.007a	0.009a	0.005a
Terpinolene (mg/100 mg)				
North aspect	0.464a	0.532a	0.422a	0.487a
South aspect	0.441a	0.647a	0.620a	0.496a

Table 1. (concluded).

Component	DMT	CBR	AM	CK
Camphor (mg/100 mg)				
North aspect	0.011 <i>b</i>	0.011 <i>b</i>	0.024 <i>ab</i>	0.053 <i>a</i>
South aspect	0.011 <i>b</i>	0.0278 <i>ab</i>	0.015 <i>ab</i>	0.038 <i>a</i>
Linalool (mg/100 mg)				
North aspect	0.017 <i>b</i>	0.015 <i>b</i>	0.057 <i>a</i>	0.023 <i>b</i>
South aspect	0.016 <i>a</i>	0.021 <i>a</i>	0.028 <i>a</i>	0.052 <i>a</i>
Longifolene (mg/100 mg)				
North aspect	0.001 <i>a</i>	0.009 <i>a</i>	0.000 <i>a</i>	0.004 <i>a</i>
South aspect	0.001 <i>a</i>	0.006 <i>a</i>	0.000 <i>a</i>	0.006 <i>a</i>
Bornyl acetate (mg/100 mg)				
North aspect	0.000 <i>b</i>	0.000 <i>b</i>	0.081 <i>a</i>	0.001 <i>b</i>
South aspect	0.000 <i>b</i>	0.000 <i>b</i>	0.015 <i>a</i>	0.004 <i>b</i>
4-Allylanisole (mg/100 mg)				
North aspect	0.235 <i>ab</i>	0.178 <i>ab</i>	0.120 <i>b</i>	0.319 <i>a</i>
South aspect	0.251 <i>a</i>	0.158 <i>ab</i>	0.102 <i>b</i>	0.271 <i>a</i>
α -Terpineol (mg/100 mg)				
North aspect	0.059 <i>a</i>	0.048 <i>ab</i>	0.043 <i>ab</i>	0.016 <i>b</i>
South aspect	0.069 <i>a</i>	0.047 <i>ab</i>	0.0412 <i>ab</i>	0.011 <i>b</i>
Nitrogen (μ g/100 mg)				
North aspect	747.88 <i>b</i>	812.16 <i>b</i>	932.47 <i>ab</i>	1011.54 <i>a</i>
South aspect	892.25 <i>a</i>	807.58 <i>a</i>	961.38 <i>a</i>	934.85 <i>a</i>
Amino nitrogen (μ g/100 mg)				
North aspect	78.69 <i>b</i>	75.65 <i>b</i>	77.66 <i>b</i>	85.50 <i>a</i>
South aspect	77.61 <i>b</i>	76.09 <i>b</i>	76.72 <i>b</i>	85.05 <i>a</i>
Sugars (mg/100 mg)				
Reducing				
North aspect	3.10 <i>ab</i>	2.61 <i>b</i>	2.80 <i>b</i>	3.58 <i>a</i>
South aspect	3.16 <i>ab</i>	2.63 <i>b</i>	3.16 <i>ab</i>	3.66 <i>a</i>
Nonreducing				
North aspect	3.24 <i>a</i>	2.12 <i>b</i>	3.11 <i>ab</i>	3.74 <i>a</i>
South aspect	3.20 <i>ab</i>	2.31 <i>b</i>	3.55 <i>a</i>	4.21 <i>a</i>
Starch (mg/100 mg)				
North aspect	21.48 <i>b</i>	21.86 <i>ab</i>	22.07 <i>ab</i>	23.40 <i>a</i>
South aspect	21.89 <i>b</i>	21.48 <i>b</i>	22.20 <i>b</i>	24.01 <i>a</i>

NOTE: Values within a row followed by the same letter are not significantly different ($p > 0.05$). *, significantly different with respect to aspect for the component ($p < 0.05$). DMT, dwarf mistletoe (*Arceuthobium americanum* Nutt. ex Engelm.); CBR, Comandra blister rust (*Cronartium comandrae* Pk.); AM, armillaria root rot (*Armillaria mellea* (Vahl. ex. Fr.) Kummer, sensu lato); CK, checks or controls, appearing to be disease free (asymptomatic).

sampled. There were differences in diameter and height, with the CKs being the tallest and largest in diameter. The DMT trees had consistently greater resin flow than the CKs (asymptomatic and apparently healthy trees).

Resin composition

The following volatiles were detected in lodgepole pine stem oleoresin: monoterpenes, oxygenated terpenes, a sesquiterpene (longifolene), and a phenylpropanoid (4-allylanisole). Table 1 contains the aspect comparisons. Table 2 combines aspect to increase the sample size for a closer look at the differences between four classes of trees. Since only camphene was found to be significantly different with respect to

aspect, the following discussion will concern the results presented in Table 2.

The monoterpene α -thujene, observed in lodgepole pine growing in northeastern Oregon (Raffa and Berryman 1982), was not detected in any of our samples. Shrimpton (1973b) did not find this monoterpene in his sample trees in British Columbia. Under the chromatographic conditions used, we were not able to detect limonene despite deliberate efforts in optimizing chromatographic conditions. The high concentration of β -phellandrene masked the limonene peak. When running the limonene and β -phellandrene standards the two would separate distinctly (retention times = 11.7 and 12.25, respectively) at low concentrations. However, when the

Table 2. Summary of selected lodgepole pine resin components with respect to associated diseases on the north slope of the Uinta Mountains, south of Evanston, Wyoming, 1989.

Component	DMT	CBR	AM	CK
Resin flow (mL/2 h)	0.47a	0.44a	0.53a	0.15b
Resin flow (mL/24 h)	1.79ab	1.72ab	2.13a	1.12b
β -Phellandrene (mg/100 mg)	17.491a	16.299a	16.477a	15.643a
Δ -3-Carene (mg/100 mg)	4.458a	5.781a	6.211a	4.752a
β -Pinene (mg/100 mg)	2.289ab	2.598a	1.413b	2.078ab
Myrcene (mg/100 mg)	0.979b	0.942b	0.994ab	1.302a
α -Pinene (mg/100 mg)	0.747a	0.684ab	0.664ab	0.614b
Terpinolene (mg/100 mg)	0.452a	0.587a	0.544a	0.491a
Sabinene (mg/100 mg)	0.512a	0.349a	0.485a	0.361a
4-Allylanisole (mg/100 mg)	0.243ab	0.168bc	0.109c	0.298a
Camphene (mg/100 mg)	0.144a	0.127ab	0.133a	0.112b
γ -Terpinene (mg/100 mg)	0.083b	0.111ab	0.151a	0.066b
Camphor (mg/100 mg)	0.011b	0.019b	0.018b	0.046a
Linalool (mg/100 mg)	0.016b	0.018ab	0.039a	0.036ab
α -Terpinene (mg/100 mg)	0.010b	0.018ab	0.025a	0.016ab
α -Terpineol (mg/100 mg)	0.064a	0.048a	0.042ab	0.014b
<i>p</i> -Cymene (mg/100 mg)	0.012a	0.011a	0.009a	0.007a
Tricyclene (mg/100 mg)	0.016a	0.011b	0.017a	0.066c
Longifolene (mg/100 mg)	0.001b	0.007a	0.000b	0.005ab
Bornyl acetate (mg/100 mg)	0.000b	0.000b	0.040a	0.002b

NOTE: Abbreviations as in Table 1. Values within a row followed by the same letter are not significantly different ($p > 0.05$).

Table 3. Summary of lodgepole pine nutrient components with respect to associated diseases on the north slope of the Uinta Mountains, south of Evanston, Wyoming, 1989.

Component	DMT	CBR	AM	CK
Nitrogen (μ g/100 mg)	820.07b	809.75b	949.34ab	974.24a
Amino nitrogen (μ g/100 mg)	78.15b	75.88b	77.11b	85.28a
Sugars (mg/100 mg)				
Reducing	3.13ab	2.62b	3.01b	3.62a
Nonreducing	3.22a	2.22b	3.39a	3.96a
Starch (mg/100 mg)	21.68b	21.66b	22.15b	23.70a

NOTE: Abbreviations as in Table 1. Values within a row followed by the same letter are not significantly different ($p > 0.05$).

proportion of β -phellandrene was increased to a level approximating its level in the oleoresin, the limonene peak fused into the β -phellandrene peak, thus precluding the detection and quantification of limonene and resulting in the overestimation of β -phellandrene.

Of the 12 monoterpenes found present in the oleoresin, three monoterpenes (tricyclene, α -pinene, and camphene) were generally lower in concentrations in CK trees than in the diseased trees, although one monoterpene (myrcene) was higher in concentration in the CK trees than in the diseased trees (Table 2). The concentration of the other monoterpenes did not vary between the CK and diseased trees.

Two oxygenated terpenes were found to be significantly different in concentrations between CK and diseased trees (Table 2). The camphor content was higher in CK trees than in the diseased trees, whereas α -terpineol content was lower in CK trees than in diseased trees. There was no difference in the level of the sesquiterpene longifolene between the CK

trees and the diseased trees. In contrast, the phenylpropanoid 4-allylanisole was significantly higher in CK trees than in diseased trees.

Phloem chemistry

No significant differences were detected with respect to aspect (Table 1). The CK trees had significantly higher starch, total nitrogen, and free amino-N contents than the diseased trees (Table 3). The CBR trees contained lower levels of reducing and nonreducing sugars than the CK, DMT, and AM trees. The CK trees contained higher levels of reducing sugars than the CBR and AM trees, and the DMT trees contained intermediate levels of reducing sugars.

Discussion

Terpenes (especially the monoterpenes) have been implicated as host resistance factors as well as bark beetle attractants.

Table 4. Response of *Dendroctonus ponderosae* to host monoterpenes.

	Pitman (1971)	Billings et al. (1976)	Conn et al. (1983)	Miller (1990)	Hobson et al.*
Myrcene	A	A	A	A	
β -Pinene	N	N	N	N	
Δ -3-Carene	N	N	N	A	
Limonene	N	N	N		
β -Phellandrene				A	
γ -Terpinene				A	
Terpinolene		N		R	
α -Pinene [†]	A(+)	N(-)	N(?)	N(?)	
4-Allylanisole					R
Camphene	A				

NOTE: A, attraction; N, no significant difference; R, repellence.

*K.R. Hobson, A.T. Whitehead, and R.A. Werner, unpublished data.

[†]Enantiomers tested: (+), (-), or unspecified (?).

Depending on the pest species each monoterpene may vary in its inhibitory capacity. Toxicity of the monoterpene components of southern pine oleoresin to SPB has been reported (Coyne and Lott 1976). Smith (1963) has, likewise, demonstrated the toxic properties of pine resin vapors to other species of *Dendroctonus*. The antibiotic or inhibitory properties of monoterpenes on the different species of bark beetle associated fungi have been demonstrated (Bridges 1987; Cobb et al. 1968; Raffa et al. 1985). Cobb et al. (1968) showed that myrcene was the most inhibitory to four species of *Ophistoma*, whereas Bridges (1987) found that 4-allylanisole, a compound not tested by Cobb et al., was the most inhibitory to all three symbiotic fungi associated with SPB. α -Pinene has been implicated as a bark beetle attractant and is known to synergize with insect-produced pheromone. α -Pinene has been hypothesized to be the most important monoterpene in the beetle's perception of a resistant host (Bordasch and Berryman 1977). Little is known about α -terpineol in relation to bark beetle associated fungi, but it has been known to serve as an attractant of some European bark beetles. Although these specific properties of individual monoterpenes are clear-cut, their functions when in combination with the other component monoterpenes have not been adequately addressed.

The response of MPB to host compounds has been investigated in field tests that combine host volatiles with beetle pheromones. When tested with *trans*-verbenol or with *trans*-verbenol and *exo*-brevicombin combined, beetle attraction is enhanced with myrcene. β -Pinene, Δ -3-carene, and limonene produced no significant effect in early studies (Pitman 1971; Billings et al. 1976; Conn et al. 1983); however, Miller (1990) in later dose-response studies did find attraction to Δ -3-carene. Miller (1990) also found attraction to β -phellandrene and γ -terpinolene. Pitman (1971) found attraction to camphene. Two compounds were found to be repellent or to interrupt MPB attraction in these studies. Terpinolene gave a moderate reduction in catch (Miller 1990) and 4-allylanisole (also known as methyl chavicol, estragol, tarragon, and by several other trivial names) gave a strong (68%) reduction (K.R. Hobson, A.T. Whitehead, and R.A. Werner, unpublished data). MPB response to α -pinene initially appeared to be variable. In Idaho stands of western

white pine, *Pinus monticola* Dougl. ex D. Don, MPB were attracted to α -pinene (Pitman 1971). In Washington stands of ponderosa pine and British Columbia stands of lodgepole pine, MPB showed no significant response to α -pinene (Billings et al. 1976; Conn et al. 1983). However, Pitman (1971) tested the *R*(+) optical isomer, the predominant isomer in western white pine. Billings et al. (1976) tested the *S*(-) enantiomer, the predominant enantiomer in ponderosa pine in the Cascades. Conn et al. (1983) and Miller (1990) do not specify the enantiomeric content of their α -pinene. The *S*(-) enantiomer is the predominant isomer in LPP in the areas where the tests were carried out (Pitman 1971). These results are summarized in Table 4.

MPB response to host compounds may be important in the host colonizing process at two points: (i) initial host selection, and (ii) aggregation on an attacked tree. All tests so far have shown significant response only when aggregation pheromones are also present. In this study it is significant to note that two of the three diseases included for comparison (CBR and AM) are the most common predisposing factors known in the Intermountain West for LPP attacked by MPB (Amman and Schmitz 1988; Tkacz and Schmitz 1986). DMT is not known to predispose trees to MPB attack.

Of the nine host compounds measured in this study with greater than 0.1 mg per 100 mg in healthy controls, one compound, 4-allylanisole, gave by far the greatest percent change from healthy to diseased trees: 43% decrease for CBR trees and 63% decrease for AM (Table 2). This compound is the strongest interruptant of MPB attraction tested so far for LPP volatiles (K.R. Hobson, A.T. Whitehead, and R.A. Werner, unpublished data). DMT trees that are not predisposed to MPB attack did not have significantly more or less 4-allylanisole than CK trees. The only other significant biochemical shift of the eight most abundant compounds measured in CBR and AM trees was myrcene, with a 28% decrease in CBR trees (AM trees were not significantly different). γ -Terpinene ranked tenth in abundance in CK trees and is attractive to MPB in field tests; γ -terpinene increased in CBR and AM trees that are predisposed to MPB attack. Compounds ranked 11th to 18th in abundance in CK trees either were not significantly different between treatments or have not been tested in the field. Of these, com-

pound 11 (camphor) perhaps offers the most promise as a factor in MPB host selection.

Nutrient levels were lower overall in the diseased trees. This may have more of an impact on tree resistance than on bark beetle and microorganism nutrition. It is well known that insects respond to nutrient-deficient hosts by feeding longer and ingesting more. Beetle associated fungi may not be impacted nutritionally by the low sugar levels because they release cellulolytic enzymes that convert cellulose to glucose (Mathre 1964; Shrimpton 1975). Thus, the diseased trees in this study would be more susceptible to attack by bark beetles as well as less able to defend themselves from the associated fungi.

Taken together, these results suggest that a biochemical basis exists for the process of MPB host selection with emphasis during endemic periods. This suggestion has not been tested here and will be difficult to test conclusively in the future because there are likely to be other factors that determine whether a host tree will be attacked. However, further studies of MPB response to host compounds and of the biochemical makeup of predisposed versus healthy trees can increase our understanding of the correlation observed here.

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