

Response of Lutz, Sitka, and White Spruce to Attack by *Dendroctonus rufipennis* (Coleoptera: Scolytidae) and Blue Stain Fungi

RICHARD A. WERNER AND BARBARA L. ILLMAN¹

Institute of Northern Forestry, Pacific Northwest Research Station,
Forest Service, USDA, Fairbanks, AK 99775-5500

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ABSTRACT Mechanical wounding and wounding plus inoculation with a blue-stain fungus, *Leptographium abietinum* (Peck), associated with the spruce beetle, *Dendroctonus rufipennis* (Kirby), caused an induced reaction zone or lesion around the wound sites in Lutz spruce, *Picea lutzii* Little, Sitka spruce, *P. sitchensis* (Bong.) Carr., and white spruce, *P. glauca* (Moench) Voss, in south-central Alaska. The effects of tree species on lesion length were nonsignificant; however, the effects of wounding versus wounding plus blue-stain inoculate were highly significant. Lesion length was significantly longer in high-flow Lutz spruce compared with low-flow trees that were wounded. There was a significant change in monoterpene composition in the induced reaction zones of wounded phloem compared with unwounded phloem. The total percentage of potential toxic monoterpenes such as limonene, myrcene, 3-carene, and beta phellandrene increased in all three host species. Egg gallery length and the area of phloem consumed by larvae outside of lesions was significantly less for trees with wounds caused by fungal inoculation compared with mechanical wounds only. Trees with fungal inoculations appeared to deter larval feeding. Hydroxystilbenes were not found in the three species of spruce; however, differences were found in the chemical content of the reaction and the nonreaction zones. Two unknown chemicals present in nonreaction zones were not found in the induced reaction zone. One chemical appears to be a dimer with a carbohydrate moiety. A low molecular weight chemical found in the induced reaction zone could not be identified by mass spectroscopy.

KEY WORDS *Dendroctonus rufipennis*, hypersensitive reaction, monoterpenes

RECURRENT OUTBREAKS OF the spruce beetle, *Dendroctonus rufipennis* (Kirby), have caused widespread mortality in stands of white spruce, *Picea glauca* (Moench) Voss, Sitka spruce, *P. sitchensis* (Bong.) Carr., and the hybrid Lutz spruce, *P. lutzii* Little, in south-central Alaska (USDA 1981, 1984, 1987; Werner et al. 1977). Lutz spruce is the preferred host of the spruce beetle, but brood development and survival are higher in white spruce (Holsten & Werner 1990). Sitka spruce extends along the coastal areas of Alaska, whereas white spruce ranges throughout interior; they hybridize in south-central Alaska where Lutz spruce is common on the Kenai Peninsula (Copes & Beckwith 1977).

Certain species of bark beetles transmit pathogenic fungi which can cause tree mortality. *Ophiostoma minus* H. & P. Sydow (= *Ceratocystis minor* (Hedgecock) Hunt) is frequently asso-

ciated with *Dendroctonus frontalis* Zimmerman attack of loblolly pine, *Pinus taeda* L., (Basham 1970, Bridges et al. 1985, Cook & Hain 1985). *Ceratocystis polonica* (Siemasko) C. Moreau and *Ophiostoma polonicum* Siemasko may be pathogenic to live Norway spruce, *Picea abies* (L.) Karst., (Hornvedt et al. 1983, Christiansen & Solheim 1990, Hornvedt & Solheim 1991). *Leptographium abietinum* (Peck) Wingfield, is commonly associated with bark beetle galleries (Kendrick 1962, Peck 1879) and has been isolated from spruce beetles in New Hampshire (Harrington 1987). In south-central Alaska, the spruce beetle is also associated with *L. abietinum*, which causes mortality of Lutz, Sitka, and white spruce when artificially inoculated or introduced into trees by the beetle (Reynolds 1992a). Safranyik et al. (1983) identified *Ophiostoma picea* (Munch) H. & P. Sydow, *O. piliferum* (Fries) H. & P. Sydow, *O. picea-perda* (Rumb.) von Arx, and *O. coeruleascens* (Munch) Nannf. as blue-stain associated with spruce beetles in British Columbia.

¹This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by USDA.

¹Forest Products Laboratory, USDA-FS, Madison, WI 53705-2398.

Table 1. Growth parameters ($\pm$ SEM) for three species of spruce trees

Tree species	Diameter, cm	Age, yr	Radial growth, mm/5 yr	Crown length, %
Lutz spruce	31.1 $\pm$ 6.4	96.1 $\pm$ 27.8	8.0 $\pm$ 2.9	80.9 $\pm$ 13.3
Sitka spruce	34.0 $\pm$ 5.4	153.1 $\pm$ 16.1	6.4 $\pm$ 2.3	61.3 $\pm$ 10.8
White spruce	28.5 $\pm$ 2.8	136.8 $\pm$ 37.4	7.2 $\pm$ 1.5	76.0 $\pm$ 11.4

The response of tree bark tissues to wounding or damage from biotic sources have been examined in gymnosperms (Mullick 1975, 1977; Mullick & Jensen 1973, 1976). An impermeable zone of tissue usually forms 2-4 wk after injury near the lesion margins by redifferentiation of healthy bark tissues (Mullick 1975). New periderm tissue forms after another 3-4 wk and seals the site of damage or injury (Mullick 1975, Mullick & Jensen 1973, 1976). This process can be altered if the lesion is caused by a pathogen that interferes with the dedifferentiation process (Barrett 1970, Wong & Berryman 1977).

Several species of conifers respond to wounding by forming an induced reaction zone around the wound. According to Shain (1979), reaction zones are necrotic tissues that are enriched with inhibitory extractives and their formation is related to a mechanism of host resistance (Shain 1967, 1971). The initial stimulus for the formation of reaction zones appears to be injury of nearby parenchyma cells caused by wounding or infection (Shain 1979). Reid et al. (1967) described the reaction zone as a rapid necrosis of cells surrounding the site of infection and accompanied by the development of traumatic resin ducts and increased concentrations of monoterpenes and phenolics. The accumulation of antifungal compounds (Kuc & Shain 1977) and the quantity of acetone-soluble extractives in trees resistant to the mountain pine beetle, *D. ponderosae* Hopkins (Raffa & Berryman 1983) found in reaction zones of lodgepole pine, *Pinus contorta* Douglas var. *latifolia* Engelm., provided evidence that this tissue constitutes a defense mechanism. Loblolly pine formed reaction zones in response to artificial wounding with and without the inoculation of fungi (Cook & Hain 1985, 1987, 1988). Grand fir, *Abies grandis* (Douglas) Lindley, also formed reaction zones when inoculated with fungal associates of the fir engraver, *Scolytus ventralis* LeConte (Berryman 1969). Changes in monoterpene content within the hypersensitive reaction zone compared with unwounded tissue have been reported in several species of Pinaceae (Reid et al. 1967, Shrimpton 1973, Russel & Berryman 1976, Raffa & Berryman 1983, Cook & Hain 1985, 1986, Paine 1984, Paine et al. 1985, Stephen & Paine 1985).

Wounding can also lead to the formation of compounds such as lignans, stilbenes, resin acids, tannins, and phenolic compounds. Some of these compounds formed in response to injury or

pathogenic attack have antimicrobial properties (Hart 1981, Hart et al. 1975; Bridges et al. 1985, Bridges 1987, Woodward & Pearce 1988a, b). Hart et al. (1975) reported that wounding of the phloem tissue of white spruce with an increment-borer had no effect on lignan concentrations in the reaction zone but increases were measured in acid pH, resin acids, and resistance to *Poria monticola* Murr. and *Coriolus versicolor* (L.) Quel.

The five objectives of this study were to (1) compare the hypersensitive reaction of Lutz, Sitka, and white spruce from mechanical wounding, wounding plus inoculation of blue-stain fungi, and wounding caused by beetle attack, (2) measure changes in monoterpene content, (3) measure differences in response of the three species of spruce to wounding as indicated by length of the reaction zone or lesion, (4) determine if stilbenes are formed in the reaction zone, and (5) compare the effect of wounding on egg gallery length and amount of phloem consumed by spruce beetles.

Materials and Methods

The field portion of this research was conducted from 1987-1990 in south-central Alaska near Soldotna, Seward, and Wasilla. Blue-stain isolates were collected from Lutz spruce near Cooper Landing, AK, that had been infested with spruce beetles after the trees were baited with frontaline. Isolates were cultured in the laboratory and tree plugs (2.5 cm in diameter) inoculated with fungal isolates as described by Reynolds (1992a).

Twenty dominant or codominant trees each of Lutz, Sitka, and white spruce were selected for inoculation of stands near Cooper Landing, Soldotna, Seward, and Wasilla, AK. These stands contained endemic populations of spruce beetle at the time of the study. Field data collected on each tree included dbh (diameter at breast height), age, radial growth at breast height for past 5 yr, and crown length (percentage of total tree height) (Table 1). Each tree was wounded the 3rd wk of May 1989 at a height of 137 cm on the bole by removing 10 circular bark plugs (2.5 cm diameter) spaced at equal distances from a band around the tree. Bark plugs were punched from the trees using specially built steel punches (2.5-cm i.d. by 15 cm long). Two treatments were randomly applied in May 1989 to the 10 wounds

and each tree received each treatment five times. Treatments consisted of wounds inoculated with *L. abietinum* and wounds without inoculation. Inoculated and noninoculated plugs were stapled in place. Each stand was baited with frontalinal plus alpha pinene to attract populations of spruce beetles into the stands.

Treatments were evaluated in late August 1989 by removing a 90-cm² rectangular section of bark centered on the site of the wound and extending 7.5 cm above and below the wound and 3 cm on either side. Bark samples consisted of the outer bark and phloem and were large enough to cover the entire reaction zone. The incidence of blue stain was recorded (Reynolds 1992a). The length of the lesion was measured from the uppermost to the lowermost points and average length calculated for each treatment. Samples of phloem from within the lesion area above and below the wound and outside the lesion area on each side of the wound were analyzed for monoterpene content. Each sample was extracted for 6 h in *n*-pentane before analysis by gas chromatography. A Hewlett Packard 588A with microprocessor using a multistage program was used. The initial temperature began at 65°C, then raised for 5 min by 4°C/min, then raised again by 7.5°C/min until final temperature of 240°C was reached. This temperature was maintained for 15 min. The percentage of the total extractable material was determined for alpha pinene, beta pinene, beta phellandrene, camphene, 3-carene, limonene, and myrcene.

Total egg gallery length (galleries excavated by parent adult beetles) and percent of phloem area consumed by larvae within and outside the lesion areas were measured from each 90-cm² bark sample.

Phloem samples from three trees were analyzed for stilbenes. Samples were air-dried for 48 h, ground to pass a 40-mesh screen on a Wiley mill and extracted with methanol for 72 h. Methanol extracts were derivatized by trimethylsilyl (TMS) as described by Hemingway et al. (1970) and analyzed by gas chromatography (Hewlett Packard Series 11-5890) and mass spectroscopy (Finnigan model 4500 with/SUPERINCOS data system). The TMS-stilbenes *trans*-4-hydroxystilbene and 4-hydroxy-diphenylmethane were used as standards because hydroxystilbenes have been found in the root bark of Sitka spruce (Woodward and Pearce 1988a, b).

The response of Lutz spruce to initial beetle attack was studied in conjunction with the wounding experiments. In May 1989, oleoresin flow was recorded in 40 codominant Lutz spruce for a 3 wk period during peak beetle flight. Trees were divided into two groups based on oleoresin flow; those with flow >2.5 ml/h and those with flow <1.5 ml/h. Both groups of trees were artificially infested with female beetles the following year. Oleoresin samples were collected in glass

vials and kept frozen until analysis for monoterpene content using the procedures described above. In May 1990, 10 trees from each group were artificially infested with female beetles. Beetles were collected from an epidemic population located near Cooper Landing, AK in early May from their overwintering sites beneath host bark at the root crown. Beetles were segregated by sex using characteristics of the seventh abdominal tergum (Lyon 1958) and stored at 5°C until needed. The bark of Lutz spruce was scored to the phloem at breast height with a no. 4 cork borer to provide entrance holes for the beetles. These entrance holes were located at four equal intervals around the circumference of the tree. Single female beetles were placed in each entrance hole which was sealed on the exterior with stainless steel insect screen.

Treatments were evaluated in late June following the normal period of beetle flight. Bark was removed, lesion length measured, and phloem samples collected as previously described from the reaction zone associated with beetle attacks. Phloem samples were placed in *n*-pentane and stored until analysis by gas chromatography.

Data within and among treatments were subjected to an ANOVA program from the CoStat (1986) statistical package. Tukey's studentized honestly significant difference [HSD] test (SAS Institute 1986) was used to test for significance ($P = 0.05$) among levels of significant main effects.

Results and Discussion

All three species of spruce responded with an induced reaction to mechanical wounding alone and wounding plus inoculation of blue-stain fungus. There were no differences in lesion length among tree species; however, the effects of wounding versus wounding plus inoculate were highly significant (Table 2). Other studies also

Table 2. Mean length of lesions from the hypersensitive reaction of three species of spruce mechanically wounded, inoculated with blue-stain fungi, or artificially infested with spruce beetles

Tree species	Treatment ^a	Lesion length, mm
Lutz spruce	W	34.6 ± 9.5d
	WI	72.7 ± 13.0c
	SBL	86.6 ± 10.5b
	SBH	99.4 ± 14.3a
Sitka spruce	W	45.3 ± 7.0b
	WI	70.7 ± 12.5a
White spruce	W	39.2 ± 13.5b
	WI	75.3 ± 29.5a

Within a column, means (±SD) for each tree species followed by the same letter are not significantly different ($P < 0.05$; Tukey's studentized [HSD] test [SAS Institute 1986]).

^aW, mechanical wounding only; WI, wounding plus inoculation with *L. abietinum*; SBL, low-flow trees infested with spruce beetles; SBH, high-flow trees infested with spruce beetles.

Table 3. Percentage of total monoterpenes in unwounded phloem, and from lesions caused by mechanical wounds and wounds with inoculation of blue-stain fungi in three species of spruce in Alaska $\pm$ SD

Tree species	Treatment*	Alpha-pinene	Beta-pinene	Camphene	Limonene	Myrcene	3-Carene	Beta-phellandrene
Lutz spruce	UW	59.62 $\pm$ 15.24a	32.50 $\pm$ 10.66a	1.23 $\pm$ 0.09a	7.32 $\pm$ 3.21c	3.15 $\pm$ 1.10b	0.42 $\pm$ 0.23b	7.03 $\pm$ 0.02b
	W	51.62 $\pm$ 12.35b	24.22 $\pm$ 8.76b	1.89 $\pm$ 0.76a	12.55 $\pm$ 3.65b	6.33 $\pm$ 2.98b	2.21 $\pm$ 0.98b	10.43 $\pm$ 0.56a
	WI	31.21 $\pm$ 9.96c	15.66 $\pm$ 4.22c	3.14 $\pm$ 1.10a	15.87 $\pm$ 4.02a	14.26 $\pm$ 4.11a	15.66 $\pm$ 3.22a	12.47 $\pm$ 3.21a
Sitka spruce	UW	60.31 $\pm$ 10.55a	37.21 $\pm$ 8.98a	2.01 $\pm$ 0.56a	10.44 $\pm$ 2.33b	4.82 $\pm$ 2.18b	2.54 $\pm$ 0.21c	11.45 $\pm$ 0.34b
	W	50.34 $\pm$ 11.34b	25.67 $\pm$ 7.23b	4.22 $\pm$ 1.45a	13.66 $\pm$ 3.32b	7.92 $\pm$ 2.22b	8.97 $\pm$ 1.01b	12.33 $\pm$ 1.87b
	WI	42.55 $\pm$ 6.31c	18.22 $\pm$ 5.67c	5.36 $\pm$ 2.04a	18.95 $\pm$ 4.67a	19.99 $\pm$ 4.12a	14.13 $\pm$ 3.24a	17.01 $\pm$ 2.15a
White spruce	UW	54.77 $\pm$ 8.23a	24.21 $\pm$ 6.22a	1.26 $\pm$ 0.10b	8.52 $\pm$ 2.23b	3.46 $\pm$ 1.34b	1.12 $\pm$ 0.77b	8.72 $\pm$ 0.56c
	W	46.24 $\pm$ 12.78b	17.89 $\pm$ 5.19b	4.43 $\pm$ 1.57ab	14.22 $\pm$ 3.56a	5.23 $\pm$ 2.67b	2.98 $\pm$ 1.10b	14.15 $\pm$ 3.33b
	WI	34.76 $\pm$ 10.99c	13.55 $\pm$ 3.44c	7.46 $\pm$ 2.89a	16.54 $\pm$ 4.45a	17.87 $\pm$ 3.03a	13.52 $\pm$ 3.39a	17.55 $\pm$ 3.12a

Within a column, means for each tree species followed by the same letter are not significantly different ($P < 0.05$; Tukey's studentized [HSD] test [SAS Institute 1986]).

* UW, Unwounded; W, mechanical wounding only; WI, wounding and inoculation with *L. abietinum*.

found that lesion length was greater in wounds associated with the inoculation of fungi than mechanical wounds (Reid et al. 1967, Wong & Berryman 1977, Coulson 1980, Cook & Hain 1985, Reynolds 1992b).

Lutz spruce with high and low oleoresin flow responded with a hypersensitive reaction to artificially induced beetle attacks. Lesions were formed around the entrance holes and egg galleries. Most egg galleries in high-flow trees were only partially constructed before exudation of resin either engulfed the beetles in the galleries or forced them out of the entrance holes. Egg galleries in low-flow trees were constructed and subsequent brood developed without interference by resin. Lesion length was significantly longer in high-flow trees than low-flow trees that were infested with beetles. Lesion length of high-flow trees was also significantly longer than lesions created by mechanical wounds or wounds with inoculated blue-stain fungus (Table 2). Tree species had no effect on lesion length. Paine and Stephen (1987) also found that lesion length was longer in loblolly pines inoculated with *C. minor* compared with sterile wounding.

Mechanical wounding and wounding plus inoculation with blue-stain fungi caused a change in the percentage of total monoterpene content in Lutz, Sitka, and white spruce. Alpha and beta

pinene were present in lower percentages in the lesion tissue of all three species of spruce when trees were mechanically wounded or wounded plus inoculated with blue stain (Table 3). Trees with lesions caused by wounding plus inoculation had lower percentages of alpha and beta pinene than trees with mechanical wounds only. Wounding and wounding plus inoculation with blue stain had no effect on the percentages of camphene in Lutz and Sitka spruce; however, these same treatments caused an increase in levels of camphene in white spruce (Table 3). Wounding plus inoculation with blue stain caused a significant increase in the percentage of limonene, myrcene, 3-carene, and beta phellandrene in all three species of trees. Wounding alone caused an increase of limonene in Lutz and white spruce but not Sitka spruce. Wounding also caused an increase in the percentage of 3-carene in Sitka spruce and beta phellandrene in Lutz and white spruce. Wounding alone had no significant effect on myrcene in any of the species. This increase in percent of monoterpenes that are reported to be toxic to other species of bark beetles (Smith 1965, 1966, 1977; Coyne & Lott 1976; Bordasch & Berryman 1977) may be an active defense against the spruce beetle. The total percentage of individual monoterpenes may play a more important role in host

Table 4. Percentage of total monoterpenes from unattacked and attacked Lutz spruce with low and high cleoresin flow $\pm$ SD

Monoterpenes	Low-flow trees		% Change	High-flow trees		% Change
	Unattacked	Attacked		Unattacked	Attacked	
Alpha-pinene	47.35 $\pm$ 12.55a	23.22 $\pm$ 7.45b	-51	60.22 $\pm$ 15.61a	29.54 $\pm$ 6.67b	-51
Beta-pinene	30.45 $\pm$ 9.91a	17.52 $\pm$ 6.53b	-42	35.67 $\pm$ 11.87a	21.37 $\pm$ 4.87b	-50
Camphene	1.05 $\pm$ 0.03a	3.23 $\pm$ 0.87a	67	1.72 $\pm$ 0.10a	4.67 $\pm$ 2.11a	63
Limonene	7.32 $\pm$ 1.67b	14.34 $\pm$ 4.76a	49	8.23 $\pm$ 1.18b	17.22 $\pm$ 4.01a	52
Myrcene	3.24 $\pm$ 1.18b	12.23 $\pm$ 3.46a	74	3.56 $\pm$ 1.45b	16.95 $\pm$ 5.02a	78
3-Carene	0.55 $\pm$ 0.18b	9.87 $\pm$ 1.14a	94	0.67 $\pm$ 0.12b	14.32 $\pm$ 2.67a	95
Beta-phellandrene	5.67 $\pm$ 1.45b	12.37 $\pm$ 1.98a	54	6.87 $\pm$ 1.79b	14.33 $\pm$ 2.99a	52

Means within rows for low-flow and high-flow trees, followed by the same letter are not significantly different ($P < 0.05$; Tukey's studentized [HSD] test [SAS Institute 1986]).

Table 5. Wounding effect on length of egg galleries and percentage of phloem consumed by larvae within and outside lesions caused by mechanical wounds and wounds with inoculation of blue-stain fungi in three species of spruce in Alaska

Tree species	Treatment ^a	Phloem area, cm ²		Egg gallery length, cm		Percent phloem area consumed	
		Within lesions	Outside lesions	Within lesions	Outside lesions	Within lesions	Outside lesions
Lutz spruce	W	14.0 ± 4.1d	76.0 ± 28.6a	2.5 ± 0.4c	14.5 ± 2.2a	5.1 ± 1.1c	84.3 ± 25.4a
	WI	29.2 ± 7.5c	60.8 ± 20.4b	0.5 ± 0.1c	6.4 ± 0.9b	0.5 ± 0.1d	55.6 ± 18.5b
Sitka spruce	W	18.0 ± 5.5d	72.0 ± 27.0a	1.2 ± 0.3c	10.7 ± 2.2a	7.5 ± 0.7c	79.1 ± 30.2a
	WI	28.4 ± 8.6c	61.6 ± 19.9b	0.5 ± 0.2c	3.7 ± 0.5b	0.0 ± 0.0d	62.2 ± 22.1b
White spruce	W	15.6 ± 5.9d	74.4 ± 30.5a	1.4 ± 0.6c	12.4 ± 1.6a	6.2 ± 1.0c	80.4 ± 34.5a
	WI	30.0 ± 9.7c	60.0 ± 22.6b	0.3 ± 0.1c	5.5 ± 0.8b	0.0 ± 0.0d	59.0 ± 24.7b

Means (±SD) for each tree species within a column and within a row for phloem area, egg gallery length, and percent phloem area consumed followed by the same letter are not significantly different ($P = 0.05$; Tukey's studentized [HSD] test [SAS Institute 19861]).

^aW, mechanical wounding only; WI, wounding and inoculation with *L. abietinum*.

defense against the spruce beetle than total monoterpene concentrations.

Beetle attack had a significant effect on the monoterpene content of Lutz spruce as the percentage of alpha pinene and beta pinene was significantly reduced compared with unattacked trees (Table 4). Beetle attack also caused an increase in percentages of limonene, myrcene, 3-carene, and beta phellandrene in low-flow and high-flow trees except camphene levels in either high-or low-flow trees were not affected by beetle attack. When comparisons were made between high- and low-flow trees, only the percentage of alpha pinene was higher in unattacked trees. Beetle attack, therefore, had more effect on levels of monoterpenes than oleoresin flow rates. High-flow trees attacked by spruce beetles can potentially use high-flow rates of oleoresin to resist beetle attack by pitching out beetles; however, the increased levels of limonene, myrcene, 3-carene, and beta phellandrene could be more important in host defense against spruce beetle attack.

The effect of tree species on egg gallery length was nonsignificant; however, egg gallery length was significantly greater outside of wound lesions than within lesions (Table 5). Adult beetles tended to deviate from the lesion tissue when constructing egg galleries and many egg galleries terminated at the edge of the lesion. The length of egg galleries was greater in phloem outside lesions that resulted from mechanical wounds compared with fungal inoculated wounds. This could imply a difference in chemistry of the phloem between wounded and inoculated trees or the fungus deters adult feeding. Differences in egg gallery length within lesions from the two different wounds was nonsignificant. Larvae consumed large amounts of phloem outside wound lesions but resisted feeding within the lesions (Table 5). The effect of tree species on larval feeding was nonsignificant but significant differences occurred among types of wounds (Table 5). Trees inoculated with fungus

seemed to deter larval feeding compared with trees with mechanical wounds only. This difference may be attributed to differences in composition of limonene, myrcene, 3-carene, and beta phellandrene associated with mechanical wounds and fungal inoculation wounds. These monoterpenes have been reported as toxic or repellent to some species of scolytids (Smith 1966, 1975; Coyne & Lott 1976; Bordasch & Berryman 1977).

The stilbenes trans-4-hydroxystilbene and 4-hydroxy-diphenylmethane were not found in the phloem of the three species of spruce; however, two unknown chemicals were present in unwounded phloem and areas outside the induced reaction zone that were not present in the induced reaction zone of wounded or wounded plus inoculated trees. The molecular mass of the unknowns was 450 and 361. The largest unknown had a carbohydrate moiety that appeared to be glucose. The presence of glycoside residues on wood extractives is not uncommon (Bate-Smith 1962), but the significance of the residue is not known. A low molecular weight unknown chemical was also found in the induced reaction zone that could not be identified by mass spectroscopy will be analyzed by nuclear magnetic resonance. More extensive studies should help to identify the unknown chemicals and provide comparisons of the major chemical differences between the tissue in the unwounded vs induced reaction zones of the species of spruce in Alaska.

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