length. Linear plots are easier to interpret than a corresponding set of beating curves and the new procedure has the following other advantages:

(a) Physical properties of pulp handsheets can be measured more accurately in the 500 to 300 CSF range.

(b) Determination of bulk is more precise than determination of CSF [see, for example, P.P.R.I.C.—Standard Reference Unbleached Eastern Softwood Kraft Pulp No. 10-65] by a factor of about 5.

(c) Regression lines can be determined of bulk *vs.* physical properties and logarithm of beating time for plups from different processes, different wood species, etc. These regression lines may lead to a reduction in the quantity of test data collected to define properly pulp physical properties and may also simplify quality control within a mill.

A larger investigation of this new procedure is being pursued on kraft pulps prepared from tree components of white spruce [*Picea glauca* (Moench) Voss]. Future publication of these results will include a discussion of what reference values of bulk should be considered in reporting test data. One suggestion is that regression line formulae, *per se*, may replace beater curves, and be used in quality control.—M. Samek and J. V. Hatton, Forest Products Laboratory, Vancouver, B.C.

The Effect of Enzyme Inhibitors on Extractives Formation of Western Red Cedar.—The ability to effect changes in extractives formation or composition would be important to the utilization of western red cedar [*Thuja plicata* Donn]. For example, a tree with no extractives would be more valuable for pulping or a tree with high extractives content would be more valuable for siding. Since extractives formation is under genetic control, any method affecting formation must interfere with genetic material (e.g. DNA) or enzymes. One such study was recently made on *Rhus* sp. and enzyme inhibitors by Hillis and Inoue (Phytochem. 5:483-490, 1966). In this work, several other compounds, growth hormones and β-thujaplicin were tested as well as seven enzyme inhibitors on western red cedar sapwood, *in vitro*, rather than *in vivo* as by Hillis and Inoue.

Freshly cut western red cedar sapwood (about 5g) was treated *in vitro* with solutions of the chemicals listed in Table 1, for 3 months in the dark at about 16 C. The solutions were then extracted with ethyl acetate and examined via paper and thin-layer chromatography for any differences between them and the control. The amounts of thujaplicatins and thujaplicatin methyl ethers (T.M.E.) present in the solution were determined paper chromatographically by the method of Swan, Jiang and Gardner (Phytochem. 8:345-351, 1969). The free and bound β-sitosterol were determined as the trimethylsilyl ether in the gas chromatograph. The column used was 5 ft x 1/8 inch stainless steel packed with 25% SE-30 on Gas Chrom Q (100-120 mesh) operating at 280 C and with nitrogen carrier gas passing at 15 ml per min. The recorder was equipped with a disc integrator, standard solutions were run consecutive to the determinations, and during each run the gas chromatograph was temperature programmed up to the isothermal temperature. During the run an internal standard (octadecane) was added to each solution in order to check on the injection efficiency. Bound β-sitosterol was similarly determined: an aliquot of the sample, octadecane, and sodium ethoxide in ethanol (1 ml of 1%) was heated under nitrogen to about 70 C for 1 hour. The solvent was evaporated, the solution taken up in dioxane-carbon tetrachloride (1:4) filtered, reacted with trimethylsilylating reagent, and made up to volume. Analyses were run on standards concurrently. Table 1 presents the data.

TABLE I

Experiments with western red cedar sapwood *in vitro*

No.	Reagent	Percent extractive yield[a]	pH[c]	Yields of extractives—ppm			
				Thujaplicatins	T.M.E.	Free β-sitosterol	Combined β-sitosterol
1	$NaAsO_2$	0.4	9	25	59	100	530
2	NaN_3	0.4	7	19	78	50	560
3	$NaOOCCH_2I$	—b	2	—b	—b	5	6
4	2,4-dinitrophenol	—b	5	—b	—b	80	750
5	NaF	0.5	6	93	100	100	640
6	KCN	0.5	11	107	61	90	640
7	NH_2OH	0.6	1	91	290	90	660
8	Kinetin	0.3	6	25	29	120	570
9	Gibberellic acid	—b	5	—b	—b	110	450
10	Naphthalene acetic acid	—b	4	—b	—b	70	520
11	β-thujaplicin	—b	4	64	—b	120	80
Control	none	0.3	4	25	30	30	30

a. Combined yields from extraction of the wood in a soxhlet and the liquor in a separatory funnel—ethyl acetate solubles.
b. Yield cannot be calculated because the inhibitor was soluble in ethyl acetate and interferred with paper chromatographic analytical method.
c. Of the solution before extraction. Adjusted to 3.0 with 6N HCl before extraction.

The table shows that the yield of heartwood lignans (thujaplicatins and T.M.E.) was affected most by $NaAsO_2$, NaN_3 and kinetin. Hydroxylamine gave at least one unique product and an extractives distribution pattern close to heartwood, other inhibitors giving unknown products were $NaAsO_2$, NaN_3, NaF and KCN. Also the solutions contained more β-sitosterol, both free and bound as a putative fatty acid ester, than the control extract.

Although these compounds have not been previously noted in western red cedar, data on the control (Table 1) shows the presence of small amounts of these ubiquitous extractives. The enhanced yields of these compounds meant that the biochemical synthesis of them was either insensitive to the enzyme inhibitors and more precursors were channelled to their syntheses or sensitive to the inhibitors but positively rather than negatively.—Eric P. Swan, Forest Products Laboratory, Vancouver, B.C.

PATHOLOGY

Residual Effects of Sunscald and Frost Injury or Young Douglas-Fir.—Frost and sunscald injury in 15- to 17-year-old Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] plantations on Vancouver Island (Foster and Johnson, Dep. Forest. Can. Pub. 1011, 1963, pp. 14-16) resulted from low temperature before hardening-off during the winter of 1955-56, and high temperature and low precipitation during the 1958 growing season. Foster and Johnson reported a high level of fungal invasion through exposed sapwood in the first 3 years after frost injury. *Haematostereum* (*Stereum*) *sanguinolentum* (Fr.) Pouzar, *Merulius* sp. and *Peniophora* sp. were isolated from about 30% of examined frost lesions, and Fungi Imperfecti from about 70%. Numerous fungi of undetermined pathogenicity were isolated from sunscald injuries. This paper reports the results of examinations of the older Douglas-fir plantation 11 years after the frost and 8 years after the sunscald injury.

Ten trees with 28 frost lesions and 10 with 31 sunscald injuries were selected from ones examined every 3 years, beginning in 1958. The sample trees were chosen from those having maximum injuries during the first examination. Each tree was cut into 1-foot lengths and the sections split to expose decay and stain; lesions were split through the face. By using the methods of Foster and Johnson (*loc. cit.*), isolations were taken from both sapwood and heartwood of those sections encompassing injuries and from all others showing evidence of decay or abnormal staining. Effect of the injuries on tree growth and form was recorded.

Three years after the original injury, the frost lesions had exposed sapwood, and sunscald injuries had dessication and lesion or canker formation. In severe injuries, bark sloughing and exposure of the sapwood usually occurred within 2 years. Multiple injuries were present on many stems and often combined to produce extensive necrotic areas. Girdling of the stem by one or more lesions frequently resulted in death of the leader above the injury; however, a permanent or continuing defect in tree form did not occur in the sample trees.

Fifty percent of the frost lesions had callused over within 6 years of the injury and 70% by 11 years. Girdling by either a

single large lesion or multiple lesions occurred in 50% of the frost-injured sample, killing 2- to 4-year-old leaders. In all cases, a branch assumed dominance and little evidence of damage could be found after 11 years.

Thirty percent of the sunscald injuries had the sapwood exposed, and all callused over in 3 years, except one, which took 8 years.

Height growth of the frost- and sunscald-injured trees was reduced for 1-2 years following the injury (Fig. 1).

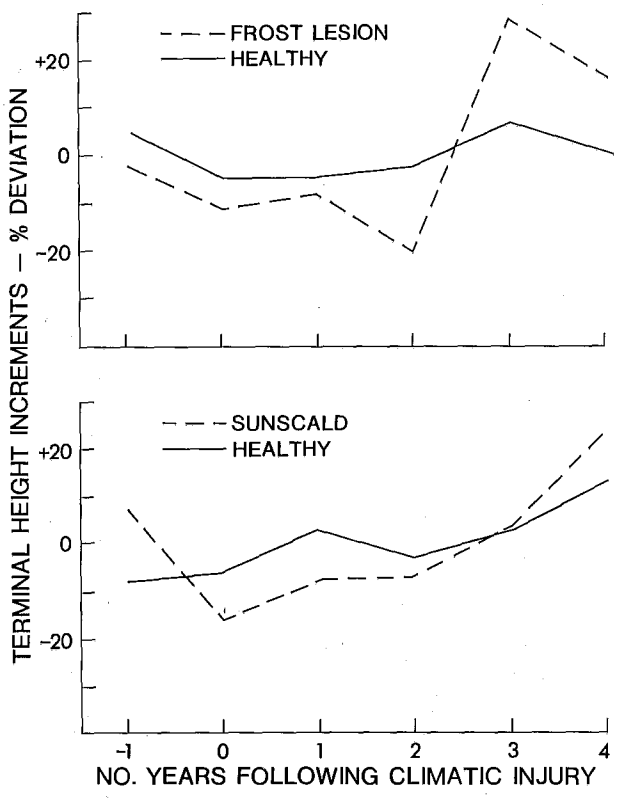

FIGURE 1. Percent deviation of terminal height increments for healthy trees, frost- and sunscald-injured trees.

Five hundred isolations were taken from the frost lesion samples and 452 from sunscald ones. Infection by wood destroying fungi was insignificant; 2% of the isolates were wood decay fungi; over 28% were non-Basidiomycetes, and over 69% failed to show any organism. No explanation is evident for the lower percentage of fungi isolated, compared with that of Foster and Johnson. *Sistotrema* (*Trechispora*) *brinkmannii* (Bres.) Erikss. was isolated from only one tree, a frost-lesion killed top, in which it was restricted to the sapwood. *Lenzites saepiaria* (Wulf. ex Fr.) Fr. was found in one sunscald lesion only; decay was confined to the scar face. *Haematostereum* (*Stereum*) *sanguinolentum* (Fr.) Pouzar was isolated from two trees; one infection originated from a 2-year-old lesion, the other from a branch stub.

This study revealed that even with severe sunscald and frost injury, Douglas-fir of these age classes recover rapidly with little lasting defect. A.L.S. Johnson, Forest Research Laboratory, Victoria, B.C.

Forceps: a Time-Saver in Agar Slant Work.—Polyfoam tube plugs, as apposed to cotton plugs, speed up the plugging process of agar slants. However, rather than follow the directions of the manufacturer (Gaymar Industries Inc., Buffalo 10, N.Y.), the use of straight 6-inch forceps with blunt serrated ends to insert the plugs will increase the plugging speed 2-3 times.

Simply pick up the plug so that most of it is held between the arms of the forceps (Fig. 1); squeeze and insert to the desired depth in the tube and, while holding the plug in position with the forefinger, withdraw the forceps. This technique can be used to replace sterile plugs by employing flame-sterilized forceps.

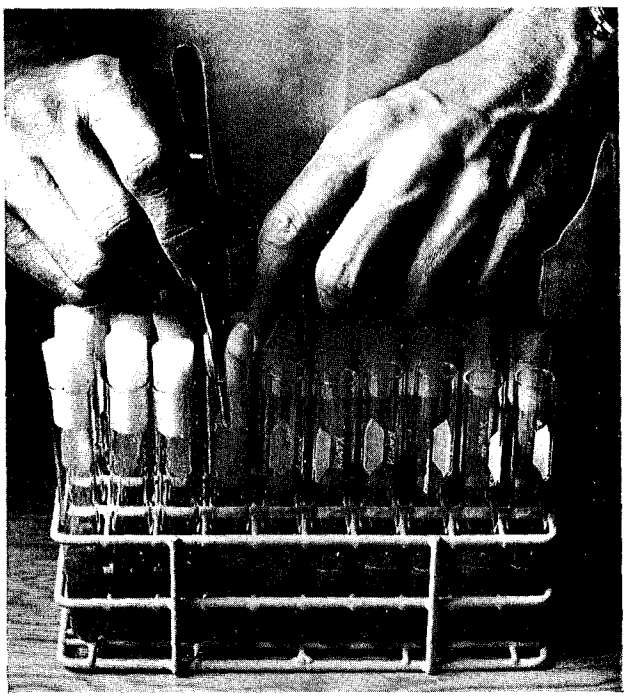

FIGURE 1. Forcep-plugging of racked agar slants with polyfoam plugs.

The method also eliminates individual handling of tubes when plugging racks of agar slants. A.L.S. Johnson, Forest Research Laboratory, Victoria, B.C.

Discoloration and Decay Following Inoculations of Yellow Birch and Sugar Maple with *Pholiota aurivella*.—In central and eastern Canada, yellow birch [*Betula alleghaniensis* Britt.] and sugar maple [*Acer saccharum* Marsh.] are often affected by the decay fungus *Pholiota aurivella* (Batsch ex Fr.) Kummer (Basham and Morawski, Can. Dep. Forest. Pub. 1072, 1964; Lavallée, Phytoprotection 50:16-22, 1969; Stillwell, Forest. Chron. 31:74-83, 1955). In the present study, trees were inoculated at Dudswell, and at Duchesnay, Quebec. At Dudswell, trees of both species were about 50 years old and 4-10 inches dbh, while at Duchesnay trees averaged 30 years of age and were 3-5 inches dbh. The inoculum was a yellow birch dowel colonized by *P. aurivella*. Controls were tested with sterile dowels.

In both hosts at Dudswell and in yellow birch at Duchesnay, the bark was surface-sterilized with ethyl alcohol (95%) before boring a 0.8 x 10 cm hole, with a sterilized bit, 2 or 4 feet above ground level at right angles to the axis of the trunk. On sugar maple at Duchesnay, a piece of bark was removed and the wood surface-sterilized before the boring was made. After inoculation, wounds were covered with a commercial dressing ("Braco").

Over a period of 2 years, trees were felled and sectioned and the sections split longitudinally. Isolations were made from discolored or decayed zones and from the surrounding unchanged wood. Chips of wood, varying in number between 15 and 116, were taken around each inoculation and placed on a 2% malt extract agar medium; a total of 4,661 chips were thus tested. Results are given in Tables 1 and 2.

Pholiota aurivella was recovered from all inoculations. The fungus was found at greater distances longitudinally from the source of inoculum in tissues close to the center of the trunk (Fig. 1A). Therefore, conditions in the central part of a living tree are more favourable for the fungus than in the outer region of the trunk. In inoculations through the bark, the surrounding dis-