

JEBIC

MONTREAL

RESEARCH NOTES

IN THIS ISSUE:

Contents of Volume 26 (1970).

Weevils and root rot in a coniferous plantation in Newfoundland.

Cut-worm damage to summer-planted white spruce tubelings.

— Successful parasitism of spruce budworm.

Chemical composition of clear and mineral-stained maple.

Erosion of wood by enzymes of wood-rotting Basidiomycetes.

Effect of solutions of nitrogen dioxide in

N-N-dimethylformamide on cellulose.

Chemical analysis of atropellis canker-infected lodgepole pine.

Vol. 26—No. 6, NOVEMBER-DECEMBER, 1970

*Published under the authority of
The Minister
Department of Fisheries and Forestry
Ottawa, Canada*

BI-MONTHLY

RESEARCH NOTES

*A selection of notes on current research conducted by the Canadian Forestry Service,
Department of Fisheries and Forestry*

CONTENTS OF VOLUME 26 (1970)

	Pages
Barton, G. M. A novel qualitative test for iron	37
Berry, A. B. Spacing of red pine affects upper stem and crown growth	50-51
Belcher, J. (see Carlson, L. W. and J. Belcher, 27)	
Belcher, J. (see Carlson, L. W. and J. Belcher, 26)	
Bhure, N. D. Nitrogen losses by volatilization from urea applied to forest soils	30-31
Bird, F. T. and J. R. McPhee. Susceptibility of spruce budworm to pure nuclear polyhedrosis virus (NPV) sprays	35
Bloomberg, W. J. Seed disease of Douglas-fir during cone storage	15-16
Bloomberg, W. J., Jack R. Sutherland, W. Lock and T. G. Dunn. Development of corky root disease in Douglas-fir transplants	14-15
Boudoux, Michel E. Effect of tube dimension on root density of seedlings	29-30
Brix, H. Effect of light intensity on growth of western hemlock and Douglas-fir seedlings	34-35
Cameron, Margaret D. (see Eidt, D. C. and Margaret D. Cameron)	
Carlson, L. W. Differential phytotoxicity complicates selection of conifer seed-treatment chemicals	37-38
Carlson, L. W. and J. Belcher. Effect of nursery soil and soil amendment extracts on growth of damping-off fungi and conifer seed germination	26
Carlson, L. W. and J. Belcher. Effect of ultrasonic root cleaning on subsequent growth of caragana seedlings	27
Chu, D. Storing of agar slants and cultures	48
Clarke, L. J. and K. E. Pardy. Biological control of the satin moth in Newfoundland	36-37
Clermont, L. P. Effect of solutions of nitrogen dioxide in N-N-dimethylformamide on cellulose	48
Cunningham, John C. Persistence of the nuclear polyhedrosis virus of the eastern hemlock looper on balsam foliage	24-25
Desai, R. L. and J. K. Shields. Yellowing of cellulose by light	3-4
Dunn, T. G. (see Bloomberg, W. J., Jack R. Sutherland, W. Lock and T. G. Dunn)	
Eidt, D. C. and Margaret D. Cameron. Pretreatment of spruce budworm eggs for counting	46-47
Fung, D. P. and R. A. Ripley. The pyrolysis of aspen and black spruce lignins	12
Girouard, R. M. Vegetative propagation of spruce	41
Gross, H. L. (see Larsen, M. J. and H. L. Gross)	
Hedlin, A. F. (see Ruth, D. S. and A. F. Hedlin)	
Honer, T. G. Bole weight to volume ratio: a constant for open-grown balsam fir	37
Howard, E. W. Dissemination and viability of seed from upland black spruce in central Newfoundland	29
Hulme, M. A. and J. F. Thomas. Erosion of wood by enzymes of wood-rotting Basidiomycetes	57
Hunt, K. and A. Kuechler. Chemical analysis of atropellis canker-infected lodgepole pine	59
Jahren, R. (see Safranyik, L. and R. Jahren 11, 19)	
Jahren, R. (see Safranyik, L. and R. Jahren 35-36)	
Johnson, A. L. S. (see Weir, L. C. and A. L. S. Johnson)	
Kamijo, K. (see Renault, T. R. and K. Kamijo)	
Kasper, J. B. An improved technique for determining the green-volume specific gravity of small wood samples	22-23
Kuechler, A. (see Hunt, K. and A. Kuechler)	
Lachance, D. Mycelial strand formation by antagonism in <i>Odontia bicolor</i>	38
Larsen, M. J. and H. L. Gross. <i>Arceuthobium americanum</i> in Ontario	40-41
Laut, J. G. (see Sutton, B. C. and J. G. Laut)	
Levitin, N. Chemical composition of clear and mineral-stained maple	57
Lindquist, O. H. Cutworm damage to summer-planted white spruce tubelings	56
Lock, W. (see Bloomberg, W. J., Jack R. Sutherland, W. Lock and T. G. Dunn)	
McMullen, L. H. Extended life cycle of Douglas-fir beetle in interior British Columbia	46
McPhee, J. R. (see Bird, F. T. and J. R. McPhee)	
Muir, John A. Dwarf mistletoe spread in young lodgepole pine stands in relation to density of infection sources	49
Murtha, P. A. Thermal image of Dutch elm disease	28-29
Nielsen, U. The influence of plot size on estimates from aerial photographs	25
Nigam, P. C. Toxicity of insecticides to sixth-instar jack-pine budworm larvae under laboratory conditions	2-3
Otvos, Imre S. and Mary E. Taylor. Avian predators of eastern hemlock looper in Newfoundland	22
Outram, Ian. Recognition of mated male spruce budworm moths	3
Page, G. Variation of site-index and basal area within the forest types of Newfoundland's Avalon Peninsula	6-7
Page, G. Variation of site-index and basal area within the forest types of western Newfoundland	49-50
Pardy, K. E. (see Clarke, L. J. and K. E. Pardy)	
Park, J. Y. Effects of field inoculation with mycorrhizae of <i>Cenococcum grandiforme</i> on basswood growth	27-28
Pollard, D. F. W. Effect of partial defoliation on leader growth	10-11
Renault, T. R. and K. Kamijo. Successful parasitism of spruce budworm in Canada by a parasite from Japan	
Retnakaran, Arthur. Preliminary results of radiation-induced sterility of the male spruce budworm	13-14
Ripley, R. A. (see Fung, D. P. and R. A. Ripley)	
Ruth, D. S. and A. F. Hedlin. Germination tests on white spruce and Douglas-fir seed from cones treated with systemic insecticides	41-42
Safranyik, L. and R. Jahren. Emergence patterns of the mountain pine beetle from lodgepole pine	11
Safranyik, L. and R. Jahren. Host characteristics, brood density and size of mountain pine beetles emerging from lodgepole pine	35-36
Shields, J. K. Some histological observations on outside-stored softwood chips	4-5
Shields, J. K. (see Desai, R. L. and J. K. Shields)	
Singh, Pritam. Armillaria root rot in a coniferous plantation in Newfoundland	5-6

Singh, Pritam. (see Warren, G. L. and Pritam Singh)
 Slater, E. (see Wilton, W. C. and E. Slater)
 Smerlis, E. Pathogenicity of *Aleurodiscus amorphus* 18
 Smith, R. B. Infection of Englemann spruce by hemlock dwarf mistletoe. 14
 Smith, R. B. Infection of western larch by hemlock dwarf mistletoe. 16-17
 Smith, R. S. Oxathiins to prevent wood decay 24
 Sterner, T. E. Butt decay in balsam fir defoliated by the spruce budworm. 17-18
 Stranks, D. W. *Trichoderma viride* enzymic degradation of wood structure. 23-24
 Sutherland, Jack R. Frost heaving of forest nursery seedlings damaged by the nematode *Xiphinema bakeri*. 48-49
 Sutherland, Jack R. (see Bloomberg, W. J., Jack R. Sutherland, W. Lock and T. G. Dunn)
 Sutton, B. C. and J. G. Laut. *Graphium penicillioides* on American elm in Manitoba and Saskatchewan. 25-26
 Swan, Eric P. Methylating enzyme specificity in western red cedar. 5
 Taylor, Mary E. (see Otvos, Imre S. and Mary E. Taylor)
 Teich, A. H. Genetic control of female flower color, and random mating in white spruce. 2
 Thomas, J. B. Lindane as a control for *Ips pini* (Say) in red pine plantations 47-48
 Thomas, J. F. (see Hulme, M. A. and J. F. Thomas)
 Tyrrell, David. Production of resting spores of some *Entomophthora* species on artificial media. 12-13
 Van Wagner, C. E. Temperature gradients in duff and soil during prescribed fires. 42
 Warren, G. L. and Pritam Singh. Hylobius weevils and armillaria root rot in a coniferous plantation in Newfoundland. 55
 Weir, L. C. and A. L. S. Johnson. Preliminary results of a study to control poria root rot of Douglas-fir. 39-40
 Wilton, W. C. and E. Slater. Effect of scarification on a non-regenerating burn. 18-19

ENTOMOLOGY

Hylobius Weevils and Armillaria Root Rot in a Coniferous Plantation in Newfoundland.—The root weevils, *Hylobius warreni* Wood and *H. pinicola* Couper, and the root rot fungus, *Armillaria mellea* (Vahl ex Fr.) Kummer, are all common root parasites of coniferous trees throughout the boreal forests of North America. Surveys have shown that these organisms have caused extensive tree mortality in pine and spruce plantations in Newfoundland but this is the first report describing the combined weevil and root-rot damage on the Island. The report also provides evidence of a relationship between the injury caused by the weevils and the incidence of the root rot.

Trees infested by the weevils exhibit characteristic symptoms of injury. Weevil larvae feed on the inner bark and cambium of the roots and root collars producing tunnels of resin and frass, frequently girdling and killing infested trees. The foliage of severely damaged trees becomes chlorotic and ultimately turns red. Trees under 1-inch stump diameter are seldom attacked and those over 8 inches, although attacked, tend to survive girdling, but become susceptible to wind-throw. Symptoms of armillaria root rot include chlorosis or browning of a few branches or whole crowns, reduced growth, and resinosis. The pathogen is recognized by mycelial fans under the bark of the infected roots, black or dark brown rhizomorphs and honey-colored fruiting bodies at or near the base of infected trees.

In 1955, a total of 6,263 seedlings of red pine [*Pinus resinosa* Ait.]; Norway spruce [*Picea abies* (L.) Karst.]; and Sitka spruce [*Picea sitchensis* (Bong.) Carr.]; were acquired from the Provincial nursery at Salmonier and planted in 64 twentieth-acre plots on a moist to wet heathland site near St. John's. Only 4,620 living and dead trees were present when the plantation was examined in

1969. Living trees were about 6 ft in height. Primary roots and root collars of all chlorotic (119), dead (103) and 452 apparently healthy trees were examined for evidence of damage by the weevils and the root rot. The percent of trees infested by the weevils or infected by the root rot was calculated from total trees examined.

Results of the survey (Table 1) show that the weevil infestation is highest in Sitka spruce (58%) and lowest in red pine (33%). However, *A. mellea* infection was highest in Norway spruce (5%) and lowest in red pine (3%). Incidence of the disease increased in trees injured by the weevils, particularly Sitka spruce, where infection was 15%, followed by 7% in Norway spruce and only 1% in red pine. Whitney (Forest. Chron., 37:401-411, 1961) found that wounds caused by these two weevils provide more infection courts for the entry of root-rotting and staining fungi than any other wound type in white spruce [*Picea glauca* (Moench) Voss]. Smerlis (Forest. Chron., 37(2):108, 1961) recorded a similar role for *H. pinicola* in balsam fir [*Abies balsamea* (L.) Mill.]. The present study indicates that the weevil injury did not contribute to the entry of *A. mellea* in the pines examined.

TABLE 1
Percent trees damaged by hylobius weevils and armillaria root rot in the Beauline Line plantation

Damaged trees	Tree Species (number of trees examined are shown in brackets)			
	<i>Pinus resinosa</i> (337)	<i>Picea abies</i> (222)	<i>Picea sitchensis</i> (115)	<i>Pinus sylvestris</i> * (38)
Weevil only				
Apparently healthy	31	17	17	53
Chlorotic	1	12	23	3
Dead	1	8	19	11
Total	33	37	58	66
Root rot only				
Apparently healthy	1	0	0	0
Chlorotic	0	1	1	0
Dead	1	5	3	0
Total	3	5	4	0
Weevils and root rot in the same tree				
Apparently healthy	<1	0	0	0
Chlorotic	0	<1	2	0
Dead	<1	6	13	0
Total	1	7	15	0
Total trees damaged				
Apparently healthy	32	17	17	53
Chlorotic	1	14	25	3
Dead	3	18	36	11
Total	36	49	77	66

**Pinus sylvestris* examined are not included in the total number of trees examined in the plantation (4,620) because these were not within the limits of the 64 plots.

Weevil infestation in all trees was higher than infection by *A. mellea*, particularly in living trees. However, the impact of damage in the plantation cannot be fully evaluated without considering the combined effect of the weevils and the fungus. Damage to Sitka spruce approached 80%, Norway spruce 50% and less than 40% in red pine. Obviously Sitka spruce is the most vulnerable of the species examined when the weevils and the root rot occur simultaneously. Recent damage was also evident in numerous apparently healthy trees, indicating the continuing progress of the problem in the plantation.

Examination of planted Scots pine [*Pinus sylvestris* L.] and natural growing white spruce adjacent to the plantation showed high population levels and abundant old damage by hylobius weevils. The initial attack by the weevils in the plantation is attributed to invasion from the infested white spruce trees. The infestation may have been accelerated by the presence of the highly susceptible Scots pine and the wet site. The incidence of *A. mellea* infection is lower in this plantation than in those examined elsewhere on the Island, possibly because of the lower proportion of spruce, the wet site, and the unusual paucity of decayed stumps. It should be pointed out that damage by the weevils and the root rot has been observed in all plantations examined regardless of tree species or site conditions and the most severe damage has always occurred on exotic species.—G. L. Warren and Pritam Singh, Forest Research Laboratory, St. John's, Nfld.

Cutworm Damage to Summer-planted White Spruce Tubelings.

—Fire destroyed about 5800 acres of mixed-wood forest near Chapleau, Ont., in early June of 1967. Foliage and smaller branches were consumed in the crown fire and the litter was partially burned. From July 10 to 31 the Ontario Department of Lands and Forests planted 160 acres of the burn with white spruce [*Picea glauca* (Moench) Voss] tubelings at a rate of 1200 per acre. In August, cutworms destroyed up to 40% of the tubelings in parts of the planting. D. Ropke, Forest Insect and Disease Survey, reported that, initially, the insects fed on sucker growth of white birch [*Betula papyrifera* Marsh.], trembling aspen [*Populus tremuloides* Michx.], maple [*Acer* spp.], mountain ash [*Sorbus americana* Marsh.], pin cherry [*Prunus pensylvanica* L. f.], and honeysuckle [*Diervilla lonicera* Mill.], as well as on aster [*Aster*], bindweed [*Polygonum*], bracken fern [*Pteridium*], interrupted fern [*Osmunda*], sarsaparilla [*Aralia*], and geranium [*Geranium*]. The white spruce seedlings were apparently eaten mainly where other foliage was sparse or where large numbers of cutworms occurred.

Two species of cutworm were involved, *Pyrrhia exprimens* Wlk. and *Mamestra curialis* Sm., in a ratio of 2:1. The robust, full-grown larvae of *P. exprimens* are about 1.3 inches long and highly variable in color, ranging from pale cream with scattered dark markings to grey-black with a network of fine white lines; some specimens have a trace of orange laterally; the head is yellow on pale larvae, brownish with heavy dark markings on dark specimens. The larvae of *M. curialis* are also variable, green to brown, with a fine, interrupted, dark-edged dorsal line, and with black-ringed spiracles set in a white or pale yellow lateral stripe.

Large-scale infestations were indicated for the following year (1968) since overwintering pupae of the two species averaged 2.5 per square foot in the ground and the incidence of parasitism was very low. Flights of adults of *P. exprimens* did occur in June and July; however, the expected hordes of cutworms did not materialize and no further injury to tubelings occurred. Tiensuu (Ann. Entomol. Fennici 11:34-38, 1945), mentions abrupt fluctuations in numbers of larvae in successive years. He attributes these fluctuations to the climatic requirements of the species in Finland and to the occurrence of a variable and sometimes prolonged diapause in the pupal stage.

Records of the Forest Insect and Disease Survey show that *P. exprimens* occurs widely across Ontario except in the south. The species was rarely collected in the 1940's and 1950's but appeared in much larger numbers from 1964-67. The common host plant is balsam poplar [*Populus balsamifera* L.] on which the larvae feed on foliage at the tips of young unshaded trees; other host plants, listed rarely, are aspen, birch, willow [*Salix* sp.], cherry [*Prunus* sp.], and wild rose [*Rosa* sp.]. In Finland the species is reported abundant on monkshood [*Aconitum*] during years of low rainfall (Tiensuu, *loc. cit.*).

The less common species in the burn, *M. curialis*, has been recorded only once previously by the Forest Insect and Disease Survey, on dogbane [*Apocynum*] near Marathon, Ont., in 1966. Specimens in the Canadian National Collection, Ottawa, are from Alberta, Ontario, Quebec, and Nova Scotia.

The origin of the 1967 infestation of cutworms is uncertain but the burned-over area may have harbored a population of pupae in prolonged diapause which emerged following the burn, or egg-laden moths from an outside source were attracted to the site of the burn. More information is required on the two cutworms, particularly with respect to the occurrence of diapause and the behavior of moths, before any recommendations can be made concerning the best planting time for tubelings relative to fire history. However, it appears that in northern Ontario summer plantings of white spruce tubelings following a spring burn are susceptible to injury by these two cutworms.—O. H. Lindquist, Forest Research Laboratory, Sault Ste. Marie, Ont.

Successful Parasitism of Spruce Budworm in Canada by a Parasite from Japan—There have already been two major attempts, both unsuccessful, to establish exotic parasites in spruce budworm populations in eastern Canada. In one attempt, parasitic flies and wasps reared from the spruce budworm [*Choristo-*

neura fumiferana (Clem.)] in British Columbia were released in large numbers in Ontario, Quebec, and the Atlantic Region in the late 1940's; in the other, 12 species collected from *Choristoneura murinana* (Hbn.) and *Cacoeciae histrionana* Froel. in Europe were released in northwestern Ontario in the early 1950's. We now describe the preliminary experiments in a further attempt to reinforce the biocontrols on the budworm. The initial idea was generated by Zwolfer's reference (Z. Angew. 61(4):448-452, 1968) to a species of *Glypta* in Japan attacking a coniferous defoliator closely related to the spruce budworm.

The junior author reported in December 1969 that:

- (a) Japanese Todo-fir [*Abies sachalinensis* Mast.] is attacked by two tortricid defoliators, *Choristoneura coniferana* Issiki and *C. diversana* Hübner;
- (b) An outbreak of *C. diversana*, the more abundant defoliator, began in 1965 and defoliation was severe in 1969;
- (c) *C. diversana* has a life history similar to the spruce budworm except that it overwinters as a first- rather than as a second-instar larva;
- (d) *Cephaloglypta laricis* Momoi is a common parasite of *C. diversana*. It is a univoltine species whose life history is very similar to the North American budworm parasite *Glypta fumiferanae* (Vier.); and
- (e) A larch tortricid [*Ptycholomoides aeriferana* H.-S.], similar in life history to *C. diversana*, is also attacked by *C. laricis* and 50% parasitism has been recorded among some host populations on larch.

On the basis of the above information, a decision was made to import an experimental shipment of *C. laricis*. The aims were: to become acquainted with the difficulties and feasibility of shipping live material from Japan; and to conduct mating and oviposition experiments under laboratory conditions.

On 9-11 June, the junior author collected ultimate-instar larvae of *C. diversana* in a 40-year-old, heavily infested plantation of *A. sachalinensis* near Asahikawa, Hokkaido. The collections were made from the upper and midcrowns of about 30 trees. The *C. diversana* larvae were reared in the laboratory and produced about 170 *C. laricis* cocoons. These cocoons were air-expressed in two lots (21 and 28 June) to the Research Institute, Canada Department of Agriculture, Belleville, Ont. The adults were screened and 116 healthy specimens were forwarded in four lots (2, 6, 9, and 14 July) to the Green River Laboratory, New Brunswick. All but 5 of the 47 females and 69 males survived the shipment.

All 43 surviving females were used in laboratory experiments to determine mating and oviposition success. Host material consisted of first- and second-instar spruce budworm larvae in hibernacula in old staminate flower cups on cut balsam fir shoots. The arrival of the Japanese parasites coincided remarkably well with the appropriate stage of larval development at Green River. One-pint mason jars were used as experimental cages, with an aqueous sugar solution as food, and strands of excelsior for resting sites. In each cage, a male and a female were provided with about 100 spun-up budworm larvae and observations were made on adult life-span and on the searching, oviposition, and resting behavior of some females. To date, results appear encouraging; of 18 lots dissected in September, 16 contained parasitized larvae, with living first-instar *C. laricis*; parasitism varied among lots from 12 to 94% (average, 52%).

Some host material from the remaining 25 experimental lots will be dissected to determine the extent of oviposition and the rest will be set outdoors to determine overwintering survival. Success in reaching maturity will be assessed next spring.

Research will be continued in 1971 if *C. laricis* can be obtained from Japan: to determine oviposition success in the field using caged trees as experimental units; and to obtain an index of the potential competition between native *G. fumiferanae* and introduced *C. laricis*, since their attack times on spruce budworm presumably coincide.—T. R. Renault, Forest Research Laboratory, Fredericton, N.B. and K. Kamijo, Hokkaido Forest Experiment Station, Japan.

FOREST PRODUCTS

Chemical Composition of Clear and Mineral-Stained Maple.

—Although the chemical composition of the clear wood of sugar maple (*Acer saccharum* Marsh.) has been reported (Clermont and Schwartz, Pulp Pap. Mag. Can. 53:142-143, 1952; Berzins, Pulp Pap. Res. Inst. Can. Res. Note 61, 1966; Freeman and Peterson, Ind. Eng. Chem. 13:803-805, 1941) there is no similar information on mineral-stained wood of this species. The only study of the chemical composition of mineral-stained maple dealt primarily with the inorganic constituents (Good, Murray and Dale, Can. J. Bot. 33:31-41, 1955).

In an attempt to relate chemical composition to cause and mechanism of mineral stain formation in maple, samples of clear and stained wood were analyzed. The samples used for analyses were of: (1) clear sapwood; (2) "heartwood" (in maple considered to be a form of protection wood); (3) lightly-stained wood from the sapwood zone; and (4) heavily-stained wood occurring in either the sapwood or "heartwood" zone. The latter was the typical green mineral-stained wood which causes considerable financial loss to industry. Table 1 gives the analyses.

Conditions for oxidation of polyphenols under alkaline conditions may exist in stained wood. The pH of the green-stained wood was 7.0 as compared with 5.4 for clear sapwood. According to Good *et al.* (Can. J. Bot. 33:31-41, 1955) the pH of stained maple increased to as much as 8.5 or 9.0 as the stain became darker or more pronounced. These high pH values were reported to be due to large amounts of potassium or calcium carbonate. They reported that, as the intensity of the stain increased, the ash content also increased; ash contents as high as 2.5 to 4.0% were not uncommon in highly-stained wood. In our work, the green-stained wood was found to contain almost five times as much ash as the clear sapwood. Extraction with water did not remove the ash from the stained wood, whereas about half of the ash was removed by water from clear sapwood. Thus, either the minerals in the stained wood were attached to the polyphenols, or they were of different composition than the minerals in clear sapwood.

Although the amounts of water extractives from clear sapwood and green-stained wood were almost the same, the extracted material from the latter was much darker in color and evidently different in chemical composition. A considerable amount of

TABLE 1
Chemical Analyses of Clear and Stained Maple

Types of Wood	Klason lignin (%)	Holo-cellulose (%)	Acetone solubles (%)	Alcohol-benzene solubles (%)	Cold water solubles (%)	Hot water solubles (%)	Hot water solubles after solvent extraction (%)	Ash (%)	Ash after hot water extraction (%)
Clear Sapwood	21.8	84.8	1.7	0.50	1.4	3.9	1.7	0.39	0.19
"Heartwood"	23.1	82.8	2.2	0.31	0.86	2.5	2.2	0.64	
Lightly stained wood	22.6	81.2	2.4	0.40	1.5	4.1	1.7	0.72	
Heavily stained wood	23.2	78.4	4.8	0.54	1.7	4.2	4.1	1.8	1.6

Acidified sodium chlorite treatments as described for holocellulose determinations (Wise, Murphy and D'Addieco, Pap. Trade J. 122:35-43, 1946) removed all of the color from the four types of wood. However, the yield of holocellulose decreased as the color intensity of the samples increased. On the other hand, the Klason lignin values varied little. Although identical treatments were used, the total of lignin and holocellulose increased to well over 100% as the color intensity of the wood decreased. It appeared to be more difficult to remove all the lignin from the clear sapwood than from the darker wood. The evidence from these analyses indicated that the more highly-colored wood contained materials which were soluble in both the acid solution of the Klason lignin determination and the acidified chlorite solution. Most probably, these materials were highly-colored polyphenolic substances.

There is further evidence that the darker color of stained wood and "heartwood" is caused by insoluble polyphenolic materials which are formed by oxidation of simpler phenolic products. Although the color of the green-stained wood was not reduced visibly by acetone extraction, the yield of extractives removed was 4.8% as compared with 1.7% from clear sapwood. The other types of wood gave intermediate values. About 70% of each of the acetone extracts was a brown solid which appeared to be a mixture of soluble lignin and a high-molecular-weight polyphenolic material. This brown material made up 3.3% of the dry weight of the green-stained wood but only 1.3% of the clear sapwood. It is suggested that this material, or part of it, is a precursor of the insoluble material which is responsible for the dark color of the stained wood.

Several white polyphenolic substances were also isolated from the green-stained wood extractives. These were present only in traces in "heartwood" extractives and were absent in sapwood. When gently heated in alkaline solution in the presence of air the white material became brown. The process was irreversible. These products are now being studied in detail, but it is suggested that the white solids may eventually oxidize to highly-colored insoluble material in the stained wood.

water solubles of sapwood was also soluble in acetone or alcohol-benzene whereas the material from green-stained wood was insoluble in organic solvents. Paper chromatography verified the presence of different materials in the water extractives of these wood samples and showed the presence of certain materials in water extractives of the green-stained wood; these were present only in traces in the "heartwood" and lightly-stained wood and entirely absent in sapwood. These materials could be glycosides which after hydrolysis and oxidation eventually became the staining material in the wood.—N. Levitin, Forest Products Laboratory, Ottawa.

Erosion of Wood by Enzymes of Wood-rotting Basidiomycetes
—Theoretical considerations of molecular sizes of enzymes causing wood decay indicate that, on *a priori* grounds, enzymatic degradation of wood appears to be confined to outside surfaces or gross capillaries (Cowling and Brown, Advan. in Chem. Ser. 95:152-188, 1969). Although direct experimental evidence is lacking for this pattern of wood degradation by isolated enzymes, we have data from studies with ball-milled wood that support the proposed mechanism.

Previously, enzymatic degradation of cellulosic substrates has been measured as the amount of material made water-soluble or the amount of specific reducing sugars formed. The average molecular size of the insoluble residue has been determined by viscometry although, with wood, the residue has been delignified prior to assay to allow solubilization in suitable solvents. We found, however, that sawdust ball-milled with Burundum cylinders for at least 2 weeks was readily soluble in cadoxen, a cellulose solvent prepared according to Henley (Svensk Papperstidn. 63:143-146, 1960) and that the changes in the molecular size of the combined wood constituents could then be followed by viscometry. Water-soluble material amounting to 14 to 18% of the oven-dry weight of the ball-milled wood was removed by stirring with cold water for 1 hour since this material would be too readily accessible to enzymatic degradation. Combustion analysis showed that ash residue, largely from the Burundum cylinders, weighed 1.5% of the oven-dry weight of the washed ball-milled wood. This same percentage of material was insoluble

in cadoxen and was routinely removed by centrifuging the solution before taking viscometric measurements. Standard cadoxen solutions with solute concentrations up to 1% were prepared by prewetting the wood with water (0.4 ml) for 15 minutes to prevent gel formation, followed by addition of cadoxen (10 ml) with vigorous agitation. After 30 minutes, specific viscosities were determined in a No. 1 Ubbelohde viscometer at 30 C, and hence the intrinsic viscosity $[\eta]$, which is directly related to the molecular size of the solute, was calculated. Huggins' constant was 0.32, a value close to that obtained for solutions of soft-coiled molecules in good solvents; this value allowed further intrinsic viscosities to be determined from a single measurement of specific viscosity (Gillespie and Hulme, *J. Appl. Polym. Sci.* 13:2031-2032, 1969).

Test enzymes were obtained by growing the typical brown rot fungus *Lenzites trabea* Pers. ex Fr. in shake culture at 28 C in an 80-ml suspension of 1% yeast extract (Difco), 0.1% glucose, and 1% 200-mesh aspen wood. Centrifuged culture supernatant was harvested after 7 days and used directly as the enzyme preparation.

In a typical assay of enzyme action, washed ball-milled aspen wood (100 mg) in pH 5.5 acetate buffer (8 ml, 0.075 M) was incubated at 50 C for a minimum of 18 hours with culture supernatant (2 ml). Incubations were terminated in an autoclave at 121 C for 20 minutes, and the wood residues were washed with water (3 x 10 ml), dried, and weighed. Intrinsic viscosities were then determined (Table 1). Reducing sugar analysis (Somogyi, *J. Biol. Chem.* 195:19-23, 1952) and paper chromatography of the supernatant from the incubations showed that the soluble mate-

TABLE 1
Degradation of ball-milled aspen wood by enzymes of *L. trabea*

Incubation time (hr)	Ash-free wood made water soluble (%)	Percentage decrease in $[\eta]$
18	18.2	13.9
18 ¹	17.9	14.2
24	25.2	20.3
72	38.1	30.6
Control ²	4.6	-3.1

¹Included to show agreement between duplicates.

²Wood incubated with autoclaved enzyme. Values were similar for all incubation periods.

rial was mostly glucose and xylose with some cellobiose and xylobiose and traces of mannose, cellobiose and xylotriose. The depolymerases were thus both cellulase and hemicellulase.

A plot of the percentage decrease in $[\eta]$ during incubation against the percentage of wood made water-soluble is linear and passes through the origin. Such a relationship is only possible for an endwise mechanism of depolymerization. Similar results were obtained with enzymes of *Polyporus versicolor* L. ex Fr., a white rot fungus.

Sharply contrasting results have been published demonstrating a random mechanism of depolymerization: when native or swollen cellulose was incubated with cellulase of *Myrothecium verrucaria* (Selby, *Biochem. J.* 79:562-566, 1961; Whitaker, *Can. J. Biochem. Physiol.* 35:733-742, 1957) or of an unidentified Basidiomycete (Rinaudo, Barnoud and Merle, *J. Polym. Sci. C* 28:197-207, 1969) the average molecular size decreased rapidly compared to the small increase in water solubility. The endwise attack observed in the present work thus suggests that lignin in the cell wall confines depolymerase activity to the ends of cellulose molecules by rendering the sides of the cellulose microfibrils inaccessible to the enzyme.—M. A. Hulme and J. F. Thomas, Forest Products Laboratory, Ottawa, Ont.

Effect of Solutions of Nitrogen Dioxide in N-N-Dimethylformamide on Cellulose.—When a solution of NO₂ in anhydrous N-N-dimethylformamide (DMF) was added to cellulose in the form of cotton batting in a weight ratio of 2.5-3 parts NO₂ per part of cellulose and the suspension shaken at room temperature, the cellulose gradually dissolved and a clear viscous solution was obtained in about 1 hr. In the case of fluffed bleached sulfite pulp, solution was complete in about 15 min at room temperature. Similar observations were also made by Schweiger (Schweiger, *Chem. Ind. (London)* 10:296, 1969) using N₂O₄ or NOCl in

DMF or dimethylacetamide. The dissolved cellulose may be recovered by precipitating in either methanol or water. It was also observed that if the cotton cellulose was soaked in pure DMF for 3 to 4 hr prior to adding the solution of NO₂ in DMF, the cellulose dissolved completely in 3 to 5 min. Such a cellulose solution was allowed to stand at constant temperature (25C) and aliquots were taken at intervals. The recovered cellulose samples were analyzed for nitrogen, carboxyl and carbonyl groups. Infrared spectra were also run. Table 1 shows the results of these analyses. The viscosity of the cellulose solution decreased with time of standing (Fig. 1).

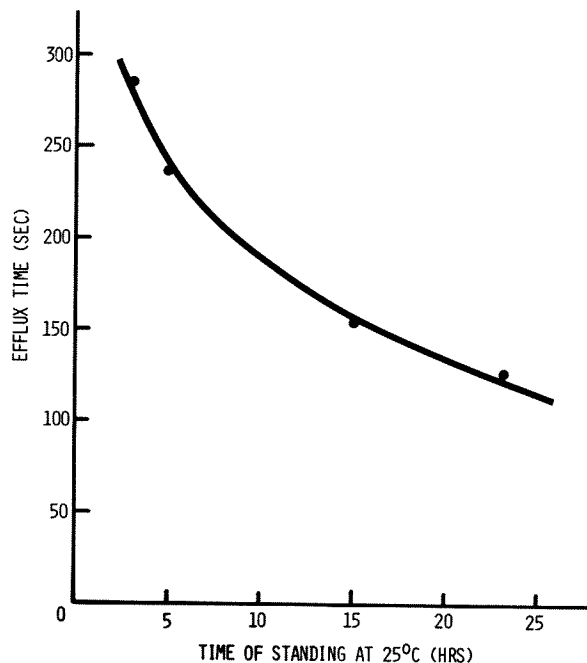


FIGURE 1. Decrease in the viscosity of a solution of cellulose in NO₂-DMF with time.

The nitrogen content of the regenerated samples increased from 0.03% after a 5-min reaction time to 1.03% after 22 hr. Infrared spectroscopy revealed the presence of a strong absorption band at 1680 cm⁻¹ in all samples, increasing in intensity with nitrogen content. Upon treatment of the samples with dilute alkali at room temperature, the band decreased in intensity. On the basis of this evidence, the nitrogen content was attributed tentatively to the formation of nitrite ester groups which are known to absorb very strongly in the 1680-1650 cm⁻¹ region.

Table 1 shows that no oxidation of alcoholic hydroxyl groups to carboxyl groups took place in this system. The small decrease in carboxyl groups is probably due to the solubilization of acidic impurities. The total carbonyl content increased slightly from 14.7, for untreated cotton, to 18.0 m-equiv/kg after 22 hr of treatment. This increase in total carbonyl groups is due to the formation of reducing end groups resulting from chain cleavage.

Infrared spectra of the regenerated cellulose samples were similar to the original cellulose in the 3600-2800 cm⁻¹ region. In the 1500-1000 cm⁻² region, absorption bands of the regenerated cellulose samples were more diffuse in character.

TABLE 1
Analyses of regenerated cellulose from solutions of NO₂ in DMF

Sample	Time of treatment (hr)	N (%)	Carboxyl (m-equiv/kg)	Carbonyl (m-equiv/kg)
Untreated Cotton			9.8	14.7
A	0.08	0.03	8.5	12.3
B	1.00	0.25
C	5.00	0.40
D	22.00	1.03	8.3	18.0

The results indicate that for short treatment times at room temperature, little or no chemical modification of the cellulose takes place in this system. It appears likely that solution takes place through solvation of the hydroxyl groups of cellulose by NO₂-DMF adducts, stable in highly polar solvents.—L. P. Clermont, Forest Products Laboratory, Ottawa, Ont.

Chemical Analysis of Atropellis Canker-Infected Lodgepole Pine.—The fungus, *Atropellis piniphila* (Weir) Lohm. and Cash, causes a canker on lodgepole pine [*Pinus contorta* Dougl. var. *latifolia* Engelm.] growing in the southern foothills of Alberta and eastern B.C. This perennial canker causes a distortion of growth with a resinous blue-black staining of both sapwood and heartwood. During the course of a literature survey on pulping of this species, it became apparent that no chemical analysis data existed for this infected wood. This note reports analytical findings that were obtained as part of the pulping studies.

Table 1 shows the chemical analyses of healthy and infected lodgepole pine. The sugar contents were determined by complete acid hydrolysis of the extracted wood, followed by reduction of the neutralized hydrolyzate and then gas-chromatographic evaluation of the acetates of these reduced sugars.

On a percentage weight basis, the infected wood contained lesser amounts of all the constituent sugars, especially glucose and mannose, while the uronic acid anhydride content (CO₂-producing material) increased almost two-fold. The increase

probably results from the presence of acidic degradation products of the fungus, which are capable of liberating carbon dioxide during the uronic anhydride analysis. The sugar analyses indicate that some glucomannan and some cellulose are the main components lost, while the tree produces abnormally large amounts of resin in an attempt to resist the fungus.

The slightly smaller amount of lignin determined for the infected wood may result from higher resin production or may be just a consequence of positional difference within or between trees.

The very high extractives content of the infected wood is important for any area where these materials are being utilized. The results obtained in this laboratory by I. H. Rogers show a crude resin acid content of 62% of the total acetone extractives, no fatty acids being detected. Turpentine yield amounted to 16.63 U.S. gallons per o.d. short ton of wood.

On an extractive-free wood basis, the carbohydrate content of the infected wood does not differ greatly from that of healthy wood. The density of the infected wood is considerably greater and, therefore, on a volume basis the pulp yield of infected wood would be expected to be normal or slightly higher than normal. Since the lignin contents of the woods are close in value, their cooking rates should be similar; however the increased acid content (sugar acids and resin acids) would mean an increase in the alkali consumption when the infected wood is cooked.—K. Hunt and A. Kuechler, Forest Products Laboratory, Vancouver, B.C.

TABLE 1
Chemical analyses of healthy and infected lodgepole pine.

Lodgepole pine	Arabinose %	Galactose %	Xylose %	Mannose %	Glucose %	Uronic Acid Anhydride %	Klason Lignin %	Alcohol: Benzene (1:2) Extractives % o.d. wood	Density lb./cu ft
Healthy	a	1.0	4.5	8.0	12.4	42.8	4.15	2.3	28
	b	0.6	2.2	4.3	5.8	20.0	1.6		
Infected	a	1.0	4.4	7.8	10.4	40.8	7.35	23.6	45
	b	0.6	2.2	4.4	5.1	20.0	2.9		

*Sugars expressed as percentage of original oven-dry, extractive-free wood.

^bSugars expressed as mole ratios.

Continued from page 00

- Kasper, J. B. and T. Szabo. 1970. The physical and mechanical properties of whitebark pine. *Forest. Chron.* 46 (August).
- Kennedy, R. W. 1970. Specific gravity of early- and late-flushing Douglas-fir trees. *Tappi* 53:1479-1481.
- Logan, K. T. 1970. Adaptations of the photosynthetic apparatus of sun- and shade-grown yellow birch (*Betula alleghaniensis* Britt.). *Can. J. Bot.* 48:1681-1688.
- MacHattie, L. B. 1970. Kananaskis valley temperatures in summer. *Amer. Meteorol. Soc.* 9:574-582.
- Marshall, Valin G. 1970. Tydeid mites (Acarina: Prostigmata) from Canada. I. New and redescribed species of *Lorryia*. *Ann. Soc. Entomol.* 15:17-52.
- McCraw, W. E. and R. M. Hallett. 1970. Studies on the productivity of skidding tractors. *Dep. Fish. Forest., Can. Forest. Serv., Pub.* 1282. 38 p.
- Moeck, Henry A. 1970. An olfactometer for the bio-assay of attractants for scolytids. *Can. Entomol.* 102:792-796.
- Morris, Oswald N. 1970. Precocious development of adult characteristics in virus-infected Lepidoptera. *J. Invertebr. Pathol.* 16:173-179.
- Morris, Oswald N. 1970. Metabolic changes in diseased insects. III. Nucleic acid metabolism in Lepidoptera infected by denonucleosis and *Tipula* iridescent viruses. *J. Invertebr. Pathol.* 16:180-186.
- Nanassy, A. J. 1970. Overlapping of dielectric relaxation spectra in oven-dry yellow birch at temperatures from 20 to 100°C. *Wood Sci. Tech.* 4:104-121.
- Nijholt, W. W. 1970. The effect of mating and the presence of the male ambrosia beetle, *Trypodendron lineatum*, on "secondary" attraction. *Can. Entomol.* 102:894-897.
- Park, J. Y. 1970. Antifungal effect of an ectotrophic mycorrhizal fungus, *Lactarius* sp., associated with basswood seedlings. *Can. J. Microbiol.* 16:798-800.

- Parker, A. K. 1970. Effect of relative humidity and temperature on needle cast disease of Douglas-fir. *Phytopathology* 60:1270-1273.
- Petty, J. A. and G. S. Puritch. 1970. The effects of drying on the structure and permeability of the wood of *Abies grandis*. *Wood Sci. Tech.* 4:140-154.
- Pnevmaticos, S. M., F. J. Petro, and I. B. Flann. 1970. Yellow birch log attributes and sawmill productivity. *Forest Prod. J.* 20(8):53-58.
- Quednau, F. W. 1970. Competition and co-operation between *Chrysocharis laricinellae* and *Agathis pumila* on larch casebearer in Quebec. *Can. Entomol.* 102:602-612.
- Quednau, F. W. 1970. Notes on life-history, fecundity, longevity, and attack pattern of *Agathis pumila* (Hymenoptera: Bracoonidae), a parasite of the larch casebearer. *Can. Entomol.* 102:736-745.
- Reid, R. W. and H. Gates. 1970. Effect on temperature and resin on hatch of eggs of the mountain pine beetle (*Dendroctonus ponderosae*) *Can. Entomol.* 102:617-622.
- Roberge, M. R. 1970. Behavior of urease added to unsterilized, steamsterilized, and gamma radiation-sterilized black spruce humus. *Can. J. Microbiol.* 16:865-870.
- Sahota, T. S. and A. Mansingh. 1970. Cellular response to ecdysone: RNA and protein synthesis in larval tissues of oak silkworm *Antheraea pernyi*. *J. Insect. Physiol.* 16:1649-1654.
- Shaw, George Gerald. 1970. Grease-film extraction of an arthropod: a modification for organic soils. *J. Econ. Entomol.* 63:1323-1324.
- Shaw, D. D. 1970. The supernumerary segment system of *Stethophyma*. I. Structural basis. *Chromosoma (Berlin)* 30:326-343.
- Smirnoff, W. A. 1970. Rhombohedral twisted crystalloids found in the cytoplasm of nuclear-polyhedrosis-infected larvae of the uglynest caterpillar. *Can. J. Microbiol.* 16:906-907.

Recent Publications

- Agnihotri, V. P. 1970. Solubilization of insoluble phosphates by some soil fungi isolated from nursery seedbeds. *Can. J. Microbiol.* 16:877-880.
- Bailey, G. R. 1970. A simplified method of sampling logging residue. *Forest. Chron.* 46 (August).
- Brown, J. E. 1970. New silvicide proves potent for thinning dense stands. *Pulp Pap. Mag. Can.* (July 3).
- Brownell, Harold H. 1970. Spruce milled wood lignin and lignin-carbohydrate complex: molecular size and shape and frequency of lignin-carbohydrate linkages. *Tappi* 53:1278-1281.
- Croome, G. C. R. 1970. A trial of selection management in a mature fir-spruce-birch forest. *Forest. Chron.* 46 (August).
- Dobie, J. and D. M. Wright 1970. The lumber recovery factor revisited. *B. C. Lumberman* (May).
- Embree, D. G. 1970. The diurnal and seasonal pattern of hatching of winter moth eggs, *Operophtera brumata* (Geometridae: Lepidoptera). *Can. Entomol.* 102:759-768.
- Evert, F. 1970. Black spruce growth and yield at various densities in the Ontario clay belt. *Forest Sci.* 16:183-195.
- Fast, Paul G. 1970. Insect lipids. In *Progress in the chemistry of fats and other lipids*. Vol. II, Part 2. R. T. Holman (ed). Pergamon Press, Oxford and New York. pp 181-242.
- Feihl, O. and V. Godin 1970. Rotary cutting of veneer from white spruce. *Dep. Fish. Forest., Can. Forest. Serv. Pub.* 1281. 12p.
- Fortescue, J. A. C. and G. G. Marten. Micronutrients: Forest ecology and systems analysis. In *Ecological studies. Analysis and synthesis*, Vol. 1. D. E. Reichle (ed). Springer-Verlag, Berlin. 1970.
- Fowler, D. P. Maternal effects in red pine, *Pinus resinosa* Ait., and their implications to provenance and progeny testing. In *Proc. Int. Union Forest Res. Organ., Sect. 22, Working Group Meet.*, at Varparanta, Finland, May 28-June 5, 1970. 11p.
- Fox, Selwyn, P. 1970. Radial stresses in curved glulam members. *Can. Constr. Eng.* (August).
- Funk, A. 1970. Fungal symbionts of the ambrosia beetle *Gnathotrichus sulcatus*. *Can. J. Bot.* 48:1445-1448.
- Funk, A., and J. Kuijt. 1970. *Dermea rhytidiformans* n. sp., the fungus associated with cork-bark of alpine fir. *Can. J. Bot.* 48:1481-1483.
- Gardiner, L. M. 1970. New northern Ontario spruce beetle compels May start on log spraying. *Can. Forest Ind.* (July).
- Godin, V. 1970. The grinding of veneer knives. *Dep. Fish. Forest., Can. Forest. Serv. Pub.* 1236 (Revised). 23p.
- Hayashi, Y., and F. T. Bird. 1970. The isolation of cytoplasmic polyhedrosis virus from the white-marked tussock moth, *Orgyia leucostigma* (Smith). *Can. J. Microbiol.* 16:695-701.
- Hayashi, Y. and S. S. Sohi. 1970. Metabolism of *Aedes aegypti* cells grown in vitro. 1. Incorporation of ³H-uridine and ¹⁴C-leucine. *In Vitro* 6:148-152.
- Hocking, D. 1970. Semesan fungicidal dip controls canker diseases of poplar cutting in Alberta. *Tree Planters' Notes.* 21(3):18-20.
- Hulme, M. A. and J. K. Shields. 1970. Biological control of decay fungi in wood by competition for non-structural carbohydrates. *Nature.* 227-300-301.

(Continued on page 59)

O.F.I.M.S.

Burmeister

DEPARTMENT OF
FISHERIES AND FORESTRY
OF CANADA