bi-monthly research notes

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ENTOMOLOGY

Mortality of Birch Casebearer Eggs.—Since its discovery in 1953 in western Newfoundland, the birch casebearer [*Coleophora fuscedinaella* Zeller] has spread throughout the Island. Defoliation of white birch [*Betula papyrifera* Marsh.] has occurred every year since 1960 in at least some areas, and branch and tree mortality has occurred occasionally.

Little is known about factors that affect the course of outbreaks of birch casebearer. Cochran (Woody Points 2: 13-14, 1969) found 12 native parasite species attacking this casebearer in Newfoundland, but in total they parasitized less than 5% of the population. More recent surveys have indicated that egg mortality may be more important than parasites in reducing population levels.

Birch casebearer eggs are laid during the month of July. They are deposited singly into patches of dense pubescence at the junction of veins on the underside of leaves. The larvae hatch after about 30 days, but the chorions and unhatched eggs remain on the leaves enabling the collection of reliable data on egg mortality.

In 1972, the intensity and cause of egg mortality was examined on infested white birch trees at Cormack and Pasadena, in western Newfoundland. Eggs were counted as living or dead on 10 leaves collected from the distal 10 inches (25.4 cm.) of a branch, occurring at the mid crown of the sample trees. At Cormack the same six trees were sampled at weekly intervals from Aug. 10 to Sept. 6. At Pasadena, 10 different trees were sampled at weekly intervals from Aug. 10 to Sept. 21.

Living eggs were yellow, opaque and rounded (Fig. 1) or they were represented by chorions from which larvae had hatched (Fig. 2). Dead eggs were either discolored (maroon or blue-black), opaque and round, or normal in color but shrivelled, or translucent-white and "collapsed" (Fig. 3). The collapsed eggs were empty, but lacked a larval emergence hole.

Before data were analysed and tests of significance performed, all egg numbers were transformed to the square root of the numbers plus one-half, and percent data to the arcsin $\sqrt{\text{percent}}$. Analysis of variance of egg counts showed that sample variances and means were not significantly different between the two study areas; therefore the data were combined. Discolored and shrivelled eggs comprised less than 1% of the total eggs and these were not included in mortality figures or in analyses.

Results of the study showed that the precent of collapsed eggs varied from a minimum of 0–18% on Aug. 10 to a maximum of 9%–60% on Aug. 30. Linear regression analysis showed a significant increase ($P=0.05$; $r = 0.79$; $Y = 4.72 + 0.57X$: where Y is the percent of collapsed eggs and X is the number of days beginning Aug. 1), from about 5% after oviposition was complete, to about 30% when all eggs were hatched (Table 1, page 6).

Although it is not known what caused the casebearer eggs to collapse, the progressive increase in the percent of collapsed eggs following the oviposition period, shows that normal eggs lost their contents after being deposited on the leaf. This suggests egg predation as the likely cause. The abundance of a small mite [*Triophydeus triophthalmus* (Ouds.)] on leaves

Figure 1. Birch casebearer egg on birch leaf.

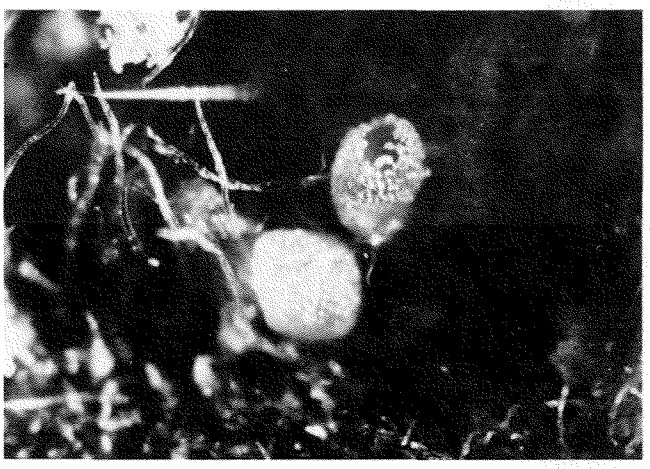


Figure 2. Birch casebearer chorion from which a larva has hatched.

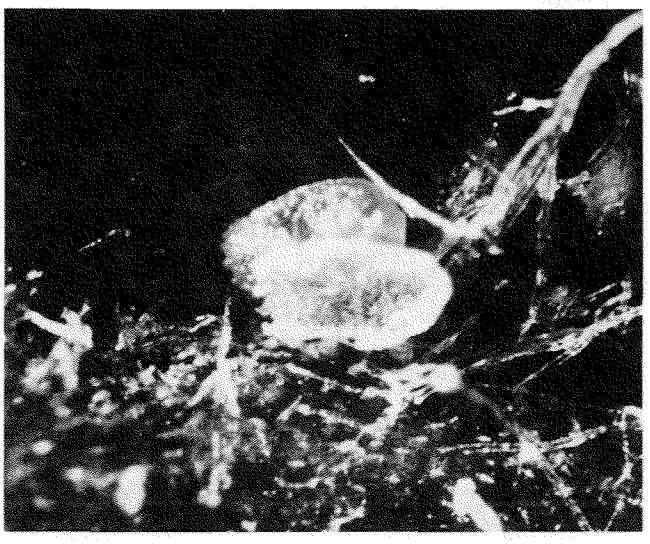


Figure 3. Collapsed birch casebearer egg.

with numerous collapsed eggs suggests that it was the predator; other known predators of insect eggs, such as mirids, chrysopids, and syrphids, were scarce on the sample trees. The feeding habits of this mite are not known but surveys in other birch stands, with averages of 40–80 casebearer eggs per leaf, showed that the mite was abundant, and that up to 99% of the eggs were collapsed. In an area where casebearer eggs averaged only 15 per leaf, the mite was less abundant and only about 5% of the eggs were collapsed. In studies on the birch casebearer in Maine, Gillespie (Maine Forest Serv., Bul. No. 7, 1932) attributed the loss of egg contents to an unidentified small mite.

The intensity of egg mortality determined in this study indicates that it is probably the major factor in terminating localized infestations of the birch casebearer in Newfoundland.—A. G. Raske, Newfoundland Forest Research Centre, St. John's, Nfld.

FIRE

A Vertical Photographic System for Small Plots.—In 1970, fire researchers at the PFRC (Pacific Forest Research Centre) tested the use of 70 mm low-level aerial photography (Lyons, Photogrammetric Eng. 30, 1964) to measure the characteristics of slash fuel complexes. These tests showed the measurements of loading and size distribution to be more than adequate for operational and most research requirements (Muraro, Can. For Serv. Publ. No. 1268, 1970).

The vertical photographic record was of definite advantage for fuel inventory and documentation of fire impact. However, the logistics of ground control and the cost of helicopter operation were not warranted for our limited requirements on widely separated study areas.

To retain the advantages of vertical aerial photography without the cost, we developed a ground based system using a 35 mm camera mounted on an aluminum mast (Fig. 1). The desired camera height, ranging from 2 m to 3.5 m, was achieved by using a commercially available aluminum extension handle similar to those used to extend paint roller or pruning shears (A, Fig. 2). The downward looking camera (B, Fig. 2) is horizontally offset from the mast by an extension arm (C, Fig. 2) hinged to the top of the mast. The hinge (D) aligns and supports the camera extension arm in the horizontal position while in use, but allows a vertical swing of 270° so it will lie flat against the mast for storage and transit.

A sliding bracket (E) fitted to the lower extension of the tube is slotted to receive a numbered plastic card 10 cm wide. In use, this sliding bracket is placed at midfield of the fuel complex to provide plot number and a constant scale. A target level to aid plumbing the mast could also be added to this sliding bracket or to the mast itself.

Figure 1. Application of the vertical photography system for fuel inventory.

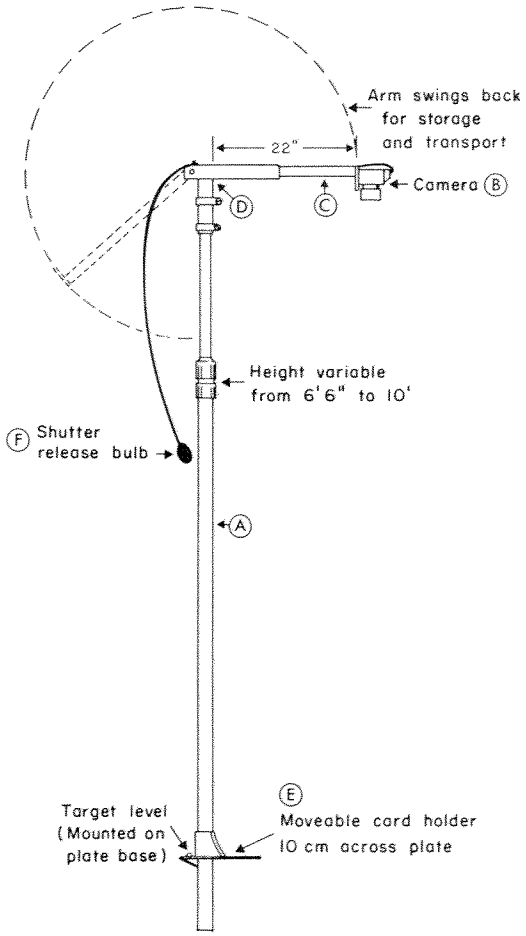


Figure 2. Generalized construction of camera mast.

The photographic equipment consists of a 35 mm camera, a remote shutter release and an automatic miniature flash gun attachment. To minimize adjustment, a camera with automatic exposure conrol would be desirable. A camera with interchangeable lens would enable one to vary the size of the area photographed, or the magnification. Motorized cameras, although desirable, are often too heavy, especially if the mast or the horizontal extension is longer than 3.3 m and 0.6 m, respectively. The camera shutter is remotely controlled (F) from the external camera shutter fitting, through the horizontal tube and down the outside of the vertical mast to a convenient height. A battery-powered trigger has been used, but the bulb system is preferred.

For our purpose, a 28 mm lens that provides a field of view of about 3.3 x 3.3 m from a height of 3.3 m is most suitable. Dull, cloudy days are best for shadow-free photographs; however, the use of an electronic flash on sunny days effectively eliminates all but the darkest shadows. Color film is used exclusively.

Construction details and Figure 2 are deliberately generalized because the design is flexible, depending on the particular needs, and limited only by the constraints of portability.

For our application, a stake marks the location of the base of the mast during the preburn inventory so as to duplicate orientation on the postburn inventory.

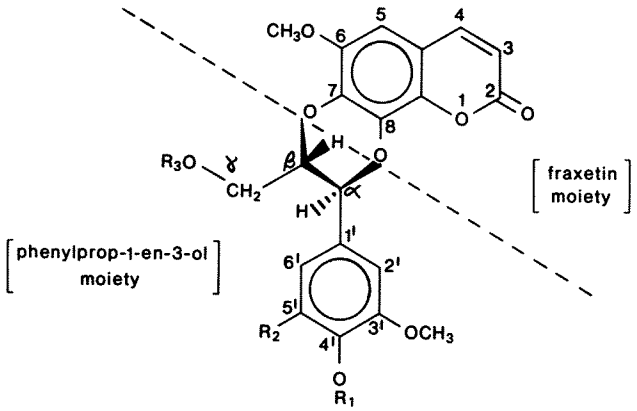
Although this system was designed to obtain permanent records of the pre and postburn fuel complex, it is adaptable for plant succession studies and to record other changing surface phenomena.—S. J. Muraro and W. L. Cave, Pacific Forest Research Centre, Victoria, B.C.

FOREST PRODUCTS

Anti-Fungal Coumarins From Mineral-Stained Maple.— Maple is subject to a discoloration commonly known as mineral stain which detracts from its appearance and value for certain purposes. As part of a continuing search for the cause and cure of such defects the phenolic compounds present in the affected area were investigated. Although a considerable amount of colored material can be removed from mineral-stained sugar maple [*Acer saccharum* Marsh.] wood by neutral solvent extraction, much of the color remains in the cell walls (Levitin, Wood Science 5:87-94, 1972) suggesting that, if phenolic compounds are responsible for the color, enzymatic oxidation and polymerization of low M.W. phenolic compounds may have taken place. Consequently, the extractives of mineral-stained maple wood were studied in an effort to isolate and identify compounds which might be precursors of the staining material.

During the present study a compound (I) and a mixture of two other compounds (II and III), all closely related in structure, were isolated from mineral-stained maple wood in approximately 1% yield (o.d. basis). They were identified as being substituted coumarins (fraxetin) (Dean, Naturally occurring oxygen ring compounds, Butterworths, London, pp. 190-1, 1963). They were not found in clear wood and were present only in traces in the protection wood of the inner portion of the tree.

These compounds were extracted with acetone-water (95:5) from mineral-stained maple wood meal. The brown powder obtained upon evaporation was chromatographed on a column of silica gel with chloroform containing 1% ethanol as the developing solvent. A yellow oil was removed first followed by a fraction containing compounds II (~80%) and III (~20%). This fraction was closely followed by compound I. Thin-layer chromatography (t.l.c.) gave R_f values (silica gel; benzene-ethanol, 150:22) of 0.26 for I and 0.38



I	$R_1 = R_3 = H$, $R_2 = OH$	(methyl-pyrogallyl)
Ia	$R_1 = R_3 = Ac$, $R_2 = OAc$	
II	$R_1 = R_3 = H$, $R_2 = OCH_3$	(syringyl)
IIa	$R_1 = R_3 = Ac$, $R_2 = OCH_3$	
III	$R_1 = R_2 = R_3 = H$	(guaiacyl)
IIIa	$R_1 = R_3 = Ac$, $R_2 = H$	

for II and III. Compound II was enriched (~ 12% of III) by rechromatographing on a column of silica gel with chloroform-ethanol (99:1). Following exhaustive trimethylsilylation of this fraction, gas-liquid chromatography (g.l.c.) (Dexsil-300, 290 C) showed two peaks which were assigned to III (36.5 min, 12.5%) and II (44.5 min, 87.5%) respectively. Nitrobenzene oxidation of the above fraction yielded a mixture in which were identified, only, syringaldehyde (70 to 75%, from II) and vanillin (10 to 15%, from III).

The basic structural features of I and II were deduced from detailed spectroscopic studies (to be published elsewhere) on both the parent compounds and their fully acetylated derivatives (Ia and IIa). Thus compound I gave a molecular ion at m/e 402 and II at m/e 416. The corresponding acetates gave molecular ions at m/e values higher by 126 and 84 atomic mass units (a.m.u.). It was therefore evident that there were three hydroxyl groups in compound I whereas compound II contained only two hydroxyl groups. Analysis of their respective nuclear magnetic resonance (n.m.r.) spectra, together with extensive nuclear magnetic double resonance experiments, indicated that these compounds were composed of a fraxetin moiety (coumarin portion) to which was fused [*trans* ($J\alpha,\beta \simeq 7$Hz)] a substituted phenylprop-1-en-3-ol moiety. Both Hα and Hβ are in axial orientations, thus the phenyl and hydroxymethyl groups are equatorial. The phenyl substitution pattern in I was observed to be 4',5'-dihydroxy-3'-methoxy (methyl-pyrogallyl); in II it was 4'-hydroxy-3',5'-dimethoxy (syringyl). Additional transitions of minor intensity were observed only in the aromatic region of the n.m.r. spectra of II and of IIa. By assigning the guaiacyl structure (III) to the minor component isolated with compound II, it is possible to account for the extra transitions in the n.m.r. spectra of II and of IIa, the two peaks observed by g.l.c., the nitrobenzene oxidation results, and the occurrence in the mass spectra of a peak at 30 a.m.u. less than the respective molecular ions of II and of IIa. This apparent loss of a.m.u. from I or Ia was not observed – nor would it be expected – for compounds II and IIa. The occurrence of a guaiacyl analogue might have been expected on the basis of the known biosynthesis of methylpyrogallyl and syringyl compounds (Higuchi, Formation

and Biological Degradation of Lignin, Advances in Enzymology 34: pp. 207-283, 1971). The infrared and ultraviolet spectra were in accord with the proposed structures. All showed a strong absorption near 1700 cm^{-1} due to a six-membered σ lactone conjugated to a double bond. Compound I had a melting point (m.p.) of 238C. The mixture of II and III was observed to be optically active, $[\alpha]_D^{25} - 15.5°$ (pyridine).

These structures are subject to refinement since the alkyl-aryl-ether linkages, which we have assigned as being 7-β and 8-α may be opposite to that shown. The β-carbon, during enzymatic phenolic oxidations, (Sarkanen, Lignins, Wiley-Interscience, pp. 95-163, 1971) can form a radical, which would couple with an oxygen radical from another molecule (fraxetin) to form a dimer, before the α-carbon can undergo reaction. The ambiguity arises since we do not know whether it is the 7 or the 8 hydroxyl of fraxetin which first undergoes a one-electron oxidation to form a free radical; however, since the 7 hydroxyl is *para* to the side chain our assignment seems reasonable. With regard to the stereochemistry at both the α- and β-carbons we are not able to deduce whether the absolute configuration is *erythro* or *threo*, but the fusion is *trans* with the bulky substituents occupying the equatorial positions.

Experiments at the Eastern Forest Products Laboratory have shown that a test solution of a 1% mixture of II and III prevents the growth of *Lenzites trabea*, a wood-rotting fungus. Compound I is being tested for biological activity. Compounds, I, II and III may be produced by the tree to protect it against infection. The significance of these compounds both as fungicides and precursors of the brown staining material is currently under study.

A full report, describing the physical parameters of these biologically active coumarins will be published elsewhere.—J. F. Manville, Western Forest Products Laboratory, Vancouver, B.C. and N. Levitin, Eastern Forest Products Laboratory, Ottawa, Ont.

MENSURATION

Site Index Formula for Major Timber Species in Ontario.—Plonski (Ont. Dep. Lands Forests, Timber Manage. Div., Rep. No. 24, 1956; Silvic. Ser. Bull. No. 2, 1960) constructed site index curves for Ontario's major timber species—black spruce [*Picea mariana* (Mill.) B. S. P.], jack pine [*Pinus banksiana* Lamb.], trembling aspen (*Populus tremuloides* Michx.), white birch [*Betula papyrifera* Marsh.], white pine [*Pinus strobus* L.], red pine [*Pinus resinosa* Ait.] and tolerant hardwoods. However, he used graphical methodology (*cf.* Bruce and Schumacher, Forest Mensuration, McGraw-Hill, New York, 1950), *i.e.*, he gave no equations for them. Thus, to utilize these site index curves, it is necessary to look up values and/or interpolate between values in the appropriate graphs. This is very tedious and often produces inconsistencies in repeated readings for the same set of site index curves.

To overcome these difficulties and to facilitate the computer processing of site index estimation, Payandeh (Can. Forest. Serv. Publ. 1318, 1973) employed stepwise multiple linear regression analysis to derive a set of equations for Plonski's site index curves, expressing site index as a function of stand age and height. These equations provide fairly accurate estimates of site indices for the range of data employed. However, they lack the flexibility and/or accuracy which might be obtained from equations based on some of the well-known exponential decay functions. That is, nonlinear functions usually produce better fit than linear models with more evenly distributed variations within the range of data, and they also behave much better upon extrapolation.

The purpose of this note is to present such a set of equations for Plonski's site index curves. These equations were derived to facilitate computer processing and to expedite interpolation between graph values.

For each species the data used consisted of 60–90 points read directly from the appropriate set of site index curves for the range of site indices and ages (by 10-year intervals) given by Plonski (1956, 1960). These values were treated as observations in a nonlinear regression analysis in which the parameters b_{1-5} were estimated by least-squares approximation techniques (*cf.* Draper and Smith, Applied Regression Analysis, John Wiley, New York, 1966). The data for each of the seven species were run with several exponential decay functions of which the following model produced the best fit:

$$S = b_1 H^{b_2} \left[1-e^{b_3 A}\right] b_4 H^{b_5}$$

where: S = site index (height at age 50), H = stand height (height of dominant and codominant trees in feet), A = stand age in years, e = base of natural logarithms, and b's = constant parameters of the model.

The estimated parameters b_1, b_2, b_3, b_4, and b_5, the R^2 value, the standard error and the maximum error of individual observations obtained for each species are given in Table 1. All species have a standard error of less than 1.4 and the maximum error does not exceed 2.9. Since it is difficult to read site index values (height at age 50) from Plonski's site index curves to the nearest foot, such errors are considered quite acceptable.

The equations presented here contain the basic limitations of the site index curves they represent.—B. Payandeh, Great Lakes Forest Research Centre, Sault Ste. Marie, Ont.

TABLE 1

Estimated parameters of a nonlinear model, expressing site index as a function of observed height and age, for the major timber types of Ontario[a]

Species	Parameters					R^2	Standard error	Maximum error
	b_1	b_2	b_3	b_4	b_5			
Black spruce	0.01024	1.75093	−0.00296	−2.98124	−0.32121	0.991	1.1	2.4
Jack pine	0.44105	1.10942	−0.02099	−3.36831	−0.32978	0.996	0.9	1.4
Trembling aspen	0.23921	1.21233	−0.01385	−3.54742	−0.35913	0.993	1.4	2.1
White birch	0.82253	0.98829	−0.03961	−3.23333	−0.16089	0.995	0.8	1.7
White pine	0.09635	1.33331	−0.02073	−3.59428	−0.10546	0.988	1.4	2.9
Red pine	0.56058	1.03965	−0.02095	−2.38568	−0.22405	0.997	0.5	1.1
Tolerant hardwoods	0.36477	1.10897	−0.02276	−2.40103	−0.12213	0.974	1.0	2.0

[a] Site index $= b_1 (\text{Height})^{b_2} \left[1-e^{b_3 \text{ Age}}\right]^{b_4} (\text{Height})^{b_5}$

PATHOLOGY

A Modified Critical Point Drying to Study Germ Tubes of Rust Fungi Under Scanning Electron Microscope.—Germ tubes of the pine stem rusts, *Endocronartium harknessii* (J. P. Moore) Y. Hiratsuka and *Cronartium ribicola* J. C. Fisch, ex Rabh., were observed without disruptive or distortive effects under a scanning electron microscope when the spores were germinated directly on specimen studs and processed with a critical point drying technique.

In the study of fragile biological material such as hyphae and germ tubes of fungi under a scanning electron microscope, it is important to avoid disruption and distortion caused by processing specimens. The critical point drying method has been used to study this type of material under transmission and scanning electron microscope (Anderson, *In* A. W. Pollister (Ed.) Physical Technique in Biological Research, Vol. 3, Part A, 1969; Royle and Thomas, Physiol. Pl. Path. 1: 345-349, 1971). With this method, specimens can be dried without the disruptive or distortive effects of surface tension during drying and their original form can be observed even after exposure to a high vacuum in a vacuum evaporator or in the specimen chamber of the electron microscope.

During a study of the germ tube morphology of pine stem rusts (*Cronartium spp.* and *Endocronartium* spp.) and other forest fungi, we developed a modified critical point drying technique for germ tubes to be observed under the scanning electron microscope. By germinating spores on specimen studs, no transfer is necessary and damage to the specimen was eliminated. Instead of flattened and distorted germ tubes (see *Fig. 1*, Hiratsuka, Can. J. Bot. 48:1692, 1970) disruption- and distortion-free images of germ tubes were observed (Figs. 3-5).

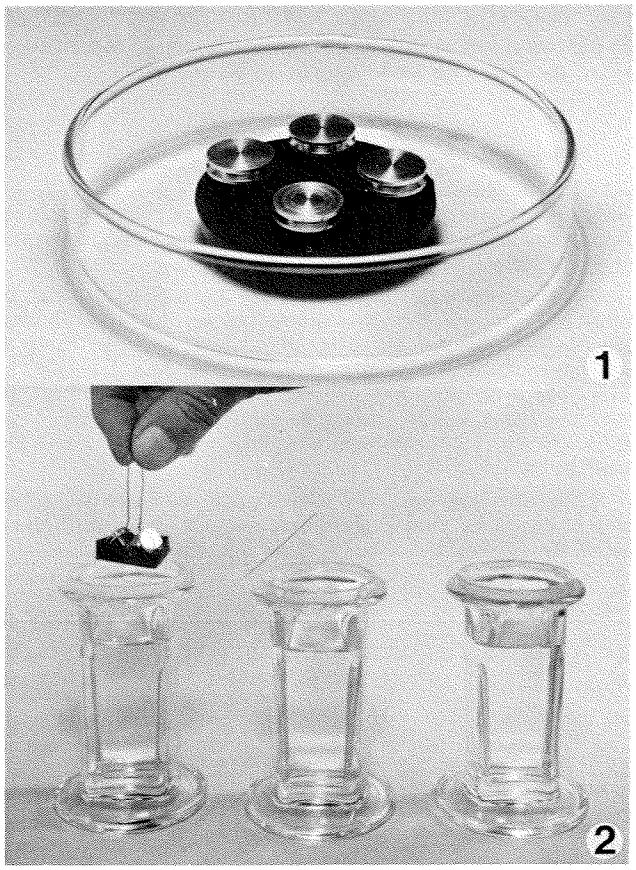


Figure 1. Specimen studs on rubber supporter in a petri dish for incubation.

Figure 2. Wire mesh mini-bracket used to transfer studs with germinated spores.

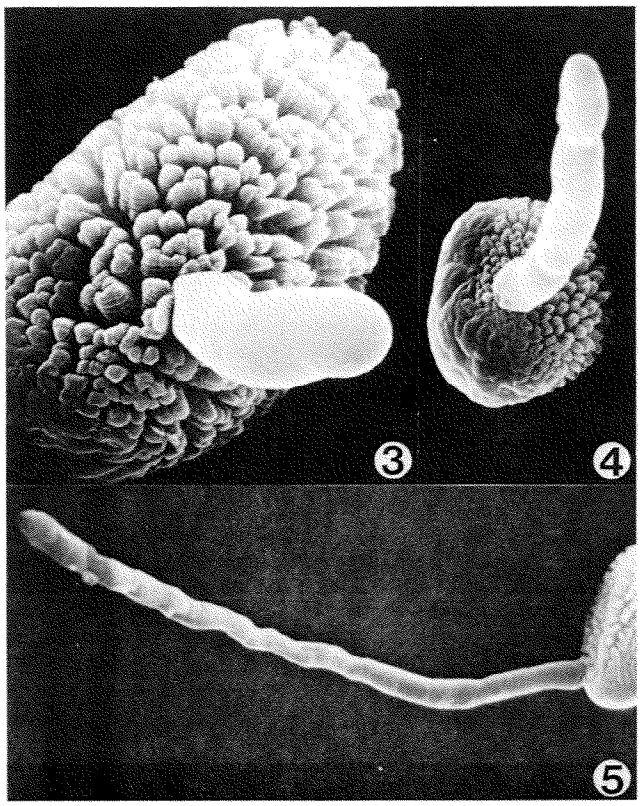


Figure 3, 4. Germ tubes *Endocronartium harknessii* telispores (peridermioid teliospores). Fig. 3 X 7600; Fig. 4. X 3200.

Figure 5. Germ tube of *Cronartium ribicola* aeciospore. X 2200.

We suggest the following procedure:

Specimen studs were mounted on a rubber support and placed in a petri dish (Fig. 1). The upper surface of the specimen studs were coated with a thin layer of 0.3% agar. After the agar cooled and hardened, aeciospores of *Cronartium ribicola* and (peridermioid) teliospores of *Endocronartium harknessii* were dispersed on the surface of the agar. A small amount of distilled water was poured into the petri dish before it was covered and incubated. After adequate time was allowed for germ tube formation, specimen studs were removed from the petri dish and dried on a slide warmer at 50°C.

Completely dried, they were put into 50% ethanol, dehydrated in 70%, 95%, and absolute ethanol, which was then replaced with 50%, 70% and 90% iso-amyl acetate solution in absolute ethanol. A wire mesh basket (Fig. 2) was used to immerse the studs in each solution for about 3 minutes. Then the studs were transferred through two changes of 100% iso-amyl acetate and stored in this solution until critical point drying.

The specimen studs in 100% iso-amyl acetate solution were quickly transferred to the critical point dryer (Denton DCP-1 Critical Point Dryer). When dried, the specimens on specimen studs were coated with a thin film of gold under high

vacuum and observed under a scanning electron microscope (Cambridge Stereoscan Mark II-A).

Our modified technique will be used for observing germination and growth of many other fungi under scanning electron microscope.—Y. Hiratsuka and P. J. Maruyama, Northern Forest Research Centre, Edmonton, Alta.

(*Continued from page 1*)

TABLE 1

Average number of birch casebearer eggs laid and percent of collapsed eggs per sample from white birch at Pasadena and Cormack, Newfoundland in 1972.

Sample date	Avg. total no. of eggs	Avg. no. of collapsed eggs	Percent of collapsed eggs
Cormack and			
Aug. 10	118.4	5.6	4.7
Aug. 17	99.9	6.6	6.6
Aug. 23	121.2	11.3	9.3
Aug. 30	121.8	46.1	37.8
Sept. 6	117.3	17.6	15.0
Pasadena only			
Sept. 13	80.4	22.0	27.4
Sept. 21	50.2	18.0	35.9

(*Continued from back cover*)

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