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).

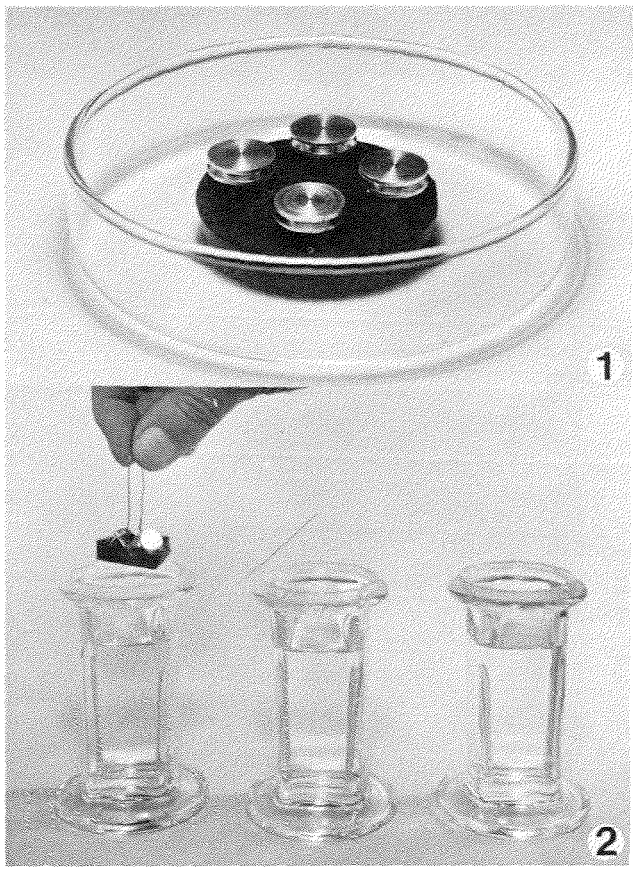

Figure 1. Specimen studs on rubber supporter in a petri dish for incubation.

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

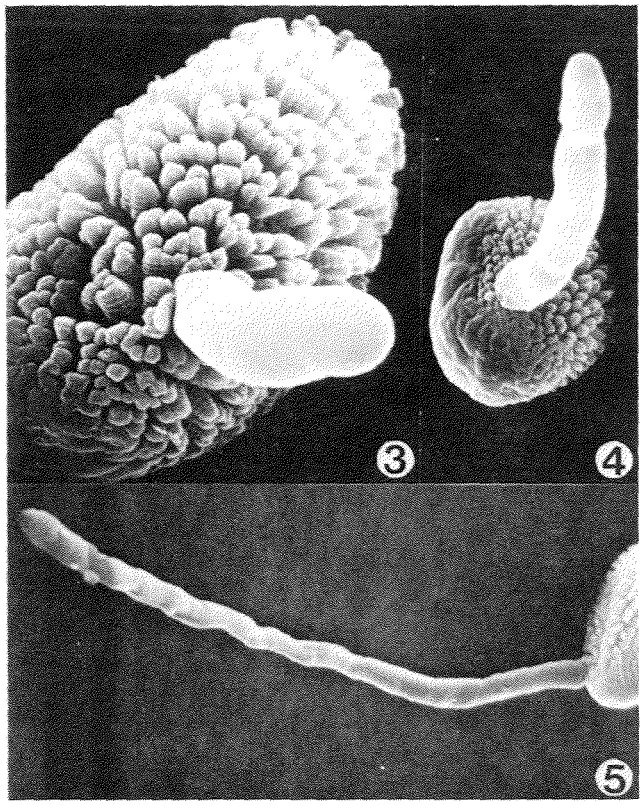

Figure 3, 4. Germ tubes *Endocronartium harknessii* telispores (peridermioid teliospores). Fig. 3 X 7600; Fig. 4. X 3200.

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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Modified critical drying

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