Histopathology and Cytology of Cronartium ribicola in Tissue Cultures of Pinus monticola

A. E. Harvey and J. Y. Woo

Plant Pathologist and Biological Laboratory Technician, respectively, USDA Forest Service, Intermountain Forest and Range Experiment Station, Ogden, Utah 84401; stationed in Moscow, Idaho, at the Forestry Sciences Laboratory, which is maintained in cooperation with the University of Idaho.

The authors acknowledge with thanks the help of John L. Grasham of this laboratory in the preparation and cultures of the tissues utilized in this study.

Accepted for publication 3 February 1971.

ABSTRACT

Intercellular ramification by mycelia of the blister rust fungus (*Cronartium ribicola*) was pronounced in 12-week-old host tissue cultures of western white pine (*Pinus monticola*). Individual hyphal cells were mononucleate and generally uniform in diameter. Constrictions of the haustoria were observed at points of entrance into host cells. Haustorial morphology varied widely. The numbers of nuclei within individual haustorial cells also varied. Mononucleate haustoria were the most common, but binucleate haustoria were rarely observed. Haustoria had well-developed encapsulation layers, and were usually associated with the host's nuclei. Host cell damage increased as the age of the infection advanced. Phytopathology 61:773-779.

Additional key words: Obligate parasitism, histology, nucleation.

Tissue cultures of western white pine (*Pinus monticola* Dougl.) provide an excellent substrate for propagating the blister rust fungus (*Cronartium ribicola* J.C. Fisch. ex Rabenh.) under defined conditions (9, 10). Infected pine tissue cultures provide a controlled system for studying the relationships of the organisms in this important host-parasite system. The following detailed histological study was undertaken in the hope that some parallels could be drawn between the histopathology of this rust and that of other, more extensively studied obligate parasites.

MATERIALS AND METHODS.—Infected pine tissue cultures used in this study were obtained as described by Harvey & Grasham (10). Twelve to 20 weeks after explanting, material was prepared and sectioned for examination under a light microscope as described by Woo (21). Parasitized tissues from naturally infected 6-year-old white pine seedlings were used for a comparison of rust morphology. A variety of standard stains were employed to elucidate specific cytological details of this host-parasite system. The material in Fig. 1-A, B, C, E, Fig. 2-I, J, Fig. 3-L, Q, and Fig. 4-T and V was stained with Johansen's quadruple stain; in Fig. 1-D, Fig. 3-M, N, O, P, and Fig. 4-U and V with Sass' Triple stain; in Fig. 2-H, K and Fig. 4-R with Bismark brown and fast green; and in Fig. 2-F and G with hematoxylin.

RESULTS.—Aerial and intercellular hyphae and haustoria of *Cronartium ribicola* were concentrated on or near the surface of the cultured host tissues (Fig. 1-A, C). Hyphae were not observed within living host cells. When individual host cells were separated from the central callus mass, hyphae aggregated on the cell surfaces, but did not penetrate except by means of haustoria. When such separated cells died, their shapes were frequently preserved by these hyphal aggregations (Fig. 1-C). Preservations of cell form were also observed within heavily parasitized host tissue masses when individual host cells died. Aggregations of intercellular hyphae were often so heavy that the total volume of an individual tissue mass was derived as much from the fungus as from the host (Fig. 1-B). The intercellular hyphae formed a closely packed mass (Fig. 1-B) of parallel, interwoven hyphal elements (Fig. 1-D). Hyphal cells were mononucleate (Fig. 1-D).

Individual host cells in infected host tissue cultures were often heavily parasitized, and many times contained numerous haustoria (Fig. 1-E). In naturally infected pine seedlings, the occurrence of more than two haustoria in a single host cell is extremely rare.

The haustoria within cultured host cells were generally typical of those in naturally infected seedlings. More variation was noted in haustorial morphology, nucleation, and septation in cultured host tissues than had been observed in seedlings. Eighty % of the haustoria in tissue cultures were a simple type, either spherical, pyriform, or reniform, and short (Fig. 2-F) or elongated (Fig. 2-G). Intermediate forms were also observed, and all forms had one basal septum. An additional 10% of the haustoria were characterized by a two-branched tip, often separated from the stalk by an additional septum (Fig. 2-H). These were the only types the authors observed in the seedling tissues used for comparison, and the only ones reported for blister rust in the literature (3, 4, 5, 14).

Four other haustorial types were found in cultured host tissues; 5% were similar to the two-branched type, except that each branch was separated from the main stalk by an additional septation (Fig. 2-I); 3% were diffuse-branched and multiseptate (Fig. 2-J); 1% were digitate and had an uncertain pattern (Fig. 2-K); and 1% were multiple-branched and characterized by a nonseptate head (Fig. 3-L).

In addition to form and septation, variation also occurred in the numbers of nuclei observed in haustorial cells. Most often, haustoria were mononucleate (Fig. 2-F, G). Approximately 1% of the haustorial cells observed were binucleate, and these generally occurred in the more complex morphological forms described (Fig. 3-M, N, O, P).

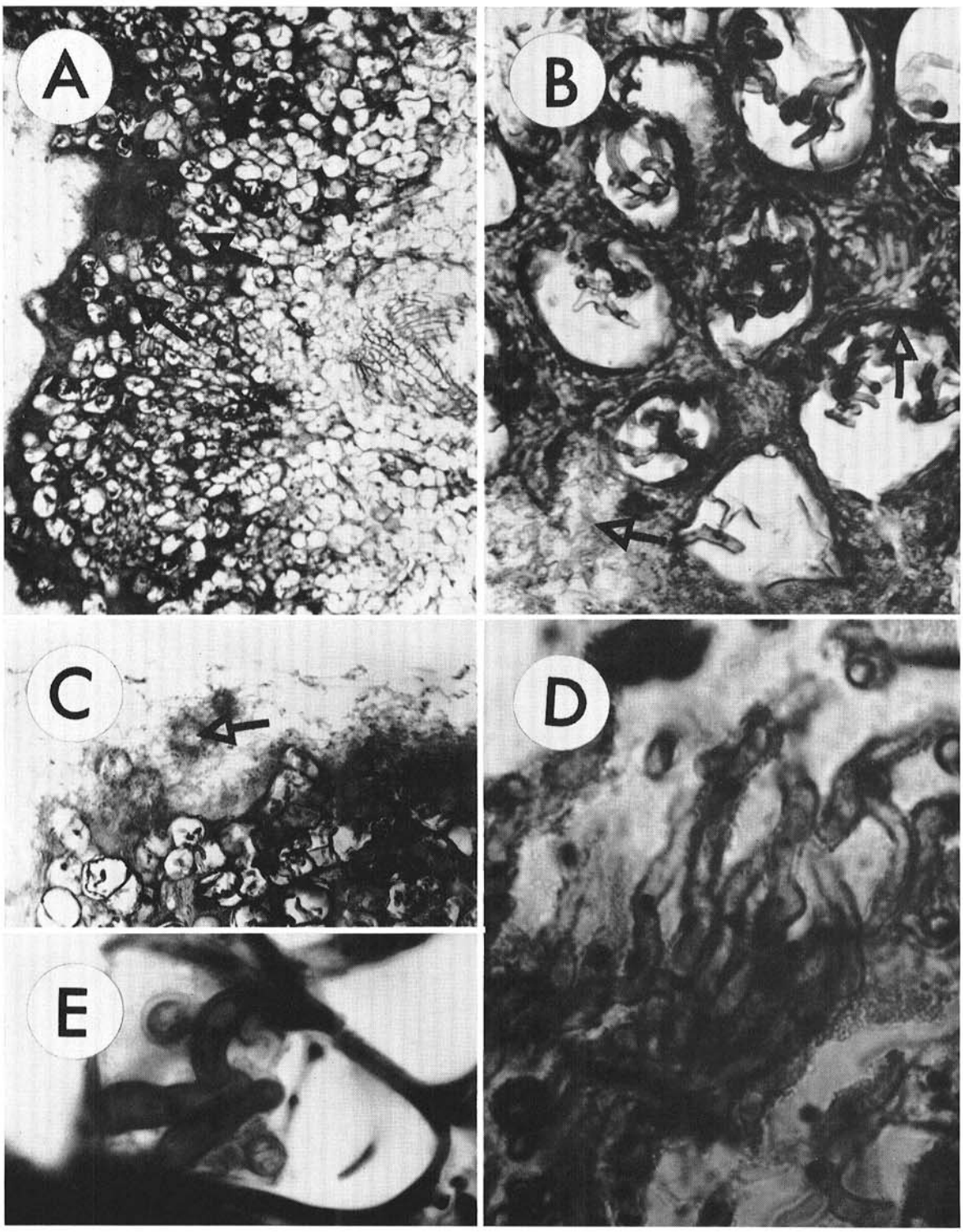

Fig. 1. Histopathology and cytology of *Cronartium ribicola* in tissue cultures of *Pinus monticola*. **A)** Ramification of host tissue culture by proliferating mycelium of the rust. Note the intercellular mycelium and haustoria concentrated on and near the surface (×400). **B)** Heavily parasitized host tissue culture. Note the large volume of fungus tissue (×900). **C)** Heavily parasitized host tissue culture. Note that the mycelial aggregations locate the former positions of individual host cells separated from the main tissue mass (×400). **D)** Mononucleate, intercellular rust mycelium (×900). **E)** Heavily parasitized individual host cell. Note the presence of at least five rust haustoria (×900).

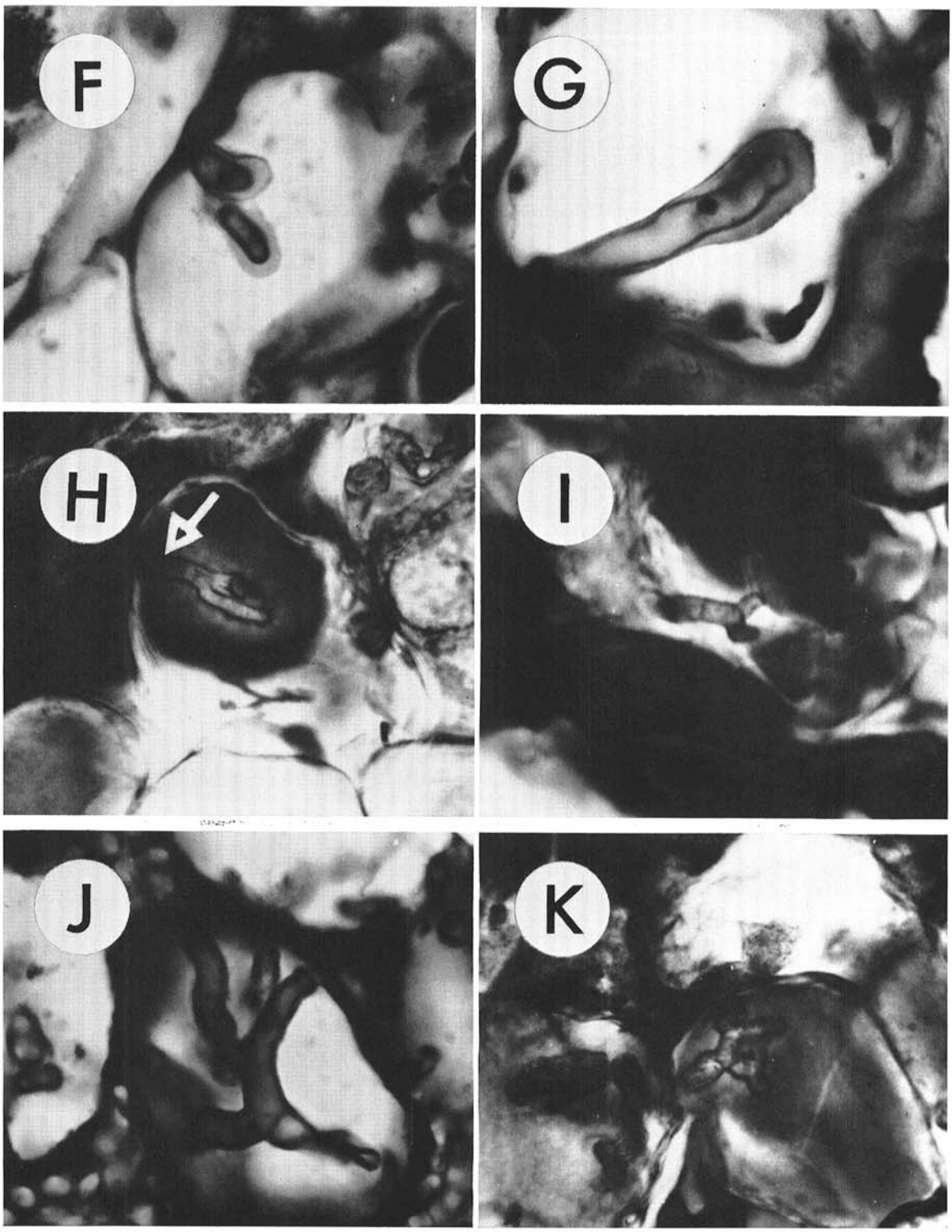

Fig. 2. Histopathology and cytology of *Cronartium ribicola* in tissue cultures of *Pinus monticola*. **F)** Typical short, unicellular haustorium. Note encapsulation (×400). **G)** Typical elongate, unicellular haustorium. Note encapsulation (×900). **H)** Typical two-branched haustorium. Note the position of the septum (×400). **I)** Two-branched, multiseptate haustorium (×400). **J)** Multiple-branched, multiseptate haustorium (×900). **K)** Multiple-branched, palmate haustorium (×400).

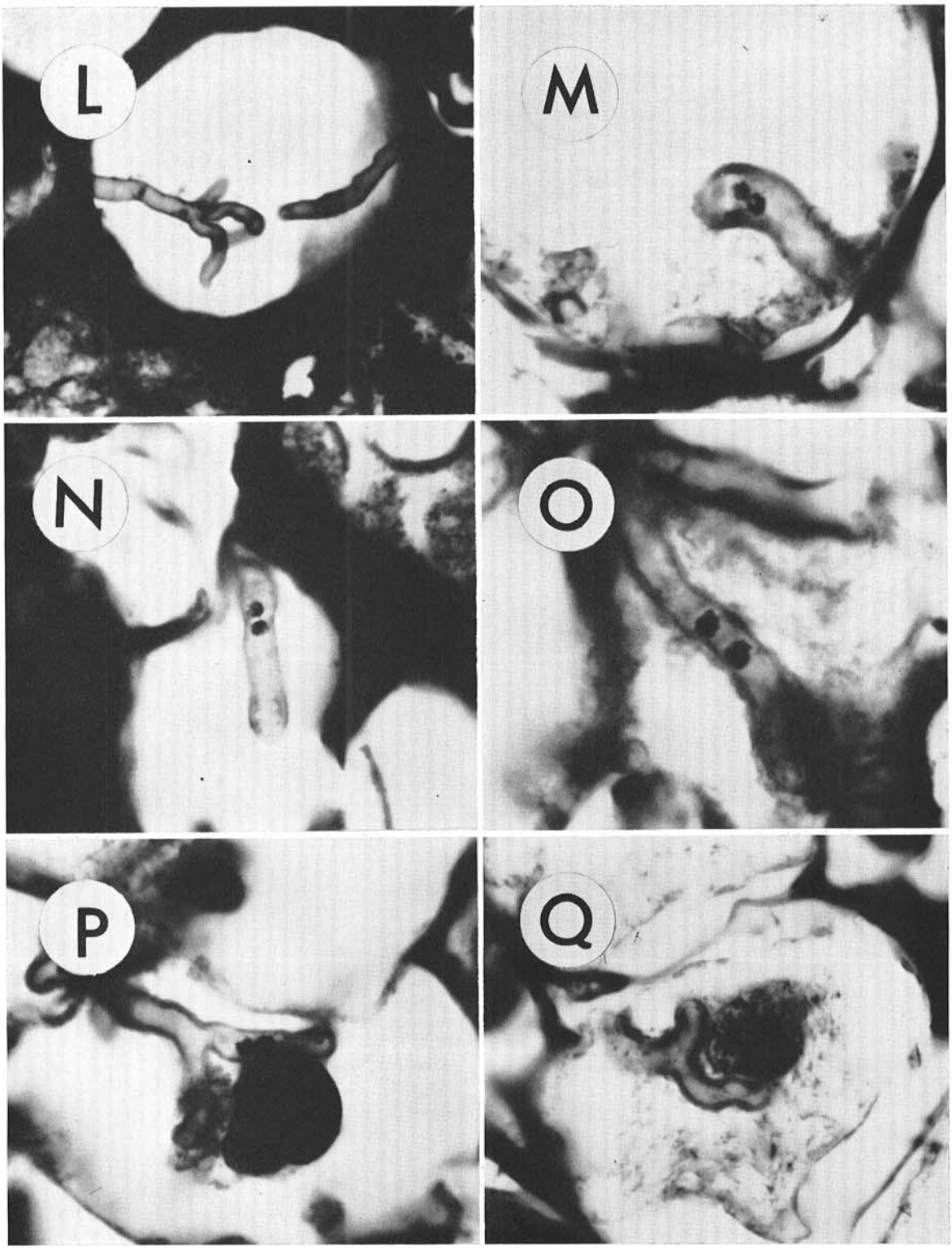

Fig. 3. Histopathology and cytology of *Cronartium ribicola* in tissue cultures of *Pinus monticola*. **L)** Haustorium with multiple-branched head (×400). **M, N, O, P)** Complex, common type haustoria in the infrequently observed binucleate condition. Note the migration of nuclei in P to the branch in closest contact with the host nucleus (×900). **Q)** A two-branched haustorium, adjacent to and around the host nucleus (×900).

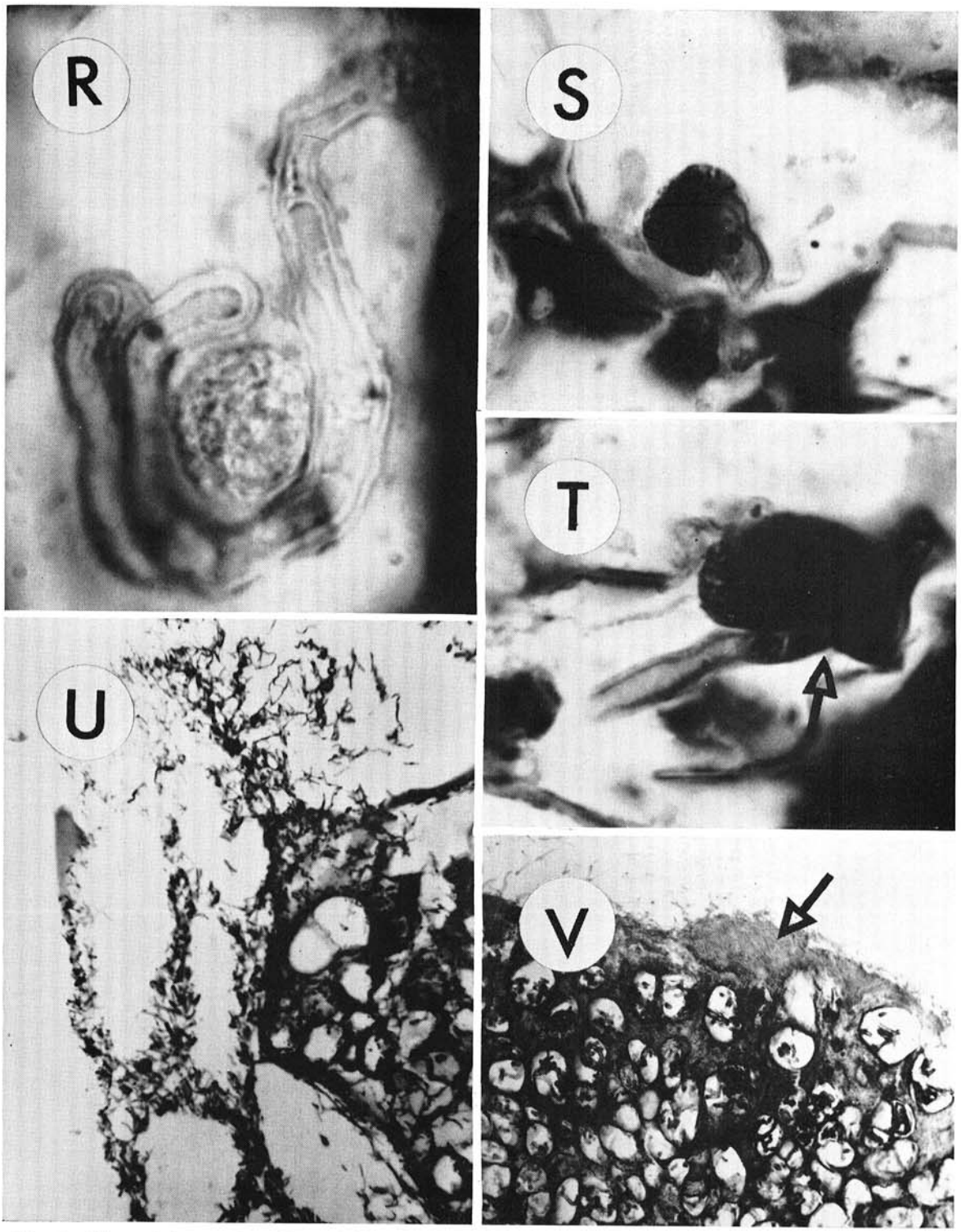

Fig. 4. Histopathology and cytology of *Cronartium ribicola* in tissue cultures of *Pinus monticola*. **R)** Curling of a multibranched haustorium around a host nucleus. Note the positions of the haustorial branches maximize the haustorium-host nucleus interface (×1,000). **S)** Host nucleus penetrated by a short, unicellular haustorium, 5-month infection (×900). **T)** Host nucleus completely penetrated by a haustorium. Note the physical disruption of the host nucleus, advanced infection (×900). **U)** Loculate, aeciumlike body (×400). **V)** Stromatic, nonloculate, aeciumlike body (×400).

Each haustorium was constricted at the point of passage through the host cell wall (Fig. 3-N), and had a well-developed encapsulation layer. This layer was particularly evident in the simple morphological types (Fig. 2-F, G). A tropic response by rust haustoria toward host cell nuclei was indicated by the frequent proximity of haustoria to host nuclei (Fig. 3-P, Q, R, S).

With increasing age, concentrated deposits of heavily pigmented (dark-brown) material appeared in the intercellular spaces (Fig. 1-A) and haustoria pierced, penetrated (Fig. 4-S), and frequently caused physical disruption of host nuclei (Fig. 4-U). After death, the host cell contents and rust haustoria disappeared, leaving large lumina within the tissue.

Pycniospores and fluid were frequently formed on parasitized host tissue cultures. The formation of aecialike bodies (10) also occurred frequently. Yellow-orange, aecialike bodies consisted of dense, pseudostromatic masses of mycelium. These structures were formed on the surfaces of heavily infected host tissue masses (Fig. 4-U, V). Some were loculate (Fig. 4-U). All mycelia contained within these structures were uninucleate. No binucleate peridial cells, aeciospore mother cells, or aeciospores were found.

Discussion.—The histological details of the host-parasite relations and the morphology of this fungus in cultured host tissues were similar to, but more variable than, those found in the natural host tissues examined or reported elsewhere (3, 4, 5, 14). In tissue cultures, the number of haustorial penetrations of single host cells was more frequent when compared to natural tissues. Haustorial morphology was more variable and the number of nuclei in certain haustorial cells was greater in cultured host tissues. The increased parasitization of cultured host cells may be an indication of enhancement in the production of metabolites utilized by the fungus or of a decrease in the defensive reactions of the host in the tissue culture environment.

Variations in haustorial morphology induced by host conditions have been reported previously (17) and were probably associated with the nutritive content of the substrate. The occasional increase in the number of septations per haustorium may be an indication that it is merely a branch of the fungal mycelium modified by the intercellular environment (8). Frequent occurrence of simple haustorial forms suggests that the one-celled haustorium is more efficient and has evolved from the multicellular type. The occurrence of the less efficient primitive forms in host tissue cultures is an indication that these cultures are highly nutritive substrates for this fungus.

The 1%-frequency of occurrence of multinucleate haustoria is interesting, in that binucleate hyphal cells have been observed but rarely in host tissues parasitized by the uninucleate phase (13, 15). Possibly, the increase in the nucleus/cytoplasm ratio may be beneficial to the function of haustorial cells, or branched-type haustoria may have been fixed during the process of division.

The well-developed haustorial sheath observed in this host-parasite combination in vitro was similar to that formed by this rust in trees (3, 5) and by pathogens in other host-obligate parasite combinations (7, 8, 16, 17). Although the fine structure was not investigated, there is no reason to believe it differed substantially from that found within natural host tissues (3).

The failure of the blister rust fungus to form the binucleate, sporulative phase of its life cycle in cultured host tissues has been reported (10, 13). The formation of loculate, aecialike bodies has not been observed previously, but earlier workers reported on the production of similar sterile structures by other rusts (1, 6). Apparently, some of the stimuli needed to initiate aeciospore production were present, but the fungus was unable to form the binucleate, aecia mother cells because other requirements were unsatisfied.

One of the most interesting aspects of this host-parasite system was the characteristic proximity of haustoria to host nuclei. Similar observations have been noted in literature (4, 5, 14, 17). A currently accepted thesis holds that plant growth regulators are involved, directly or indirectly, in the host-parasite interactions of this (2, 12) and of other obligate parasites (18, 19). The primary site of action of these substances apparently is mediated by the nucleus and/or nuclear products (20). Although many other factors could be responsible, the involvement of plant growth-regulating compounds seems plausible.

Blister rust cultures fail to differentiate haustoria or associated structures when separated from cultured host tissues by cellophane membranes (11). This failure is a clear indication that neither these structures nor their physical association with host nuclei are required for the completion of the vital growth processes of this fungus. Therefore, they appear to be adaptations advantageous to but not necessarily required for the utilization of a unique substrate.

LITERATURE CITED

1. Allen, R. F. 1929. Concerning heterothallism in Puccinia graminis. Science 70:308-309.
2. Boyer, M. G. 1967. The relation of growth regulators to the development of symptoms and the expression of stem resistance in white pine infected with blister rust. Can. J. Bot. 45:501-513.
3. Boyer, M. G., & P. K. Isaac. 1964. Some observations on white pine blister rust as compared by light and electron microscopy. Can. J. Bot. 42:1305-1309.
4. Clinton, G. P., & F. A. McCormick. 1919. Infection experiments of Pinus strobus with Cronartium ribicola. Conn. Agr. Exp. Sta. Bull. 214:428-459.
5. Colley, R. H. 1918. Parasitism, morphology, and cytology of Cronartium ribicola. J. Agr. Res. 15:619-659.
6. Craigie, J. H. 1931. An experimental investigation of sex in the rust fungi. Phytopathology 21:1001-1040.
7. Ehrlich, H. G., & M. A. Ehrlich. 1963. Electron microscopy of the host-parasite relationships in stem rust of wheat. Amer. J. Bot. 50:123-130.
8. Fraymouth, J. 1956. Haustoria of the Peronosporales. Brit. Mycol. Soc. Trans. 39:79-107.
9. Harvey, A. E. 1967. Axenic cultures of western white pine cambial explants infected with blister rust. Phytopathology 57:1005 (Abstr.).
10. Harvey, A. E., & J. L. Grasham. 1969. Growth of the rust fungus Cronartium ribicola in tissue cultures of Pinus monticola. Can. J. Bot. 47:663-666.

11. HARVEY, A. E., & J. L. GRASHAM. 1970. Growth of Cronartium ribicola in the absence of physical contact with its host. Can. J. Bot. 48:71-73.
12. HARVEY, A. E., & J. L. GRASHAM. 1969. Effects of plant growth regulators on Cronartium ribicola in tissue cultures of Pinus monticola. Phytopathology 59:1029-1030 (Abstr.).
13. HARVEY, A. E., & J. Y. WOO. 1969. Some cytological characteristics of Cronartium ribicola in tissue cultures of Pinus monticola. Phytopathology 59:12 (Abstr.).
14. HIRT, R. R. 1964. Cronartium ribicola, its growth and reproduction in tissues of eastern white pine. New York State Univ. Coll. Forestry Syracuse Univ. Tech. Pub. 86. 30 p.
15. JEWELL, F. F., & N. M. WALKER. 1965. Normal and abnormal mycelial characteristics of Cronartium quercum in shortleaf pine. Phytopathology 55:1325-1327.
16. MOORE, R. T. 1965. The ultrastructure of fungal cells, p. 95-115. *In* C. C. Ainsworth & A. S. Sussman [ed.] The fungi, an advanced treatise. Vol. I. Academic Press, N.Y.
17. RICE, M. A. 1927. Haustoria of rust fungi. Torrey Bot. Club Bull. 54:63-153.
18. SEQUEIRA, L. 1963. Growth regulators in plant disease. Annu. Rev. Phytopathol. 1:5-30.
19. SHAW, M. 1966. Cell biological aspects of host-parasite relations of obligate fungal parasites. Can. J. Bot. 45:1205-1220.
20. VAN OVERBEEK, J. 1966. Plant hormones and regulators. Science 152:721-731.
21. WOO, J. Y. 1970. Techniques for sectioning and staining tissue cultures of western white pine. USDA Forest Serv. Res. Note INT-116. 4 p.