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# ENDOPHYTIC FUNGUS IN ROOT CORTICAL CELLS OF THE FERN *LYGODIUM JAPONICUM* SWARTZ

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## ABSTRACT

Cross-sections of the enlarged branch roots of *Lygodium japonicum* Swartz show a diarch stele of small diameter surrounded by three or four layers of cortical cells, each cell having a diameter approximating that of the stele. It is shown that the outer cortical cells contain starch grains while the cortical cells in contact with the stele contain fungal hyphae and clumps of darkly staining material. This finding is at variance with Clarke (1936), who reports only the presence of starch in the cells of the cortex. Positive identification of the fungus is to be the subject of a subsequent study.

## INTRODUCTION

The presence of endophytic fungi in the Schizaeaceae was reported in the gametophyte and young sporophyte of *Schizaea pusilla* Pursh by Britton and Taylor in 1901. Since then, mycorrhizae have been found common to the gametophytes studied within this family (Bierhorst, 1965-1968). Clarke (1936) reported on the morphology and anatomy of *Lygodium japonicum* Swartz but did not mention the presence of fungal hyphae in either the gametophyte or the sporophyte. Bierhorst (1965-1968) has given thorough reports on gametophyte habits and gametophyte-sporophyte relationships for several members of this family, but not for species of *Lygodium*. In 1966 he noted the continuation of hyphae from the gametophyte of *Schizaea melanesica* Selling into the cortex of the sporophyte root.

In this paper first mention is made of the presence of an endophytic fungus in the root cortical cells of *Lygodium japonicum* Swartz. The gametophyte was not studied. Presently, gametophytes and sporophytes of this species are being cultured in the laboratory for the purpose of isolating the fungus. This work is being done by Norman Meyer, a graduate student working under Dr. E. T. Browne, Jr.

## MATERIALS AND METHODS

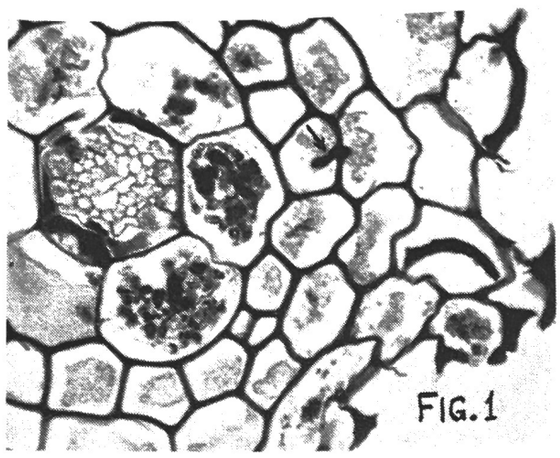
Branch roots used in the study were taken from a root and rhizome complex collected by Dr. E. T. Browne in 1968. The specimen from which it was taken was growing naturally in Thomasville, Georgia. The root-rhizome complex was fixed immediately in Allen's modification of Bouin's Solution II (Sass, 1958), and specimens received by me were processed by standard methods. The roots were embedded in Tissuemat and sectioned at 10 microns. The several staining procedures used were as follows:

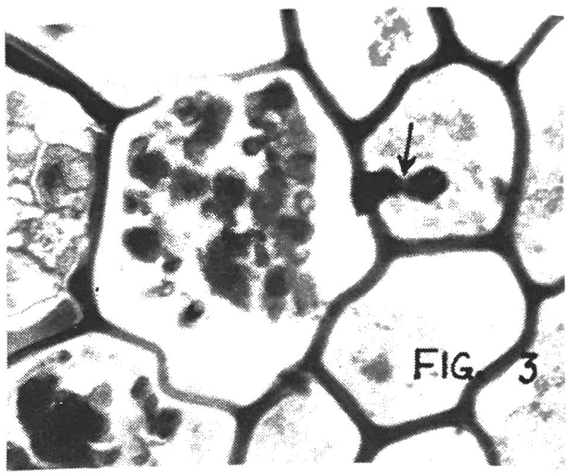
| *Stain* | *Staining Time* |
|---|---|
| 1. 1% thionin in 5% aqueous phenol (Johansen, 1940) | 45 minutes |
| 2. 0.3 g. $I_2$, 1.5 g. KI in 100 distilled HOH (Johansen, 1940) | few seconds |
| 3. 1% thionin | 45 minutes |
| counterstained with saturated solution of Orange G in 99% isopropanol (modified from Stoughton, 1930) | 30 seconds |

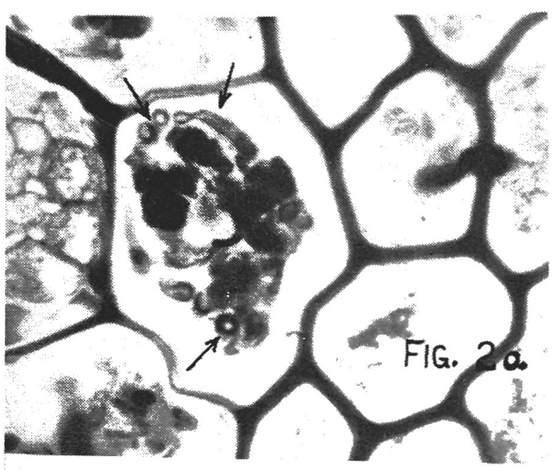
All stained sections were mounted in Piccolyte S-100. Selected photographs of Procedure 3 are included in this paper.

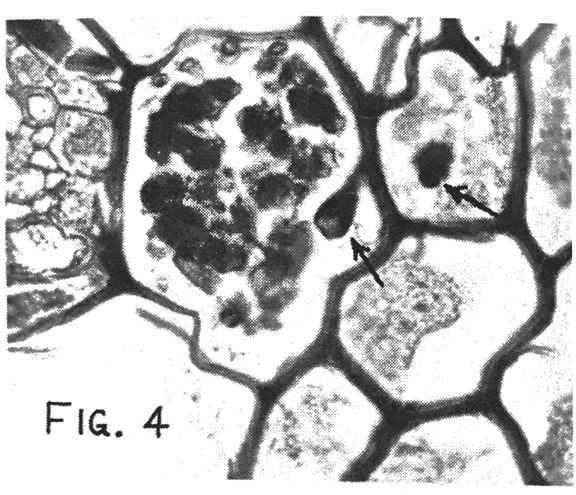
## RESULTS

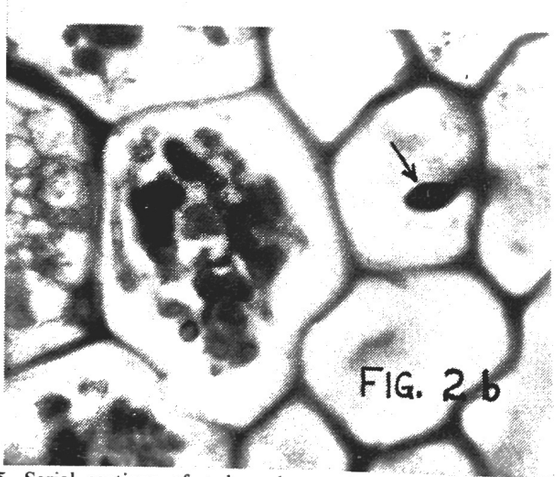
Single staining with thionin showed the innermost cells of the cortex to be congested with a dense material having strong affinity for thionin, while the outer cortical cells were unstained and free of this substance. Single staining with $I_2$-KI positively identified starch in the outer cortical cells, while the inner cells remained unstained. Thionin counterstained with Orange G showed darkly staining clumps of material and sections of hyphae within the innermost cortical cells, and also showed large hyphae extending from one cortical cell into another (Figs. 1-5).

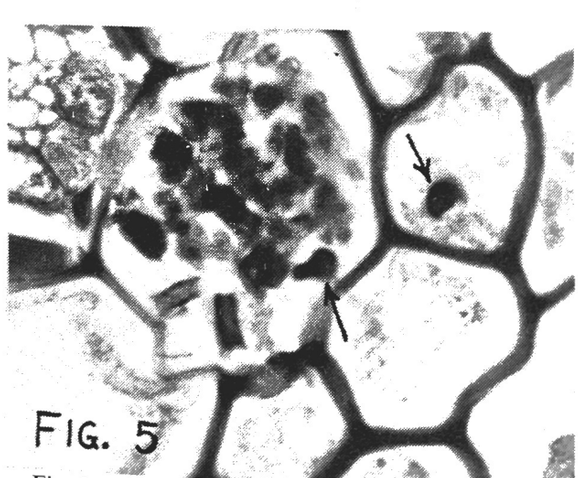



Figs. 1-5. Serial sections of a branch root from *Lygodium japonicum* Swartz showing hyphae and heavily stained clumps within innermost cortical cells and showing the progression of large, darkly stained hyphae through two cortical cells. Stained with thionin and Orange G.

Magnification: Fig. 1, 10X objective, print X 125.
Figs. 2-5, 97X oil objective, print X 1210.

Fig. 1. Low power view showing thionin-stained clumps filling some cortical cells next to the stele. A large hypha penetrates a cell wall (arrow).

Fig. 2a. Higher magnification of Fig. 1. Both longitudinal and cross sections of hyphae can be seen (arrows).

Fig. 2b. As Fig. 2a but focused to show enlarged hypha within a cortical cell (arrow).

Fig. 3. Arrow indicates branching of the large hypha or contact between two hyphae.

Figs. 4 and 5. Parts of the large hyphae may be followed in two cortical cells (arrows). Longitudinal and cross sections of other hyphae may be seen in the cortical cell next to the stele.