

## Histology of Witches'-Broom Caused in Cacao by *Crinipellis pernicioso*

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### ABSTRACT

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Mycelium of *Crinipellis pernicioso* was found throughout the cortex, phloem, ray, and pith of mature, green, and dry witches'-brooms of cacao (*Theobroma cacao*). The mycelia in green and dry brooms were composed of intercellular, uninucleate hyphae 4–9  $\mu\text{m}$  wide that were branched, sometimes distorted and swollen, and septate with conspicuous walls. Dry brooms contained granular mycelia composed of binucleate and often

intracellular hyphae (2–8  $\mu\text{m}$  wide, with clamp connections) that originated by branching from a uninucleate mycelium. Host cells were in disarray in dry brooms, and uninucleate mycelium appeared to be intermixed with host cells. No mycelium was found in healthy tissues to which brooms were attached. Arthrospores (6–7  $\times$  5  $\mu\text{m}$ ) were observed in dry brooms.

*Additional key words:* *Marasmius perniciosus*.

Witches'-broom, caused in cacao (*Theobroma cacao* L.) by *Crinipellis pernicioso* (Stahel) Singer (11), was first reported in Surinam in 1895 (1,2). At the present time, the disease is widespread in the tropical lowlands of northern South America and the Caribbean islands of Trinidad, Tobago, and Grenada, but it still is absent from cacao-growing areas in Central America, Bahia (Brazil), and Africa (1,2). Yield losses are estimated to range from 20 to 80% (7,9).

Incomplete knowledge of the etiology and epidemiology of the witches'-broom disease has contributed to failure to develop adequate control measures. Two aspects of the disease that have not been resolved are the relation of the fungus to broom tissue formation and the genetic constitution of the infectious form(s) of the causal agent. This report describes the location and nuclear condition of *C. pernicioso* mycelia in host tissue.

### MATERIALS AND METHODS

Vegetative and/or cushion brooms in several stages of development were collected from cacao plantings at Pichilingue, Ecuador. Small segments were taken at 3-cm intervals along the entire broom and processed by routine procedures for killing, fixing, paraffin embedding, and sectioning (12,13,19). Sections were stained with Conant's quadruple stain (3) except that they were kept in the safranin only 1 min. Nuclei also were stained with a modified HCl-Giemsa technique (10,18). Tissues were hydrolyzed in 3N HCl for 20 min at room temperature, washed 10 min in phosphate solution (pH 7.2), stained in Giemsa for 10 min, and mounted in phosphate buffer. Giemsa-stained tissues remained suitable for examination for 4–6 hr.

### RESULTS

Intercellular mycelium composed of uninucleate hyphae 2–3  $\mu\text{m}$  wide with conspicuous walls and infrequent nuclei was found only in the inner portion of the cortex in the proximal half of very young brooms. No mycelium was found in the distal half of such brooms. Uninucleate mycelium was found in the cortex, phloem, ray, and pith tissues throughout the entire length of mature, green brooms. The mycelium was composed of branched, septate, swollen, and distorted hyphae in some places (Fig. 1).

Binucleate mycelium was observed to arise by hyphal branching from the uninucleate type in mature, green brooms (Fig. 2). The binucleate mycelium, often with clamp connections that were nearly translucent, was more granular than the uninucleate form, was regularly intracellular, and appeared to be fragile. Hyphae in the binucleate mycelium was 2–8  $\mu\text{m}$  wide.

Host tissues were partially disrupted in the distal portions of mature brooms, and arthrospores (6–7  $\times$  5  $\mu\text{m}$ ) (5) were observed occasionally in the distal portions of dry brooms.

### DISCUSSION

Mycelium of *C. pernicioso* has been reported previously (8,17,20,21) to be morphologically variable. We determined that mycelium of the fungus invades the entire length of mature brooms. Baker and Crowdy (1) postulated that mycelium spread from brooms into healthy tissues to initiate formation of lateral brooms. The authors have found no report that the pathogen has been observed in or isolated from tissues to which brooms are attached. Healthy branches, flowers, and pods are commonly seen growing next to broom attachment sites. No mycelium was found in healthy tissues adjacent to brooms.

The uninucleate mycelium of *C. pernicioso* in mature brooms followed an intercellular pattern of development with the same morphological characters observed in young brooms so long as infected tissues remain organized. In host tissues that were seriously disrupted, mycelium was intermixed with disorganized tissue, but the mycelium was always intercellular.

The monokaryotic character of the mycelium in living and dead, diseased tissues is a unique feature of *C. pernicioso*, since other hymenomycetes have no parasitic monokaryophase. Pegus (17) reported that the monokaryophase of the fungus was restricted to living, diseased material and suggested that only the monokaryophase of the organism is pathogenic. He postulated the existence of a dikaryophase based on his observation of mycelium with clamp connections, but exceptions to this association have been reported (14–16). We were able to find the dikaryophase in dry broom material.

Our observations indicated that infection by *C. pernicioso* is confined to hypertrophied tissues. During a considerable period while the broom is being formed and after hypertrophied tissues become necrotic, the mycelium is strictly uninucleate, intercellular, and without clamp connections. At some later time, the binucleate, intracellular mycelial form appears. This change in nuclear condition occurs by some undetermined mechanism during broom development prior to drying. Both mycelial forms were found in mature, green, and dry brooms.

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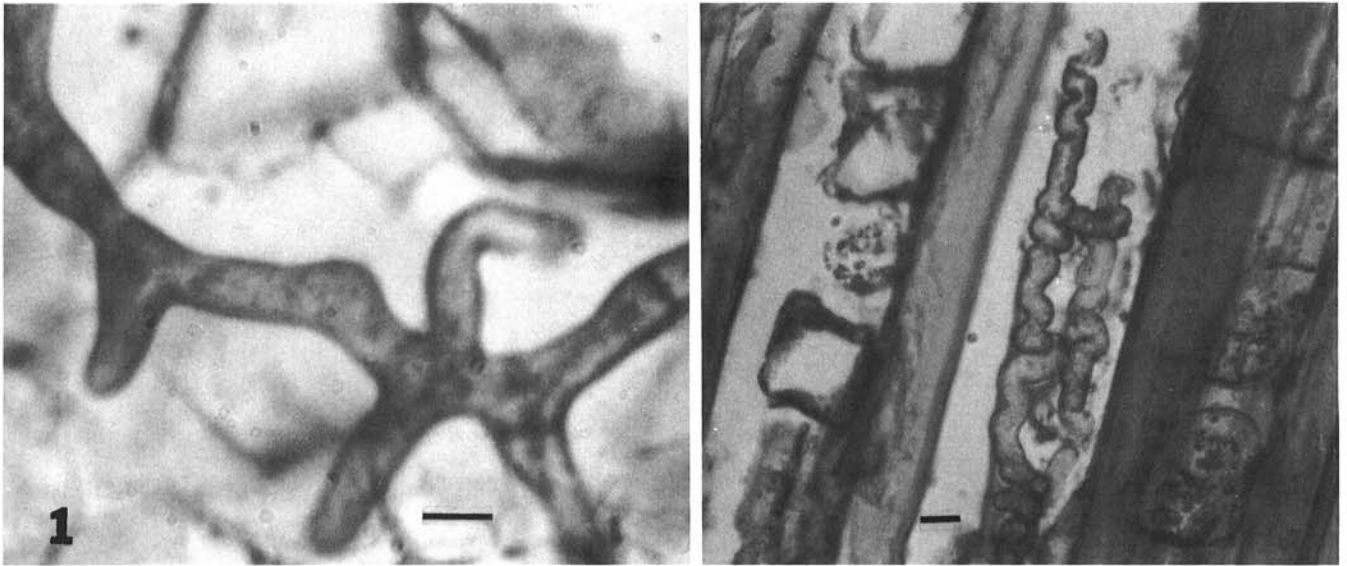


Fig. 1. *Crinipellis pernicioso* mycelia in witches'-broom of cacao. Intercellular, branched, septate mycelium in the cortical (parenchymatous) tissue of a broom (left); and convoluted, intercellular mycelium in ray tissue. Bars equal 10 µm.

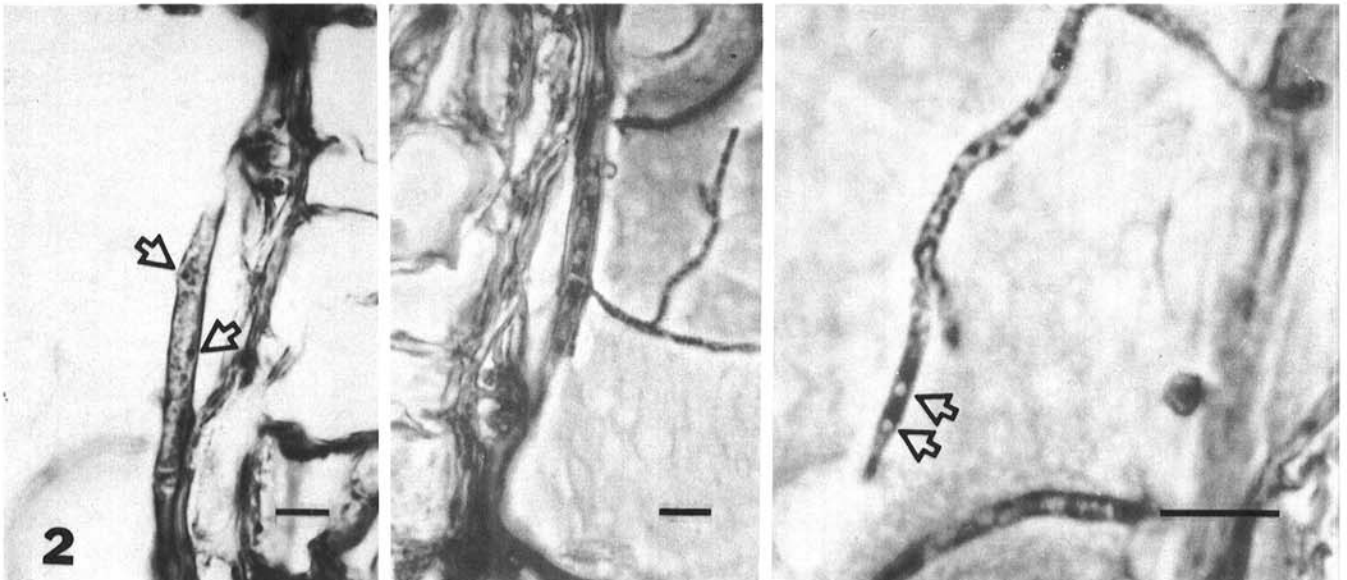


Fig. 2. Evidence of differing nuclear status of the two mycelial types of *Crinipellis pernicioso* and the transition from uninucleate to binucleate. Larger diameter, uninucleate mycelium (left) with nuclei indicated by arrows (see cross wall between nuclei); branching of large-diameter mycelium to smaller-diameter (binucleate) mycelium (middle); and adjacent nuclei (arrows) in small-diameter binucleate mycelium (right). Two photos on right are phase contrast. Bars equal 10 µm.

The presumed homothallism of the pathogen is based on the dikaryotization of originally monokaryotic mycelium derived from single, uninucleate basidiospores (4,6). We believe that additional studies of the pathogen are needed to resolve the conflicting evidence of homogeneity of the fungus in culture and the persistent presumption of pathogen variability (pathotypes) in the field. The mechanism(s) for production of arthrospores also may be determined by laboratory studies, but their role will remain unknown until the disease cycle is better understood.

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