Arthroconidia Formation in Cultures of *Marasmius perniciosus*

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ABSTRACT

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Binucleate arthroconidia developed when specific single-basidiospore isolates of *Marasmius perniciosus* from Trinidad and Surinam were paired in culture. Arthroconidia developed from dikaryotic hyphae with clamp connections and germinated to produce similar hyphae. Symptoms were not induced in cacao seedlings inoculated with arthroconidia.

Additional key words: Crinipellis perniciosa, witches' broom of cacao.

Witches' broom of cacao (*Theobroma cacao* L.) was observed first in 1895 in Surinam (2). In 1915, Stahel (15) attributed the disease to *Marasmius perniciosus,* a previously unknown agaric, assumed to be endemic to the Amazon Valley which also is considered to be the center of origin for cacao. [The fungus has been reclassified as *Crinipellis perniciosa* by Singer (10) but many cacao workers still retain the original name given by Stahel.] The disease is now widespread in the tropical lowlands of South America where, in some areas, it causes crop losses up to 70% (2, 10). It is restricted to continental South America, and the Caribbean Islands of Trinidad, Tobago, and Grenada but is still absent from Bahia, Brazil, the main cacao producing region of Latin America. Possible introduction of the pathogen into new areas where cacao is grown is a continuous threat despite geographical barriers and recent severity of witches' broom disease in Ecuador has led to speculation concerning the development of new pathotypes of the fungus.

Asexual reproduction is common in basidiomycetes (6, 11). Many hymenomycetes have been reported to produce one or more kinds of asexual conidia, including chlamydospores or arthroconidia (improperly named oidia), but neither an imperfect stage nor any form of resting structure has been reported for *M. perniciosus* (2, 10).

MATERIALS, METHODS, AND RESULTS

Arthroconidial formation was originally observed in paired cultures utilizing two single-basidiospore isolates and, more specifically, when the TB-11 (Trinidad) culture was grown with the SB-1 or SB-8 (Surinam) isolates. Formation of arthroconidia also was observed later in mass-basidiospore cultures derived from Surinam material.

The arthroconidia observed were hyaline, short, and cylindrical with thin walls. They ranged in size from 5-9 × 1.5 - 3.0 μm, and were formed in chains by fragmentation of hyphae or hyphal branches bearing clamp connections (Fig. 1). Staining with 1% aniline blue revealed that the arthroconidia were binucleate (Fig. 2 A-B).

The process of arthroconidium formation in *M. perniciosus* apparently is similar to that observed in other hymenomycetes (6). Segmentation usually takes place in a basipetal manner beginning near the apex of a hypha. The first indication of fragmentation is the development of a thin, almost imperceptible, refractive, transverse line in the protoplasm near the apex of the hypha which does not undergo further elongation. Conidial ontogeny was determined using the slide culture technique of Riddell (14). Arthroconidia are formed only on aerial hyphae, and remain attached forming chains of variable length, but they are easily dislodged by drops of water running over tilted plate cultures.

Arthroconidia germinated readily, sometimes even while still attached in the chains (Fig. 3), under conditions of high relative humidity. Many arthroconidia germinate by a single germ tube within 24 hours.

Cultures maintained their capability to produce arthroconidia through several transfers when appropriately paired with other isolates or when transfers were made from paired cultures that produced arthroconidia. Hyphae bearing clamp connections were formed on plates seeded with arthroconidial suspensions and in cultures obtained from single arthroconidia.

Witches' broom symptoms were not observed within 16 weeks after inoculation of 30-day cacao seedlings with arthroconidial suspensions or with disks from agar cultures. Also, infection was not obtained when 4-day-old cacao seedlings were inoculated by dipping them in a suspension of arthroconidia (3×10^5) although this procedure has been used with consistent success for basidiospore inoculation (9). Symptom development was induced in these studies only on plants inoculated with basidiospore suspensions.

DISCUSSION

It is well known that the mycelia of several fungi occasionally break into chains of arthroconidia when the food supply of the medium has been exhausted or under other conditions unfavorable for growth (6). In the studies herein reported, production of arthroconidia by *M. perniciosus* seemed to be a specific function of actively growing mycelium that occurred in varying degrees on different media containing high or low amounts of

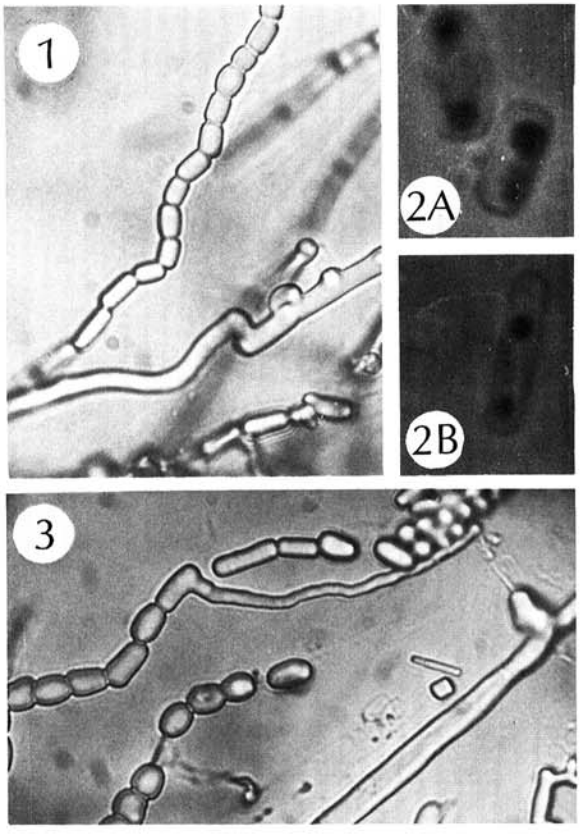

Fig. 1-3. Catenulate formation of arthroconidia from mycelium of *Marasmius perniciosus* with hyphal clamp connections (× 1,000). **2-A and B)** Binucleate arthroconidia of *Marasmius perniciosus* (× 2,500). **3)** Germination of arthroconidia of *Marasmius perniciosus* (× 1,000).

nutrients. Culture conditions may have affected the growth of the mycelium, but not the process of arthroconidial formation. It was observed that arthroconidium-forming isolates, in all cases regardless of media or incubation conditions, always produced arthroconidia.

Although arthroconidial formation is common in hymenomycetes (7, 11, 12), its occurrence has been limited to the monokaryotic mycelium of heterothallic species. Very few species have been found that produce from dikaryotic mycelium binucleate arthroconidia which later fragment to become monokaryotic arthroconidia (3, 4, 5, 6, 7, 16). Monokaryotic hyphae of *M. perniciosus* has not been observed in culture (1, 2, 13), because this stage apparently is ephemeral based on the observations reported by Delgado and Cook (8). Since arthroconidia were formed in cultures bearing clamp connections, it could be anticipated that the fragmentation of the dikaryotic hyphae of *M. perniciosus* would result in binucleate arthroconidia and stained preparations demonstrated this to be the case. A function as diploidizing agent is the most important role that has been attributed to uninucleate arthroconidia in the hymenomycetes (3, 6, 7, 16). In the case of *M. perniciosus* it would seem that arthroconidia probably function only in ordinary vegetative reproduction.

The observation that arthroconidial formation by *M. perniciosus* occurred in only a few cultures (of paired single-basidiospore isolates) may support the observation by Lyman (12) that asexual spores formed by some hymenomycetes are found only in vitro. However, the fact that arthroconidial production always was associated with cultures obtained from mushrooms produced on brooms from Surinam could be an indication of genetic significance concerning capability for variation in the fungus. Surinam is the presumed center of origin for the fungus and where the disease was first observed. It remains to be established if arthroconidia of *M. perniciosus* are produced under field conditions where they could serve to disseminate and propagate the pathogen.

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