Ultrastructural Examination of the Puccinia graminis-Darluca filum Host-Parasite Relationship

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ABSTRACT

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Uredial sori of *Puccinia graminis* infected with *Darluca filum* were examined by transmission and scanning electron microscopy to determine the ultrastructural morphology of the host-parasite interface. We concluded that uredospores are penetrated directly by nonspecialized hyphae, with penetration largely dependent on the enzyme-producing capacity of the hyperparasite. In its association with *P. graminis, D. filum* may be described as a penetrating (nonhuastorial) destructive biotrophic mycoparasite.

Additional key words: mycoparasite, hyperparasite.

Darluca filum (Biv.-Bern. ex Fr.) Cast., the imperfect state of *Eudarluca caricis* (Fr.) O. Eriks., (11) is a cosmopolitan hyperparasite associated with many species of rust fungi (1, 10, 14, 16, 19). *Darluca filum* is most commonly observed as clumps of shiny, black, spherical pycnidia situated among the spores of uredial sori, where it presumably derives nutrients by direct hyphal penetration of uredospores (19). *Darluca filum* also has been reported to penetrate pycnial, aecial, and telial spore stages (1, 16), though some disagreement exists on these points (20). Direct penetration of sub-basal cells of uredial sori (19) and rust mycelium (12) has been reported, but these observations are also in dispute (1). In uredial sori, however, spore production is disturbed and in some cases completely stopped (1, 4), suggesting *Darluca filum* may possess potential in the biological control of rusts (4, 12, 14).

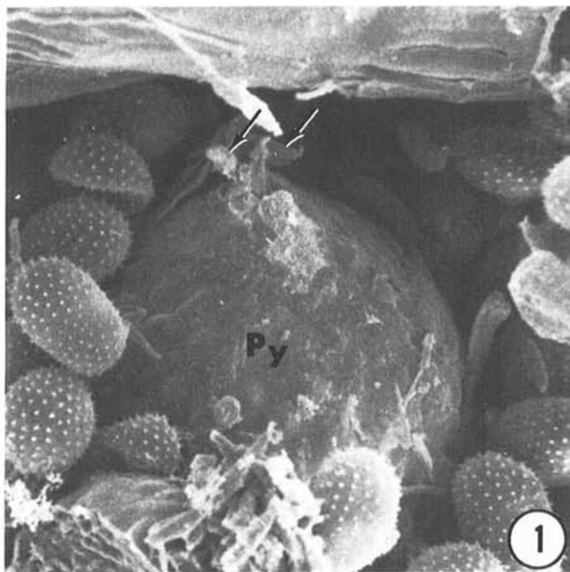
Since hyperparasitic mechanisms are diverse and complex, studies on the parasitic mode of many mycoparasites (6) have concentrated on the morphology of nutrient-securing structures (3, 17). Similar studies have not been done with *D. filum,* and would be more difficult considering the type of host tissue parasitized and the fact that its rust hosts are obligate parasites. This study reports the ultrastructural morphology of the *P. graminis-D. filum* host-parasite interface, and describes the nutrient-securing structures and the effects of parasitism on the fungal host.

MATERIALS AND METHODS

Samples were collected from a field plot of orchardgrass (*Dactylis glomerata* L.) infected with rust (caused by *Puccinia graminis* Pers.), many sori of which were infected with *Darluca filum*. Controls consisted of uredial sori free of *D. filum* infection. For transmission electron microscopy (TEM), *D. filum*-infected and *D. filum*-free uredial sori were excised from orchardgrass leaves. The samples were fixed with 3% glutaraldehyde-3% acrolein in a 0.1 M phosphate buffer, pH 6.8, for 4 hours at room temperature. Following several rinses in buffer, the samples were postfixed with 2% OsO_4 in the same buffer for 3 hours at room temperature. They were rinsed with several changes of buffer, dehydrated in a graded acetone series, and embedded in Spurr's low viscosity medium (21). Ultrathin sections were cut with a diamond knife on a Reichert Om-U2 ultramicrotome, then stained with uranyl acetate and lead citrate (18). Transmission micrographs were obtained with an RCA EMU-3G electron microscope operating at 100 kV. Specimens employed for scanning electron microscopy (SEM) were prefixed with glutaraldehyde-acrolein as described, rinsed with buffer, and postfixed with buffered 2% OsO_4 for a minimum of 6 hours. Fixatives, buffer vehicles, and fixation temperatures were identical to those used for TEM samples. Following fixation, SEM samples were rinsed with distilled water and dehydrated in a graded ethanol series. The absolute ethanol was replaced by amyl acetate with a graded ethanol-amyl acetate series and the specimens were critical-point dried (2). The dry samples were mounted on specimen holders with conductive cement, coated with gold in a high vacuum evaporator, and examined with a JEOL JSM-S1 scanning electron microscope operating at 10 kV.

RESULTS

Scanning microscopy of infected uredial sori clearly demonstrated the intimate association of *D. filum* and its host. Developing or mature pycnidia were immersed in the pustule (Fig. 1) and two-celled conidia with conspicuous terminal mucilaginous appendages were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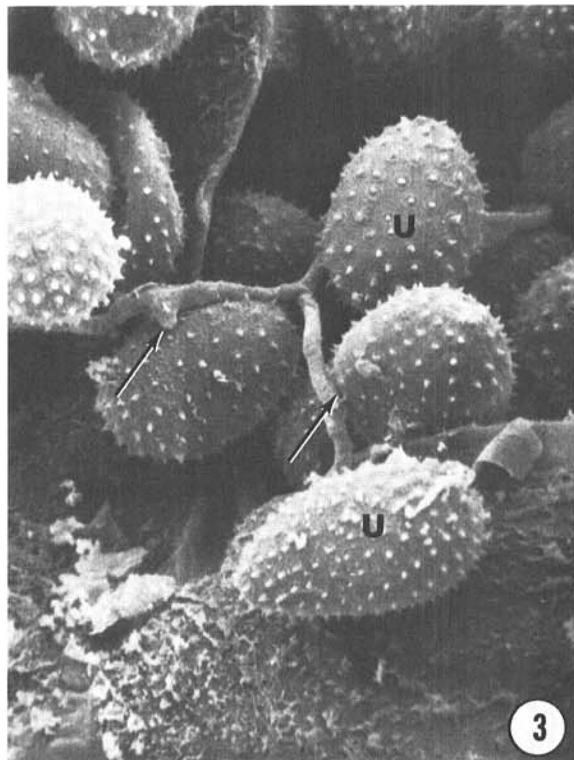
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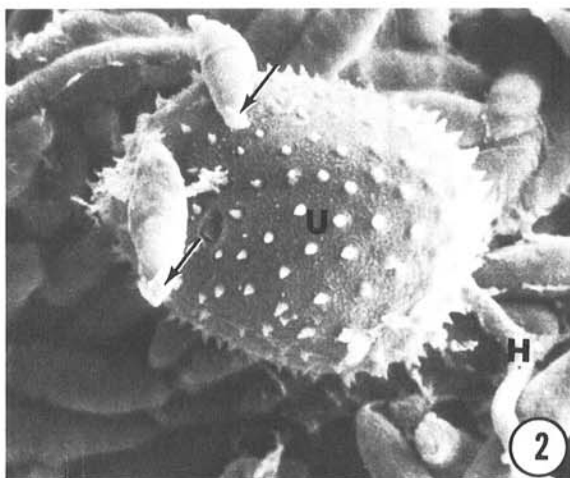
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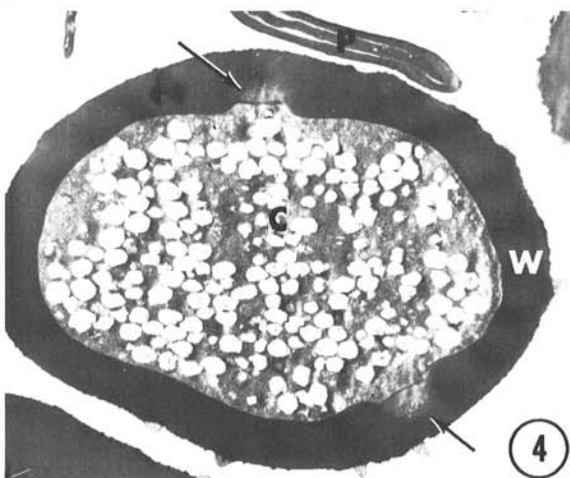
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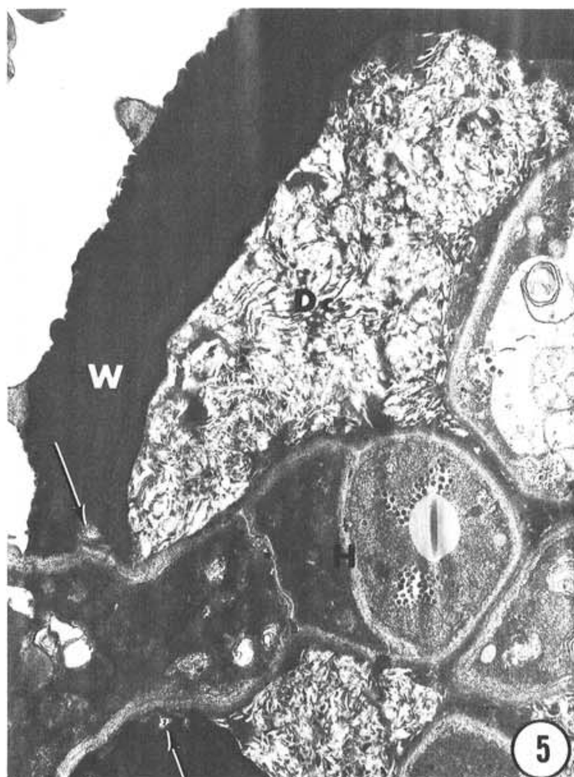
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abundantly produced (Fig. 2). Occasionally, uredial sori were completely overgrown with mycelium, pycnidia, and conidia of the hyperparasite. Hyphae of *D. filum* were observed on the pustule surface (Fig. 3), but the majority of mycelial development occurred below exposed layers of uredospores.

Transmission microscopy of cross sections of uredial pustules revealed widespread penetration of uredospores by *D. filum*. However, penetration of sub-basal cells or rust mycelium was not observed. Penetration of uredospores occurred at random locations on the spore surface and penetration sites were distinct from germ pore plugs which were observed in both healthy and infected spores (Fig. 4). Penetration of uredospore walls resulted in the production of electron-lucent cavities or channels adjacent to the walls of the penetrating hyphae and may indicate enzymatic degradation of host wall components. In some cases, fragments of uredospore wall material appeared separated from the wall by this network of channels. Following wall penetration, total destruction of cytoplasmic contents occurs (Fig. 5). The extreme disorganization of cytoplasm in penetrated spores suggests rapid diffusion of the toxic substance, possibly an enzyme, resulting in loss of spore viability. Penetration of uredospore walls by *D. filum* hyphae apparently can be initiated from outside or inside the spore wall, as was indicated by sequential sections through many other infected spores. This disagrees with reports on other mycoparasites which state that penetration does not occur from within infected host tissues (7, 8). Growth of the hyphae within the uredospore wall appeared unrestricted and nondirectional. This observation, along with the failure to observe any specialized penetrating structures such as appressoria, penetration pegs, etc., indicates direct penetration of a nonspecific type rather than a more specific, haustorial type.

DISCUSSION

In most investigations of parasitism the fungal parasite is usually classified with respect to its effect on the host and the mode of parasitism. However, descriptive terminology has been inconsistent and is confusing. Gäumann (13) classified all parasites as either biotrophic (deriving nutrients from living host tissue) or necrotrophic (deriving nutrients from cells which have died) usually in response to the approaching parasite. A system devised by Bessey (5) and modified by Lilly and Barnett (15), further subdivided biotrophic parasites into either balanced or destructive biotrophs. Balanced biotrophs live in relative harmony with their hosts, doing little or no damage. They are capable of mechanical penetration but are weak producers of enzymes and/or toxins. In contrast, destructive biotrophs cause severe damage to host tissues, are strong producers of enzymes, but are relatively weak mechanical penetrators. Further subdivision of each category may be made on the basis of parasitic mode (6, 9).

Fitting *D. filum* into this classification scheme has proved difficult because the necessary preliminary ultrastructural work had not previously been done. Sappin-Trouffy (19) reported the morphology of the host-parasite interface between *Puccinia porii* and *D. filum,* describing penetration of uredospores and sub-basal cells by *D. filum*. Subsequent investigations of *D. filum* on a number of the rust hosts reported conflicting data (1, 20) which suggested that only spores are penetrated. The present study demonstrates that infection of *P. graminis* uredospores by *D. filum* results in spore wall penetration and destruction of cytoplasm. Wall penetration may be due to combined mechanical and enzymatic processes, with enzymatic digestion presumably playing the major role. The absence of specialized penetration structures indicates that direct penetration may be accomplished by any vegetative hypha. Penetration is accomplished by digestion of wall material and it appears to be unrestricted judging from the random orientation of hyphae within spore walls. This suggests that both degraded wall material and cytoplasmic constituents may be nutritionally beneficial to the invading fungal hyperparasite. Based on these observations, we consider *D. filum* in its association with *P. graminis* to be a destructive biotrophic mycoparasite which enters host cells by direct hyphal penetration.

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Fig. 1-5. Electron micrographs of *Darluca filum* on *Puccinia graminis*. **1)** Portion of a uredial pustule of *P. graminis* containing a pycnidium (Py). Conidia (arrows) are present at the ostiole of the pycnidium (× 750). **2)** Conspicuously ornamented uredospore (U) in conjunction with hypha (H) and typical two-celled conidia of *D. filum* bearing mucilaginous appendages at their tips (arrows) (× 2,500). **3)** Portion of uredial sorus depicting uredospores (U) in contact with a hypha of *D. filum*. Points of intimate contact (arrows) indicate sites of potential hyphal penetration (× 1,500). **4)** Uninfected uredospore of *P. graminis* showing the osmiophilic spore wall (W) and germ pore plugs (arrows). Spore cytoplasm (C) contains many electron-lucent vacuoles. Paraphysis (P). (× 5,800). **5)** Uredospore of *Puccinia graminis* showing the point of penetration by a hypha of *Darluca filum*. The cytoplasm of such spores (Dc) is disorganized and normal organelles are lacking. The abundance of *D. filum* hyphae suggests rapid and extensive intraspore development of the parasite. Electron-lucent areas (arrows) within the uredospore wall adjacent to the penetrating hypha (H) suggests enzymatic digestion of spore wall components (× 12,750).

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