Widespread Distribution of *Delacroixia coronata* and other Saprophytic Entomophthoraceae in Plant Detritus

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Delacroixia coronata (Cost.) Sacc. & Syd. (*1, 2*) is one of the few species in the Entomophthoraceae which, unlike the many insect parasites belonging in that family, develop well on various artificial media commonly used for cultivating microscopic fungi. When planted on Petri plates of a fairly transparent medium like maize meal agar it reveals vigorous mycelial growth, abundant formation of large globose conidia singly on aerial conidiophores, forcible projection of these conidia some distance from their place of origin, frequent germination of a conidium by emission of a vegetative germ tube, and equally frequent germination by production of a globose secondary conidium on a short, stout aerial outgrowth. It often shows also, though in greatly varying measure, a more distinctive type of reproduction wherein a large globose conidium will simultaneously put forth plural—usually about a dozen—short outgrowths, and on them will give rise terminally to a corresponding number of spores, all markedly and in about equal degree smaller than their parent. Although this multiplicative reproduction, which led Costantin to erect a separate genus for the fungus, may have lost some value as a diagnostic character through its observed occurrence also in *Conidiobolus brefeldianus* Couch (*3*), it has gained significance with respect to the basic morphology of the family through the discovery of two parasitic species, *Meristacrum asterospermum* Drechsl. (*4*) and *Ballocephala sphaerospora* Drechsl. (*5*), that reproduce asexually by giving rise only to small conidia. In disclosing the large conidium as a sporangium with a manner of spore development somewhat like that of *Cunninghamella,* the multiplicative reproduction of *D. coronata* establishes the small-spored forms as primitive members of the Entomophthoraceae. The resemblances of *M. asterospermum* to *Gonimochaete horridula* Drechsl. (*6*), which produces endogenous immotile spores in aerial hyphal outgrowths, seem suggestive of remote phylogenetic connections of the family with lagenidiaceous types, such as *Protascus subiliformis* Dang. (*7*) and *Haptoglossa heterospora* Drechsl. (*4*), that form endogenous immotile spores, presumably homologous with zoospores, in submerged intramatrical thalli.

D. coronata has been encountered at firsthand so infrequently that it is generally regarded by mycologists as a rare species. During 30 years it has appeared adventitiously in my cultures only four times. More recently, however, a procedure designed especially for isolating microscopic fungi that commonly produce their spores in a short time and soon shoot them off forcibly has shown the species to be virtually ubiquitous in leaf mold and in other kinds of plant detritus resulting from exposure of vegetable materials to slow decay in moist contact with the ground. In this procedure samples of detritus are first freed of their coarser components. Portions of the finer friable residue are then fastened with soft agar to the underside of a lid from a sterilized Petri dish. By gently kneading the mixture of detritus and agar all particles of decaying material are thoroughly moistened and thus made to adhere firmly enough to remain in position when the lid is turned right side up. The whole central area of the lid may advantageously be

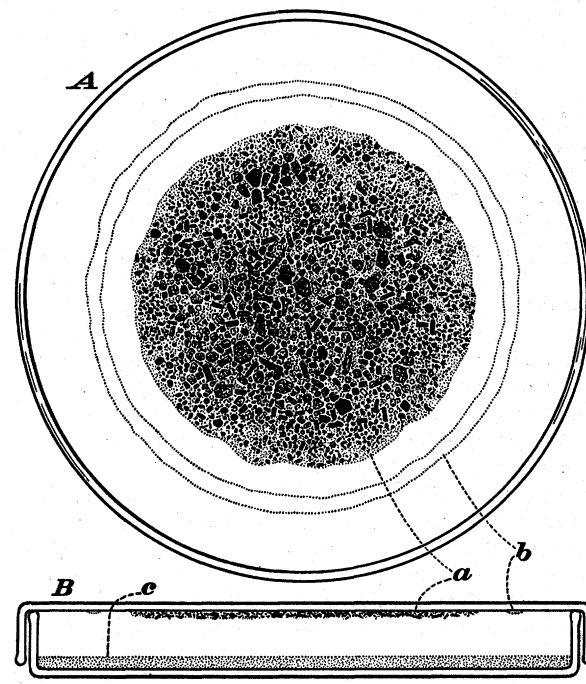

FIG. 1. Petri plate culture with canopy of leaf mold, showing bottom view, *A*, of lid, and horizontal view, *B*, of culture with lid in position: *a*, adhering puddled mixture of leaf mold and soft agar; *b*, circular barrier of soft vaseline or heavy mineral oil; *c*, layer of agar culture medium. Approx × .75.

planted with detritus (Fig. 1, *a*), but an outer zone about 15 mm wide should be left clean and as nearly sterile as possible. If small particles have fallen into the clear zone the whole outer area should be wiped with a sterile cotton plug slightly moistened in alcohol. Should the detritus appear rather badly infested with mites or other minute crawling animals it may be expedient to restrict their wanderings by applying soft vaseline or heavy mineral oil in a circular band (Fig. 1, *b*) around the central area. The lid is then gently placed on the lower half of a sterilized Petri dish containing a firm plate of moderately transparent, sterile agar culture medium (Fig. 1, *c*). Wherever *D. coronata* is present in the canopy of moist vegetable detritus it produces conidiophores from which conidia are forcibly propelled. After falling on the agar below, many of the conidia germinate vegetatively, thereby giving rise to scattered mycelia. As

these mycelia are usually free of alien organisms they readily yield pure cultures when transferred to sterile agar slants. More than a dozen pure cultures, all originating from separate conidia, may often be obtained from a single Petri plate within 2 days after it has been covered with a detritus canopy.

In addition to *D. coronata* other members of the Entomophthoraceae capable of "saprophytic" growth develop in canopied plate cultures, though in lesser quantity. The genus *Conidiobolus* would seem only meagerly represented in the collection of cultures isolated from leaf mold taken up in different localities in Maryland and Virginia late in the autumn of 1951. A species of *Basidiobolus* has been obtained rather frequently from dark, soggy leaf mold taken from wet, wooded areas near Beltsville, Md. When it is grown on maize meal agar the species produces numerous zygospores with the curious paired beaklike protuberances long familiar in textbook illustrations of *B. ranarum* Eidam (*8*), though the thick wall surrounding the individual zygospore seems always completely colorless. Its asexual reproduction has so far not been observed directly, yet some production and forcible discharge of conidia must in each instance precede its appearance in a canopied agar plate culture. Moreover, when a sizable slab of maize meal agar occupied by its vegetative mycelium is affixed adhesively to the ceiling of a Petri dish containing sterile agar culture medium, the fungus will begin growing in the agar below quite as certainly, though not as promptly, as will *D. coronata* under similar conditions. By such affixture above a sterile agar plate entomophthoraceous mycelia that in a canopied culture have become contaminated with bacteria or been overgrown by alien fungi may often be easily purified.

At temperatures near 20° C mycelial strands extended downward here and there from a canopy of detritus, mainly by members of the Mucorales, will usually reach the agar floor in 2½ or 3 days after the culture was prepared. The culture thereupon becomes rather quickly overgrown by miscellaneous microorganisms and soon is greatly impaired in usefulness, if not utterly ruined. Success in isolating entomophthoraceous forms by the procedure here recommended must be achieved early. Newly collected moist detritus should, as a rule, provide most rapid development. However, material collected in an air-dry or nearly air-dry condition has given very satisfactory results for several months—far better results than moist or wet material that after being collected was kept in a tight container in a warm laboratory for 2 or 3 weeks before it was used. The pronounced deterioration of the material in the tight container evidently resulted from excessive development of species of *Trichoderma, Aspergillus,* and *Penicillium,* as well as of members of the Mucorales, during the brief period of storage. Where moist plant detritus cannot be used immediately the saprophytic entomophthoraceous forms and other components of its natural microflora, including many predaceous and parasitic fungi destructive to rhizopods and eelworms, can be preserved fairly well by allowing it to dry out slowly at relatively low temperatures.

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Comments and Communications

Antibiotics and Immunodesensitization in the Treatment of Human Brucellosis

The new antibiotics—streptomycin, aureomycin, chloromycetin (Cloramphenicol), and terramycin—which have a strong inhibitory action against *Brucella,* have been used in the treatment of human brucellosis with satisfying and prompt, but not lasting results. The authors believe that an antibiotic's success in the treatment of any infectious disease depends upon an efficient and prompt immunological response of the body against the infection, in order to continue and consolidate the inhibitory action when the antibiotic is stopped. In brucellosis the natural immunological response to the infection is slow and late; for this reason, when the action of the antibiotic is suspended, the infection advances anew, regardless of the strength of the drug, and acute relapses occur or the disease takes a chronic course. For these reasons the authors have considered it necessary to stimulate artificially the development of immunity simultaneously with the administration of the antibiotic (Chloramphenicol). In brucellosis there is a condition of allergy; hence it is not possible to give the antigen in doses as large and as frequent as would be desirable for a prompt immunological response, without causing violent hypersensitivity reactions. Thus it is necessary to give small, slowly absorbed, progressively increasing doses of a species-specific antigen, in order to desensitize and at the same time stimulate the development of immunity. The authors give the name of immunodesensitization to this method.

In 480 cases of brucellosis so treated, the allergy to *Brucella,* as shown by the intensity of the skin reaction to the intradermal injection of the antigen, decreased notably or eventually disappeared, as compared with an equal number of nontreated cases in which the allergy persisted or increased indefinitely.