

MYCOLOGY.—*Morphological features shown in Aphanomyces isolations from roots of spinach and flax.* CHARLES DRECHSLER, United States Department of Agriculture: Plant Industry Station, Beltsville, Md.

Nearly 20 years ago I noted in a brief abstract (Drechsler, 1935) the isolation of a species of *Aphanomyces* from discolored roots of spinach plants (*Spinacia oleracea* L.) originating in New Jersey and Virginia and from discolored flax roots (*Linum usitatissimum* L.) collected in northern Wisconsin. Reference was made in the abstract to resemblances that the isolations from spinach and flax bore to *A. cladogamus*, a species I had previously described (Drechsler, 1929) from cultures obtained from tomato rootlets (*Lycopersicon esculentum* Mill.). Subsequently (Drechsler, 1946) in setting forth its antagonistic behavior toward different species of *Pythium* I designated my saprolegniaceous fungus from spinach as a strain of *A. cladogamus*, though an *Aphanomyces* culture isolated from spinach in the Netherlands had under the designation *A. euteiches* P. F. 2 (Meurs, 1928) earlier been assimilated to the congeneric parasite causing root rot of peas (*Pisum sativum* L.). As members of the Saprolegniaceae have been found associated with root rot or root discoloration only in a few of the crop plants grown in greenhouse, garden, or field, the reported occurrence of two congeneric water molds on any one cultivated plant species appears in excess of normal expectations. Unfortunately the *Aphanomyces* culture that Meurs obtained from spinach was not available for comparison with my own isolations. Because of restrictions on the importation of pathogenic material a side-by-side comparison of any similar European culture with isolations obtained in the United States might not be readily accomplished in the future. Under the circumstances it may in some degree be helpful to set forth the morphological features that led me to assign my isolations from spinach, as also my conspecific isolations from flax, to *A. cladogamus*.

The morphological features distinguishing the several root-rotting species of *Aphanomyces* are displayed mainly in their sexual reproductive apparatus. Important differences are expressed in the presence or

absence of a visible and fairly close mycelial connection between the oogonium and its attendant antheridia; in the measure and manner in which the sexual branches, together often with some concomitant hyphal ramifications, are interlocked or intertwined; in the dimensions of the oogonium and oospore, including not only the diameters of these bodies but also the thickness of the envelopes or walls surrounding them. Since the shape and posture of antheridia are likely to be disturbed more or less in the manipulations necessary for proper microscopic examination of sexual apparatus developed in water cultures, all reproductive units herein figured were taken from maize-meal-agar plate cultures of a consistency firm enough to endure manipulation, yet not too hard for antheridia to grow to their normal size without evident impediment or noticeable deformity. The distribution of fine maize-meal particles through the agar substratum seems helpful in encouraging abundant sexual reproduction, and is more especially advantageous in promoting normal development inside the oogonia, with resultant formation of oospores having the correct internal organization necessary for longevity. Judged from appearances the substances that here promote normal oospore development would seem largely to remain undissolved in the maize-meal particles until these particles are acted upon by mycelial branches close by. At all events the fungi herein discussed, like some species of *Pythium*, will commonly extend numerous hyphal branches among clustered maize-meal particles, and in the somewhat densely permeated regions will then give rise to reproductive units rather close together. Consequently the more or less complicated hyphal relations frequent in sexual reproductive development are often brought into being in regions where relatively intricate hyphal ramification has already come about from nutritional needs. On the upper surface, besides, even in cultures in which growth of aerial mycelium has been meager, some aerial branches will often arise from positions close

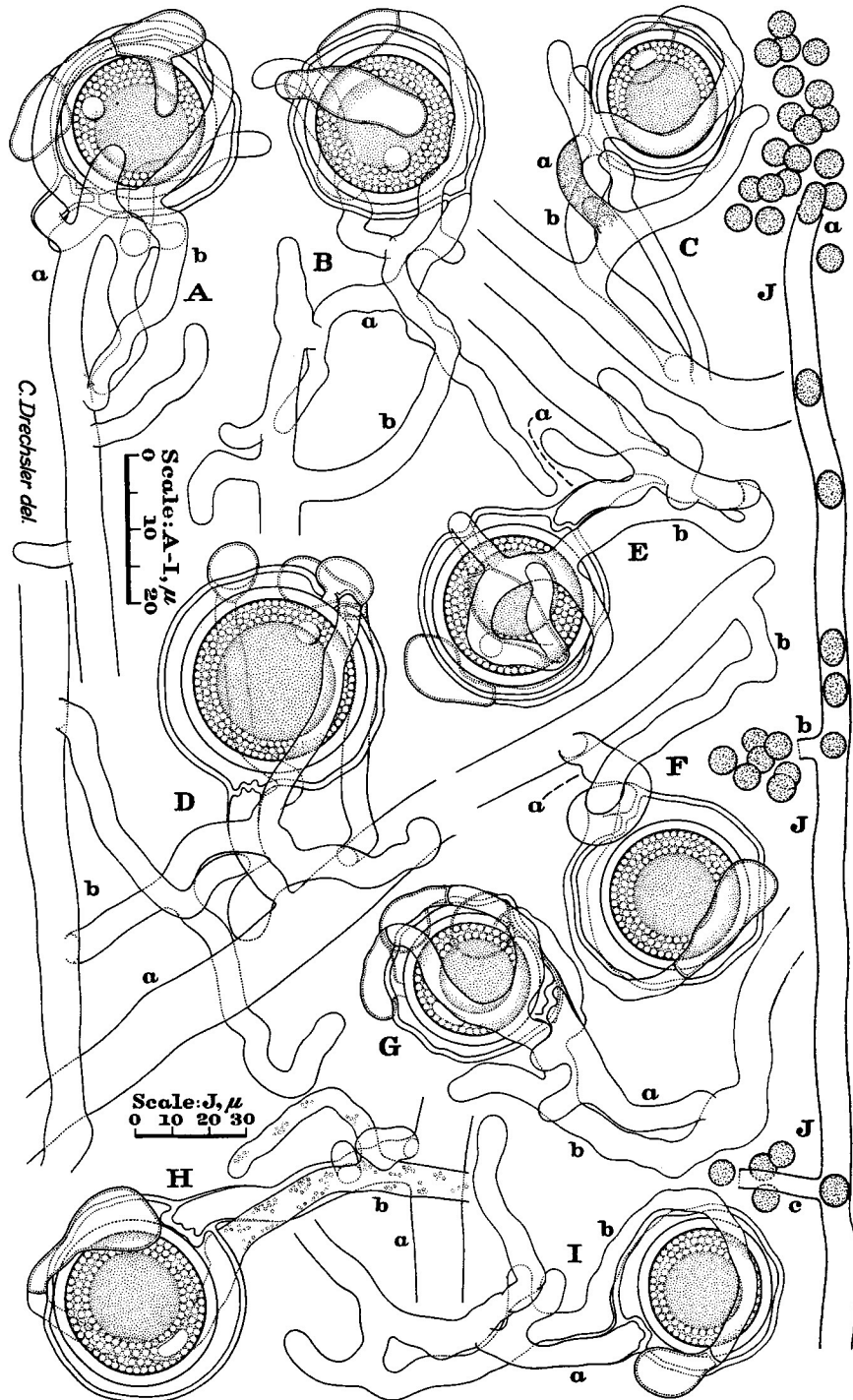


FIG. 1.—*Aphanomyces cladogamus* isolated from spinach. A-I, Mature units of sexual reproductive apparatus produced in maizemeal-agar plate cultures and drawn at a uniform magnification with the aid of a camera lucida; a, branch or hypha supplying the oogonium; b, branch or hypha supplying the male complement; $\times 1,000$. J, Distal portion of evacuation tube showing encysted zoospores near the 3 openings, a-c; $\times 500$.

to oogonia and antheridia, and thereby will add to the apparent intricacy of the sexual apparatus. In preparing the accompanying figures of sexual reproductive units it has been deemed justifiable to obviate the added complexity resulting from aerial growth by using thin slices cut from the under side of maize-meal agar plate cultures, and to avoid the complexity deriving from nutritional exigencies by selecting mostly the reproductive units scattered with convenient sparseness between the more crowded regions where maize-meal particles had been clustered. Among such units many are clearly discernible in all their parts, even where four or five antheridia are present, and where some of the antheridia together with some supporting branches are applied to the under side of the oogonium. Reproductive units with six, seven, or eight antheridia are, however, usually too complicated to be shown accurately at the moderate magnification herein used for all figures of sexual apparatus.

MORPHOLOGY OF THE APHANOMYCES ISOLATED FROM SPINACH ROOTS

In the spinach root-rot fungus, much as in the tomato rootlet fungus on which the original description of *Aphanomyces cladogamus* was based, the oogonium and its attendant antheridia sometimes have a close mycelial connection but at other times lack such connection. Unusually simple monoclinous reproductive units come into being in instances where the oogonial stalk (Fig. 1, F, a) and the single attendant antheridial branch are both given off in positions rather close together, from the same mycelial filament. Frequently in such units the oogonial stalk entwines the antheridial branch spirally, thereby establishing firm contact without the aid of accessory hyphal parts (Fig. 1, F); and frequently too, the antheridial branch (Fig. 2, H, b) bears a few short spurs which interlock variously with the twining oogonial stalk (Fig. 2, H, a). Sometimes in relatively simple monoclinous reproductive units both the oogonial stalk (Fig. 1, I, a; Fig. 2, F, a) and the antheridial branch (Fig. 1, I, b; Fig. 2, F, b) give off one or more interlocking spurs, so that firm contact is provided without entwinement. All terminal antheridial branches enwrap the oogonium rather extensively whether they are supplied (Fig. 1, A, b; B, b; C, b; E, b;

G, b. Fig. 2, A, b; C, b; D, b) from the same hypha as the oogonial stalk (Fig. 1, A, a; B, a; C, a; E, a; G, a. Fig. 2, A, a; C, a; D, a) or are given off by a mycelial filament (Fig. 1, D, b; H, b. Fig. 2, B, b; E, b; G, b; I, b) not closely connected with the filament (Fig. 1, D, a; H, a. Fig. 2, B, a; E, a; G, a; I, a) supplying the oogonium. Reproductive units with 4 or 5 antheridia (Fig. 2, C) consequently have a more or less intricate appearance. After its contents have contracted to form the oospore, the oogonial envelope, unlike that of *Aphanomyces euteiches*, commonly relaxes in noticeable degree, and in a fully mature state often presents an irregular undulating profile.

Two hundred reproductive units selected at random in maize-meal agar plate cultures of the New Jersey strain of the spinach *Aphanomyces* gave measurements for oogonial diameter, expressed in the nearest integral number of microns, with the following distribution: 20 μ , 2; 21 μ , 10; 22 μ , 21; 23 μ , 29; 24 μ , 35; 25 μ , 35; 26 μ , 37; 27 μ , 17; 28 μ , 7; 29 μ , 6; 30 μ , 1. The 200 oospores of correct internal organization in these units gave measurements for diameter distributed as follows: 14 μ , 1; 16 μ , 1; 17 μ , 9; 18 μ , 29; 19 μ , 39; 20 μ , 55; 21 μ , 29; 22 μ , 28; 23 μ , 8; 25 μ , 1. The reserve globule varied in diameter from 8.2 to 14.4 μ . With respect to thickness the oogonial envelope measured 0.5 to 1.1 μ and the oospore wall 1.1 to 2 μ . The measurements for diameter of oogonium averaged 24.4 μ ; those for diameter of oospore, 19.8 μ ; those for diameter of reserve globule, 11.1 μ ; those for thickness of oogonial envelope, 0.7 μ ; and those for thickness of oospore wall, 1.5 μ .

In the tomato-rootlet fungus on which the description of *Aphanomyces cladogamus* was based the branched sporangium was often found discharging its zoospores through plural openings in the distal portion of the evacuation tube. Sporangial discharge through plural openings is likewise frequent in the isolations obtained from spinach (Fig. 1, J, a-c), though in many instances all the zoospores are released from the single opening at the axial tip. Immediately after their release the spores round up and encyst. The encysted zoospores found grouped irregularly around the hyphal openings (Fig. 1, J, a-c) from which they have emerged commonly measure about 7.5 μ in diameter. It seems probable that the relatively small number of encysted zoospores between 9 and 10 μ in diameter may owe their

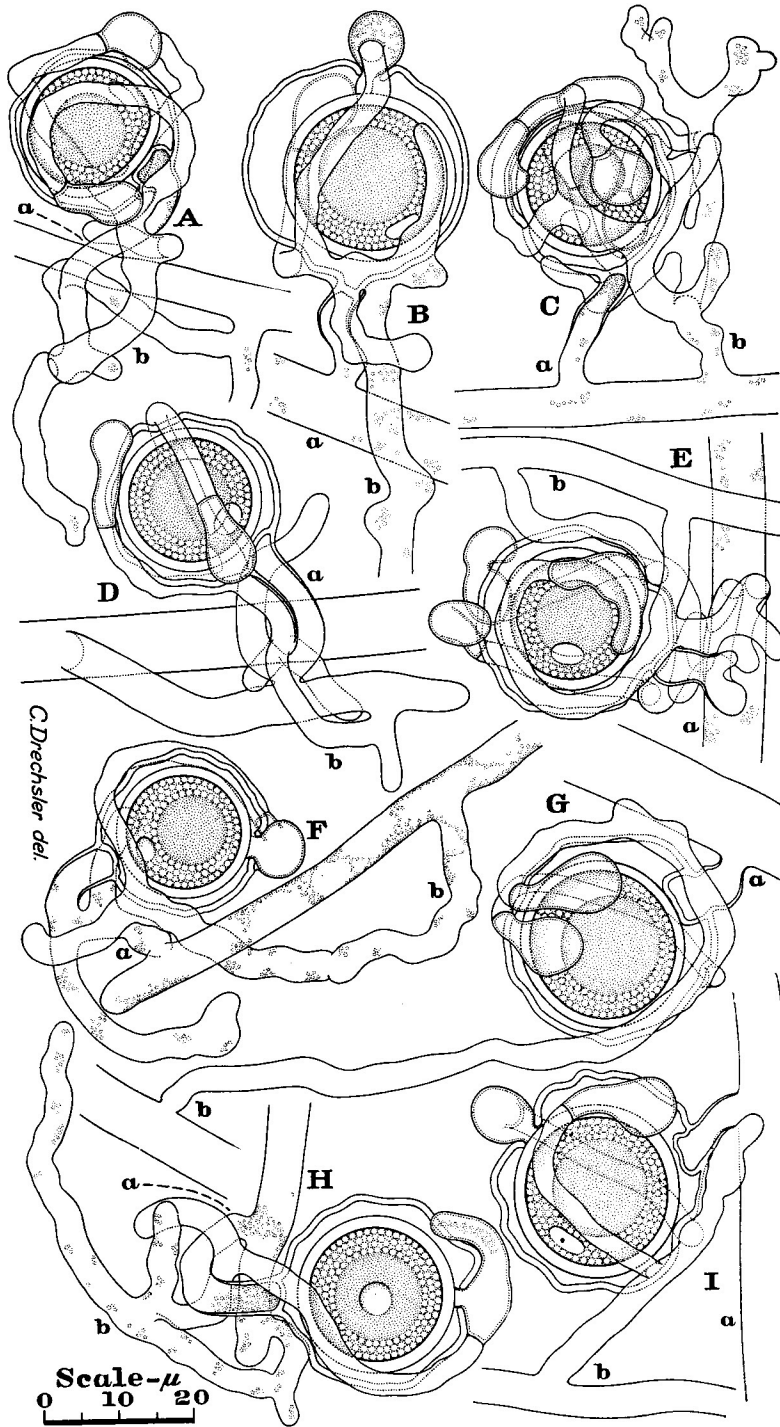


FIG. 2.—*Aphanomyces cladogamus* isolated from spinach; $\times 1,000$ throughout. A-I, Mature units of sexual reproductive apparatus produced in maize meal-agar plate cultures and drawn at a uniform magnification with the aid of a camera lucida; *a*, branch or hypha supplying the oogonium; *b*, branch or hypha supplying the male complement.

larger size to incomplete protoplasmic cleavage within the sporangium.

MORPHOLOGY OF THE APHANOMYCES ISOLATED FROM FLAX ROOTS

The flax plants that yielded the *Aphanomyces* cultures reported earlier (Drechsler 1935) were taken from a small stand of flax immediately adjacent to a larger area of field peas (*Pisum sativum* L. var. *arvense* (L.) Poir.) in northern Wisconsin on July 10, 1931. As the ground at the time had for several weeks been excessively wet the foliage of the peas was rather markedly yellowed, and the lower portions of the stems, as also the roots, showed extensive cortical decay of the type common in garden peas (*P. sativum*) after attack by *A. euteiches*. The flax likewise had a generally unthrifty appearance which could for the most part have resulted directly from the very unfavorable condition of the soil. No injury was observed in the flax stems above the ground level but when individual plants were pulled up the main root in many instances showed patches of yellowish, orange, and reddish discoloration. When pieces of root thus discolored were placed on sterile maize-meal agar, mycelia of an *Aphanomyces* grew out promptly in many instances. To determine whether a plant not belonging in the Leguminosae was here being attacked by the water mold known mainly for its injurious action on peas, some of the mycelia were used for starting pure cultures. All the cultures readily formed sexual reproductive apparatus, which, as is evident from the 19 units figured herein (Fig. 3, A-J; Fig. 4, A-I), differed conspicuously from that of *A. euteiches*, but resembled closely the reproductive apparatus formed in the *Aphanomyces* cultures obtained from spinach roots.

The oogonium and attendant antheridia of the flax-root fungus are frequently of monoclinous origin. In especially simple reproductive units with only a single antheridium both the oogonial stalk (Fig. 3, C, a; J, a) and the antheridial branch (Fig. 3, C, b; J, b) may be borne subterminally on a main mycelial hypha (Fig. 3, C) or on a short ramification of a main hypha (Fig. 3, J). The oogonial stalk in such units may entwine in noticeable measure either the male branch itself (Fig. 3, C) or a spur borne on it (Fig. 3, J). Oogonia borne on a stalk arising subterminally from a main hypha (Fig. 3, H, a; Fig. 4, D, a; E, a; G, a; I, a) are frequently

supplied with plural antheridia of monoclinous origin, the male cells being borne sometimes on ramifications of a single subterminal branch (Fig. 3, H, b. Fig. 4, D, b; G, b; I, b) and sometimes on plural branches given off separately in subterminal positions (Fig. 4, E, b, c). Similarly in monoclinous units formed in other than subterminal positions the oogonial stalk (Fig. 3, A, a; D, a; E, a; F, a; I, a. Fig. 4, A, a; C, a; F, a) arises laterally from a main hypha from which is given off nearby the male branch that bears a single attendant antheridium (Fig. 3, I, b) or more frequently ramifies to supply plural antheridia (Fig. 3, A, b; D, b; E, b; F, b. Fig. 4, A, b; C, b; F, b). In such monoclinous units, also, plural attendant antheridia may be borne on two (Fig. 4, B, b, c) or more branches arising separately from the same hypha as the oogonial stalk (Fig. 4, B, a). Diclinous reproductive units, in which the hypha (Fig. 3, B, a; G, a. Fig. 4, H, a) bearing the oogonial stalk and the hypha (Fig. 3, B, b; G, b. Fig. 4, H, b) supplying the male complement have no close mycelial connection, are formed freely in cultures of the flax-root fungus, much as in the conspecific isolations from spinach roots.

Two hundred mature reproductive units taken at random in maize-meal agar plate cultures of the flax-root fungus gave measurements for diameter of oogonium, expressed in the nearest integral number of microns, with a distribution as follows: 17 μ , 1; 19 μ , 1; 21 μ , 6; 22 μ , 9; 23 μ , 9; 24 μ , 12; 25 μ , 38; 26 μ , 26; 27 μ , 35; 28 μ , 25; 29 μ , 20; 30 μ , 8; 31 μ , 4; 32 μ , 2; 33 μ , 2; 35 μ , 2. The 200 oospores of correct internal structure in these units gave measurements for diameter which were distributable thus: 14 μ , 1; 16 μ , 1; 17 μ , 1; 18 μ , 5; 19 μ , 15; 20 μ , 30; 21 μ , 58; 22 μ , 50; 23 μ , 25; 24 μ , 8; 25 μ , 3; 26 μ , 2; 27 μ , 1. Measurements for diameter of reserve globule ranged from 8 to 14 μ ; those for thickness of oogonial envelope, from .5 to 1.2 μ ; those for thickness of oospore wall, from 1.1 to 1.9 μ . From the several sets of measurements averages were computed as follows; for diameter of mature oogonium, 26.3 μ ; for diameter of oospore, 21.3 μ ; for diameter of reserve globule, 10.9 μ ; for thickness of oogonial envelope, 0.9 μ ; for thickness of oospore wall, 1.5 μ .

In sporangia of the flax-root fungus, just as in sporangia of the fungus isolated from spinach, the hypha destined to serve as evacuation tube often is furnished below its bluntly rounded tip (Fig. 4,

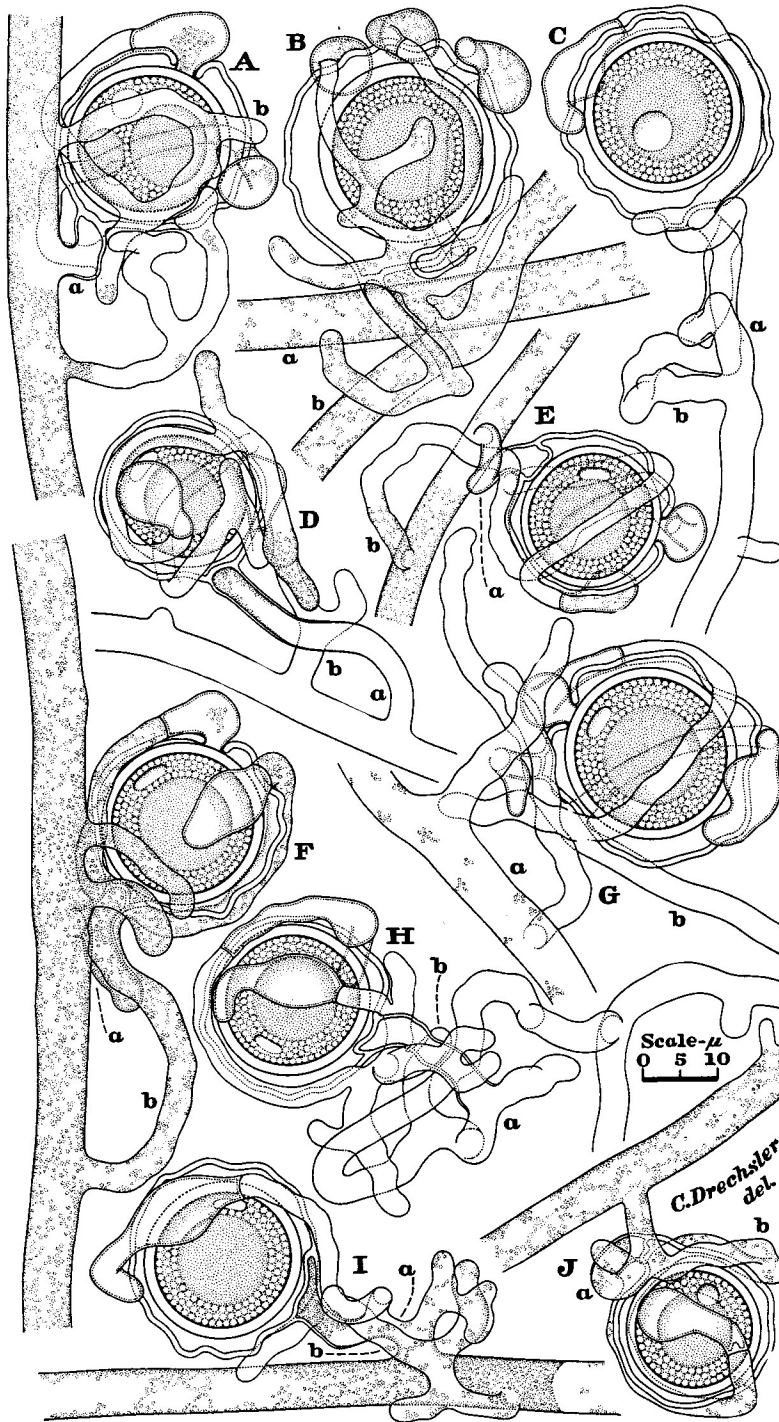


FIG. 3.—*Aphanomyces cladogamus* isolated from flax: $\times 1,000$ throughout. A-J, Mature units of sexual reproductive apparatus produced in maize-meal-agar plate cultures and drawn at a uniform magnification with the aid of a camera lucida; a, branch or hypha supplying the oogonium; b, branch or hyphae supplying the male complement.

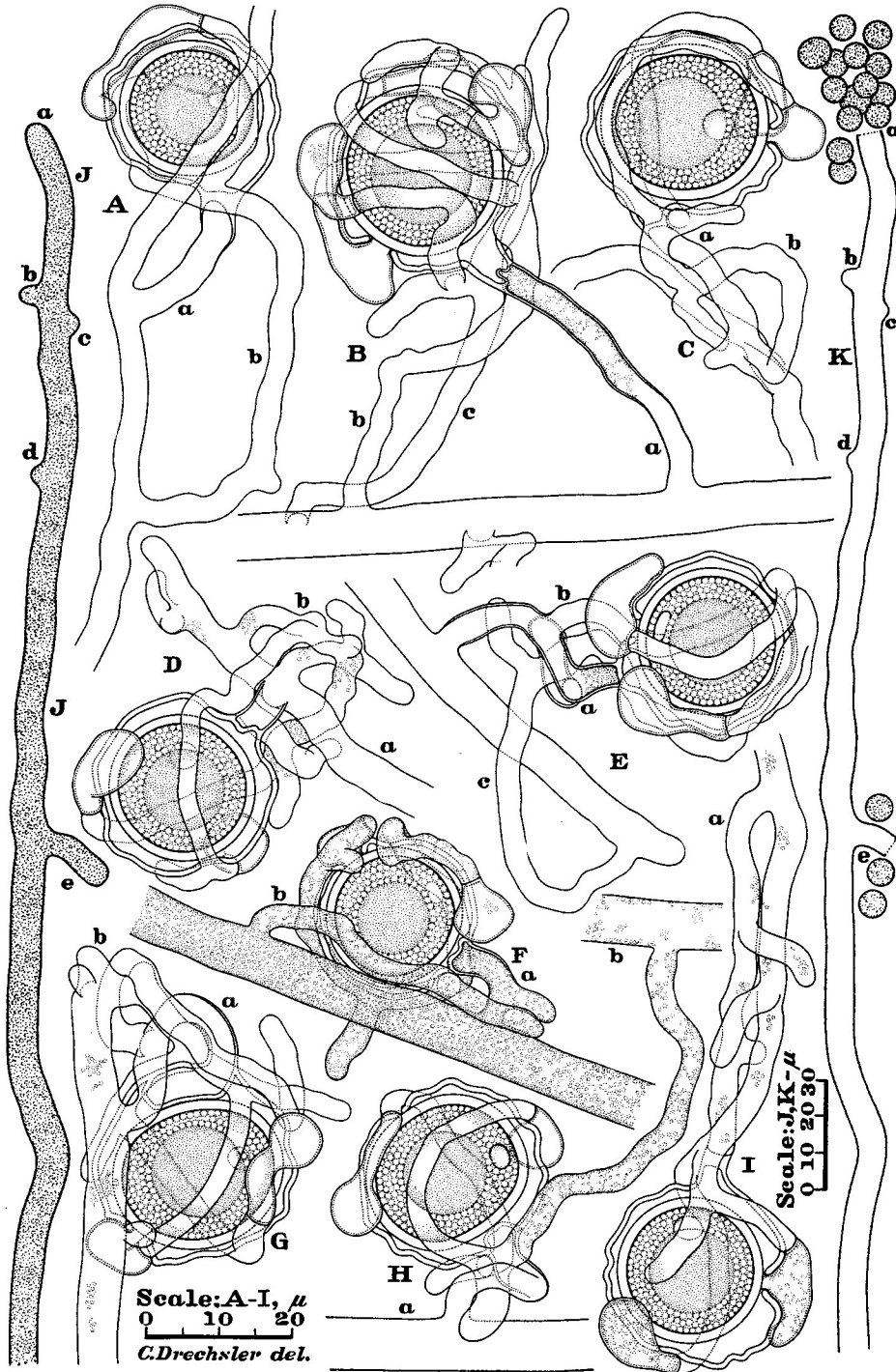


FIG. 4.—*Aphanomyces cladogamus* isolated from flax. A-I, Mature units of sexual reproductive apparatus produced in maize-meal-agar plate cultures and drawn at a uniform magnification with the aid of a camera lucida; *a*, branch or hypha supplying the oogonium; *b* (also *c* in B and E), branch or hypha supplying the male complement; $\times 1,000$. J, Distal portion of evacuation tube; *a*, tip; *b-d*, lateral protuberances; *e*, short branch; $\times 500$. K, Same after sporangium was discharged, showing some encysted zoospores near the openings at *a* and *e*; *b-d* remained closed; $\times 500$.

J, a) with a few protuberances (Fig. 4, J, b-d) or short spur-like branches (Fig. 4, J, e). Frequently when the axial tip (Fig. 4, K, a) yields to allow emergence of the individualized zoospores one (Fig. 4, K, e) or more of the lateral protrusions likewise gives way apically, so that discharge of the sporangium takes place through plural openings. In many instances some protrusions remain closed (Fig. 4, K, b-d). Where all protrusions remain closed, or where no protrusions are present, the zoospores necessarily are discharged from the single opening at the axial tip. After they have rounded up and encysted near the openings (Fig. 4, K, a, e) the zoospores commonly measure about 7.5μ in diameter.

The isolations from roots of spinach and flax thus agree satisfactorily with *Aphanomyces cladogamus* in the morphology of their sporangia and zoospores. They are held properly referable

to that species more especially, however, because of close resemblances in their sexual reproductive apparatus—resemblances evident in all main dimensions, in the frequent monoclinous origin of reproductive units, and in the arrangement of the oogonium and its attendant antheridia as well as of the hyphae or branches supplying these organs.

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