

MYCOLOGY.—*Aphanomyces euteiches* from pea roots and “*Aphanomyces euteiches* P. F. 2.” CHARLES DRECHSLER, United States Department of Agriculture, Plant Industry Station, Beltsville, Md.

The original account (Jones and Drechsler, 1925) of my *Aphanomyces euteiches* was based entirely on the saprolegniaceous parasite that occurs during wet seasons as a causal agent of serious root rot in garden peas (*Pisum sativum* L.). Soon after the account was published Linford (1927) reported having found oospores typical of the fungus also in diseased roots of narrow-leaved vetch (*Vicia angustifolia* L.) seedlings as well as in diseased seedlings of alfalfa (*Medicago sativa* L.) and sweet clover (*Melilotus alba* Desr.). He further observed several varieties of sweet peas (*Lathyrus*

odoratus L.) greatly weakened from spontaneous attack by the parasite, and in inoculation trials successfully infected nine additional leguminous species. Mainly because of similarities shown by oospores found in their decaying roots he considered four nonleguminous plants including barley (*Hordeum vulgare* L.) and oats (*Avena sativa* L.) subject to invasion by *A. euteiches*. Subsequent study of a culture isolated by Linford from an oat root, however, revealed distinctive morphological and developmental features; wherefore it was used as type material of a separate species, *A.*

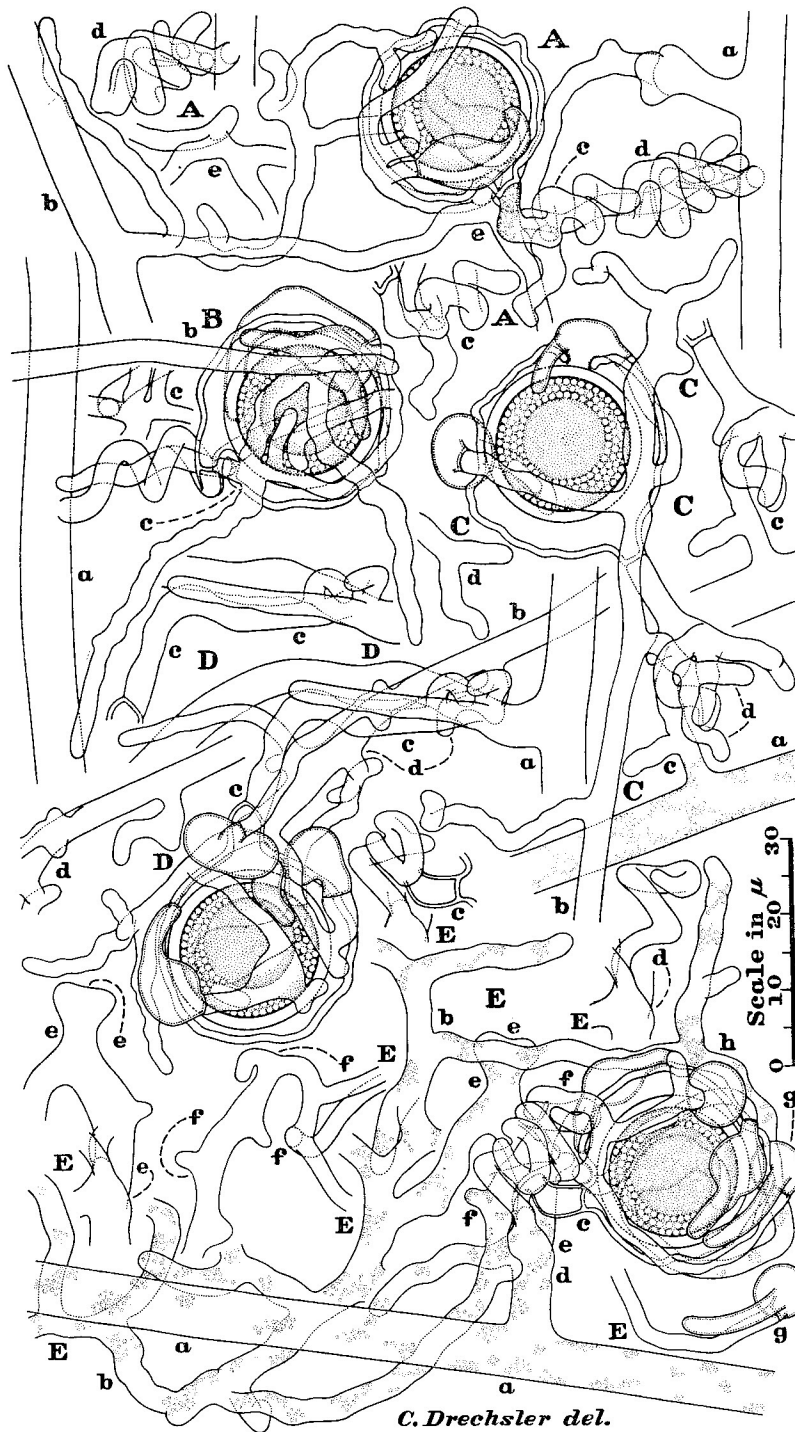


FIG. 1.—Mature sexual reproductive apparatus of the isolation received from the Centraalbureau voor Schimmelcultures at Baarn, Netherlands, as *Aphanomyces euteiches* P. F. 2; $\times 1,000$ throughout. A–D, Diclinous reproductive units, with one mycelial hypha, a, supplying the oogonium, and another hypha, b, supplying the attendant antheridia. E, Reproductive unit with traceable mycelial connection between the stout mycelial filament, a, that supplies the oogonium and the hypha, b, that supplies the 5 attendant antheridia. For greater clearness some of the more complicated portions of the several units (A, c–e; B, c; C, c, d; D, c, d; E, c–h) are shown separately as well as in proper position.

camptostylus Drechsler (1929). Similarly a number of cultures isolated from tomato (*Lycopersicon esculentum* Mill.) roots in 1926, which at first had been referred (Drechsler, 1927) to *A. euteiches*, were on closer examination found to represent a different species that I then presented (Drechsler, 1929) under the binomial *A. cladogamus*. More recently Doran, Guba, and Gilgut (1942) in Massachusetts reported that *A. euteiches* often causes damping-off of celery (*Apium graveolens* L.). Detailed evidence concerning the relationship of *A. euteiches* to any *Aphanomyces* cultures that may perhaps have been isolated from celery or barley in the United States has not so far been supplied.

In the scanty relevant European literature a few nonleguminous plants are cited as hosts of *Aphanomyces euteiches* or are mentioned as having yielded cultures referable to that species. Buisman (1927, p. 45) in the Netherlands isolated from decaying roots of pansies (*Viola tricolor* var. *hortensis* Hort.) an *Aphanomyces* which to her looked very similar to *A. euteiches* but which in her inoculation experiments did not attack pea roots. She was unable to try it out on pansies and thus did not establish whether her fungus was the cause of an acute root rot which she mentioned as appearing in her experimental garden. In cross-inoculation experiments later carried out by Meurs (1928) an isolation of *A. euteiches* was found capable of infecting peas, but incapable of infecting either *Viola tricolor* or *V. cornuta* L.; whereas the pansy-root *Aphanomyces* was found incapable of infecting peas, but capable of infecting both *V. tricolor* and *V. cornuta*. Meurs, like Buisman, held the pansy fungus to be morphologically identical with the parasite causing pea root rot, though he recognized that it presented some differences in growth as well as in parasitism. He therefore considered it a physiological form of the same species, and designated it as *A. euteiches* P. F. 2. Further he concluded that it was the cause of an acute root rot of pansies in Holland. It seems noteworthy that Meurs came to this conclusion even though he had obtained from the pansy other fungi that might have been regarded

as likely root parasites. Of nine cultures he isolated from *Viola tricolor* he assigned two to *Pythium intermedium* DeBary and four to *P. irregulare* Buisman, and listed two as belonging to undetermined species. Besides *V. tricolor* he listed two other plants, *Spinacia oleracea* L. and *Arabis alpina* L., as having each yielded a culture of *Aphanomyces euteiches* P. F. 2.

A decade later Van Eek (1938) reinvestigated pansy root rot in the Netherlands with results strongly divergent from those of Meurs. In repeating the tests described by Meurs and employing the same strain of "*Aphanomyces euteiches* P. F. 2," Van Eek obtained only slight evidence of pathogenicity. He concluded accordingly that *A. euteiches* P. F. 2 could not be the causal agent of severe root rot of pansies. In view of the possibility that the original strain of "*A. euteiches* P. F. 2" might have lost its virulence during the years it had been kept in culture Van Eek reisolated the strain from a successfully infected plant, but the recovered isolation likewise showed only meager aggressiveness. Apparently he obtained no new culture that was referable to *Aphanomyces*. However, despite the general infrequency of saprolegniaceous fungi as plant pathogens he isolated two such fungi—his *Brevilegnia gracilis* and his *B. macrospora*—from diseased pansy plants and found both to be strongly parasitic.

Soon after Meurs had designated as *Aphanomyces euteiches* P. F. 2 the water mold he and Buisman had isolated from pansies a culture contributed by him under that designation was supplied to me from the Centraalbureau voor Schimmelcultures at Baarn, Netherlands. In observance of precautions advisable with organisms of foreign origin the culture was never used in inoculation trials of any kind either outdoors or in the greenhouse. However, it was compared side by side on several different agar substrata with *A. euteiches* from pea roots, with several congeneric isolations obtained from pansies in and near the District of Columbia (Drechsler, 1934), and with the strains of *A. cladogamus* obtained from roots of flax (*Linum usitatissimum* L.) and spinach (*Spinacia oleracea* L.) in the United States (Drechsler, 1935). The

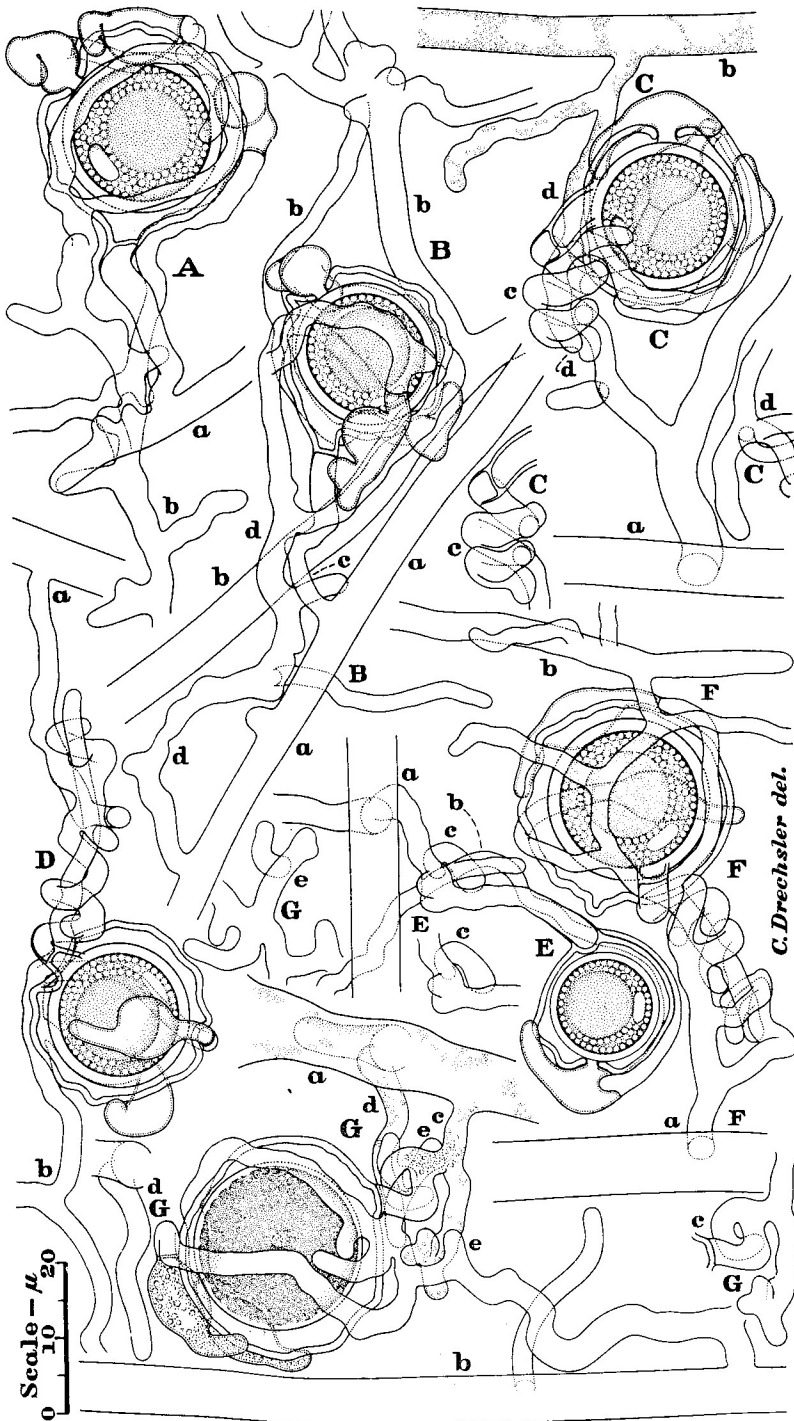


FIG. 2.—Sexual reproductive apparatus of the isolation received as *Aphanomyces euteiches* P. F. 2; $\times 1,000$ throughout. A-G, Dielicious reproductive units, with one mycelial hypha, a, supplying the oogonium, and another hypha, b, supplying one (B, E) or more (A, C, D, F, G) attendant antheridia; in B two antheridia of monoelicious origin (upper left, lower middle) are present besides, these being supplied from a branch, d, that is given off by the same hypha as the oogonial stalk, c. Oospore in early stage of development in G, but fully mature in A-F. For greater clearness some of the more complicated parts in three of the units (C, c, d; E, c; G, c-e) are shown separately as well as in proper position.

Dutch isolation displayed generally greater intricacy in the make-up of its sexual reproductive apparatus than any of the isolations with which it was compared. Despite the rather complicated appearance of the 15 reproductive units figured herein (Fig. 1, A-E; Fig. 2, A-G; Fig. 3, A-C) to show the postural and positional relations of the sex organs, these units hardly reveal the full scope of complexity displayed by the fungus.

With respect to the hyphal connection of its sex organs the culture designated as *Aphanomyces euteiches* P. F. 2 resembled *A. cladogamus* more closely than *A. euteiches*. While in many of its reproductive units the hypha (Fig. 1, A-D:a; Fig. 2, A-G:a) bearing the oogonial stalk showed no visible connection with the hypha (Fig. 1, A-D:b; Fig. 2, A-G:b) supplying an antheridial branch, it commonly gave rise here and there to monoclinal or androgynous units in which a connection between the female branch (Fig. 3, A-C:a) and the male branch (Fig. 3, A-C:b) could be traced readily. In more complicated units it was often difficult to follow even a fairly short mycelial connection (Fig. 1, E, d, e) between an oogonial stalk (Fig. 1, E, c) and the wide axial filament (Fig. 1, E, a) from which it was supplied, or to make out an existing connection between the oogonial stalk (Fig. 1, E, c) and the hypha (Fig. 1, E, b) from which was given off the branch or branches (Fig. 1, E, f, h) supplying one or more attendant antheridia (Fig. 1, E, g). Occasionally a branch (Fig. 2, B, d) given off by the same mycelial hypha (Fig. 2, B, a) as the oogonial stalk (Fig. 2, B, c) was found to supply one or more attendant antheridia (Fig. 2, B, upper left, lower middle) apart from any antheridia (Fig. 2, B, lower right) supplied from a neighboring hypha (Fig. 2, B, b).

The complicated make-up frequent in sexual reproductive apparatus of "*Aphanomyces euteiches* P. F. 2" came about in large measure from entwining of hyphal parts. Sometimes the oogonial stalk entwined a ramification of the antheridial branch (Fig. 1, B). Sometimes an antheridial branch (Fig. 1, E, f) or the terminal portion of a hypha bearing one or more antheridial

branches (Fig. 2, C, D, F) was entwined not only by the oogonial stalk but also by short ramifications borne on it. Often, again, a prolongation of a hypha (Fig. 1, A, e) bearing antheridial branches was entwined by a branch (Fig. 1, A, c) given off from the oogonial stalk, or even by a branch (Fig. 1, A, d) from the mycelial filament (Fig. 1, A, a) bearing the oogonial stalk. Reproductive units with little or no intertwining of hyphal parts acquired in many instances an intricate appearance through interlocking of spurs extended from the branches bearing the apposed sex organs (Fig. 1, C, c, d; Fig. 2, E, c).

Two hundred oogonia of "*Aphanomyces euteiches* P. F. 2" taken at random in maize-meal agar plate cultures 30 days old gave measurements for diameter, expressed in the nearest integral number of microns, with the following distribution: 18 μ , 1; 20 μ , 5; 21 μ , 6; 22 μ , 16; 23 μ , 18; 24 μ , 33; 25 μ , 40; 26 μ , 35; 27 μ , 19; 28 μ , 13; 29 μ , 6; 30 μ , 3; 31 μ , 3; 32 μ , 1; 33 μ , 1. The 200 oospores of correct internal organization within these oogonia gave measurements for diameter distributed thus: 16 μ , 4; 17 μ , 13; 18 μ , 26; 19 μ , 38; 20 μ , 47; 21 μ , 38; 22 μ , 20; 23 μ , 11; 24 μ , 3. Averages of 25.0 μ and 19.8 μ were computed for oogonial diameter and oospore diameter, respectively. In the 200 reproductive units the oogonial envelope varied from .5 to 1.3 μ in thickness, averaging .8 μ in this dimension; while the oospore wall measured 1 to 1.9 μ in thickness and averaged 1.5 μ . Measurements for diameter of the reserve globule in the individual oospores ranged from 9 to 16 μ , and gave an average of 11.4 μ .

Thus in its main dimensions the culture isolated in the Netherlands would not seem to have differed markedly from *Aphanomyces cladogamus*. Owing to the difficulty of ascertaining from a single culture the importance of hyphal entwining attendant to sexual reproduction it appears about equally uncertain whether "*A. euteiches* P. F. 2" should be included in *A. cladogamus* or should be kept separate from that species. Study of additional cultures from pansies in the Netherlands may be necessary before a sound decision can be reached as to the proper disposition

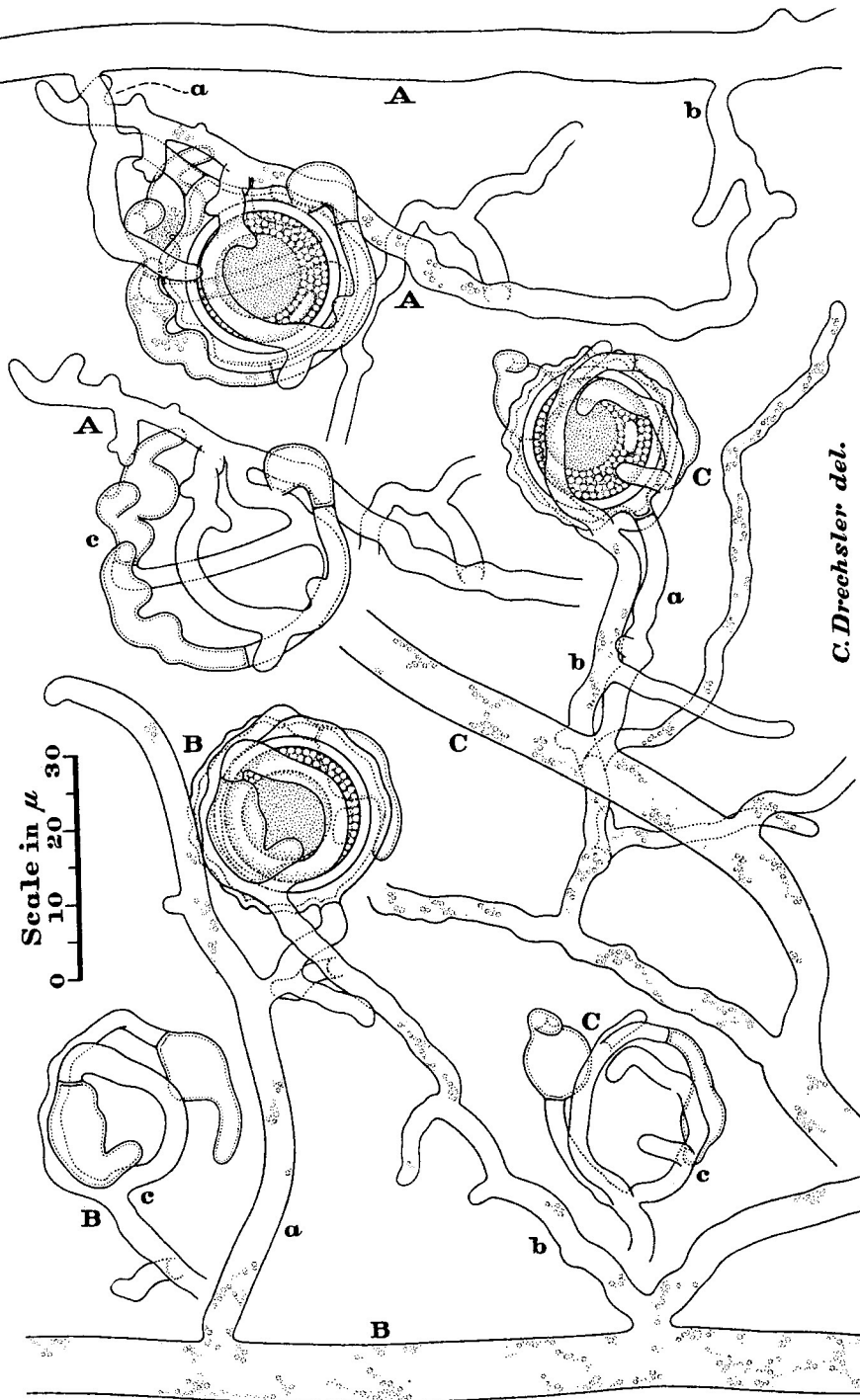


FIG. 3.—Mature sexual reproductive apparatus of the isolation received from the Centraalbureau voor Schimmelcultures at Baarn, Netherlands, as *Aphanomyces euteiches* P. F. 2; all reproductive units formed in maize-meal agar plate cultures, and drawn at a uniform magnification with the aid of a camera lucida; $\times 1,000$ throughout. A-C, Monoclinous reproductive units, showing mycelial connection between the hyphal branch, a, supplying the oogonium and the hyphal branch, b, supplying the attendant antheridia; for greater cleanness the entire male complement of each unit is shown besides in a separate drawing, c.

of the fungus dealt with by Buisman and by Meurs.

Whatever its relation to *Aphanomyces cladogamus* the fungus received as *A. euteiches* P. F. 2 was assuredly not to be held conspecific with *A. euteiches* occurring in pea roots. Its oogonial envelope, being relatively thin and pliable, would usually shrink more or less irregularly after the oospore had been formed, and consequently in mature reproductive apparatus most often showed a somewhat undulating profile. In true *A. euteiches*, on the other hand, the oogonial envelope is conspicuously thicker (Fig. 4, A-F). After formation of the oospore it behaves more nearly like a rigid shell, and in agar culture usually maintains its smoothly spherical outer contour for many weeks without evident change in size and shape.

Pronounced helicoid intertwining of hyphal parts is not characteristic of sexual reproduction in *Aphanomyces euteiches* from pea roots. In maize-meal agar plate cultures of the pea-root parasite the mycelial connection between an oogonium and its attendant antheridia is generally too remote to be traced. Sometimes, however, the oogonium of one reproductive unit (Fig. 4, A, a) and the male complement of a neighboring reproductive unit (Fig. 4, A, b) are found supplied by the same mycelial filament (Fig. 4, A, c) from positions perhaps less than 50μ apart; the other sex organs of the two units being then usually contributed by two separate mycelial filaments (Fig. 4, A, d, e). Most often one hypha gives rise to the oogonium either distally (Fig. 4, B, a) or on a short lateral stalk (Fig. 4, C, a; D, a; E, a), while another hypha near by (Fig. 4, B, b; C, b; D, b; E, b) gives off the antheridial branch or branches that bear terminally all the attendant male cells. Rather commonly the oogonial stalk and antheridial branch appear in some measure interlocked by means of lateral spurs. In instances where an oogonium is supplied with antheridia from 2 or 3 mycelial hyphae (Fig. 4, F, b, c, d) only one of these hyphae (Fig. 4, F, b) is usually found interlocked with the mycelial filament (Fig. 4, F, a) giving off the oogonial stalk.

Aphanomyces euteiches was listed by

Dennis and Foister (1942) as the cause of a root rot of *Viola* spp. (including the pansy and violet) observed by them in 5 regions comprising the central and eastern sections of Scotland. A typewritten circular (Anonymous, 1931) issued earlier from the Royal Botanic Garden in Edinburgh stated that *Violas* grown on the same land for a number of years often contract a disease from which the stem turns brown and shrivels, and the leaves turn yellow; death of the individual plants ensuing after the stem has decayed for an inch or two. On microscopical examination of the stem, collar, and decaying root numerous spherical bodies with slightly thickened walls were observable, being found mainly in the vascular elements. These bodies were identified as oospores of the causal agent of the root rot, a species of *Aphanomyces* similar to *A. euteiches*. Yet no species of *Aphanomyces* is mentioned by Chesters and Hickman (1939, 1944) in either of the 2 papers setting forth some fungus parasites they observed through examination of numerous varieties of *Viola* and pansy received from all parts of Britain and found affected with a soft rot of the stem or of the root system. In the final stages of the disease complex discussed by Chesters and Hickman, much as in the disease described in the Edinburgh circular, the leaves wilt and shrivel, and the whole plant collapses.

The occurrence of *Aphanomyces* oospores in the woody cylinder—a feature noted also in the roots of severely diseased pansies in and near the District of Columbia—suggests that the *Aphanomyces* attacking cultivated violets and pansies in Scotland may be identical with the species that attacks pansies in the United States rather than with the species that causes root rot in peas.

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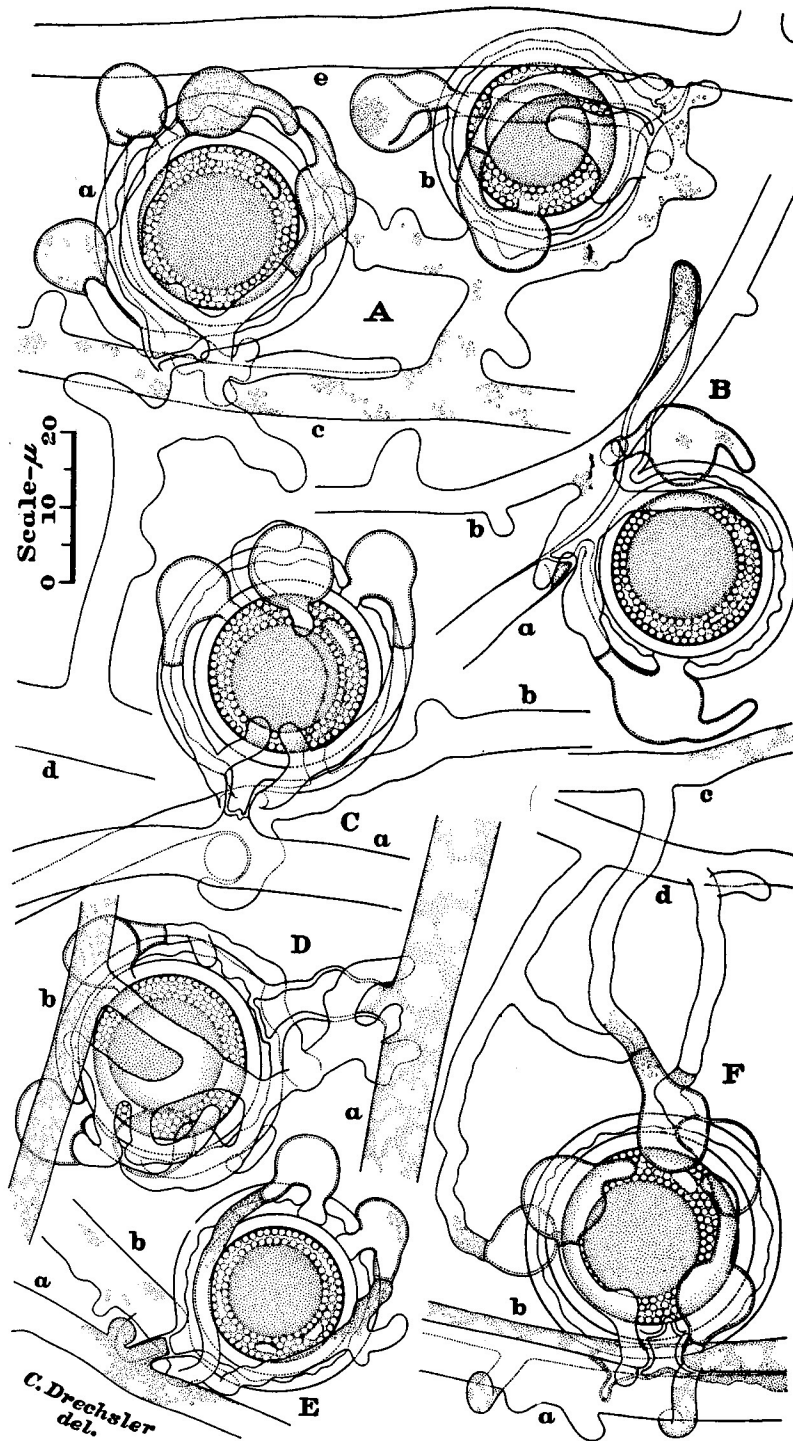


FIG. 4.—Mature sexual reproductive apparatus of the pea root-rot parasite, *Aphanomyces euteiches*; all reproductive units formed on the under side of maize-meal agar plate cultures; $\times 1,000$ throughout. A, Two connected reproductive units, a and b; c, mycelial hypha supplying the oogonium of unit a and the antheridia of unit b; d, mycelial hypha supplying antheridia of unit a; e, mycelial hypha supplying oogonium of unit b. B-E, Declinous reproductive units, each showing origin of oogonium from one mycelial hypha, a, and origin of attendant antheridia from a neighboring hypha, b. F, Declinous reproductive unit showing oogonium supplied by one mycelial hypha, a, and its male complement of 5 antheridia supplied conjointly by 3 neighboring hyphae, b-d.

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