

BOTANY.—*A crown-rot of hollyhocks caused by Phytophthora megasperma n. sp.*¹ CHARLES DRECHSLER, U. S. Department of Agriculture.

On May 15, 1931, a specimen of diseased hollyhock, *Althaea rosea* Cav., from a garden in the District of Columbia and reported to be illustrative of a trouble that had led to the loss of other plants in the same plot, was submitted to the writer for examination. Additional specimens were received during the ensuing two weeks. Early in June, perhaps because of the advent of drier weather conditions, but more probably because all the diseased plants had by that time succumbed, the destruction came to a halt in the garden referred to, though on June 5 a dying specimen from a small experimental planting

¹ Received November 16, 1931



Fig. 1. A.—Hollyhock plant affected with crown-rot, as collected in Washington, D. C. showing decay throughout short over-wintered stem and in the discolored proximal portions of the fleshy roots, $\times \frac{2}{3}$. B.—Longitudinal section of hollyhock crown attacked by *Phytophthora megasperma*, showing sharp line of demarcation between decayed and healthy parts at bases of new shoots, $\times \frac{2}{3}$. Photographed by Lillian A. Guernsey.

of hollyhocks at Arlington Experiment Farm, Rosslyn, Va., was found to be affected with the same disease. While definite information as to the distribution of the malady is not available, there is reason to believe that it is fairly widespread, a grower in the vicinity of Racine, Wis., for example, stating that he had lost his entire planting of hollyhocks during the early part of June 1931, the destruction evidently having taken precisely the same course as was observed in the District of Columbia.

As the seat of the malady is very largely in the underground parts (fig. 1, A), the trouble makes itself manifest at the beginning sometimes through poor growth of the new shoots, but in other instances, as notably in the specimen at Arlington Experiment Farm, a dozen robust shoots between 1.5 and 2.0 meters high may be produced before any sign of abnormality is noticeable. Ordinarily no premonitory changes in coloration or turgidity of leaves or stalks are apparent when one after another the shoots fall to the ground, where they soon wither and die. In the course of a few days the entire aerial growth from a well developed crown may be killed. The manner of destruction thus shows a strong similarity to that pertaining to the foot-rot of various species of *Lilium* by the fungus generally designated as *Phytophthora cactorum* (Leb. & Cohn) Schroet., or to the foot-rot of rhubarb caused by *P. parasitica* Dast.

When the overwintered underground parts of a dying plant are examined, the short stem is usually found to be completely involved in a decay that extends downward into many if not into all of the large fleshy roots, often for distances of from 5 to 10 cm. and sometimes for distances of from 15 to 20 cm. Outwardly the decay is evidenced in a buff or sepia or darker brown discoloration, while internally the tissues, in addition to being more or less discolored, are softened to such a degree that the fibrous and woody elements are readily separated into longitudinal shreds. The decay is found usually to extend only a very short distance from the overwintered stem into the new shoots, the line of demarcation between diseased and healthy tissues being here rather sharply marked by a darkly discolored marginal zone (fig. 1, B). Apparently the shoots obtain water for a considerable period through the vascular elements of the completely killed overwintered parent stem, and fall to the ground only when the supporting tissues are weakened mechanically in such measure as no longer to be capable of supporting the weight of the aerial structures.

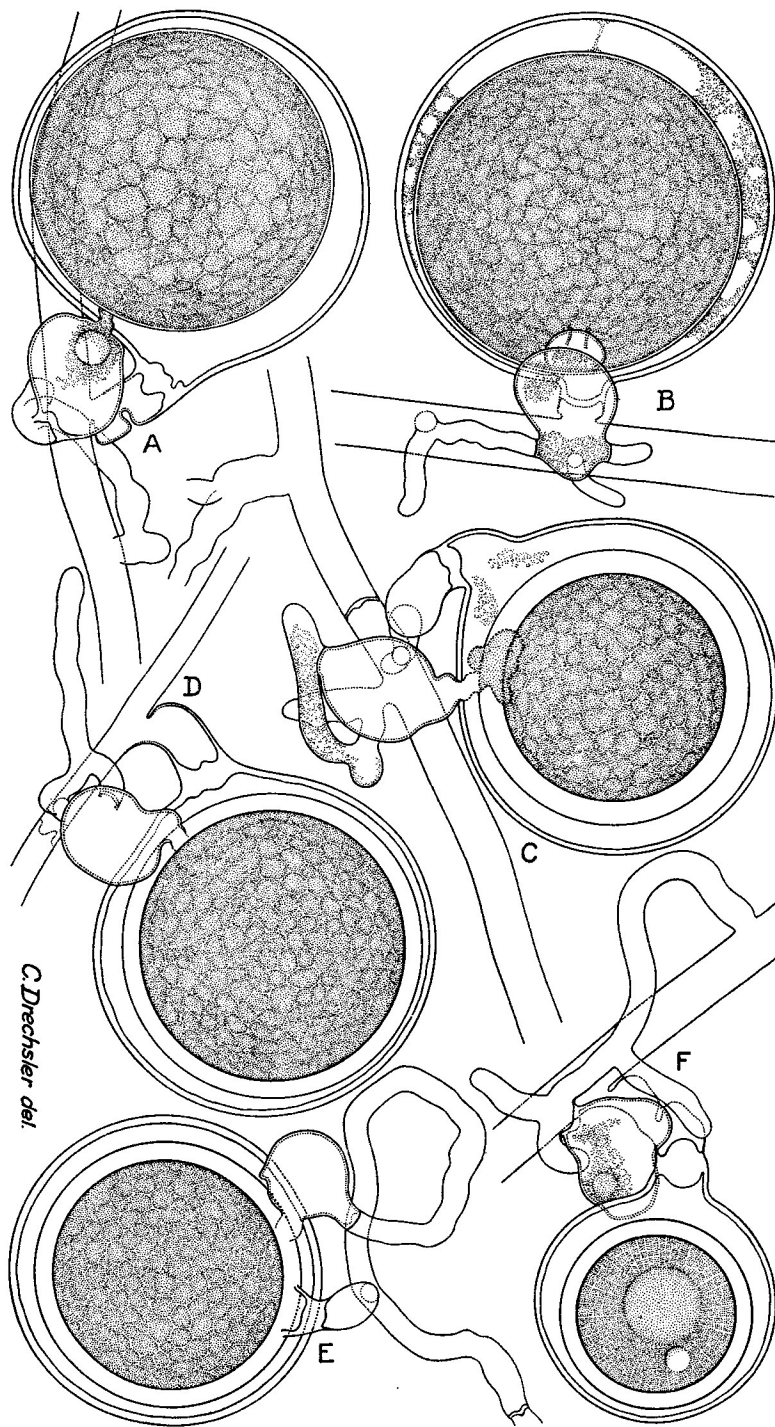


Fig. 2. Sexual apparatus of *Phytophthora megasperma*, drawn with camera lucida, $\times 1000$,

In the greater bulk of the decaying tissues of the diseased hollyhock plants examined was found a moderate quantity of intercellular mycelium, which being usually devoid of protoplasmic contents, could not usually be made to yield new growth. New growth and subsequently pure cultures on artificial media were obtained fairly readily from pieces of newly invaded tissue from the margins of the diseased parts by employing the method set forth in an earlier paper (4), and with very satisfactory regularity when the washing of the infected material was continued until the gelatinous substance oozing in extraordinary quantity from the irrigated hollyhock tissue had been largely removed.

The fungus thus obtained displays in its mycelium the vegetative features generally associated with species of *Phytophthora*. Its stinging, openly branching habit and the substantial appearance of the granular contents of its hyphae at once indicate a member of that genus rather than of the related genus *Pythium*. As in many other species of *Phytophthora* and, indeed, in various species of *Pythium*, septa make their appearance in the originally continuous hyphae with the withdrawal of the granular contents. In completely evacuated mycelium the rather thick cross-walls are present usually in considerable number.

A high degree of distinctiveness attaches, however, to the sexual apparatus, which is produced promptly and abundantly on nearly all substrata ordinarily employed (figs. 2-4). Aside from its ready production, the sexual apparatus is distinguished by the unusually large dimensions of oogonium and oospore. Thus 200 oogonia produced on maize-meal agar plates and chosen at random 10 to 15 days after planting, yielded measurements of diameters distributed according to values expressed to the nearest micron as follows: 33 μ , 1; 34 μ , 1; 37 μ , 1; 39 μ , 2; 40 μ , 3; 41 μ , 5; 43 μ , 6; 44 μ , 7; 45 μ , 19; 46 μ , 24; 47 μ , 28; 48 μ , 19; 49 μ , 27; 50 μ , 24; 51 μ , 17; 52 μ , 8; 53 μ , 3; 54 μ , 2; 56 μ , 2; 57 μ , 1. Measurements of the diameters of the 200 oospores contained within these oogonia yielded values distributed as follows: 26 μ , 1; 28 μ , 1; 30 μ , 1; 32 μ , 1; 33 μ , 2; 34 μ , 3; 36 μ , 2; 37 μ , 6; 38 μ , 2; 39 μ , 17; 40 μ , 32; 41 μ , 26; 42 μ , 26; 43 μ , 30; 44 μ , 22; 45 μ , 13; 46 μ , 8; 47 μ , 5; 49 μ , 1; 52 μ , 1. These values from which averages for diameter of oogonium and diameter of oospore of 47.4 μ and 41.4 μ respectively were computed, may presumably be regarded as fairly representative of the species, having been obtained from material in which the bodies in question were present

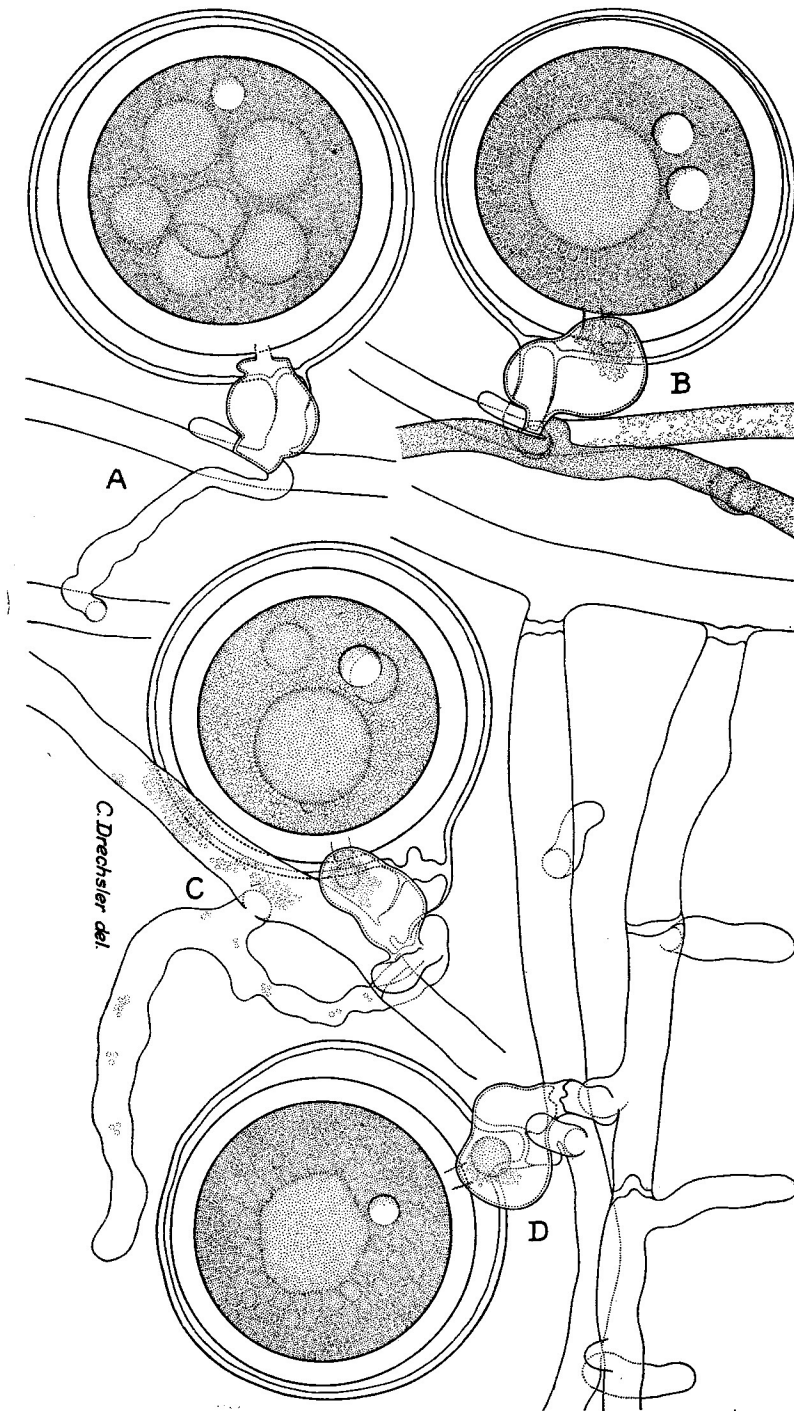


Fig. 3. Sexual apparatus of *Phytophthora megasperma* drawn with camera lucida, $\times 1000$.

in large number and showed practically no tendency toward degeneration. The oospores in substantially all cases showed the structure normal for the stage of maturity in which they were found. Naturally more extreme values not included in the ranges found in the course of the random selections came under observation. Thus in the sex apparatus shown in Figure 4, E, among the smallest seen in an irrigated lima-bean agar preparation, in which somewhat smaller dimensions are wont to prevail than in dry maize-meal agar plate cultures, the oogonium measures only 16μ in diameter, while the oospore measures only 11μ ; and again the very largest oogonium seen in any maize-meal agar culture was found to measure 61μ in diameter, and contained an oospore 54μ in diameter.

The pertinent literature contains few records of oogonia and oospores equalling or closely approaching in size those of the hollyhock parasite. In a recently published comparative study of the genus *Phytophthora*, Tucker (9) states that the oogonia and oospores of *P. erythroseptica* Pethyb. with average diameters of 36.3μ and 31.4μ respectively, and hence fully 10μ smaller in these dimensions than the hollyhock fungus, exceed in size those of any congeneric form, and held this superiority in size to be diagnostic for that species. In this connection it must be mentioned that Tucker reports that on transferring sterile mycelium of *P. erythroseptica* to Petri's mineral solution he found after one week a few large oogonia measuring 30.1 to 64.3μ (average 45.1μ) in diameter. As, however, oospores were not observed in these oogonia there were, indeed, excellent grounds for not regarding the large structures as representative of the species. Somewhat similarly disturbing considerations pertain to Petri's report (7) of the production of oogonia varying in diameter from 57 to 62μ and oospores "non ancora ben differenziate" measuring between 50 and 56μ by *P. cambivora* (Petri) Buism. grown on carrot-agar acidulated with malic acid, when previously (6) oospores of the chestnut parasite in the tissues of the diseased host had been found to measure only 20 to 27μ . Ashby (2) more recently reported the discovery of several oogonia and oospores in a pure culture of *P. cambivora*, which with respect to size more nearly approximated those of the hollyhock parasite. It is to be noted that whereas the antheridia of both *P. erythroseptica* and *P. cambivora* developing in pure culture have always been found to be of the amphigynous type, those of the hollyhock fungus are predominantly paragynous.

A really close approximation to the hollyhock parasite in size of oogonium and oospore is to be inferred from Alcock's summary (1) of the morphological features of the fungus held by her to be responsible for the "Lanarkshire strawberry disease" prevalent of late years in Scotland and England: "Oogonia average about 46μ to 47μ in diameter; oospores average about 33μ to 47μ diameter; oospore wall about 4μ thick; sporangia about 50μ by 30μ ." Antheridia of the amphigynous type were stated to have been made out in the cells of the host in a few instances, failure to obtain the fungus in pure culture having precluded more complete observations of the sexual apparatus. According to a later note (3) of somewhat indefinite authorship, presumably the same parasite is "characterized by a large sporangium, by oospores of the two types and as far as has been ascertained is similar to *Phytophthora cinnamomi*." It is not certain whether the latter quotation is to be interpreted as implying that the strawberry parasite produces large globose resting bodies of the type described by Rands (8) for his *P. cinnamomi*; but assuredly no bodies of such type have ever been seen in cultures of the fungus isolated from diseased hollyhocks.

As has been mentioned the antheridia of the hollyhock fungus are predominantly paragynous. In plate cultures of maize-meal agar approximately 99 out of every hundred sexual units exhibit a paragynous relationship of the male organ (figs. 2, A-F; 3, A-D; 4, A-C), the amphigynous relationship occurring only rather rarely. On irrigated lima-bean agar preparations useful in the study of asexual reproduction the proportion of amphigynous antheridia (fig. 4, D-F) is much larger, varying often between 25 and 35 in every hundred. The fungus is very obviously homothallic, for in many instances when it is neither too remote nor leads into one of the knotted hyphal tangles present here and there, the mycelial connection between antheridium and oogonium may be readily traced. In some cases (figs. 2, D; 4, C) the combined lengths of antheridial branch, oogonial stalk and intervening portion of hypha is not in excess of 40μ . The usual somewhat irregularly orbicular or broadly elliptical shape of the antheridium is sometimes modified by the presence of a distal protuberance or lobe (figs. 2, B, C, D; 4, B) by which contact with the oogonium is established.

In its asexual reproduction the hollyhock parasite is rather similar to *Phytophthora cryptogea* Pethyb. & Laff., *P. cinnamomi* and *P. cambivora*. The sporangium is as a rule ovoid (fig. 5, A-F) but frequently the development of a distal lobe or protuberance (fig. 5, K, L, N, O) brings

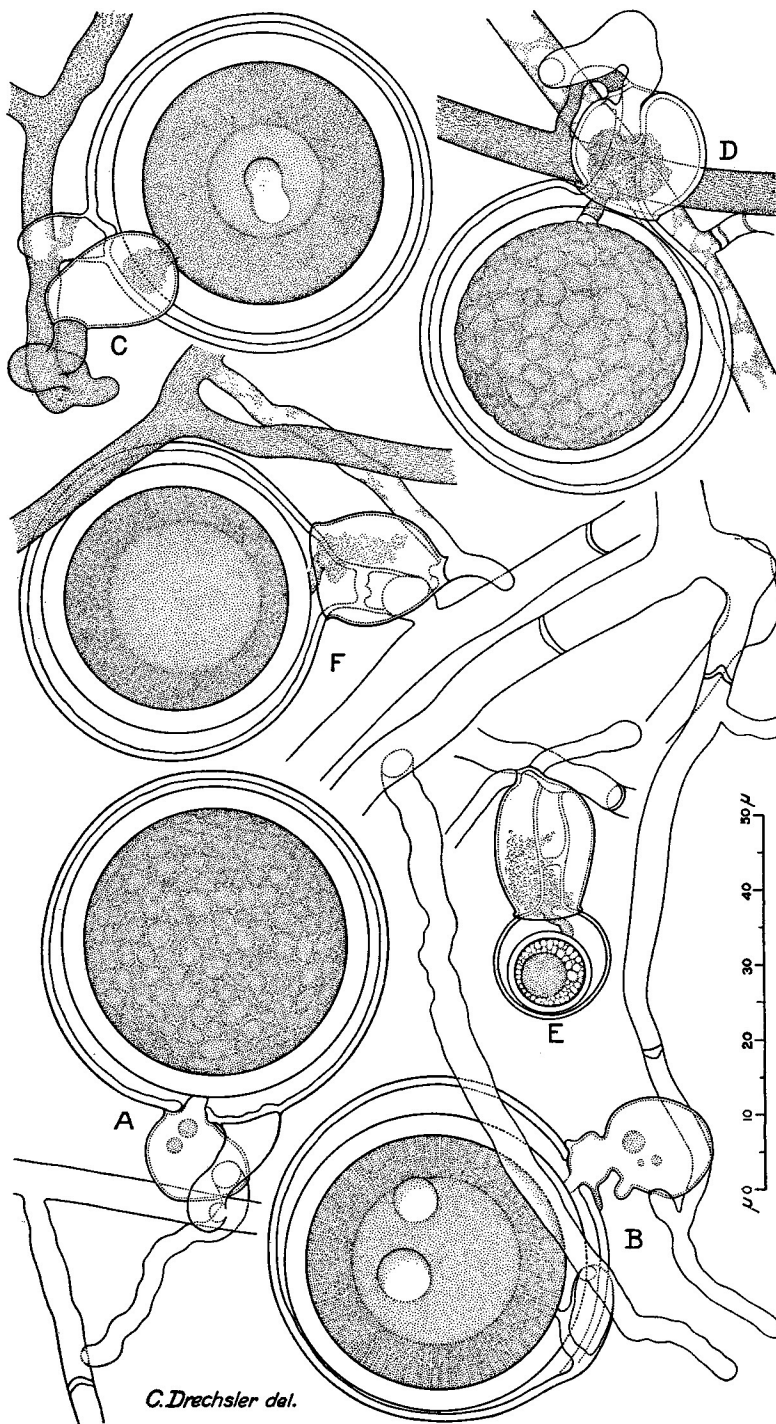


Fig. 4. Sexual apparatus of *Phytophthora megasperma* drawn with camera lucida, $\times 1000$.

about a modification in its outward form suggesting the distal modifications sometimes present on antheridia. A papilla of dehiscence protruding beyond the apical contour is not produced, although quite obviously the wall at the apex of the sporangium becomes transformed into a noticeably thickened refringent cap through the yielding of which discharge is effected. Dehiscence is accompanied by a perceptible shrinkage of the sporangial envelope, as may be seen by a comparison of Figure 4, F with Figure 4, E, the contraction apparently being only in part accounted for by the relaxed contour of the empty membrane. The hypha supporting the sporangium is proliferous, so that a second or a third sporangium may be borne on the same axis, either within the empty envelope of the primary one, or beyond the latter, depending on the lengths of the intervening increments of growth of the sporangiophore (fig. 5, G-M).

The sporangia obtained in irrigated preparations are too variable for profitable statistical metric treatment. Those formed earliest in a fresh preparation measure usually from 35 to 60 μ in length by 25 to 45 μ in diameter. The larger individuals like the one shown in Figure 5, A, yield between 35 and 45 zoospores; slightly smaller examples like those shown in Figure 5, B, C, yield between 30 and 35; the medium-sized specimen represented in Figure 5, E, yielded 18 by accurate count; the small one shown in Figure 5, D, was seen to deliver 6 zoospores. As the preparations become older and in part exhausted, the sporangia decrease in size until specimens make their appearance with minimum dimensions; that is, with the length between 15 and 20 μ , and width between 6 and 8 μ . Such diminutive structures as, for example, the

Fig. 5. Asexual reproductive structures of *Phytophthora megasperma*, drawn with camera lucida, $\times 500$. A, B, C.—Fully grown primary sporangia. D, E.—Sporangia immediately preceding dehiscence. F.—Empty envelope of sporangium shown in E. G, H, I, J, K, L, M.—Sporangia and supporting hyphae showing proliferation. O, P.—Evacuated sporangial envelopes within which are retained empty cyst walls with membranous parts of papillae of dehiscence, and in P, in addition, some encysted zoospores without evidence of repetitional development. Q.—Sporangium after frustrated dehiscence showing one zoospore cyst wall evacuated by means of a papilla of dehiscence, one discharged miniature sporangium on a germ sporangiophore perforating the wall of the primary sporangium, and 10 zoospores in various stages of repetitional development by production of miniature germ sporangia. R, a-i.—Zoospores after rounding up. S.—Two zoospores each giving rise to a germ sporangium, a showing beginning of development, b showing miniature sporangium delimited by basal septum. T, a-f.—Zoospores, each provided with a papilla of dehiscence. U, a-h.—Evacuated cyst membranes after escape of zoospores in second swimming stage. V, a-c.—Zoospores germinating by vegetative germ tubes. W.—A zoospore in motile stage.

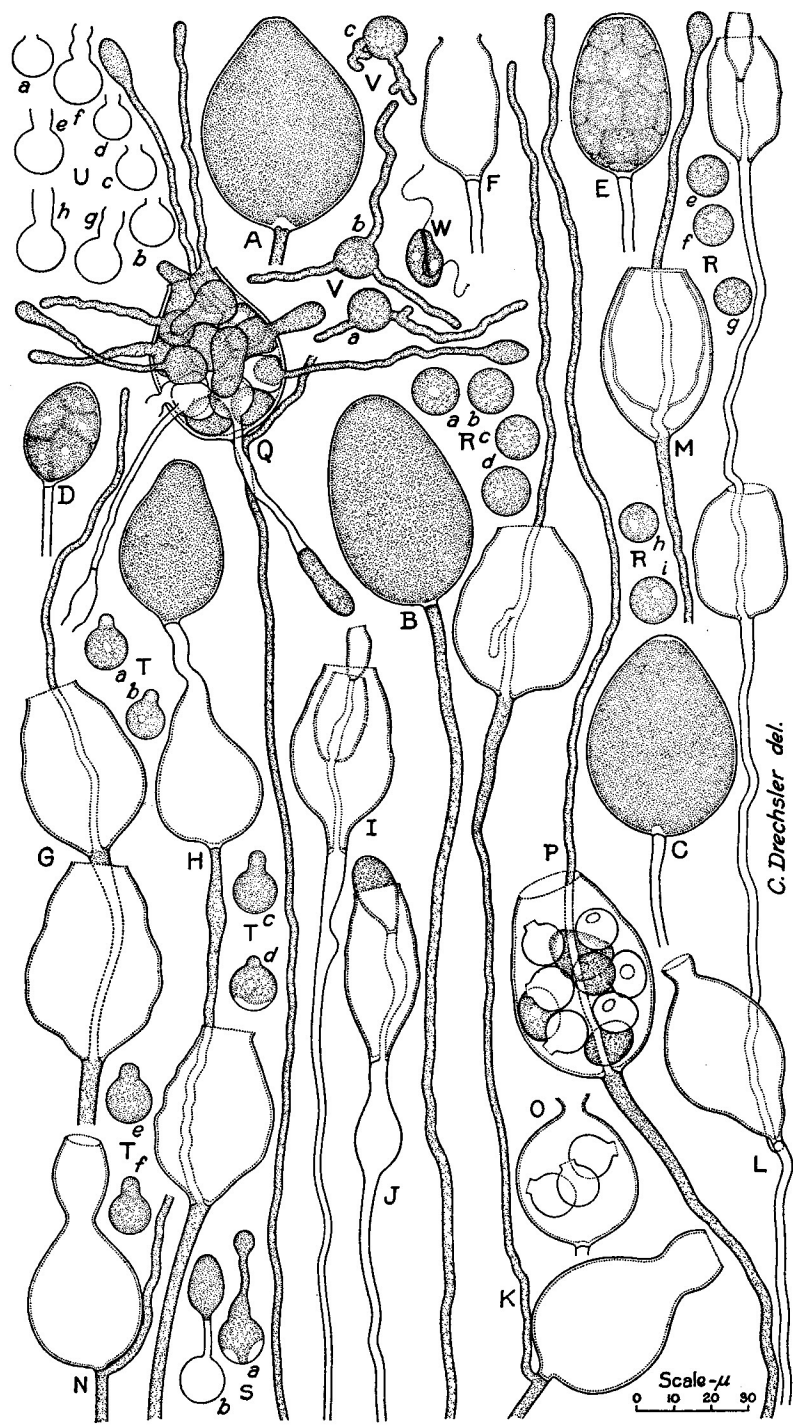


Fig. 5. *Phytophthora megasperma*. For explanation see page 522.

tertiary sporangium shown in Figure 5, I, give rise to only a single zoospore.

The zoospores are of the biciliate, longitudinally grooved, reniform type usual in the genus (fig. 5, W), which after a period of swarming round up (fig. 5, R, *a-i*). A second period of motility frequently ensues, this being accomplished through both of the courses of repetitional development set forth for a number of congeneric forms in an earlier paper (5). In most instances the encysted zoospore produces a wide papilla (fig. 5, T, *a-f*) which ultimately yields at the apex to liberate the full-fledged secondary swimming spore. Usually the cylindrical modification on the evacuated cyst wall is relatively short (fig. 5, U, *a-d*) but in some instances modifications of more considerable lengths (fig. 5, U, *e-h*) remain behind as evidence of exceptionally long papillae. This type of development prevails also among zoospores retained in relatively small number within sporangia (fig. 5, O, P) the dehiscence of which has been partially frustrated. In fewer instances a properly liberated zoospore gives rise to the second swimming stage by the production of a miniature sporangium on a delicate germ sporangiophore (fig. 5, S, *a, b*). Yet in cases where the dehiscence of the ordinary large sporangia has been frustrated more nearly completely, so that the imprisoned zoospores are packed rather closely within the containing envelopes, the escape of the protoplasts in this species as in congeneric forms is usually accomplished through the latter type of repetitional development (fig. 5, Q).

As far as the writer is aware the literature contains no record of any species of *Phytophthora* combining oogonia and oospores having dimensions approaching those characteristic of the hollyhock parasite with predominantly paragynous antheridia, proliferous, non-papillate sporangia and the absence of large globose "chlamydozoospores." The fungus under consideration is therefore described as a new species for which a specific term descriptive of the large oospore is deemed appropriate.

***Phytophthora megasperma* n. sp.**

Mycelium intercellular in tissues of host; on artificial substratum of somewhat radiating aspect, composed of freely branching hyphae from 3 to 8 μ in diameter; continuous in actively growing stage, later, on becoming evacuated, developing numerous, rather thick septa; producing aerial growth in small or moderate quantity; under aquatic conditions extramatrical growth meager.

Sporangium regularly formed terminally on a long, simple or sparingly branched, extramatrical filament measuring mostly 50μ to 2 mm. in length and 2 to 2.5μ in diameter, though often expanding in the distal portion to a diameter of 3 to 5μ ; later often coming into a lateral position through continued elongation of the supporting filament from immediately below the delimiting septum; regularly ovoid, but occasionally bearing distally a protuberance or lobe of variable size; measuring 6 to 45μ in transverse diameter by 15 to 60μ in length; on dehiscence opening broadly at apex without formation of an outwardly protruding papilla; after evacuation proliferous in moderate measure, both by formation of sessile or nearly sessile secondary or often tertiary sporangia within primary one and by repeated growth of the supporting filament through the orifices of the empty envelope to produce additional sporangia externally. Zoospores produced from 1 to 45 in a sporangium; reniform, longitudinally grooved, biciliated, after rounding up measuring 10 to 13μ in diameter; individually germinating by germ-tubes usually 1 to 3 in number, or often, whether properly liberated or retained within the sporangial envelope owing to frustrated dehiscence, often giving rise to a secondary zoospore,—the repetitional development taking place either by direct discharge of contents through an evacuation tube 3.5 to 5.5μ in diameter and 1 to 10μ in length, or by the production of an elongated miniature sporangium mostly 6 to 10μ in diameter and 16 to 22μ in length on a germ sporangiophore mostly 1.5μ in diameter and 10 to 60μ in length.

Oogonium borne terminally on a stalk usually 5 to 15μ in length; smooth, subspherical, measuring 16 to 61μ , mostly 42 to 52μ (average 47.4μ) in diameter; provided with a wall 0.5 to 1.7μ (average 1.2μ) in thickness. Antheridium single; irregularly orbicular or prolate ellipsoidal, sometimes provided with a distal protuberance or lobe; measuring usually 10 to 18μ in diameter by 14 to 20μ in length; in some (1 to 35 out of 100) cases amphigynous, but more often paragynous, in latter event usually applied near base of oogonium and often in intimate contact with oogonial stalk; borne laterally or terminally or in intercalary relationship on a branch mostly 5 to 50μ in length, the branch sometimes arising from a hypha not demonstrably connected with the oogonial hypha, but sometimes having close mycelial connection with the oogonium, the total length of filamentous parts between the septa delimiting the sex organs occasionally not exceeding 40μ . Oospore colorless or more often distinctly yellowish; smooth, subspherical, measuring 11 to 54μ , mostly 37 to 47μ (average 41.4μ) in diameter; provided with a wall 0.8 to 4.6μ (average 3.6μ) in thickness, and containing a reserve globule measuring at early maturity 6.5 to 24.0μ (average 17.6μ) in diameter.

Causing a destructive decay of the stem and roots of *Althaea rosea* Cav. in Washington, D. C. and at Rosslyn, Va.

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