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REPETITIONAL DIPLANETISM IN THE GENUS PHYTOPHTHORA[1]

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INTRODUCTION AND HISTORY

In a recent abstract (*10*)[2] concerning a prominently papillate species of Phytophthora different from *P. erythroseptica* Pethyb., as the cause of at least occasional instances of pink rot of potato (*Solanum tuberosum* L.) tubers in the United States, reference was made to the frequent manifestation of diplanetism by the fungus. Secondary motile zoospores were set forth as being delivered directly through an evacuation tube, or liberated from a 1-spored sporangium produced terminally on a delicate germ sporangiophore. Mention was made of the rather striking reproductive arrangement often resulting when zoospores imprisoned within an ordinary sporangium exhibit the latter type of development, as well as of the prevalence of diplanetism in congeneric forms, an appropriate instance being cited in another species which had been isolated from diseased potato tubers in the United States, and which was similarly capable of causing pink rot.

In spite of considerable study devoted to species of Phytophthora during more than a half century, the production of a second swimming spore from a previous one, without the interposition of a vegetative phase, appears, as far as the writer is aware, to have been recorded only once—in Sawada's recent account (*22*) of his *Phytophthora melongenae*. To be sure, according to Gäumaun (*11, p. 80*), Murphy observed

> in *Phytophthora infestans* the shedding of the membrane, as in Dictyuchus; the zoospores which have found no suitable substrate come to rest and surround themselves with a membrane. After a certain time they again slip out with the same reniform appearance and swarm further.

From the publication (*16, p. 459*) cited, it is not apparent that the text permits such interpretation, as Murphy seemingly is not describing either any condition suggestive of Dictyuchus or emergence of a secondary swimming spore from an encysted stage. His account of the production of secondary conidia on germ tubes of zoospores is interesting nevertheless in relation to the phenomenon of repetitional diplanetism:

> When the zoospores germinate under favorable conditions, the germ tube is of such enormously greater capacity than the original spore, and continues increasing in length (even though not completely filled with protoplasm), or at least remains alive and vigorous-looking so long, that a saprophytic existence is at once suggested. * * * They, too, have the faculty of producing "secondary" conidia, an observation which does not seem to have been recorded previously. * * * Subsequently in a preparation originally set up under sterile conditions and kept for five days at 10°–15° C., in which there had been abundant zoospore formation, the almost universal production of "secondary" conidia by zoospores situated near the edge of the cover glass was observed. * * * These new conidia

[1] Received for publication Nov. 13, 1929; issued March, 1930.
[2] Reference is made by number (italic) to "Literature cited," p. 572.

were borne on the ends of long spirally twisted tubes. They contained all the protoplasm, even of the branches, where such were present. In form they exactly resembled those produced by conidia being asymmetrical and provided with prominent papillae and yellow oil-drops, but they were much smaller, although having a considerably greater volume than the original zoospore.

The increased volume of the secondary conidium implies, apparently as an essential feature of the kind of development discussed, an expansion of material that could scarcely be attributed to purely reproductive processes.

A presumably similar course of development was noted later by Godfrey (*12*) in a study of one of the rhubarb (*Rheum rhaponticum* L.) foot-rot parasites described by him as *Phytophthora parasitica* var. *rhei:*

Within an hour or two after motility ceases zoospores often begin to germinate. * * * Germination in water sometimes results in the formation of small conidia, normal in appearance, as shown in F. These may produce zoospores, or may germinate by germ tubes and continue the vegetative growth.

The small conidium illustrated in the Figure F referred to is represented by a structure that would seem to exceed in volume the zoospore from which it originated by no less than eight times. The widening of the more distal portion of filament suggests too that the product of the zoospore may have attained such extension that it might perhaps have been more appropriately designated as a young mycelium rather than as a germ tube.

The reproductive development of the zoospores of *Phytophthora melongenae* described by Sawada does not appear to have been seriously disturbed through the intervention of vegetative growth. As the original account is in Japanese, a quotation from a somewhat free translation of the text [3] may be appropriate:

After swimming about for 30 minutes or an hour the zoospore loses its cilia, comes to rest, and rounds up as a spherical body, 10 to 11μ in diameter. On resting approximately 30 minutes it germinates by a single delicate germ tube, though in one instance two tubes were found produced. The germ tube either develops extensively, or it ceases to elongate and gives rise to a terminal conidium. The latter measures usually 11 to 12 by 8 to 9μ, though examples measuring as much as 13 to 16 by 10 to 13μ occur. Probably the former contains a single zoospore while the latter contains two. These small conidia open at the apex to liberate zoospores.

The zoospore which fails to make its way out of the conidium comes to rest inside and germinates, the germ tube sprouting through the papilla which already is open, or penetrating the conidial wall. In case the tube penetrates the wall, it frequently soon ceases elongation to produce a terminal conidium which germinates by liberating a zoospore.

Among the illustrations accompanying Sawada's text are three figures showing zoospores that have germinated by delicate germ sporangiophores each bearing terminally a minute, obviously 1-spored sporangium. In one of these figures (*22, pl. 3, fig. 3*) the structures are lying free; in another (*22, pl. 3, fig. 6*) the zoospores, four in number, imprisoned within the primary sporangial membrane, have each thrust their germ sporangiophore through the papillary opening to produce a minute sporangium externally; and in the third (*22, pl. 3, fig. 7*) four zoospores out of a larger number of imprisoned encysted structures have again each produced a minute zoosporangium internally, the germ sporangiophore here, however, in all instances perforating the wall of the primary sporangium.

[3] The translation was prepared by Sabura Katsura.

To another figure (*22, pl. 3, fig. 4*) showing two evacuated zoospore membranes, each with an ample papillary opening, is attached the explanatory legend: "Resting zoospore producing a zoospore directly."

TREATMENT FAVORING DIPLANETIC DEVELOPMENT

As repetitional diplanetism seems to be of some importance in the biology of various species of Phytophthora, the present discussion supplementing Sawada's observations may not be excessive. The conditions favoring the production by encysted zoospores of a second swimming stage without the interposition of a vegetative phase apparently are the same as those that favor zoospore formation generally. In order that the necessary development might take place on a scale large enough to permit ready observation, it was found advantageous, after inducing the production and discharge of a generous supply of ordinary sporangia, to maintain conditions suitable for continued discharge. This could not usually be accomplished by mounting aerial sporangiferous material from artificial cultures on a microscope slide and covering with a cover glass, or by placing the material in a sealed hanging-drop preparation, or by cultivating in a Van Tieghem cell.

With the forms discussed in the present paper more satisfactory results were obtained through direct use of growth in Lima-bean agar, though for other types, like *Phytophthora cryptogea* Pethyb. and Laff., and *P. cinnamomi* Rands, this substratum was obviously less suitable. Portions of plate culture containing actively growing mycelium were removed to sterile Petri dishes, cut into pieces of convenient area, and irrigated with sterile distilled water in such a way that the upper surface of the agar was moistened without being inundated. As a result of such procedure, the papillate potato tuber parasite, for example, gave rise in the course of 12 to 15 hours to an extraordinarily heavy crop of sporangia, these being comparable in uniformity in size and shape with the sporangia produced in nature by *P. infestans* (Mont.) De Bary or *P. phaseoli* Thaxter. As long as the cover of the Petri dish was kept in place zoospore production occurred only in negligible quantity. On the removal of the cover the familiar changes recorded by numerous observers—cleavage of protoplasm, writhing movement, progressive individualization, swelling of the papillary substance—began and proceeded simultaneously in practically all the fully grown sporangia, with the result that after about a half hour very rapid liberation of zoospores lasting about 10 or 15 minutes ensued everywhere.

As an access of fresh air seemed to be almost the only consequence of the removal of the cover, Murphy's observation on the importance of oxygen in zoospore production in *Phytophthora infestans* was thus accorded confirmation in the behavior of the papillate pink-rot parasite. A similar response to ventilation was shown in preparations of most of the other congeneric organisms included in the present study. Material kept under observation and hence subjected to illumination from a microscope lamp gave rise to the swimming stage with noticeably greater rapidity than similar material in the same container but not exposed to special illumination.

SEQUENCE OF EVENTS IN DIPLANETIC DEVELOPMENT

With an abundance of zoospores available at the beginning, and additional quantities supplied by the dehiscence of succeeding crops of sporangia, the development leading to a secondary swimming stage could be looked for several hours later, suitable conditions being maintained meanwhile by admitting, if need be, fresh air from time to time, and by cautious replacement of water lost by evaporation. An early stage in such development of the papillate pink-rot organism is represented in Figure 1, A, *h* and *i*, the zoospores, after rounding up and becoming encysted as spherical bodies 8 to 12μ in diameter, having produced a dome-shaped protrusion measuring mostly 3 to 4.5μ in basal diameter and generally approximately the same in length. Until the protrusion attains its definitive size, it is filled, like the structure from which it originates, with protoplasm containing fine granules in rather open arrangement.

Since in considering the direct delivery of secondary motile zoospores the various forms included in the present study in which such delivery has been demonstrated exhibit no departure from a same uniform sequence of events, the series of stages (fig. 1, K, *l–n*) drawn from material of the rhubarb foot-rot fungus, *Phytophthora parasitica* var. *rhei* Godfrey, will illustrate the pertinent developmental stages revealed in the papillate pink-rot parasite as well as in other congeneric types. The first internal change readily noticeable consists in the retraction of the granular contents from the protuberance so that the latter appears to be filled only with clear liquid. (Fig. 1, E, *l*.) Soon thereafter, if not at the same time, the protoplast can be

EXPLANATORY LEGEND FOR FIGURE 1

A.—Unnamed form with papillate sporangia isolated from decaying potato tubers, possibly referable to *P. parasitica: a*, Extensive development of miniature sporangia from zoospores imprisoned within the second of a series of three sporangia; *b–e*, miniature sporangia produced from free encysted zoospores; *f*, an empty miniature sporangium; *h, i*, encysted zoospores with evacuation tubes; *j–s*, empty cyst envelopes after escape of secondary swimming spores.

B.—A nonpapillate species isolated from diseased Idaho potato tubers, possibly to be referred to *P. erythroseptica: a–f*, Encysted zoospores, each showing a vacuole and an evacuation tube; *g*, motile zoospore at moment of liberation from cyst envelope; *h, i*, evacuated cyst envelopes.

C.—*P. melongenae: a*, Large sporangium containing an evacuated cyst envelope; *b*, sporangium containing imprisoned zoospores, of which all except one have given rise to germ sporangia; *c–j*, germ sporangia produced by free encysted zoospores; *k*, free germ sporangium after dehiscence; *l*, encysted zoospore with evacuation tube; *m, n*, evacuated cyst envelopes.

D.—*P. parasitica* derived from diseased cotton boll: *a*, Extensive development of miniature sporangia from imprisoned zoospores; *b*, sporangium containing two empty cyst membranes, one manifesting direct dehiscence, the other the production of a germ sporangium; *c*, sporangium with two evacuated cyst envelopes, and a secondary motile spore again to be retained within parent sporangium; *d*, sporangium with extensive development of germ sporangia from imprisoned zoospores; *e, f*, successive stages in development of germ sporangium; *g*, germ sporangium borne on an unusually long sporangiophore; *h, i*, germ sporangia after dehiscence; *j–n*, encysted zoospores with evacuation tubes; *o*, encysted zoospore immediately before liberation of secondary spore; *p*, escape of secondary zoospore from cyst envelope; *q–z, a′, g′*, evacuated cyst envelopes.

E.—*P. parasitica* var. *rhei: a*, Sporangium containing two evacuated cyst envelopes; *b*, sporangium showing extensive production of miniature sporangia by imprisoned zoospores; *c*, sporangium with four imprisoned zoospores, three having produced germ sporangia, the other having discharged a secondary motile spore directly; *d–j*, germ sporangia after dehiscence; *k–n*, successive stages in development of secondary zoospore directly within cyst wall; *o*, zoospore with unusually long evacuation tube; *p*, another zoospore in same state as *l*, immediately preceding evacuation; *q–x*, evacuated cyst envelopes.

F.—Unnamed form isolated from diseased Honeydew melon: *a*, Germ sporangium produced by encysted zoospore; *b*, evacuated germ sporangium; *c–n*, evacuated cyst envelopes.

G.—Unnamed form isolated from diseased sugar-beet root in Utah: *a–c*, Encysted zoospores with broad protuberances of dehiscence; *d–m*, evacuated encysted envelopes.

H.—Unnamed form isolated from diseased tomato fruit in California: *a–e*, Encysted zoospores with developing evacuation tubes; *f–o*, evacuated cyst envelopes.

I.—*P. fagi: a*, Secondary motile spore after emergence being held fast by cilium adhering to inner surface of cyst wall; *b–e*, evacuated cyst envelopes.

J.—*P. citrophthora: a–c*, Germ sporangia produced by free encysted zoospores; *d, e*, evacuated germ sporangia; *f*, encysted zoospore with well-developed evacuation tube; *g, h*, evacuated cyst envelopes

K.—*P. hibernalis: a–f*, Germ sporangia produced by encysted zoospores; *g–p*, evacuated germ sporangia.

L.—*P. cactorum: a*, Profuse development of germ sporangia by encysted zoospores imprisoned in a large sporangium; *b–d*, germ sporangia produced by free zoospores; *e*, germ sporangium after evacuation; *f*, encysted zoospore with evacuation tube; *g–k*, evacuated cyst envelopes.

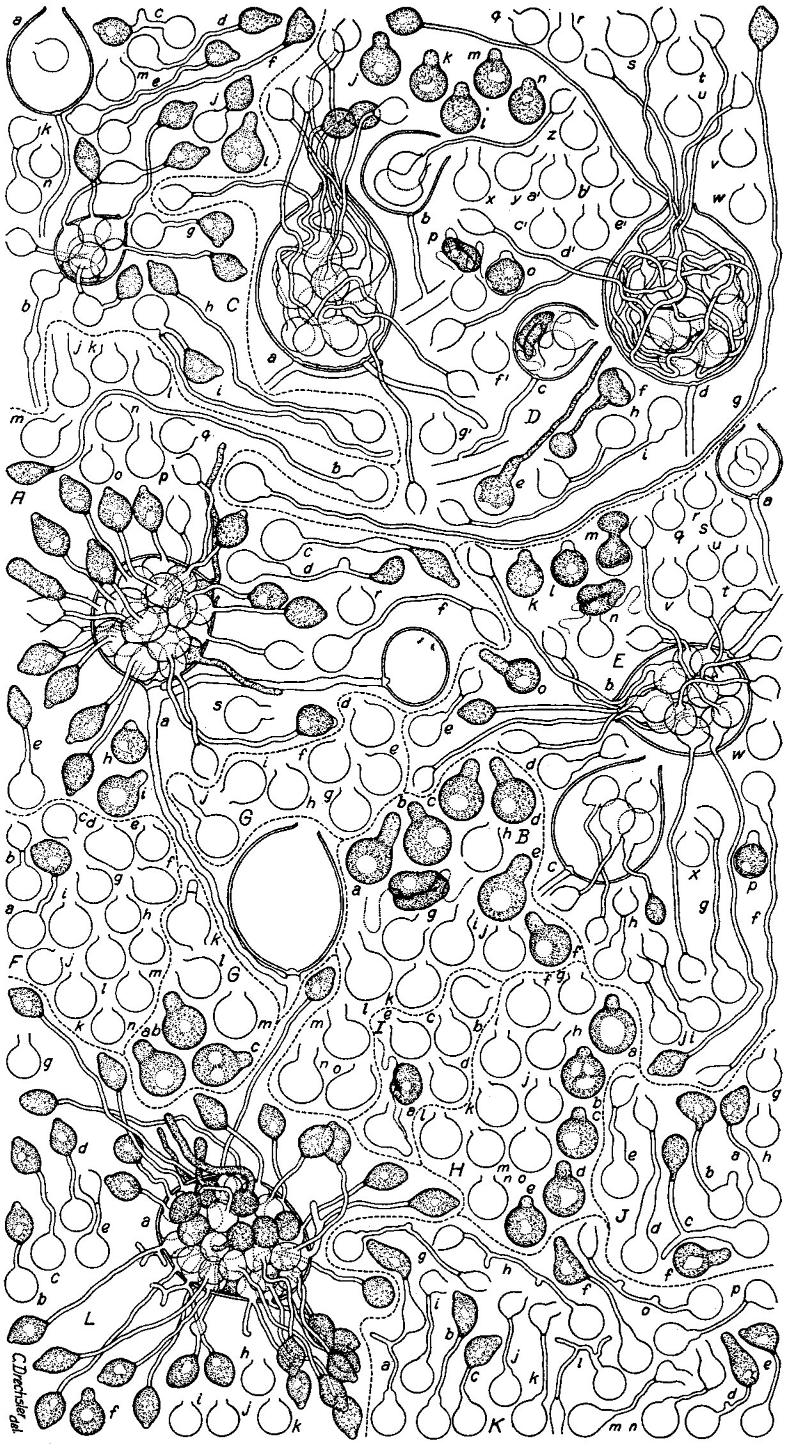

FIGURE 1.—Repetitional diplanetism in various species of Phytophthora. All parts drawn with the aid of the camera lucida. × 500
(For explanatory legend see opposite page)

observed to have drawn away slightly here and there from the cyst membrane. Writhing movements similar to those that are associated with sporogenesis in the large sporangia distinctive of the genus, now become apparent. Some time after movement becomes perceptible, usually not exceeding 10 or 15 minutes, the tip of the protrusion gives way and the protoplasmic contents pass out through the isthmus (fig. 1, E, *m*), becoming integrated at the orifice as a biciliate zoospore of the same type as that from which it was derived (fig. 1, E, *n*). The passage of the protoplast through the relatively wide neck is ordinarily accomplished in 3 or 4 seconds, while the pause between its delivery and its swimming away as a motile zoospore occupies about 1 second as a rule, rarely more than 2 seconds.

As the zoospore produced is not apparently distinguishable from those coming directly from an ordinary large sporangium, only the empty cyst membrane with its papillary orifice or open evacuation tube—a structure much more familiar to students of the Saprolegniaceae than to investigators dealing with the general run of fungi pathogenic to economic plants—is left behind as visible evidence of the developmental phenomenon described, which for convenience might be referred to as "direct" diplanetism. The evacuation tube, of course, represents the membrane surrounding the cylindrical portion of the protuberance of dehiscence, and is generally relatively short, often measuring about 1μ in length. (Fig. 1, A, *n*, *q*, *r*.) Greater lengths, however, are not rare (fig. 1, A, *k*, *l*, *m*, *o*, *p*, *s*), although a condition like that shown at A, *j*, the most extreme encountered in any of the forms studied, in which the length of the tube equals or perhaps slightly exceeds the diameter of the spherical part, must be regarded as decidedly unusual. Obviously the empty cyst envelopes remaining as evidence of the occurrence of direct diplanetism correspond to the two structures shown in Sawada's Plate 3, Figure 4, to which reference has already been made.

The other kind of repetitional diplanetic development illustrated by the Japanese author and described in his text somewhat more fully, was, as has been stated earlier, also abundantly represented in material of the papillate pink-rot fungus. The encysted primary zoospore served as origin of a filament from 1 to 1.3μ in diameter growing out either immediately from its periphery (fig. 1, A, *c*) or more frequently from the tip of a much stouter protuberance (fig. 1, A, *b*, *d*, *e*) similar to the modification involved in the direct dehiscence already described. After attaining a length ordinarily varying between 5 and 25μ, though sometimes exceeding 50μ, the delicate filament ceased to elongate and swelled terminally into an expansion which increased in size with the migration into it of granular materials. Eventually the protoplasmic contents were completely transferred to the terminal structure, which then invariably became delimited from the filament by a septum. The distinctive shape, often obpyriform, and the terminal papillate modification that soon became apparent at once characterized the structure externally as a miniature sporangium. This characterization was consistently borne out when, after perceptible retraction of the contents from the confining membrane, especially in the apical region, and writhing movements of the usual duration, the apical beak yielded, and the protoplast squeezed through the orifice and swam away from the empty sporangium (fig. 1, A, *f*) as a biciliate zoospore. The latter was not distinguishable from zoospores of primary origin, although in pronounced instances it seemed to be marked by perceptible inferiority in size. The developmental feature incident to the production of a secondary motile zoospore formed singly within a miniature sporangium borne on a delicate germ sporangiophore arising directly from an encysted zoospore or from an ineffective protuberance of dehiscence may conveniently be termed "indirect" diplanetism.

As has been suggested, indirect diplanetism is often revealed conspicuously in the case of zoospores that have failed to escape from the large primary sporangia usual in species of Phytophthora. Incomplete discharge of the latter structures, to which reference is made by numerous investigators, is observable in some measure in almost every lot of material of any member of the genus. While the reasons for frustrated dehiscence are not always obvious, in the writer's experience instances became especially numerous when the upper surface of the irrigated Lima-bean agar over which the sporangia were distributed either became too dry or was flooded to excess. It seems probable, therefore, that deficiency in moisture on the one hand and deficiency in oxygen on the other are frequently causes. In any case the imprisoned zoospores, after moving about within the sporangium for a considerable period with such vigor as their number and the limited space permitted, finally came to rest and rounded up inside.

According to observations of numerous students, further development proceeds by the production of vegetative germ tubes that perforate the sporangial wall and thus gain access to the exterior. Such vegetative development is common, especially when the structures concerned remain submerged rather deeply. Under more favorable circumstances, as has been mentioned, imprisoned zoospores of the papillate pink-rot fungus were found to give rise individually to single germ sporangiophores that perforated the sporangial wall, ordinarily without evident diminution in diameter, or passed through the papillary orifice. A terminal miniature sporangium that subsequently liberated a single secondary swimming spore was produced in each case. In instances where the imprisoned zoospores numbered a score or more, or where only a relatively small proportion had been discharged normally, the encysted bodies were packed so closely that their membranes partly adhered to one another, and the germ sporangiophores were thrust forth in bristling array. (Fig. 1, A, *a*.) The resulting arrangement provided a reproductive apparatus of distinctive appearance.

Direct diplanetism, while usually not as abundantly manifested in the case of imprisoned zoospores of the papillate pink-rot fungus as indirect, nevertheless is observed here in considerable measure. As the circumstances attending such occurrence are evidently similar in all species where it has been demonstrated, figures drawn from congeneric forms may be taken as adequately illustrative. When only one (fig. 1, C, *a*) or a few (fig. 1, E, *a*) zoospores are imprisoned, so that the cysts come to lie within the sporangial envelope under conditions not greatly different, with respect to crowding, from those encountered outside, all of the secondary swimming bodies may be directly discharged by way of an evacuation tube and gain access to the exterior of the sporangial wall by passing through the papillary opening. Direct repetitional development may occur even in cases where the papillary opening happens to be effectually blocked by one of the cysts, as for example, in the instance represented in Figure 1, D, C. The obstructing cyst readily discharged its protoplast by the happily oriented evacuation tube directly through the papillary orifice, while the secondary zoospore from the less fortunately placed cyst was prevented from egress and as a result after protracted effort rounded up inside. In most instances, perhaps, the majority of the zoospores imprisoned within a sporangium give rise to a secondary swimming stage by the indirect method of development, while one or a few, especially of those most favorably situated with reference to crowding and accessibility to the papillary opening, manifest direct dehiscence. (Fig. 1, D, *b*, and E, *c*.)

PARTICULARS OF DIPLANETISM IN VARIOUS SPECIES OF PHYTOPHTHORA

Diplanetism is not restricted to a few members of the genus Phytophthora. Its occurrence in a fungus isolated from diseased potato tubers originating in Idaho in 1922 was recorded earlier. Mainly because of the identity of the host material from which it was derived, its ready pathogenicity to potato tubers on inoculation into wounds, and the absence of any papillate modification in the ovoid sporangia, the fungus was tentatively referred to *P. erythroseptica*. The American nonpapillate pink-rot fungus was never found to produce sexual structures in any substratum on which it was tried out, though parallel cultures of the organism received from the Centraalbureau voor Schimmelcultures at Baarn, Netherlands, under the label "*P. erythroseptica*" (presumably authentic, inasmuch as it was contributed originally by G. H. Pethybridge), often yielded an abundance of normal oospores. The sporangia of the American form exceeded those of the European parasite, especially in length, measurements of 200 of these bodies developed on irrigated Lima-bean agar giving an average length of 53.5μ and an average diameter of 30.2μ as compared with 44.5μ and 29.7μ, respectively, calculated for the organism obtained from abroad. A rather strong proliferous tendency manifested in the American fungus by the sporangiophore growing through the base of one sporangium after its evacuation, to produce another farther on, often yielded a series of three or four empty sporangial envelopes borne on the same axial sporangiophore. The zoospores of the fungus are relatively large, measuring in the subspherical encysted form about 12μ in diameter. Development leading to a second swimming stage (fig. 1, B, *g*) is begun by the production of a papilla of dehiscence usually 4 to 6.5μ in diameter and 2 to 8μ in length (fig. 1, B, *a–f*), the empty envelope being provided with a rather wide evacuation tube of variable length (fig. 1, B, *h–o*). Indirect diplanetism through the development of a miniature 1-spored germ sporangium has never been observed in the fungus under consideration.

Irrigated pieces of Lima-bean agar culture of the parasite isolated by Ocfemia (*17*) from diseased eggplant (*Solanum melongena* L.) fruits in the Philippines, and treated by him as being identical with *Phytophthora melongenae*, provided an abundant display of diplanetism. (Fig. 1, C, *a–n*.) Production of miniature sporangia (fig. 1, C, *c–j*) appeared much more frequently than direct liberation of the secondary motile bodies, both when the encysted primary zoospores were free and when they were imprisoned within the large sporangia.

(Fig. 1, C, *a*, *b*.) The secondary miniature sporangia produced by two of the seven imprisoned zoospores shown in Figure 1, C, *b*, did not give rise to a swimming stage directly, but gave rise instead to tertiary sporangia. Additional irregularities were found in occasional branching of the germ sporangiophore, the short diverticulum not set off by a cross wall and the relatively long element eventually delimited by a septum, shown in Figure 1, *C*, *c* and *i*, respectively, providing representative examples.

Abundant diplanetism was evident in irrigated material of a fungus communicated by S. F. Ashby as a strain of *Phytophthora parasitica* Dastur derived from cotton bolls in Monserrat. It, presumably, was the same organism further referred to in a publication (*1*) as having been isolated by E. M. Wakefield. (Fig. 1, D, *a–e'*.) Many of the larger sporangia of the fungus, suggesting chlamydospores in their almost subspherical shape, are provided with a relatively thick envelope. Apparently because of its substantial character, the latter structure is not readily perforated by the germ sporangiophores of imprisoned zoospores, which consequently are constrained to wind about extensively before emerging either by eventually piercing the wall or more often by passing through the papillary opening. (Fig. 1, D, *a*, *d*.) Failure to reach the exterior is rare, since the germ sporangiophores, even when not confined, often attain an unusual length. (Fig. 1, D, *g*.) The broad basal protuberance from which many (fig. 1, D, *e*, *f*, *g*, *i*), though not all (fig. 1, D, *h*), of the germ sporangiophores arise give an impression as if direct development of a second swimming stage is first attempted, followed in case of failure by the more indirect development. That the acquisition of cilia within the membrane of the encysted structure (fig. 1, D, *o*) and the escape of the biciliate zoospore (fig. 1, D, *p*) are not rare is attested by the presence of numerous empty cyst envelopes with evacuation tubes of variable length, abundantly scattered about free in appropriately treated material, as well as within empty primary sporangia (fig. 1, D, *b*, *c*).

Close similarity to the cotton-boll fungus in structures associated with diplanetism as well as in cultural characteristics and morphology of the sporangium was revealed in eight cultures that had been isolated from the tomato, five having been obtained from fruits affected with buckeye rot, two from stems affected with decay near the soil line, and the other from a rootlet showing decay at the tip. Such similarity is in harmony with the assignment of the buckeye-rot parasite, originally described from the tomato as *Phytophthora terrestris* Sherb., to *P. parasitica*. About the same similarity was evident also in a fungus derived from rhubarb affected with foot rot in the District of Columbia in September, 1928, and presumably to be identified with the form described by Godfrey as *P. parasitica* var. *rhei*. Here, also, frustrated dehiscence of the zoospores was provided for through the appearance of a secondary swimming stage, by direct discharge of the motile body (fig. 1, E, *a*), by production of a germ sporangium (fig. 1, E, *b*), or by both types of development (fig. 1, E, *c*). The sporangial wall usually was not as thick as the more strongly indurated homologous structures of the cotton-boll parasite, its perforation by the germ sporangiophores entailing only relatively little coiling of the filamentous structures. Otherwise production of the germ sporangia (fig. 1, E, *d–j*) took place much as in the cotton-boll fungus, and the direct liberation of zoospores from the encysted bodies (fig. 1, E, *k–n, p*), following a sequence of stages already described, yielded evacuated cyst envelopes in large numbers (fig. 1, E, *q–x*).

A moderate display of diplanetism was observed in irrigated Lima-bean agar preparations of the species of Phytophthora, the isolation of which from a honeydew melon (*Cucumis melo* var. *inodorus* Naud.) fruit affected with decay was reported in a brief abstract (*9*). Production of germ sporangia (fig. 1, F, *a, b*) occurred less frequently than liberation of the secondary motile spores from the primary encysted ones (fig. 1, F, *c–n*). When the latter were somewhat irregular in shape, often as a result of rounding up under crowded conditions in insufficient water, their capacity to give rise to a second swimming stage was not noticeably impaired. (Fig. 1, F, *d, f*.)

A species of Phytophthora isolated by C. M. Tompkins from a mature sugar beet (*Beta vulgaris* L.) root originating at Price, Utah, in 1927, and greatly resembling the form isolated from affected Idaho potato tubers already discussed with respect to cultural characters and to morphological features pertaining to the sporangium, resembled the latter fungus also with respect to repetition of the swimming stage. The relatively large encysted primary zoospore, measuring usually about 12μ in diameter, put forth a wide papillary protuberance (fig. 1, G, *a–c*) that functioned in permitting the direct liberation of a secondary motile spore, leaving the empty cyst envelope (fig. 1, G, *d–m*) provided with a usually short evacuation tube. Germ sporangia arising from encysted zoospores were never observed. The funnel-shaped evacuation tube shown in G, *j*, and the short cylindrical diverticulum with the distal part delimited by a septum shown in G, *k*, represent departures from more regular development. In the absence of a more adequate description of the beet parasite, measurements of 50 sporangia produced on irrigated Lima-bean agar gave a range in length of 34 to 75μ, with an average of 58.3μ, and a range in width of 27 to 40μ, with an average of 33.1μ. After the evacuation of the somewhat narrow ovoid zoosporangium by means of an apical modification not protruding from the general contour as a recognizable papilla, the sporangiophore often continued growth through the empty envelope to produce another sporangium farther on. Repetition of such renewal of growth frequently resulted in three or four empty envelopes being found in series on a single axial supporting filament.

A species of Phytophthora isolated by G. B. Ramsey in October, 1928, from California tomato (*Lycopersicum esculentum* Mill.) fruits with brownish or brownish-purple lesions on the distal region showed great similarity in its manifestation of diplanetism to the congeneric fungi from Utah sugar beets and Idaho potatoes. The primary zoospore, measuring usually from 10 to 12μ in diameter after encystment, produced a protuberance of ample size (fig. 1, H, *a–e*) by means of which the motile secondary spore was directly liberated, leaving behind the empty cyst envelope with open evacuation tube (fig. 1, H, *f–o*). The fungus with its ovoid nonpapillate sporangia having an average length of 47.3μ and an average diameter of 30.9μ—these values being calculated from 50 measurements, using irrigated Lima-bean agar—was apparently specifically distinct from any hitherto reported on the tomato. The absence of protruding papillae from the sporangia readily sets it apart from the types assignable to

Phytophthora parasitica (*P. terrestris* and the stem-girdling fungus reported by Reddick (*20*)), as well as from *P. infestans* (Mont.) De Bary and *P. mexicana* Hotson and Hartge (*13*). Compared with an authentic culture of *P. cryptogea* Pethyb. and Laff. received from the Imperial Bureau of Mycology, the California fungus showed a considerably faster rate of growth, a noticeably smaller diameter of mycelial threads, and a vastly greater production of sporangia. Its close affinity and possible identity with the parasites from sugar beets and from Idaho potatoes were indicated by similarity in beheavior and appearance when the three organisms were given similar treatment in parallel cultures and preparations.

Repetitional diplanetism in moderate measure was revealed in irrigated Lima-bean agar preparations of a fungus received in artificial culture from the Centraalbureau voor Schimmelcultures as *Phytophthora fagi* Hartig. The secondary zoospore in all cases observed was formed directly within the cyst envelope. In a number of instances the protoplast, after its passage through the papilla of dehiscence, was held tethered near the orifice for several minutes by one of the cilia adhering to the inner surface of the empty cyst membrane apparently with sufficient firmness to resist the pull exerted by the other cilium. (Fig. 1, I, *a*.) Since *P. fagi* has frequently been regarded as probably identical with *P. cactorum* (Cohn and Leb.) Schroet., and indeed resembles it closely in shape and size of sporangium, it may be of interest to note that in the fungus under consideration a secondary swimming stage was not observed to arise indirectly through the production of a miniature germ sporangium—a type of development exceedingly frequent in *P. cactorum*. The encysted zoospores of the fungus from the Centraalbureau and the empty envelopes left behind after their evacuation (fig. 1, I, *b–f*) appeared generally slightly larger than the corresponding structures of the related organism, the average diameter being about 10μ in the former, as compared with 9μ in the latter. Differences in cultural features were also evident, the fungus received from the Netherlands growing less than one-fourth as rapidly in linear extension as the other when cultivated, for example, on Lima-bean agar, and its thallus revealing a densely branching habit on that medium as compared with an openly disposed one. Any trustworthy opinion regarding the relationship of *P. fagi* and *P. cactorum* should, however, be based on a study of a dozen or more cultures of the beech (*Fagus sylvatica* L.) seedling parasite, preferably from sources not too close together geographically.

Several strains of *Phytophothora citrophthora* (Sm. and Sm.) Leon., all derived presumably from California lemon (*Citrus limonia* Osbeck) fruits affected with brown rot, exhibited repetitional diplanetism in ample measure in irrigated Lima-bean agar preparations. The secondary motile stage was developed both indirectly by the production of germ sporangia (fig. 1, J, *a–e*) and directly by the acquisition of cilia within the cyst membrane. The evacuation tube associated with the latter process was usually of moderate proportions. (Fig. 1, J, *f–h*.) As in certain other species, the germ sporangiophore frequently was present as a delicate prolongation of a broader basal protuberance, the latter evidently being an unsuccessful evacuation tube. (Fig. 1, J, *a*, *c–e*.)

Diplanetism was abundantly manifested in irrigated Lima-bean agar preparations of a fungus received from the American Type Culture

Collection as *Phytophthora hibernalis* Carne, and representing, presumably, an authentic type of the organism described recently (*3*) as the cause of brown rot of citrus in Australia. The secondary swimming stage was brought about through the production in extraordinary numbers of miniature germ sporangia (fig. 1, K, *a–f*) and their subsequent evacuation (fig. 1, K, *g–p*). No instances of development of motility directly within the cyst envelope were observed, though the frequent origin of the delicate germ sporangiophores from a broader basal protuberance (fig. 1, K, *a–c, f, m, n, p*) suggested the possibility that such development may have been attempted but came to naught, owing perhaps to unsuitable conditions. The production of a tertiary germ sporangium from the secondary one shown in Figure 1, K, *a*, and a certain tendency toward branching evident in some sporangiophores (fig. 1, K, *a, d, g, h, l, o*) constitute irregularities.

Reference has been made to the frequent exhibition of diplanetism in irrigated preparations of *Phytophthora cactorum* through the production of miniature sporangia. The important part played by such development in the liberation of swimming zoospores in instances of frustrated dehiscence of the large sporangia is illustrated in Figure 1, L, *a*, showing 28 germ sporangia borne on pedicels thrust through the confining sporangial wall from a corresponding number of imprisoned cysts. Similar development ensues in quantity also with primary zoospores that have encysted unconfined in their surroundings after a normal period of active swimming. (Fig. 1, L, *b–e*.) The secondary zoosporic stage may arise, too, through the fashioning of the motile body within the cyst membrane, as shown by the presence of a papillate protuberance in many encysted structures (fig. 1, L, *f*), later persisting on the evacuated envelope as a cylindrical modification of varying length (fig. 1, L, *g–k*). While all strains of *P. cactorum* show close similarity in diplanetic development, it may not be amiss to record that the illustration shown in Figure 1, L, was made from a Lima-bean agar preparation of one of the several strains from diseased stems of *Lilium pyrenaicum* Gouan, the isolation of which was reported earlier in a brief note (*7*).

COMPARISON WITH DIPLANETIC DEVELOPMENT IN RELATED FUNGI

Although diplanetism has been observed in additional representatives of Phytophthora, no features associated with the phenomenon have come to light which are not amply illustrated in the dozen forms mentioned. If the papillate pink-rot fungus be assigned to *P. parasitica*, which, in spite of certain differences, it would seem to resemble more closely than any other of the better established species, and the fungi from Idaho potato tubers, Utah sugar-beet roots, and California tomato fruits be regarded as conspecific, the assortment of forms would be resolved presumably into about eight species. In all of the forms considered, the fashioning of the secondary zoospore took place directly within the cyst envelope or within the miniature sporangium, accompanied by noticeable writhing movements with retraction of protoplasm here and there from the confining wall, especially at the papilla or evacuation tube. The biciliate body after its passage through the open protuberance immediately swam away, except, as has been mentioned, in a few instances where a cilium adhered to the inner surface of the cyst wall. The manner of discharge was therefore essentially the same as that generally characteristic of the ordinary sporangia produced throughout the genus.

The evident parallelism between the primary and the secondary swimming stages with respect to the fashioning and discharge of the motile zoospores in species of Phytophthora suggests analogous development in the genus Pythium. In discussing the germination of the zoospores of his *Pythium dictyosporum*, a species with filamentous sporangia parasitic on *Spirogyra insignis* Hass, Raciborski (*19, p. 284*) stated that if the germ tubes do not reach the Spirogyra filament, a new, small sporangium, which contains only one zoospore, is formed at the tip of the germ tube.

The small sporangia to which reference is made correspond apparently to the miniature germ sporangia produced by most of the Phytophthora forms discussed here. No details concerning the escape of the secondary zoospores were given, nor is it evident that the manner of dehiscence was observed.

Butler (*2*), however, gave a more satisfactory illustrated account of the diplanetic development manifested by his *Pythium diacarpum*. His description is confirmed by the writer's own observations on diplanetism in a number of species of Pythium, including, for example, *P. butleri* Sub., the parasite often associated with decay of various commercial cucurbitaceous fruits and of snap beans (*Phaseolus vulgaris* L.) in transit and on the market. The somewhat large primary zoospore of this fungus, after encystment measuring mostly 10 or 11μ in diameter, produces an evacuation tube usually 1.9 to 2.2μ in diameter and 3 to 25μ in length, the protoplasmic contents in the meantime displaying a vacuole of increasing size. Finally the refringent tip of the evacuation tube yields and the contents flow out to collect at the orifice, the streaming requiring usually from 30 to 45 seconds. The discharged protoplast soon begins to exhibit writhing movements, which, as cilia make their appearance and a grooved reniform shape is assumed, become increasingly energetic though restricted within a confined space. Usually about 20 minutes after the cessation of streaming the now violently active zoospore dashes away as a free-swimming body. The restriction in amplitude of movement of the secondary spore previous to its ultimate liberation is interpreted as evidence of the presence of a confining vesicle. Direct though somewhat dubious visible evidence of such a vesicle is provided now and then in indications of an approximately circular contour coinciding with the limits of the field of movement and never more than faintly discernible even in proximity to the mouth of the evacuation tube. Since in preparations of the same fungus the vesicles associated with the smallest sporangia of vegetative origin, producing 2, 3, or 4 zoospores, are often nearly if not quite as difficult to make out, the parallelism between the production of primary and of secondary zoospores is, indeed, little less than complete. While *Pythium butleri* represents a species with lobulate sporangia, similar diplanetic development was observed in extraordinary abundance in a congeneric form with undifferentiated filamentous sporangia isolated from diseased roots of sugarcane (*Saccharum officinarum* L.).

Cornu (*4*) mentioned *Pythium proliferum* De Bary and its varieties as examples of saprolegniaceous forms, the zoospores of which may emit zoospores similar to themselves as an alternative to putting forth vegetative germ tubes. The details of the repetitional development were not discussed further. Cornu described development of zoospores in *P. proliferum* as taking place often within a vesicle formed at the extremity of the sporangium and at other times within the sporangium itself, the ready-fashioned zoospores in the latter case escaping directly when discharge occurred, since, although a vesicle appeared, it persisted only a few instants. The departure from the course of development generally prevalent in the genus Pythium is interesting when considered in connection with Butler's statement that in some cases in *P. diacarpum* much of the sporangial contents becomes divided at the mouth of an urn-shaped modification in the absence of a vesicle.

A more striking parallel with the condition set forth by Cornu is provided in the literature dealing with *Pythiomorpha gonapodioides* Petersen. According to the accounts of Petersen (*18*), Minden (*15*), and Kanouse (*14*), the fashioning and discharge of zoospores in the latter fungus would seem to be similar to that among species of Phytophthora. The vesicle described by Minden is evidently an ephemeral, poorly developed structure, bursting at an early stage in the evacuation of the sporangium to allow the completely formed zoospores to swim away without much delay—not differing greatly, therefore, from the bladderlike membrane noted by Dastur (*5*) in *Phytophthora parasitica*, and by Rosenbaum (*21*) in *P. cactorum* and *P. arecae* (Colem.) Pethyb. In Kanouse's account a second swarming was mentioned, the protoplasm passing slowly out of the cyst wall, though cilia were not observed, and no activity of the liberated naked cell other than the emission of a germ tube was represented in the accompanying illustration.

Because of the dearth of detail concerning the development of locomotive organs, it is not possible to refer the diplanetic development in question either to the type prevailing in the genus Phytophthora or in Pythium. In any case the mere presence of such development can hardly be advanced as a strong argument in favor of the distinctiveness of any form or group from either of these genera.

Although a fungus received in artificial culture from the Centraalbureau voor Schimmelcultures as having been contributed originally by Kanouse under the name *Pythiomorpha gonapodioides* has yielded no structures relating to diplanetism in the few irrigated preparations examined by the writer, a number of undoubtedly closely related species have revealed such structures in moderate abundance. Of these species, one isolated from diseased rootlets of ragweed (*Ambrosia trifida* L.) and referred to in a brief abstract (*8*) manifestly gives rise to the secondary swimming stage, not only by the fashioning of the motile body within the cyst wall and its direct delivery through an evacuation tube, but also indirectly by the production of miniature sporangia. The former mode of development is shown by the presence of empty spherical cyst envelopes usually measuring 11 to 16μ in diameter, each provided with an open evacuation tube 3 to 6μ in diameter and 5 to 15μ in length. When miniature sporangia are produced they are borne on germ sporangiophores mostly 2μ in diameter and ranging from 40 to 285μ in length. Frequently an empty cyst envelope with an open evacuation tube is provided with one or two narrower filamentous outgrowths suggesting abortive germ sporangiophores. Although unfortunately the fashioning and liberation of the secondary motile bodies were not observed, the occurrence of two types of development paralleling those observed in various species of Phytophthora is not without interest.

However little tenable ground the writings on Pythiomorpha offer for the maintenance of that genus as a separate taxonomic entity, the fungus discussed by Kanouse as *P. gonapodioides* does not seem referable to Phytophthora. In the hands of the present writer the presumably identical organism never produced zoospores within the sporangium to discharge them full fledged through an orifice provided by a uniformly sessile papilla. On the contrary, development took place as in typical representatives of Pythium, by the discharge of undifferentiated contents into a vesicle in which division into zoospores was accomplished in 15 or 20 minutes. The papilla of dehiscence was sometimes sessile and at other times surmounted an evacuation tube 5μ or more in length. In versatility of asexual reproduction the fungus thus recalls Cornu's early description of similar behavior in *Pythium proliferum*. A larger measure of distinctiveness is embodied apparently in the somewhat unusual shape of the antheridium and its manner of application to the oogonium. In Kanouse's drawing of the sexual apparatus, the male organ is represented as an elongated clasping structure evidently applied lengthwise to the oogonium. A similar shape and manner of application is shown in Dissmann's figures (*6, figs. 19 and 33*) of the antheridium of his *Pythium proliferum*. As far as the writer is aware, no antheridum of similar type and of similar disposition with reference to the female structure has been recorded for any fungus definitely assigned to Phytophthora.

SUMMARY

In many species of Phytophthora, the zoospores after encystment give rise to secondary swimming spores similar to themselves except for a slight inferiority in size. The repetitional development follows either one of two courses. A papilla or tube of dehiscence, usually relatively broad and of variable length, may be produced, which on yielding at the apex permits the immediate escape of the completely formed secondary zoospore fashioned in the meantime from the protoplast with accompanying writhing movements visible in retractions here and there from the cyst wall. Or a slender but often rather long germ sporangiophore, terminating in a miniature papillate sporangium delimited by a basal septum, may be put forth, either from the periphery of the cyst or from an unsuccessful evacuation tube. The contents of the miniature sporangium are eventually discharged from the papillary orifice as a completely developed motile zoospore.

Only development of the more direct type was observed in *Phytophthora fagi* and in three unnamed congeneric fungi with non-papillate, proliferous sporangia, one originating from a diseased Idaho potato tuber, one from a mature sugar-beet root in Utah, and the other from a decaying tomato fruit in California. In *P. hibernalis* only the second indirect type of repetitional development was noted. Both types were found in all strains referable to *P. parasitica* in a somewhat broad sense, including *P. parasitica* var. *rhei* and possibly a papillate form isolated from decaying potato tubers. *P. cactorum, P. citrophthora, P. melongenae* and a fungus

previously reported as having been isolated from a diseased Honeydew melon represent other congeneric parasites exhibiting direct as well as indirect diplanetism.

When dehiscence of the ordinary sporangia is partly or wholly frustrated, the imprisoned zoospores after encystment often give rise to a free-swimming stage through repetitional development. The germ sporangiophores perforating the sporangial wall or passing out through the papillary orifice to bear the miniature sporangia externally often lend a distinctive bristling aspect to the resulting reproductive arrangement.

Diplanetism was observed in various species of Pythium with filaments and with lobulate sporangia, as, for example, *P. butleri*, the encysted structure discharging its undifferentiated contents through a slender evacuation tube into a small vesicle where they are fashioned into a single motile spore. In Pythium as in Phytophthora, therefore, repetitional development follows the course generally characteristic of zoospore formation in the genus. In certain allied forms distinguished by elongated clasping antheridia closely applied lengthwise to the oogonium, and by mostly terminal and often proliferous sporangia, the occurrence of empty cyst envelopes and of germ sporangiophores terminating in miniature sporangia provide proof of the prevalence of diplanetic development.

LITERATURE CITED

(1) ASHBY, S. F.
1922. OOSPORES IN CULTURES OF PHYTOPHTHORA FABERI. Roy. Bot. Gard. Kew, Bul. Misc. Inform. 1922: [257]-262.

(2) BUTLER, E. J.
1907. AN ACCOUNT OF THE GENUS PYTHIUM AND SOME CHYTRIDIACEAE. India Dept. Agr. Mem., Bot. Ser. 1: 1-161, illus.

(3) CARNE, W. M.
1927. A BROWN ROT OF CITRUS IN AUSTRALIA (PHYTOPHTHORA HIBERNALIS N. SP.). Jour. Roy. Soc. West. Aust. 12: 13-41, illus.

(4) CORNU, M.
1872. MONOGRAPHIE DES SAPROLÉGNIÉES, ÉTUDE PHYSIOLOGIQUE ET SYSTÉMATIQUE. Ann. Sci. Nat., Bot. (5) 15: [5]-198, illus.

(5) DASTUR, J. F.
1913. ON PHYTOPHTHORA PARASITICA NOV. SPEC., A NEW DISEASE OF THE CASTOR OIL PLANT. India Dept. Agr. Mem., Bot. Ser. 5: [177]-231, illus.

(6) DISSMANN, E.
1927. VERGLEICHENDE STUDIEN ZUR BIOLOGIE UND SYSTEMATIK ZWEIER PYTHIUMARTEN. Arch. Protistenk. 60: 142-192, illus.

(7) DRECHSLER, C.
1926. FOOT-ROT OF LILIUM CANDIDUM AND LILIUM PYRENAICUM CAUSED BY PHYTOPHTHORA CACTORUM. Phytopathology 16: 51-53.

(8) ———
1927. A PECULIAR TYPE OF PYTHIUM. (Abstract) Phytopathology 17: 55.

(9) ———
1929. A FRUIT ROT OF HONEY DEW MELONS DUE TO A SPECIES OF PHYTOPHTHORA. (Abstract) Phytopathology 19: 85.

(10) ———
1929. A DIPLANETIC SPECIES OF PHYTOPHTHORA CAUSING PINK ROT OF POTATO TUBERS. (Abstract) Phytopathology 19: 92.

(11) GÄUMANN, E. A.
1928. COMPARATIVE MORPHOLOGY OF FUNGI. Transl. and rev. by C. W. Dodge. 701 p., illus. New York and London.

(12) GODFREY, G. H.
1923. A PHYTOPHTHORA FOOTROT OF RHUBARB. Jour. Agr. Research 23: 1-26, illus.

(13) HOTSON, J. W., and HARTGE, L.
1923. A DISEASE OF TOMATO CAUSED BY PHYTOPHTHORA MEXICANA, SP. NOV. Phytopathology 13: [520]–531, illus.

(14) KANOUSE, B. B.
1925. PHYSIOLOGY AND MORPHOLOGY OF PYTHIOMORPHA GONAPODIOIDES. Bot. Gaz. 79: [196]–206, illus.

(15) MINDEN, M. VON.
1916. BEITRÄGE ZUR BIOLOGIE UND SYSTEMATIK EINHEIMISCHER SUBMERSER PHYCOMYCETEN. *In* Falck, R. Mykologische Untersuchungen und Berichte 1: 146–255, illus.

(16) MURPHY, P. A.
1920–22. THE BIONOMICS OF THE CONIDIA OF PHYTOPHTHORA INFESTANS (MONT.) DE BARY. Roy. Dublin Soc. Sci. Proc. (n. s.) 16: 442–466.

(17) OCFEMIA, G. O.
1925. THE PHYTOPHTHORA DISEASE OF EGGPLANT IN THE PHILIPPINE ISLANDS. Philippine Agr. 14: 317–328, illus.

(18) PETERSEN, H. E.
1910. AN ACCOUNT OF DANISH FRESHWATER-PHYCOMYCETES, WITH BIOLOGICAL AND SYSTEMATICAL REMARKS. Ann. Mycol. 8: [494]–560, illus.

(19) RACIBORSKI, M.
1892. PYTHIUM DICTYOSPORUM, NIEZNANY PASCRZYT SKRETNICY. (PYTHIUM DICTYOSPORUM, EIN NEUER PARASIT DER SPIROGYRA.) Bul. Internatl. Acad. Sci. Cracovie 1891: 283–287.

(20) REDDICK, D.
1920. A FOURTH PHYTOPHTHORA DISEASE OF TOMATO. Phytopathology 10: [528]–534.

(21) ROSENBAUM, J.
1917. STUDIES OF THE GENUS PHYTOPHTHORA. Jour. Agr. Research 8: 233–276, illus.

(22) SAWADA, K.
1927. DESCRIPTIVE CATALOGUE OF THE FORMOSAN FUNGI. PART III. Japan Dept. Agr., Gov. Research Inst. Formosa, Rpt. 27, 62 and 7 p., illus. [In Japanese, English translation on file in Bureau of Plant Industry.]