

BOTANY.—*Antagonism and parasitism among some oomycetes associated with root rot.*¹ CHARLES DRECHSLER, Bureau of Plant Industry.

Among the many species of *Pythium* that may be isolated, especially in wet seasons, from softened, discolored, or decaying portions of the roots, stems, or basal leaves of herbaceous cultivated plants, some prove ineffective, under ordinary circumstances, for bringing about the root rot, stem rot, or crown rot with which they were found associated. When these relatively innocuous fungi are not attended by more strongly pathogenic forms, their occurrence in diseased plants is usually held to derive from some limited capacity for parasitism whereby they are enabled to attack phanerogamic hosts that have become much weakened, or in part moribund, as the result of unfavorable external conditions. On the other hand, when, as very often comes to pass, an innocuous species is found accompanied by a demonstrably pathogenic form—or, perhaps, even by two or three such forms—there is reason to presume usually that it entered the plant as a secondary invader, and then propagated itself saprophytically by drawing nourishment from tissues already killed by an earlier invader. However, a more complicated system of biotic relationships would seem to obtain in many cases of root rot where any one of the three echinulate species I described under the names *P. oligandrum*, *P. acanthicum*, and *P. periplocum* (9) is present as secondary invader, since on transparent agar media these species freely display destructive parasitism on many root-rotting forms congeneric with them.

When, for example, *Pythium ultimum* Trow and *P. oligandrum* both grow out from a piece of decaying pea (*Pisum sativum* L.) root into a Petri plate of maize-meal-agar culture medium—as, indeed, has often happened in subjecting diseased pea roots from Maryland, Delaware, New Jersey, and New York, to procedure suitable for isolation of oomycetes—the former is attacked by the latter in spectacular manner. The same parasitic development can be brought to

light conveniently by planting the two fungi some distance apart on a maize-meal-agar plate. Along the line where the two growing mycelia meet, the advance of *P. ultimum* is abruptly halted and its hyphae become enveloped in innumerable places by intricately ramifying branches of *P. oligandrum* (Fig. 1). Soon these branches penetrate into the enveloped hyphae and extend prolongations longitudinally within them to assimilate the degenerating protoplasmic contents. Here and there the internal filaments send out ramifications that attack other hyphae of *P. ultimum*. Conidia and young oogonia of *P. ultimum* are also attacked, though apparently with less readiness than young vegetative hyphae. Often the destruction is so rapid and thoroughgoing that in only scattered portions of the *ultimum* mycelium is sexual reproduction permitted to reach a stage where the thick oospore wall affords reliable protection.

Pythium debaryanum Hesse and *P. irregulare* Buism. (3), which occur as casual agents of root rot and damping-off almost as frequently as *P. ultimum*, have likewise been observed undergoing violent attack by *P. oligandrum*, not only in dual cultures prepared purposely for such observation, but also in isolation plate cultures where manifestly the destruction, taking place in the transparent medium merely continues the destruction spontaneously begun in such natural substrata as tomato (*Lycopersicon esculentum* Mill.) roots, pansy (*Viola tricolor* L.) roots, sugar-beet (*Beta vulgaris* L.) seedlings, and peach (*Prunus persica* Sieb. & Zucc.) seedlings. *P. mammillatum* Meurs (13), often found in discolored rootlets of field tomatoes in Maryland and Virginia, is attacked by *P. oligandrum* in dual cultures no less severely than the three familiar damping-off species with which it belongs taxonomically as a member of an intimately interrelated series. In the same series must be included also an apparently undescribed species found occurring abundantly during May, 1939, on pansies seriously affected with root rot in the District

¹ Received July 16, 1942.

of Columbia; the fertilization of its large oogonia, which often measured 28 to 33 μ in diameter, by an antheridium consisting frequently of an adjacent hyphal segment, relating it more particularly to *P. ultimum*. This species, too, is very destructively parasitized by *P. oligandrum* in dual cultures, penetration of its hyphae being accomplished after they have been closely enveloped by ramifications of the spiny form (Fig. 2, A). Again, when *P. splendens* Braun, a species less intimately related to those commonly causing damping-off, is grown in dual culture with *P. oligandrum*, it suffers elaborate envelopment of its hyphae, which then are permeated longitudinally by narrow filaments of the spiny form and expropriated of their degenerating contents (Fig. 2, B). *P. salpingophorum* Drechsl. (9) similarly is attacked with spectacular effect, while *P. butleri* Subr., *P. graminicolum* Subr., and *P. arrhenomanes* Drechsl., as also the three interrelated prolific species I described under the names *P. helicoides*, *P. oedochilum*, and *P. palingenes* (9) suffer less severely in encounters with *P. oligandrum*.

As *Pythium acanthicum* and *P. periplocum* have only occasionally been obtained from softened or discolored roots, isolation plate cultures have afforded little opportunity for observing the behavior of these echinulate species toward the congeneric forms known to cause root troubles. However, when the two species are grown in dual cultures with various congeners pathogenic to phanerogamic plants, parasitic activity similar to that of *P. oligandrum* comes to light: *P. ultimum*, *P. debaryanum*, *P. irregulare*, *P. mammillatum*, *P. splendens*, *P. salpingophorum*, and the *ultimum*-like form found prevalent in pansy roots, being attacked in a most destructive manner, whereas, in general, *P. butleri*, *P. graminicolum*, *P. arrhenomanes*, *P. helicoides*, *P. oedochilum*, and *P. palingenes* incur less ruinous injury. That aquatic congeners are subject to similar adverse action is evident from the readiness with which, in dual cultures, delicate ramifications of *P. acanthicum* (Fig. 2, C, a; D, a) as well as of *P. periplocum* (Fig. 2, E, a) invest the hyphae of

P. marsipium (Fig. 2, C, b; D, b; E, b), a species I recently described (12) from decaying leaves of the white waterlily, *Nymphaea tuberosa* Paine.

The three species of *Pythium* thus given to attacking other members of the genus are distinguished, even when growing alone, by a delicate mycelial habit achieved through unusually abundant development of slender branches that arise laterally from axial filaments of moderate width. No such copious production of slender branches suitable for envelopment of alien hyphae occurs in the vegetative growth of *P. megalacanthum* de Bary *sensu* Buisman (3), a form associated with flax (*Linum usitatissimum* L.) scorch in The Netherlands, or of the two closely related American species, similarly provided with large oogonia, which I described (9) as *P. mastophorum* and *P. polymastum*. The separateness of the two spiny series, indicated by marked differences both in make-up of sexual apparatus and in mycelial texture, is further evidenced by ready parasitism of the three delicate species on all three of the coarse species. The parasitism of *P. acanthicum* on *P. mastophorum*, initiated by extensive envelopment of *mastophorum* filaments (Fig. 2, F), has in some instances led to more severe injury than usually eventuates in any of the other eight combinations of host and parasite that are possible between the two series. Since the oogonia of the coarse forms are often invaded even during relatively late stages in their growth, their capacious spiny envelopes often come to surround from three to six alien echinulate oogonia, each usually containing a mature oospore of normal structure.

Pythium anandrum, which I originally described from decaying underground buds of rhubarb (*Rheum rhaponticum* L.) in Maryland (9), and which more recently was also found associated with crown rot of rhubarb in California (14), has its oogonia ornamented with tapering protuberances that in general shape resemble the oogonial spines of *P. oligandrum*, but its mycelium lacks any extensive development of fine ramifications, being rather similar in coarseness and manner of branching to the myce-

lium of *P. debaryanum* or of *P. irregulare*. As might be expected, in view of such similarity, the species is attacked by *P. oligan-*

drum, though for the most part not with much severity. However, *P. acanthicum* and *P. periplocum* ordinarily show more pro-



Fig. 1.—*Pythium oligandrum* attacking *P. ultimum* in dual culture on maize meal agar; approximately $\times 400$. Photomicrograph taken by Marguerite S. Wilcox.

nounced aggressiveness in their attack on *P. anandrum*, many of the hyphae enveloped by them being subsequently invaded and expropriated of contents. *Pythiogeton autossytum*, a pythiaceous fungus that I isolated and described (10) from softened leaf-sheaths of the common cat-tail, *Typha latifolia* L., also is attacked only feebly by *P. oligandrum*, yet suffers appreciable injury when grown in dual culture with either *P. acanthicum* or *P. periplocum*. Wherever a filament of *P. periplocum* (Fig. 2, G, a; H, a) encounters one of *P. autossytum* (Fig. 2, G, b; H, b) it envelops the latter with branches whose somewhat lobate, rounded tips evidently become affixed by means of an adhesive secretion, after the manner of appressoria.

While *Pythium oligandrum* thus is inimical in varying degree to many pythiaceous fungi, it is itself affected unfavorably by a number of oomycetes found associated with root rot. When grown in dual culture with the congeneric *P. complens* Fischer [= *P. gracile* Schenk *sensu de Bary* (1, 2), *P. gracile* (de Bary) *sensu Ward* (16), *P. torulosum* Coker & Patterson (4)], which occurs widely in the decaying roots of numerous phanerogamic plants, including, for example, pansies, peas, sugar beets, beans (*Phaseolus vulgaris* L.), spinach (*Spinacia oleracea* Mill.), and sugar cane (*Saccharum officinarum* L.), a few of its hyphae are attacked and invaded for short distances (Fig. 3, A). It suffers much more severe injury from *Plectospora myriandra* Drechsl., a saprolegniaceous fungus originally isolated from tomato rootlets (7). A growing mycelium of *P. oligandrum* is abruptly halted in its advance wherever it encounters a growing mycelium of *P. myriandra*. Everywhere in the zone of encounter the hyphae of *P. oligandrum* are elaborately enveloped by ramifications put forth from axial filaments of *P. myriandra* (Fig. 3, B). An increased opaqueness of the enveloped hyphae soon announces the onset of progressive disorganization within them. Some of the affected hyphal parts are penetrated and invaded lengthwise, with consequent disappearance of their degenerating protoplasmic materials; though, on the whole, utilization of

such materials would seem hardly commensurate with the expenditure entailed in enwrapping the *Pythium* filaments.

Injury from encounter with *Plectospora myriandra* is incurred likewise by *Pythium periplocum* and *Pythium acanthicum*, and in varying measure also by many other congeneric species less intimately related to *Pythium oligandrum*, including those most frequently found responsible for damping-off, root rot, stem rot, and fruit rot. Moreover, destructive behavior toward pythiaceous fungi is not limited to *P. myriandra*, but is displayed with equally telling effect by three root-rotting strains of *Aphanomyces* obtained from infected roots,—two of the strains in question having been isolated from discolored roots of flax and spinach, respectively (11), several years before the third was isolated from softened cortex of a pansy root dug up in Arlington, Va., on May 4, 1939. Judging from the origin and positional relationships of their antheridial branches, the flax and spinach strains appear certainly referable to *A. cladogamus*, a species I based originally on cultures obtained from tomato rootlets (8); and the pansy strain, despite some aberrance, would seem better referable to this species than to any other hitherto described. At all events the three strains, when grown in dual culture with numerous species of *Pythium*, show decided parallelism in their strongly antagonistic behavior. Thus, when the spinach strain encounters the pythiaceous form from decaying waterlily leaves, which, owing to its production of very large globose intramatrical reproductive bodies, appears identical with the form that Dissmann (6) assimilated to *P. undulatum* Pet., it puts forth elaborate ramifications to envelop and destroy the alien hyphae (Fig. 3, C) much after the same manner in which the pansy strain puts forth elaborate ramifications to envelop and destroy hyphae of *P. dissotocum* Drechsl. (Fig. 3, D), a species causing important damage to sugar cane under unfavorable conditions (15).

Less aggressive antagonism is usually displayed by *Aphanomyces cochlioides* Drechsl., a water mold often causing damping-off and root rot of sugar beets in wet fields (8).

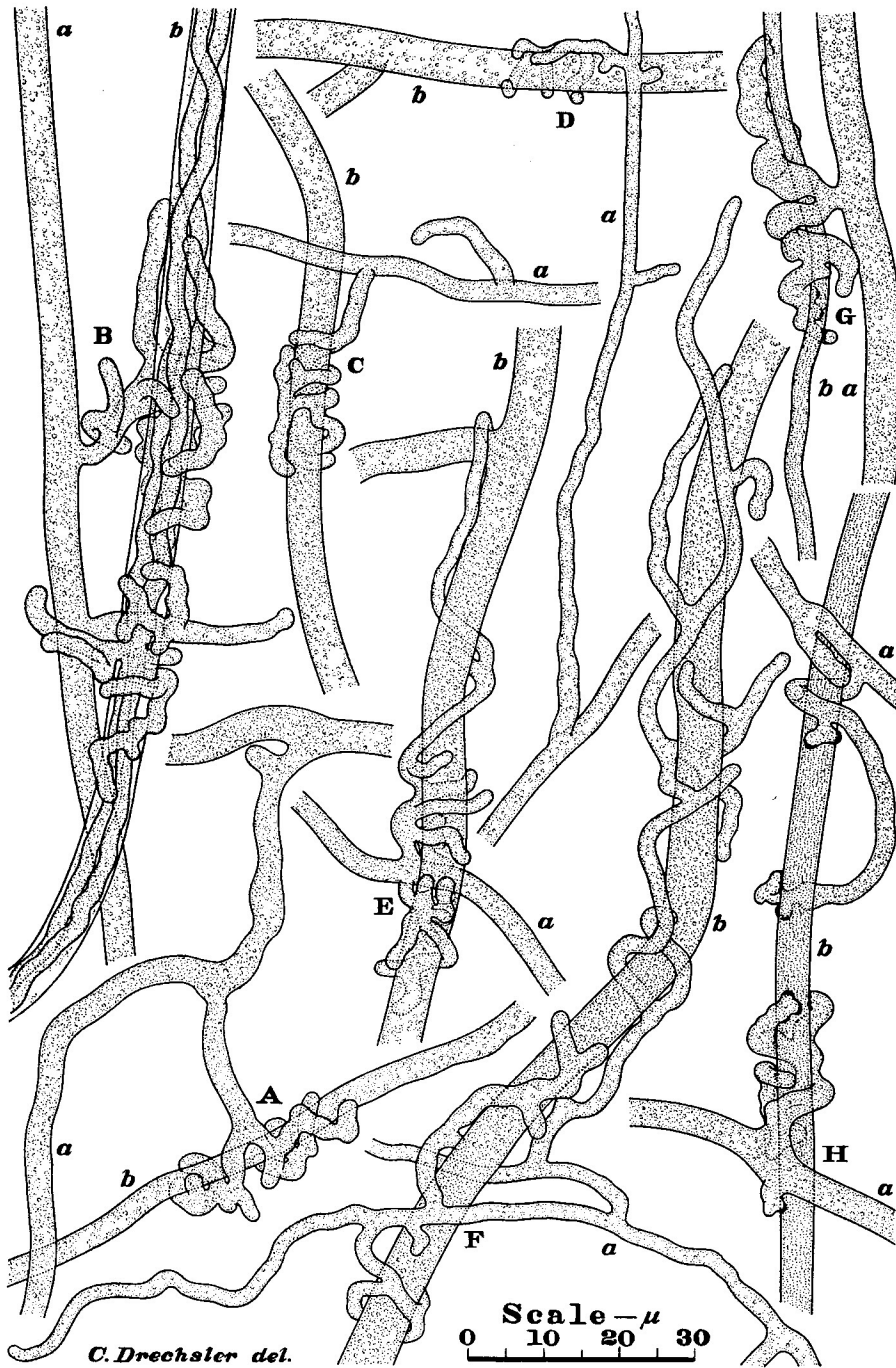


Fig. 2.—Attack of three echinulate species of *Pythium* on several pythiaceous fungi in dual cultures; all parts drawn to a uniform magnification with the aid of a camera lucida; $\times 1000$. A, *Pythium oligandrum*, a, enveloping a filament of an *ultimum*-like congeneric species from pansy roots, b. B, *P. oligandrum*, a, enveloping and invading a filament of *Pythium splendens*, b. C, D, *Pythium acanthicum*, a, enveloping *Pythium marsipium*, b. E, *Pythium periplocum*, a, enveloping a filament of *P. marsipium*, b. F, *Pythium acanthicum*, a, enveloping a filament of *Pythium mastophorum*, b. G, H, *Pythium periplocum*, a, enveloping *Pythiogeton autossytum*, b.

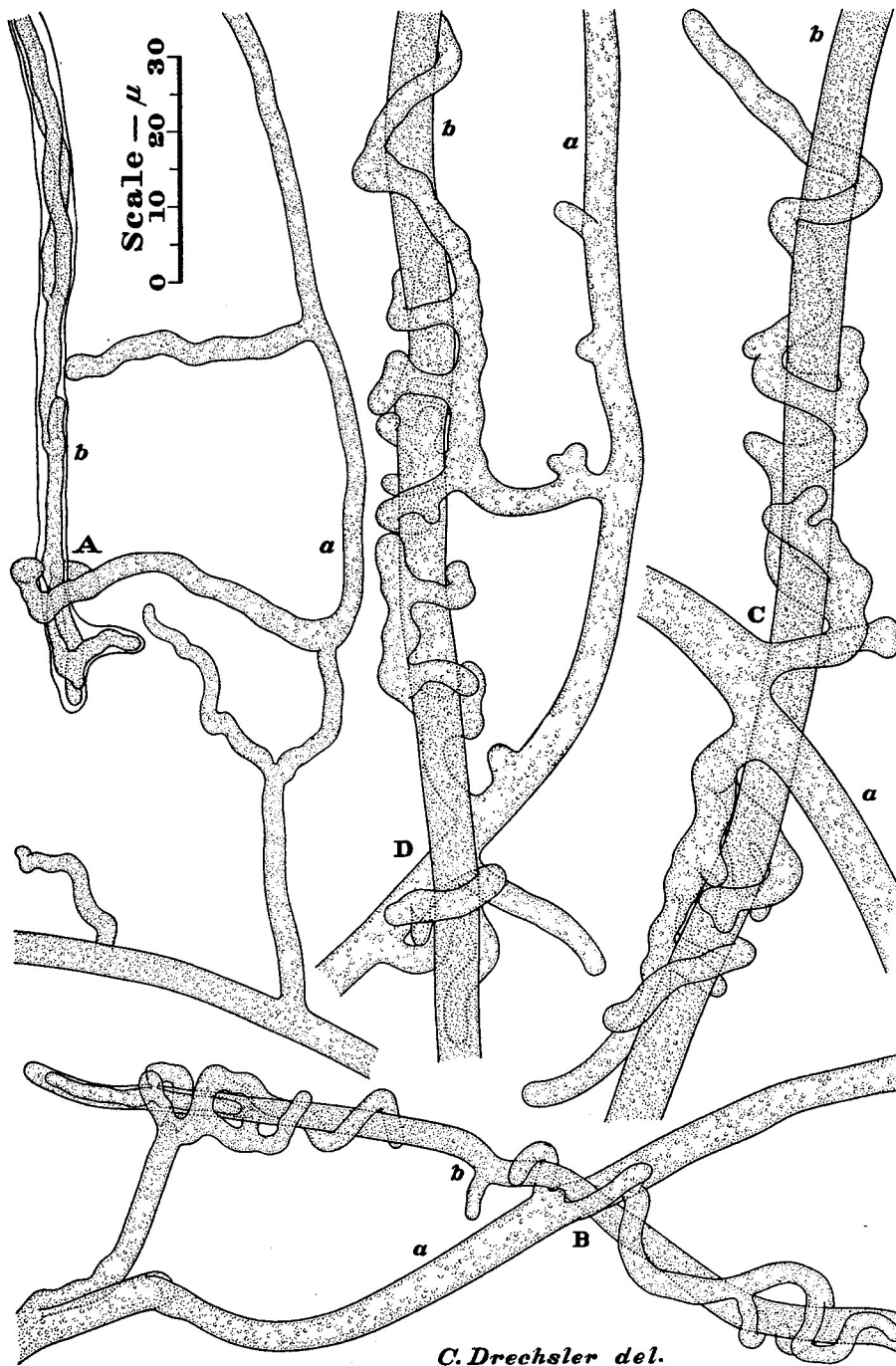


Fig. 3.—Harmful relationships between various oomycetes; all parts drawn to a uniform magnification with the aid of a camera lucida; $\times 1000$. A, *Pythium complens*, a, attacking and invading *Pythium oligandrum*, b. B, *Plectospora myriandra*, a, attacking *P. oligandrum*, b. C, Spinach strain of *Aphanomyces cladogamus*, a, attacking a filament, b, of *Pythium undulatum sensu Dissmann*. D, Pansy strain of *A. cladogamus*, a, attacking *Pythium dissotocum*, b.

When this saprolegniaceous parasite encounters *Pythium debaryanum* or *P. mammillatum* in dual culture, it abruptly halts the advance of the alien mycelium and causes protoplasmic degeneration in terminal portions of alien hyphae along the zone of encounter; the injury evidently coming about, for the most part, from mere proximity, since often no special involvement of alien hyphae can be detected. Growing in opposition to *P. myriotylum* Drechsl., which frequently is responsible for field decay of watermelon fruits in Florida, *A. cochlioides* has sometimes been observed extending rangy hyphae to wind loosely about alien filaments here and there, and occasionally has, in addition, been seen putting forth short branches to promote protoplasmic degeneration in these filaments, or even to invade them internally on a small scale.

From their pathogenic behavior under experimental conditions there is reason to believe that both *Aphanomyces cochlioides* and *A. cladogamus* operate mainly as direct parasites on the phanerogamic plants in which they occur habitually. The direct parasitism of *Pythium periplocum* and *P. acanthicum* in causing blossom-end rot of watermelon (*Citrullus vulgaris* Schrad.) fruits could not readily be questioned even if experimental evidence of their infective capabilities were lacking, for when occurring in specimens of this rot the two echinulate species are usually unaccompanied by other likely agents of decay. In the ecological assemblage of oomycetes here under consideration, a capacity for bringing about disease in higher plants can manifestly coexist with a capacity for attacking and injuring other members of the assemblage. However, as the several saprolegniaceous fungi hitherto found attacking higher plants all have smooth oogonia, it appears probable that the spiny *A. exoparasiticus*, described by Couch (5) as being parasitic on various phycomycetes, may not be pathogenic to any species of phanerogams.

The parasitic and antagonistic relationships between oomycetes associated with root rot come into strongest expression where both of the fungi concerned are in a

high state of vegetative vigor. In dual cultures that have been started by planting the two species some distance apart hyphal envelopment and hyphal degeneration, if present, is always most pronounced in the narrow zone where the growing mycelia encounter each other, that is, in the zone where, without exception, young vigorous hyphae of the aggressor come upon equally young hyphae of the opposing form. Dual cultures incubated at a temperature of 28°C., which is fairly close to the optimum temperature for mycelial growth in many species of *Pythium*, usually show more extensive hyphal envelopment than similar cultures incubated at 18°C. Hyphal envelopment is usually more abundant when rather soft maize meal agar, containing 15 grams of agar-agar to the liter, is employed than when the medium used contains 25 grams of agar-agar to the liter. As *Pythium oligandrum*, *P. acanthicum*, and *P. periplocum* initiate and conclude sexual reproduction earlier than most congeneric forms, and as they usually exhaust their mycelia almost completely in producing sexual apparatus, areas in dual cultures first occupied by these species may later be invaded by other species of *Pythium* without much hindrance except, perhaps, from accumulated staling products.

LITERATURE CITED

- (1) BARY, A. DE. *Untersuchungen über die Peronosporaeen und Saprolegnieen und die Grundlagen eines natürlichen Systems der Pilze.* Abh. Senckenb. Naturf. Ges. 12: 225-370. 1881.
- (2) ———. *Zur Kenntnis der Peronosporaeen.* Bot. Zeit. 39: 521-530, 537-544, 553-563, 569-578, 585-595, 601-609, 617-625. 1881.
- (3) BUISMAN, C. J. *Root rots caused by phycomycetes.* Meded. Phytopath. Lab. "Willie Commelin Scholten" Baarn 11: 1-51. 1927.
- (4) COKER, W. C., and P. M. PATTERSON. *A new species of Pythium.* Journ. Elisha Mitchell Sci. Soc. 42: 247-250. 1927.
- (5) COUCH, J. N. *Notes on the genus Aphanomyces with a description of a new semiparasitic species.* Journ. Elisha Mitchell Sci. Soc. 41: 213-227. 1926.
- (6) DISSMANN, E. *Vergleichende Studien zur Biologie und Systematik zweier Pythium-*

- Arten. Arch. Protistenk. **60**: 142-192. 1927.
- (7) DRECHSLER, C. *Two water molds causing tomato rootlet injury.* Journ. Agr. Res. **34**: 287-296. 1927.
- (8) ———. *The beet water mold and several related root parasites.* Journ. Agr. Res. **38**: 309-361. 1929.
- (9) ———. *Some new species of Pythium.* Journ. Washington Acad. Sci. **20**: 398-418. 1930.
- (10) ———. *A species of Pythiogeton isolated from decaying leaf-sheaths of the common cat-tail.* Journ. Washington Acad. Sci. **22**: 421-449. 1932.
- (11) ———. *Occurrence of a species of Aphanomyces on roots of spinach and flax.* Phytopathology **25**: 14-15. 1935.
- (12) ———. *Three species of Pythium with proliferous sporangia.* Phytopathology **31**: 478-507. 1941.
- (13) MEURS, A. *Wortelrot veroorzaakt door schimmels uit de geslachten Pythium Pringsheim en Aphanomyces de Bary,* 95 pp. Baarn, 1928.
- (14) MIDDLETON, J. T. *Crown rot of rhubarb caused by Pythium spp.* Phytopathology **31**: 863. 1941.
- (15) STEVENSON, J. A., and R. D. RANDS. *An annotated list of the fungi and bacteria associated with sugarcane and its products.* Hawaiian Planters' Rec. **42**: 247-313. 1938.
- (16) WARD, H. M. *Observations on the genus Pythium (Pringsh.).* Quart. Journ. Micr. Soc. (n. ser.) **23**: 485-515. 1883.