# *Pythium* Species Pathogenic to Onion Seedlings Grown on Organic Soils in New York

W. L. Bruckart and J. W. Lorbeer

Graduate research assistant and professor, respectively, Department of Plant Pathology, New York State College of Agriculture and Life Sciences, Cornell University, Ithaca 14853.
Accepted for publication 9 June 1981.

## ABSTRACT

Bruckart, W. L., and Lorbeer, J. W. 1982. *Pythium* species pathogenic to onion seedlings grown on organic soils in New York. Phytopathology 72:469-475.

*Pythium irregulare* and *P. coloratum* isolated from onion seeds and seedlings grown in natural organic (muck) soils in New York were strongly pathogenic to onion seedlings. Five of six other species (*P. ultimum*, *P. sylvaticum*, *P. torulosum*, *P. paroecandrum*, and *P. rostratum*) from the same sources were weakly pathogenic and isolated infrequently. *P. irregulare* caused significantly more damping-off than did *P. coloratum* and was isolated from organic soils cropped to both onion and lettuce. *P. coloratum* was associated most frequently with one soil having a history of lettuce culture.

*Additional key words*: *Allium cepa*, *Lactuca sativa*.

Although Pythium damping-off of onion (*Allium cepa* L.) seedlings grown on New York organic (muck) soils has been reported (8), detailed studies on the identification and relative pathogenesis of the species of *Pythium* involved have not been conducted previously. Detailed investigations may not have occurred because broad spectrum fungicides that controlled onion smut generally also controlled Pythium damping-off (8,9) and obviated the need for such studies.

On a worldwide basis, 10 species of *Pythium* have been isolated from onion and two of these (*P. irregulare* Buisman and *P. coloratum* Vaartaja) were found to be pathogenic (6,13–15). The objective of the present study was to identify species of *Pythium* occurring naturally in New York organic soils cropped to onions and lettuce and capable of causing damping-off of onion.

## MATERIALS AND METHODS

**Source of isolates.** Isolates of *Pythium* spp. were obtained from organic soils collected at commercial onion and lettuce (*Lactuca sativa* L.) fields in several areas of New York. These fields were located at the Bostic (Bos) and LaScalla (Las) farms at Pine Island and Middletown in Orange County, and the Chillemi Brothers (Ira) farm at Ira in Cayuga County. All of these fields have had a long continuous history of onion culture, but had not been studied previously for damping-off potential. Another organic soil was collected from a field planted with lettuce for several years at the Sorbello (Sor) farm at Fulton in Oswego County.

**Isolation procedures.** Isolates of *Pythium* spp. were obtained from all of the natural organic soils sampled (Table 1). This was accomplished by collecting five soil samples from the top 15.2 cm (6 in.) for each field, bulking and thoroughly mixing the samples, and placing subsamples of each bulked sample in 10-cm (4 in.)-diameter plastic pots. Twenty-five pesticide-free onion seeds (cultivar Buccaneer) were planted in 40 pots containing soil from all fields sampled (10 pots for each field). Twenty-five pesticide-free lettuce seeds (cultivar Ithaca) were planted in 10 additional pots containing Sor soil samples. Half of all of the pots were incubated at 14 C and half at 25 C. All pots were watered twice weekly. All of the onion and lettuce seeds or seedlings in one pot were sampled at 1, 2, 4, 7, and 14 days after planting, and isolations of *Pythium* attempted from all of the seeds or seedlings recoverable, regardless of their condition. The isolation data for both temperatures of incubation were combined and listed under each source of soil (Table 1).

The seeds and seedlings were washed for 1 hr in running tap water, surface sterilized in 0.5% sodium hypochlorite, and plated onto cornmeal agar (CMA) amended with pimaricin, vancomycin, and pentachloronitrobenzine to form a medium (PVP) selective for Phycomycetes (11). The recipe for the PVP medium was: Difco CMA, 17 g; pimaricin (Gist-Brocades N.V., Delft, The Netherlands), 5 mg; vancomycin HCl (Eli Lilly and Co., Indianapolis, IN 46206), 300 mg; terraclor 75 WP (Olin Corp., Little Rock, AR 77203), 130 mg; and tap water, 1 L. The plates were incubated at 21 C for 48 hr. All fungi growing on the PVP medium

TABLE 1. Recovery of different *Pythium* spp. from seed and seedlings of onion and lettuce in natural organic field soil collected from four different farms in New York

| *Pythium* species | Number of *Pythium* isolates from soil | | | | | |
|---|---|---|---|---|---|---|
| | | | | Sor[b] | | |
| | Bos[a] | Las[a] | Ira[a] | Lettuce[c] | Onion[c] | Total |
| *P. irregulare* | 72[d] | 38 | 26 | 11 | 4 | 151 |
| *sylvaticum* | 35[d] | 5 | 3 | 2 | 3 | 48 |
| *coloratum* | 3 | 0 | 0 | 14 | 9 | 26 |
| *ultimum* | 2 | 1 | 0 | 8 | 7 | 18 |
| *acanthicum* | 16[e] | ... | ... | ... | ... | 16 |
| *rostratum* | 0 | 0 | 1 | 1 | 2 | 4 |
| *paroecandrum* | 0 | 0 | 0 | 0 | 1 | 1 |
| *torulosum* | 0 | 0 | 1 | 1 | 0 | 2 |
| Totals | 128 | 44 | 31 | 37 | 26 | 266 |

[a] Bos = Bostic Farm, Pine Island, Orange County, NY; Ira = Chillemi Brothers Farm, Ira, Cayuga County, NY; Las = LaScalla Farm, Middletown, Orange County, NY. These fields had a long continuous history of onion culture.

[b] Sor = Sorbello Farm, Fulton, Oswego County, NY. This soil was collected from a field with a recent history of lettuce culture.

[c] Lettuce = isolates recovered from lettuce seed and seedlings used as bait. Onion = isolates recovered from onion seed and seedlings used as bait.

[d] In addition to the 72 and 35 isolates, nine isolates of *P. irregulare* and 19 isolates of *P. sylvaticum* were obtained by a soil dilution procedure utilizing the PVP medium. This involved mixing 1 g of soil (dry weight equivalent) in 10 ml of 0.2% water agar and mixing for 2 min on a Vortex mixer. In one series, 0.1 ml of this suspension was added to each of three plates of PVP (15 ml) and incubated at 21 C. In a second series, 0.5 ml of suspension was added to each of three plates of PVP. After either 24 or 48 hr, all colony types of *Pythium* which appeared on the six plates were isolated and stored on cornmeal agar slants under mineral oil at 2 C. This isolation procedure was repeated three times. No other species of *Pythium* were isolated using this method.

[e] All isolates of *P. acanthicum* were obtained by using acidified potato dextrose agar (APDA) for plating seeds and seedlings. APDA was not used for isolating *Pythium* spp. from the other soils.

0031-949X/82/05046907/$03.00/0

were transferred individually to water agar or PVP plates 24 or 48 hr after plating. A procedure developed by Sleeth (16) was modified to remove bacterial contaminants. This involved inverting the medium in the plates to which the fungi had been transferred and allowing the fungi to grow upward through the medium and emerge on the surface free of contaminants. Hyphal tips were transferred to CMA slants and, once established, they were covered with sterile mineral oil and stored at 2 C until identified and tested for pathogenicity.

**Identification of isolates.** For identification, all isolates of *Pythium* were grown for 2 wk at 21 C in the dark on either hemp seed agar (HSA; consisting of 5 g hemp seed per liter of water) or oatmeal agar (OMA; consisting of 0.2 g rolled oats per liter of water). Isolates were examined by placing one drop of acid fuchsin in lactophenol directly onto the fungus growing on the agar, placing a coverslip over the stained area, and examining the structures with a compound microscope. The isolates were identified by using the monograph of Middleton (7) and the papers by Waterhouse (20,21). Two of the species of *Pythium* required additional tests for their identification. *P. sylvaticum* Campbell and Hendrix (a heterothallic species) was identified by making crosses and studying the structures that resulted from compatible reactions. The procedures utilized by Emerson (2) for production of sporangia and zoospores of *Pythium* spp. were followed to obtain sporangia and zoospores of *P. coloratum*.

**Pathogenicity tests.** Inoculum for pathogenicity tests was

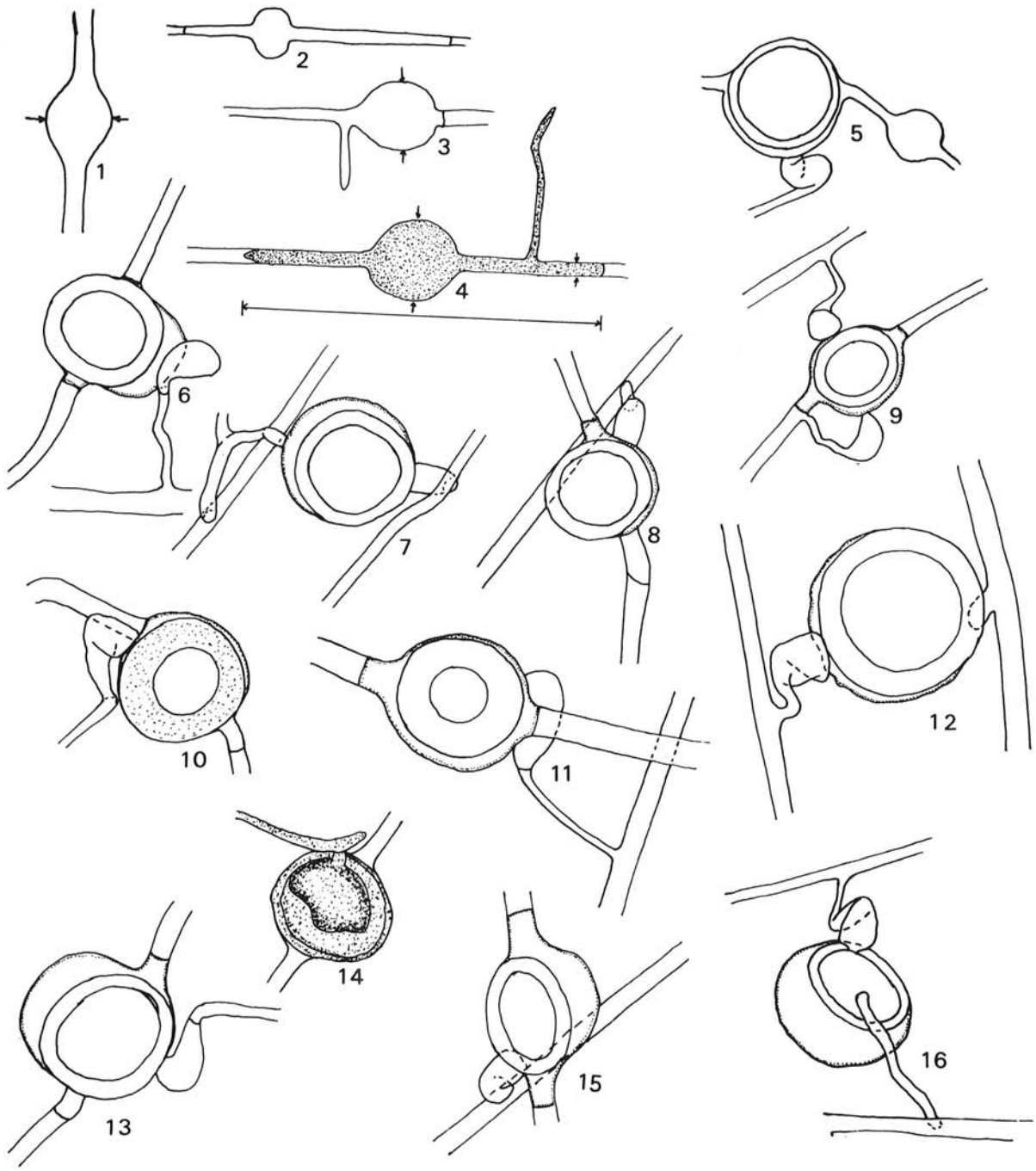


**Fig. 1.** Reproductive structures of *Pythium coloratum* after growing for 2 wk at 21 C in the dark on 0.2% oatmeal agar. Stippling indicates portions of structures stained with acid fuchsin. **1–3,** Filamentous structures with intercalary swellings, each is void of cytoplasm. Diameters of 1 and 3 at arrows are 16.6 and 21.3 $\mu$m, respectively. **4,** Filamentous structure with an intercalary swelling and a branch. Dimensions are 22.2 $\mu$m for the diameter of the swollen portion at arrows, 5.5 $\mu$m diameter for the hypha at arrows, 165 $\mu$m length (as indicated by bar). **5–16,** Antheridia and oogonia. Measurements of oospores (diameter, wall thickness [$\mu$m]) are: **5)** 16.6, 1.2; **6)** 21.3, 2.4; **7)** 19.0, 2.4; **8)** 16.6, 3.0; **9)** 19.0, 3.5; **10)** 19.0, 4.7; **11)** 19.0, 5.4; **12)** 23.7, 3.5; **13)** 19.0, 3.6; **14)** 21.3, no wall measurement (oospore was not fully formed in this oogonium); **15)** 19.0 × 16.6, 3.6; **16)** 14.2 × 19.0, 2.4.

prepared by growing each isolate of *Pythium* spp. studied on autoclaved oat seeds for 8 days in the dark at room temperature. Four infested oat seeds were evenly distributed (one centered in each quadrant) at the same level on sterile greenhouse mix (equal parts of sand, topsoil, and peat moss) in each of three 10-cm (4-in.) plastic pots per isolate. Twenty-five onion seeds were evenly distributed at this same level in each pot. The seed then was covered with additional greenhouse mix to a depth of 12.5 mm (0.5 in.). Three pots without infested oat seed were prepared as the control for each pathogenicity test. All pots were placed in a growth

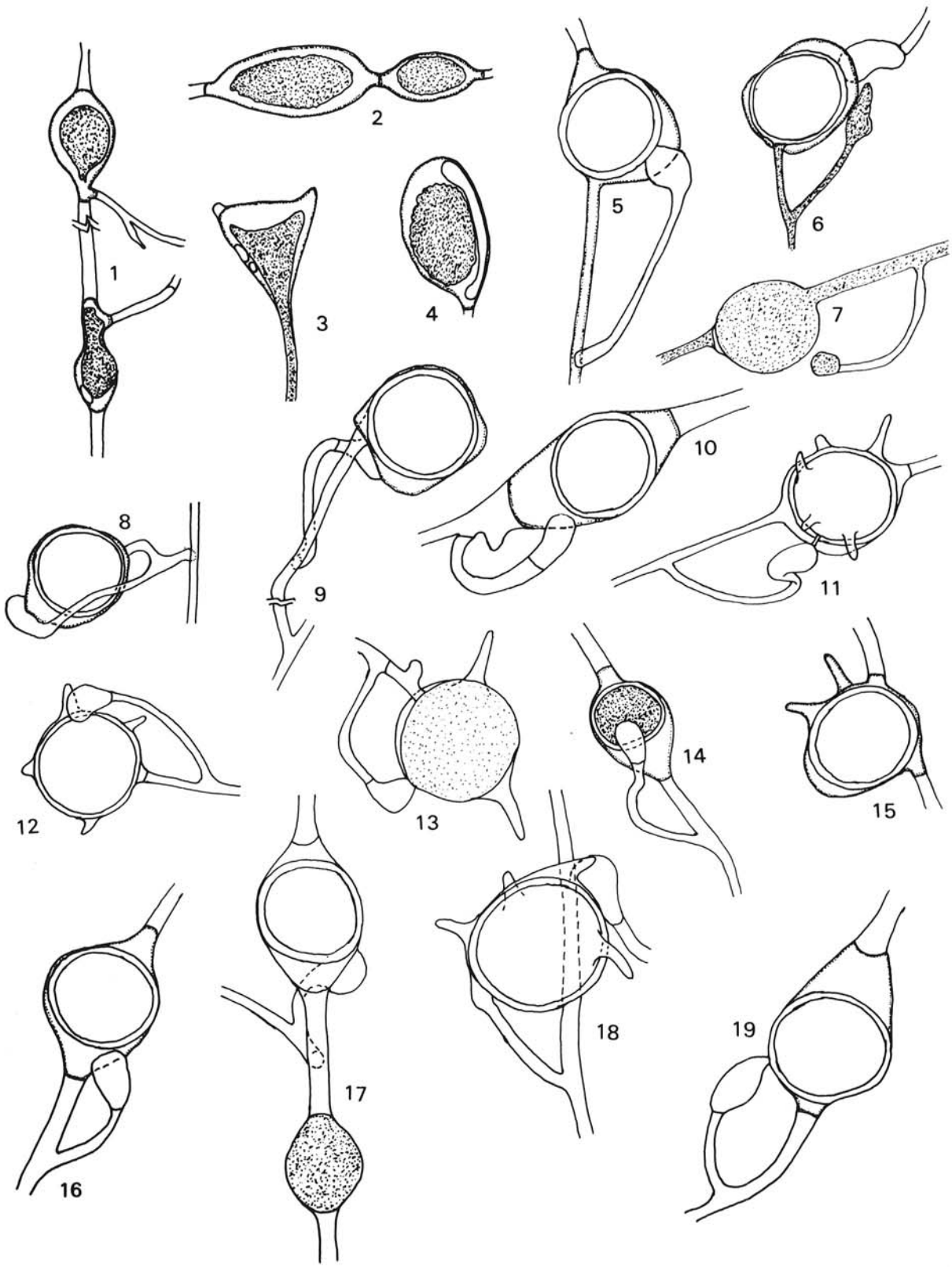


**Fig. 2.** Reproductive structures of *Pythium irregulare* grown at 21 C for 2 wk in the dark on 0.2% oatmeal agar. Stippling indicates portions of structures stained with acid fuchsin. **1–4**, Sporangia; **5–19**, antheridia and oogonia (**5, 6, 8–12, 14–19,** oospores fully formed within oogonia. **17**, Oogonium and sporangium on same hyphal element). Measurements of sporangia (width × length [$\mu$m]) and oospores (diameter [$\mu$m]) are: **1)** 18.4 × 28.8 (upper) and 11.5 × 26.3 (lower); **2)** 18.5 × 40.3 (left sporangium); **3)** 22.2 (at widest width) × 18.5; **4)** 22.2 × 33.3; **5)** 14.4; **6)** 11.9; **7)** no measurement; **8)** 14.2; **9)** 14.2; **10)** 16.6; **11)** no measurement; **12)** 16.6; **13)** 16.6; **14)** 14.2; **15)** 14.2; **16)** 16.6; **17)** 16.6; **18)** 16.6; **19)** 16.6.

chamber with a 24 C (day) and 18 C (night) regime, 21,500 lux (2,000 ft-c) lighting, ambient air RH, and watered twice weekly (and more frequently, if necessary). Stand counts were made 2 wk after planting. Stand data were compared according to confidence intervals based upon Student's *t*-distribution.

## RESULTS

**Species isolated.** Eight *Pythium* species were isolated from onion and lettuce seeds and seedlings used as bait in natural New York organic soils cropped to onion and lettuce (Table 1). Only *P.*

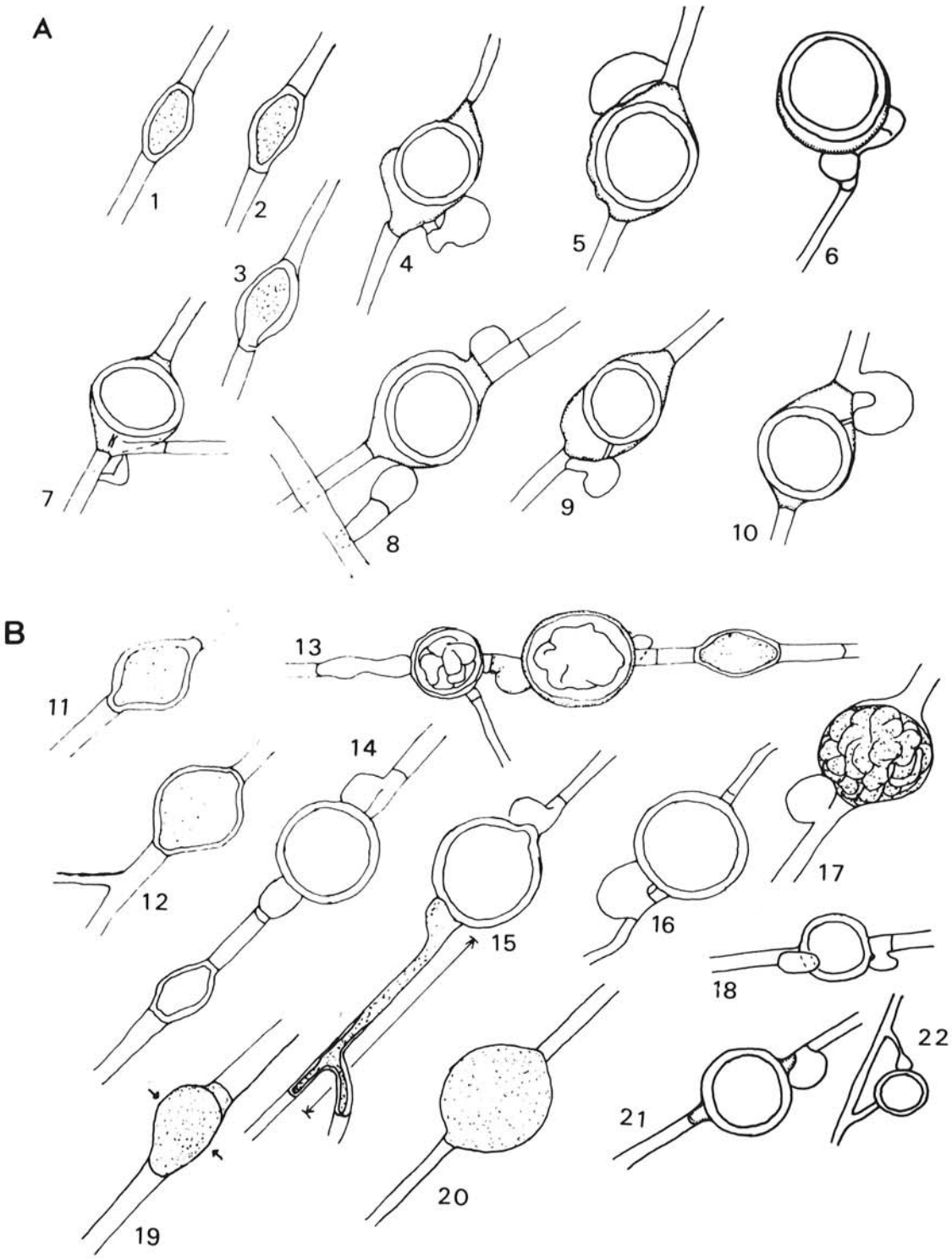


**Fig. 3.** Reproductive structures of *Pythium paroecandrum* and *P. rostratum* after growing for 2 wk at 21 C in the dark on 0.2% oatmeal agar. Stippling indicates portions of structures stained with acid fuchsin. **A**, Structures of *P. paroecandrum*: **1–3**, intercalary sporangia. **4–10**, Antheridia, oogonia, and oospores. Measurements of sporangia (width × length [$\mu$m]) and oospores (diameter, wall thickness [$\mu$m]) are: **1)** 9.5 × 19.0; **2)** 8.3 × 13.1; **3)** 13.1 × 21.3; **4)** no measurement; **5)** 19.0, 2.0; **6)** 16.6, no wall measurement; **7)** 13.0, 1.0; **8)** 17.8, 2.0; **9)** 19.0, 2.0; **10)** 16.6, 1.5. **B**, Structures of *P. rostratum*: **11–12**, intercalary sporangia. **13**, Swellings represent (left to right) two oogonia and one sporangium. **14**, One intercalary sporangium (void of contents) and one oospore. **15, 16, 18, 21, 22**, Intercalary oogonia with mature oospores. **17**, Intercalary oogonium with immature oospore. **19–20**, Intercalary sporangia or unfertilized oogonia. Measurements of sporangia (width × length or diameter [$\mu$m]) and oospores (unless otherwise indicated) (diameter [$\mu$m]) are: **11)** 11.9 × 17.8; **12)** 16.6; **13)** (left to right) 11.9, 19.0, 9.5 × 11.9; **14)** (oospore) 19.0; **15)** 22.2, length of stippled structure 55.5; **16)** 15.4; **17)** (oogonium) 14.2; **18)** no measurement; **19)** (at arrows) 7.1; **20)** 14.2; **21)** 16.6; **22)** 22.2.

*irregulare* and *P. sylvaticum* were associated with all of the soils. The remaining species occurred less frequently and were not isolated from all of the soils.

All species of *Pythium* isolated grew on the PVP medium. However, the isolates of *P. acanthicum* Drechsler were isolated by using acidified potato-dextrose agar, a medium used on only one occasion in obtaining isolates from the Bos soil. *P. acanthicum* was not isolated on the PVP medium even though the fungus grew on PVP.

**Description of species as isolated.** *Pythium acanthicum*—SPORANGIA usually intercalary, filamentous with occasional subspherical swellings; OOGONIA intercalary, spherical, wall covered with many (more than 20) spiny protuberances (sensu Waterhouse) less than 5 μm in length; ANTHERIDIA usually monoclinous, stalked, one per oogonium; OOSPORES plerotic, spherical, smooth-walled.

*Pythium coloratum*—SPORANGIA filamentous with occasional swollen sections; OOGONIA intercalary, spherical (21.8 μm diameter), smooth-walled; ANTHERIDIA usually diclinous, two to three per oogonium; OOSPORES aplerotic, spherical, 18.7 μm diameter, wall thick (3.0 μm) and smooth (Fig. 1).

*Pythium irregulare*—SPORANGIA usually intercalary, variable in shape (spherical [22.1 μm diameter], limoniform [17.1 × 26.9 μm] or obovate); OOGONIA usually intercalary, limoniform, or spherical, usually smooth-walled but projections (sensu Waterhouse) seen occasionally (variable with isolate); ANTHERIDIA monoclinous or diclinous, stalked when monoclinous, one (two or three) per oogonium; OOSPORES aplerotic, spherical, 16.0 μm diameter, wall average thickness (1.0 μm) and smooth (Fig. 2).

*Pythium paroecandrum* Drechsler.—SPORANGIA intercalary, variable in shape (usually limoniform, but spherical, obovate); OOGONIA usually intercalary, spherical (limoniform), smooth-walled; ANTHERIDIA monoclinous or diclinous (when monoclinous arising adjacent to oogonium), one to two per oogonium; OOSPORES aplerotic, spherical, 17.4 μm diameter, smooth-walled (Fig. 3A).

*Pythium rostratum* Butler.—SPORANGIA terminal or intercalary, spherical or ellipsoidal; OOGONIA usually intercalary, spherical or limoniform, smooth-walled; ANTHERIDIA monoclinous, very short or reduced to a single cell swelling laterally from the hyphal strand immediately adjacent to the oogonium, one or two per oogonium; OOSPORES aplerotic (nearly filling the oogonium), spherical, 18.4 μm diameter, smooth-walled (Fig. 3B).

*Pythium sylvaticum*—SPORANGIA terminal or intercalary, variable in shape (spherical, limoniform, asymmetrical); OOGONIA terminal, spherical; ANTHERIDIA diclinous, one to three per oogonium; OOSPORES aplerotic, spherical, 20 μm diameter, wall moderately thick (2.0 μm) and smooth (Fig. 4).

*Pythium torulosum* Coker and Patterson—SPORANGIA lobate; OOGONIA terminal or intercalary, spherical, smooth-walled; ANTHERIDIA usually monoclinous, arising adjacent to the oogonium, one or two per oogonium; OOSPORES plerotic, spherical, 15.5 μm diameter, wall thick (2.2 μm) and smooth (Fig. 5A).

*Pythium ultimum* Trow.—SPORANGIA few, terminal, spherical, or occasionally small, limoniform or barrel-shaped, intercalary; OOGONIA terminal, smooth-walled; ANTHERIDIA mostly monoclinous, one per oogonium, arising adjacent to oogonium; OOSPORES aplerotic, spherical, 17.7 μm diameter,

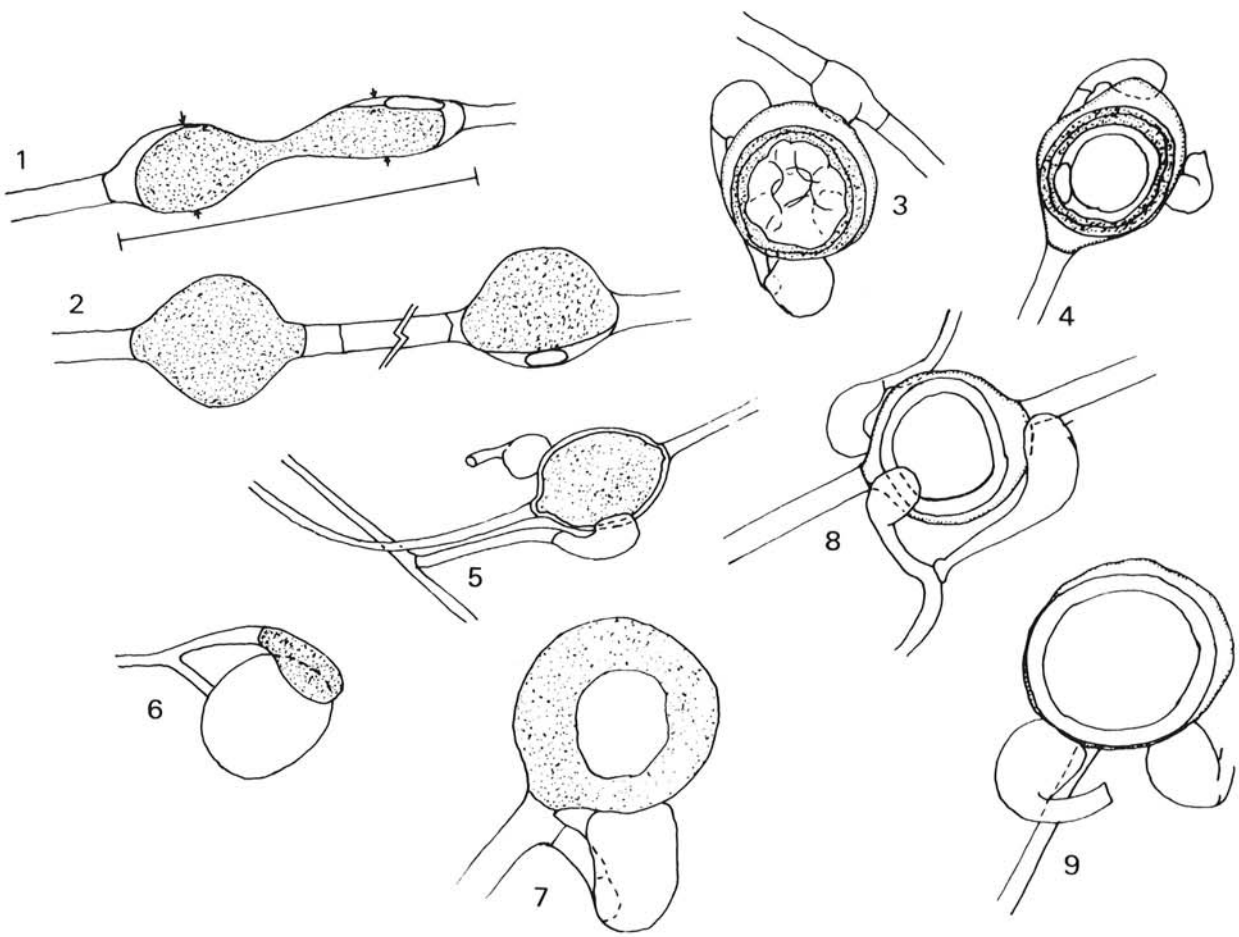


**Fig. 4.** Reproductive structures of *Pythium sylvaticum* after growing compatible mating types for 2 wk at 21 C on 5% hemp seed agar in the dark. Stippling indicates portions of structures stained with acid fuchsin. **1–2**, Intercalary sporangia. **3–9**, Antheridia, oogonia, and oospores. Measurements of sporangia (width × length [μm]) and oospores (unless otherwise indicated) (diameter [μm]) are: **1)** 18.5 (left set of arrows), 16.7 (right set of arrows) × 66; **2)** 25.9 × 33.3 (left sporangium), 22.2 × 29.6 (right sporangium); **3)** 21.3; **4)** 20.2; **5)** (oogonium) 18.5; **6)** (oogonium) 19.2; **7)** 18.5; **8)** no measurement; **9)** 16.6.

wall thick (2.3 μm) and smooth (Fig. 5B).

**Isolation frequency**. *P. irregulare* was isolated most frequently in soils from the three fields with a history of onion culture (Table 1). Of the 203 isolates from these soils, 136 were *P. irregulare*, and 43 were *P. sylvaticum*. *P. coloratum*, *P. ultimum*, *P. rostratum*, and *P. torulosum* were isolated infrequently.

The same *Pythium* species were recovered from the Sor soil, but the occurrence frequencies of the species differed in this soil compared to the other three soils (Table 1). Of the 63 isolates from this soil, *P. coloratum* occurred most frequently (23 isolates) followed by *P. irregulare* and *P. ultimum* (15 isolates each). Less common were *P. sylvaticum* (five isolates), *P. rostratum*, and *P. paroecandrum*. *P. coloratum*, and *P. ultimum* occurred in greater proportion in this soil than in the soils from the onion fields, but the proportion of *P. irregulare* was less.

**Pathogenicity**. Although most isolates of *P. irregulare* caused significantly more damping-off than any of the other species isolated, a number were weakly pathogenic. The average stand associated with the virulent isolates of *P. irregulare* (146 of the 160 isolates) was 14.2% of the 25 onion seeds planted, which is a reduction of 61% from the controls (Table 2). *P. coloratum* caused significantly more damping-off than all other species except *P. irregulare*. The average stand reduction associated with these isolates in pathogenicity tests was 49%. Six of the seven other species also caused a significant reduction in onion seedling stands compared with the controls, but the reduction was less than 25%. Only stands associated with *P. acanthicum* did not differ significantly from the controls.

## DISCUSSION

*P. irregulare* and *P. coloratum* appear to be the causal agents of Pythium damping-off of onion in New York based upon their pathogenesis to onion seedlings and their frequency of isolation from seedlings grown in soils collected from onion and lettuce fields. Robertson (13) reported that *P. coloratum* was more

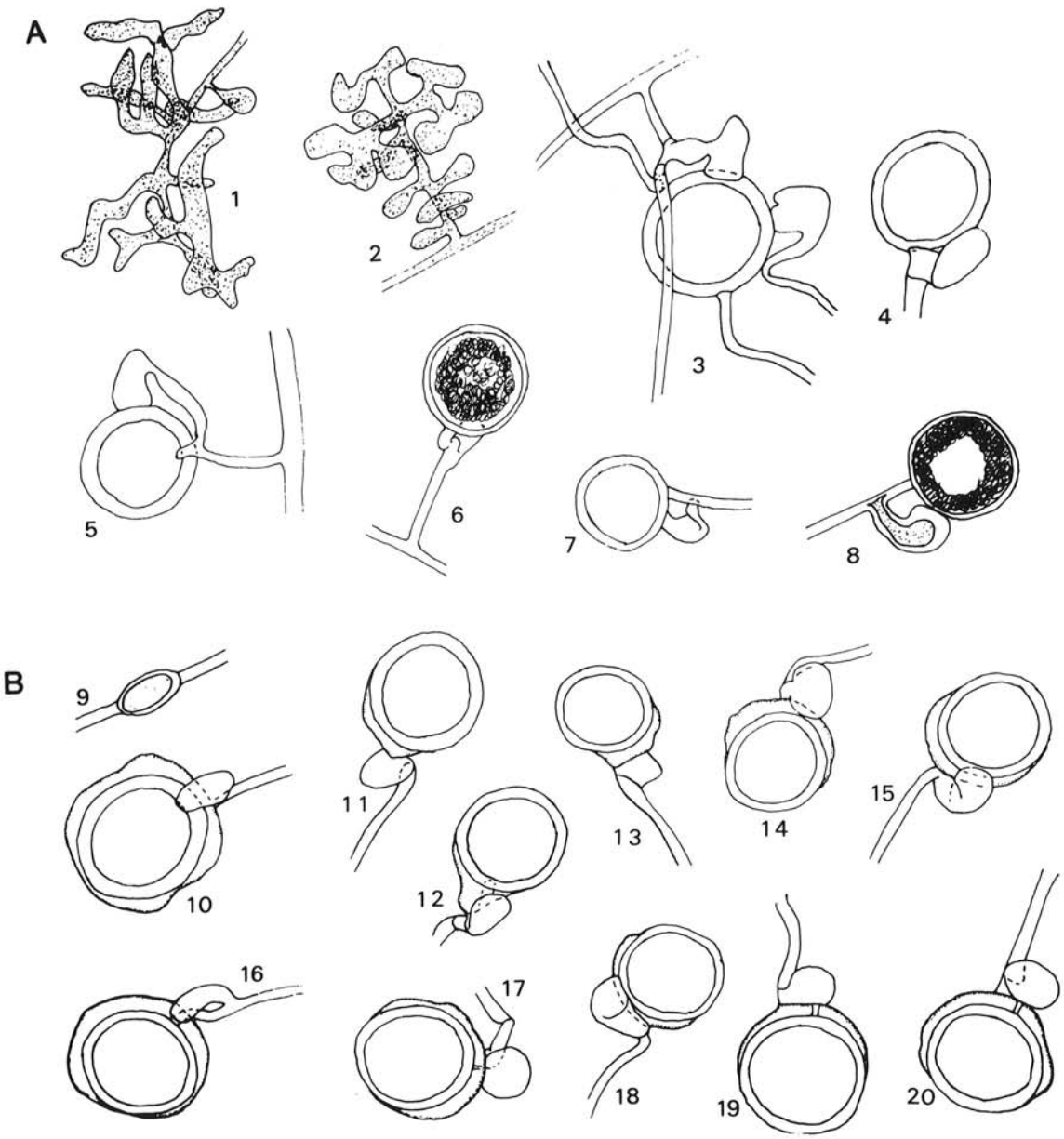


**Fig. 5**. Reproductive structures of *Pythium torulosum* and *P. ultimum* after growing for 2 wk at 21 C in the dark on 0.2% oatmeal agar. Stippling indicates portions of structures stained with acid fuchsin. **A**, *P. torulosum*: **1–2**, lobate sporangia. **3–8**, Antheridia and oogonia (**3–5, 7**, oospores are fully developed). Measurements of oospores (unless otherwise indicated) (diameter, wall thickness [μm]) are: **1–2**) no measurements; **3**) 17.8, 2.4; **4**) 16.6, no measurement; **5**) 15.4, 2.0; **6**) 16.6, wall not formed; **7**) 17.8, 2.0; **8**) 16.6, wall not formed. **B**, *P. ultimum*: **9**, intercalary conidium (sensu Trow). **10–20**, antheridia, oogonia, and oospores. Measurements of conidium (width × length [μm]) and oospores (diameter, wall thickness [μm]) are: **9**) 9.5 × 11.9; **10**) 15.4, 2.4; **11**) 16.6, 2.4; **12**) 16.6, 2.4; **13**) 16.6, 2.4; **14**) 17.8, 2.4; **15**) 19.0, 2.0; **16**) 19.0, 2.4; **17**) 17.8, 2.4; **18**) 20.4, 1.5; **19**) 19.0, 2.4; **20**) 16.6, 2.0.

frequently isolated from onion in Australia than *P. irregulare*, but that each species caused nearly the same amount of damping-off in pathogenicity tests. *P. coloratum* seems to be widely distributed since it occurred in each of the areas of Australia sampled and also was isolated from carrots grown in British Columbia, Florida, Minnesota, and Wisconsin (5). In the present investigation, the fungus was isolated from onion and lettuce seed and seedlings grown in the Sor soil, which was cropped to lettuce. It was not recovered from the Las and Ira soils but was isolated at a low frequency from the Bos soil. Additional studies are necessary to determine the extent of the distribution of *P. coloratum* in New York organic soils cropped to vegetables. Presently, it appears that cropping history or location (possibly both) regulate the distribution of this fungus in the agricultural organic soils of New York.

*P. irregulare* was reported by Semeniuk and Melhus (14,15) to cause a disease of onion seedlings in Iowa. McKeen (6) reported the fungus caused damping-off of onion seedlings grown from seed under commercial greenhouse conditions and used as transplants. *P. irregulare*, like *P. coloratum*, appears to be widely distributed. Hendrix and Campbell (3) recovered *P. irregulare* more frequently in North American soils than any other species of *Pythium* by using soil dilution plates directly as well as apple slices as bait. Starr and Mai (17) reported that *P. polymorphon* from an organic soil at Carlisle, NY, was pathogenic to celery, and in the presence of *Meloidogyne hapla* Chitwood, root necrosis by this species was increased. Since *P. polymorphon* is regarded as synonymous with *P. irregulare* (19), it follows that *P. irregulare* was described prior to the present study as occurring in New York organic soils. Since *P. irregulare* was isolated frequently from all of the organic soils sampled in the present study, it can be concluded that the fungus is widely distributed in those organic soils cropped to onion and other vegetable crops in New York.

In addition to *P. irregulare* and *P. coloratum*, seven species of *Pythium* have been isolated from onion plants by other workers: *P. debaryanum* Hesse, *P. mamillatum* Meurs, *P. graminicola* Subramanian, and *P. paroecandrum* (15); *P. spinosum* Sawada (7), and *P. afertile* Kanouse and Humphrey and *P. ultimum* (13). None of these were reported as pathogenic to onion seedlings (7,13,15). *P. ultimum* and *P. paroecandrum* were isolated from onion seed and seedlings grown in natural organic soils in the present study, but the other five species were not. Species of *Pythium* isolated from onion for the first time in the present study were *P. sylvaticum*, *P. acanthicum*, *P. rostratum*, and *P. torulosum*. The heterothallic *Pythium* species isolated from onion by Robertson (13) in Australia may be the same as the heterothallic *P. sylvaticum* isolates obtained from onion seeds and seedlings grown in natural organic soils in the present study.

TABLE 2. Pathogenicity of *Pythium* species isolated from onion and lettuce seeds and seedlings used as bait in the organic soils that were sampled

| *Pythium* species | Isolates tested (no.)[a] | Stand (%)[b] $\overline{X}$ CI |
|---|---|---|
| *P. irregulare* | | |
| virulent | 146 | 14.2 ± 1.7 |
| weakly pathogenic | 14 | 62.8 ± 6.0 |
| *coloratum* | 26 | 26.4 ± 6.4 |
| *ultimum* | 18 | 50.4 ± 5.6 |
| *sylvaticum* | 67 | 58.8 ± 3.6 |
| *acanthicum* | 16 | 70.4 ± 5.6 |
| Miscellaneous[c] | 7 | 56.0 ± 8.8 |
| Unidentified | 38 | 48.8 ± 8.0 |
| Controls (30 tests) | ··· | 75.2 ± 4.4 |

[a] The total number of isolates obtained from all soils sampled.
[b] $\overline{X}$ = average percentage of onions standing 2 wk after planting (based on 25 seeds planted initially) for all isolates of *Pythium* spp. tested. CI = confidence interval, $P = 0.05$.
[c] Miscellaneous species include *P. rostratum, P. paroecandrum,* and *P. torulosum.*

Oogonia, antheridia, oospores, and oidialike cells were formed in compatible crosses between isolates of *P. sylvaticum* in the present study. These structures were of the same shapes and dimensions as those described by Campbell and Hendrix (1), Papa et al (10), and Pratt and Green (12) for *P. sylvaticum*. Compatible reactions were observed on OMA as well as HSA in the present study, but not on water agar. Hendrix and Campbell (4) stated that HSA was satisfactory for oospore production between compatible isolates of *P. sylvaticum* because of the sterols contained in the medium, but that other media such as CMA or potato-dextrose agar were unsatisfactory because of the absence of sterols.

The identification of *P. coloratum* was facilitated by growing isolates of the fungus on boiled grass blades in a mixture of autoclaved pond water and distilled water (2). Sporangia were abundant when the fungus was grown in this manner, but were not observed on agar. When the mycelium was remounted in this water, the lavender color of the oospore wall was distinct. This is an important feature in identification of *P. coloratum* (18,20) and was not distinct when *P. coloratum* was grown on solid media.

## LITERATURE CITED


1. Campbell, W. A., and Hendrix, F. F., Jr. 1967. A new heterothallic *Pythium* from southern United States. Mycologia 59:274-278.
2. Emerson, R. 1958. Mycological organization. Mycologia 50:588-621.
3. Hendrix, F. F., Jr., and Campbell, W. A. 1970. Distribution of *Phytophthora* and *Pythium* species in soils in the continental United States. Can. J. Bot. 48:377-384.
4. Hendrix, F. F., Jr., and Campbell, W. A. 1973. Pythiums as plant pathogens. Annu. Rev. Phytopathol. 11:77-98.
5. Howard, R. J., Pratt, R. G., and Williams, P. H. 1978. Pathogenicity to carrots of *Pythium* species from organic soils of North America. Phytopathology 68:1293-1296.
6. McKeen, C. D. 1950. Preliminary studies on a Pythium rootrot of spanish onion seedlings. Sci. Agric. 30:123-131.
7. Middleton, J. T. 1943. The taxonomy, host range and geographic distribution of the genus *Pythium*. Mem. Torrey Bot. Club 20:1-171.
8. Newhall, A. G. 1939. Muck crop diseases to date in New York State. Plant Dis. Rep. 23:204-205.
9. Newhall, A. G. 1943. Onion seed treatments. Plant Dis. Rep. Suppl. 145:39-45.
10. Papa, K. E., Campbell, W. A., and Hendrix, F. F., Jr. 1967. Sexuality in *Pythium sylvaticum*: Heterothallism. Mycologia 59:589-595.
11. Pieczarka, D. J. 1977. Ecology and biology of *Pythium* species associated with snap bean roots and soils in New York State. Ph.D. thesis. Cornell University, Ithaca, NY. 91 pp.
12. Pratt, R. G., and Green, R. J., Jr. 1971. The taxonomy and heterothallism of *Pythium sylvaticum*. Can. J. Bot. 49:273-279.
13. Robertson, G. I. 1976. *Pythium* species in market gardens and their pathogenicity to fourteen vegetable crops. N.Z. J. Agric. Res. 19:97-102.
14. Semeniuk, G., and Melhus, I. E. 1942. Yellow dwarf, pink root and other onion diseases in Iowa. Pages 143–144 in: Report on Agricultural Research for the Year Ending 30 June 1942. Agric. Exp. Stn., Iowa State College of Agriculture and Mechanic Arts 1941/1942 (Part 1).
15. Semeniuk, G., and Melhus, I. E. 1943. Yellow dwarf, pink root and other diseases in Iowa. Pages 128–129 in: Report on Agricultural Research for the Year Ending 30 June 1943. Agric. Exp. Stn., Iowa State College of Agriculture and Mechanic Arts 1942/1943 (Part 1).
16. Sleeth, B. 1945. Agar medium and technique for isolating *Pythium* free of bacteria. Phytopathology 35:1030-1031.
17. Starr, J. L., and Mai, W. F. 1976. Effect of soil microflora on the interaction of three plant-parasitic nematodes with celery. Phytopathology 66:1224-1228.
18. Vaartaja, O. 1965. New *Pythium* species from South Australia. Mycologia 57:417-430.
19. Vaartaja, O. 1966. Environmental variation in antheridial characteristics used in separating *Pythium irregulare* and *P. polymorphon*. Can. Dep. For., For. Entomol. Pathol. Branch, Bi-monthly Progr. Rep. 22(2):2-3.
20. Waterhouse, G. M. 1967. Key to *Pythium* Pringsheim. Mycological Pap. 109, Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England. 15 pp.
21. Waterhouse, G. M. 1968. The genus *Pythium* Pringsheim. Mycological Pap. 110, Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England. 71 pp.