Pythium Species Associated with Strawberry Roots in Japan, and Their Role in the Strawberry Stunt Disease

Tsuneo Watanabe, Koji Hashimoto, and Masanao Sato

Respectively, Department of Plant Pathology and Entomology, National Institute of Agricultural Sciences, Nishigahara, Kita-ku, Tokyo, Japan; Plant Pathology Laboratory, Saitama Horticulture Experiment Station, Kuki, Saitama, Japan; and Department of Plant Protection, Shizuoka Agriculture Experiment Station, Kitaando, Shizuoka, Japan.

Accepted for publication 15 April 1977.

ABSTRACT

WATANABE, T., K. HASHIMOTO, and M. SATO. 1977. Pythium species associated with strawberry roots in Japan, and their role in the strawberry stunt disease. Phytopathology 67:1324-1332.

Of 58 fungus genera identified among 2,011 fungus isolates from roots of "stunted" and healthy strawberry plants collected in May 1973 to December 1974 in Japan, 60% were species of *Fusarium*, *Pythium*, and *Rhizoctonia*. Fourteen species were identified among the 291 isolates of *Pythium* spp. the most dominant of which were *P. sylvaticum* complex (138 isolates), *P. ultimum* (46 isolates), *P. spinosum* (42 isolates), and *P. oedochilum* (40 isolates). For eight of the species (*P. afertile*, *P. angustatum*, *P. apleroticum*, *P. echinulatum*, *P. inflatum*, *P. myriotylum*, *P. paroecandrum*, and *P. torulosum*) this appears to be the first record of isolations from strawberry plants throughout the world. Isolations from the rhizosphere of the strawberry yielded ten species of *Pythium*, the most dominant of which were *P. carolinianum* (49 isolates), *P. aphanidermatum* (14 isolates), *P. spinosum* (11 isolates), and *P. sylvaticum* complex (nine isolates). *Pythium aphanidermatum*, *P. carolinianum*, and *P. intermedium* were isolated only from the rhizosphere soil in this study. *Pythium ultimum* was found to be a primary pathogen of the stunt disease of strawberry among several *Pythium* spp. tested at temperatures below 20 C. *Pythium echinulatum*, and other *Pythium* species also were pathogenic on strawberry roots at 26 C. Implication of *P. ultimum* and *Pythium* spp. in the occurrence of the stunt disease of strawberry in Japan is discussed in this study.

The forcing of strawberry plants (*Fragaria chiloensis* Duch. var. *ananassa* Bailey) in Japan has been commonly practiced in drained rice paddy fields as the second crop from autumn to late spring. The strawberry plants are grown for cold protection in vinyl houses or sometimes vinyl tunnels, together with polyethylene film mulch.

A disease of strawberry plants which is characterized by poor growth, root deterioration, and low yields, and known as "stunting" recently has become serious in Japan. The disease appears in October about 20 days after plants are transplanted to fields and is most severe by January or February. Temperatures are the lowest throughout the year about that time. Soil temperatures of strawberry fields, for example, were 2-8 C at the minimum, 7-15 C at the maximum in the open field, and 10-18 at the minimum, 21-29 at the maximum in the vinyl tunnel field with polyethylene film mulch in February, 1965 in Shizuoka prefecture (14).

The disease may be the same as blackroot complex in California (15, 32), black root in Michigan (22), root rot in Illinois, and Canada (9, 16, 25), and progressive decline in Italy (2).

Ceratobasidium sp. (*Rhizoctonia fragariae*) (32), *Idriella lunata* (15), and *Mycelium radicis atrovilens* (31) all have been implicated in the blackroot complex in California. *Verticillium* sp., *Rhizoctonia* sp., *Pythium* sp., *Fusarium* sp., and *Cylindrocarpon* sp. have been associated with the disease in Italy (2). Nemec (16) recorded representatives of 81 genera of fungi isolated from both diseased and healthy strawberry roots of two cultivars in Illinois, and Gourley (3), in a study of cold-stored, apparently-healthy, strawberry plants of nine cultivars in Canada, and found representatives of 43, and 38 genera of fungi from crowns and roots, respectively.

No critical survey has been conducted of the fungal flora associated with stunted strawberry plants in Japan. During the course of such a study, 2,011 fungal isolates from both diseased and healthy strawberry roots produced mainly, *Fusarium*, *Pythium*, and *Rhizoctonia* (about 60% of the total isolates). The significance of *Fusarium* spp. and *Rhizoctonia* spp. to strawberry plants in Japan has been pointed out many times, but only a few reports have been concerned with taxonomy and pathology of *Pythium* spp. on strawberry.

The purpose of this study was to identify the fungi associated with roots of the diseased and healthy strawberry plants, with particular attention to *Pythium* species, and to find the cause of the stunt disease by testing the pathogenicity of selected *Pythium* species on strawberry plants.

MATERIALS AND METHODS

Isolations.—Strawberry plants used for this study were collected on ten different occasions from six different prefectures in Japan from May 1973 to December 1974. Stunted plants with characteristic symptoms such as yellow leaves or roots with red-stele were not included as samples because of the chance that such "stunting" might be caused by *Fusarium oxysporum* f. sp. *fragariae* or *Phytophthora fragariae*. Forty-four plants were used

including two plants each of diseased, and healthy plants of cultivar Donner (Saitama prefecture); one diseased, and one healthy plant each of Fukuba (Kanagawa prefecture), and Harunoka (Shizuoka prefecture); three diseased, and one healthy plant of Hogyoku (Mie prefecture); and 18 diseased, and 14 healthy plants of Hokhowase (Shizuoka, Shimane, and Tottori prefectures).

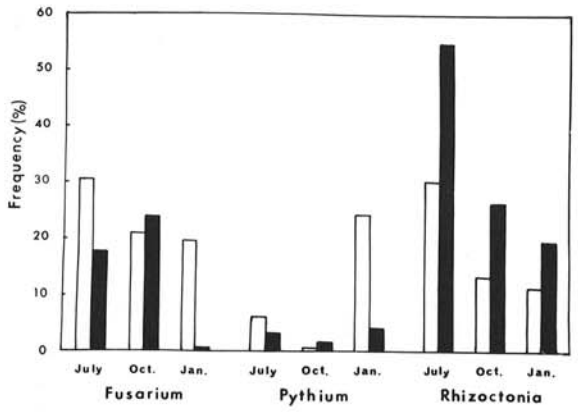


Fig. 1. Frequency (%) of isolation of *Fusarium* spp., *Pythium* spp., and *Rhizoctonia* spp. from diseased (solid bars) and healthy strawberry plants (blank bars) of cultivar Hokhowase sampled at the Shizuoka (Japan) Agricultural Experiment Station in the growing season.

Root segments (5 mm long) were dissected from random roots, washed in running tap water for over 2 hr, blotted, and placed without surface sterilization on water agar in plates. Hyphal tips were selected at random from the fungal growth from each segment after incubation for 1 to 3 days at 20 C, and transferred to potato-dextrose agar plates.

Rhizosphere soil was collected from the plants grown in Shizuoka, Mie, Shimane, and Tottori prefectures. Fungi were isolated directly from soil particles placed on water agar (26), or indirectly, by baiting with potato disks (4 mm diameter by 3 mm thick) that were placed in contact with the soil for 24 hr, washed under running tap water for 2 hr, and then placed on water agar plates (10). Hyphal tips were selected at random after 1 to 3 days of incubation at 20 C.

Inoculations.—Thirteen isolates of eight species of *Pythium*, *P. aphanidermatum*, *P. artotrogus*, *P. carolinianum*, *P. echinulatum*, *P. myriotylum*, *P. spinosum*, *P. sylvaticum* complex, and *P. ultimum* were tested for pathogenicity under the greenhouse conditions at 9(0-26), 17 ± 2, 20 ± 2, and 26(20-38) C. Inocula consisted of washed mycelial mats produced in potato-dextrose broth. One young runner plant with two new leaves was transplanted into autoclaved soil (about 900 g of air-dried soil) in a 15-cm diameter clay pot with one mat of a given fungus per pot. This runner plant had been rooted in chloropicrin-treated soil. The experiments were conducted at least twice with three to five plants (cultivar Donner) per treatment, and were terminated after 50 or

TABLE 1. Dominant fungus genera associated with strawberry roots of five different cultivars sampled in six prefectures of Japan

Cultivar	Location	Total isolates (no.)	Fungus genera	Isolates (no.)	Frequency (%)
Hokhowase	Shizuoka	1,090	*Rhizoctonia*	331	30.4
			Fusarium	214	19.6
			Pythium	66	6.1
Hokhowase	Shimane & Tottori	129	*Fusarium*	51	39.5
			Rhizoctonia	50	38.8
			Pythium	8	6.2
Donner	Saitama	402	*Pythium*	127	31.6
			Rhizoctonia	81	20.1
			Cylindrocarpon	72	17.9
			Fusarium	52	12.9
			Mortierella	24	6.0
Hogyoku	Mie	238	*Pythium*	78	32.8
			Fusarium	47	19.7
			Humicola	14	5.9
			Curvularia	13	5.5
			Pyrenochaeta	12	5.0
			Mortierella	12	5.0
Fukuba	Kanagawa	84	*Rhizoctonia*	39	46.4
			Cylindrocarpon	12	14.3
			Cladosporium	5	6.0
			Gliocladium	5	6.0
Harunoka	Shizuoka	68	*Fusarium*	29	42.6
			Cephalosporium	6	8.8
			Dactylella	6	8.8
			Penicillium	6	8.8
			Pythium	4	5.9

60 days. Root discoloration was rated 0 (healthy roots), 1 (up to 10% root discoloration), 2 (11-25% root discoloration), 3 (26-50% root discoloration), or 4 (more than 51% discoloration for the individual tap roots). Data were obtained by counting numbers of leaves of each plant, and weighing fresh weights of total plants. Measurements also were made on the sizes of the petioles of each plant, and longest, and widest portion of the three leaflets of the largest mature leaf 60 days after planting. The test fungi always were reisolated from the inoculated plants.

RESULTS

Fungus genera associated with strawberry roots and occurrence of Fusarium, Pythium, and Rhizoctonia.—A total of 2,011 isolates from roots of both "stunted" and healthy strawberry plants of five different cultivars were identified into 58 genera, excluding unknowns. The dominant fungus genera among these fungi are listed in Table 1.

Of 2,011 isolates, *Rhizoctonia*, *Fusarium*, and *Pythium* were the most frequent, and made up 25.2%, 19.6%, and 14.5% of the isolates, respectively. These three genera were isolated from healthy as well as diseased plants, and their occurrence at different times during the growing season was analyzed using cultivar Hokhowase, sampled in Shizuoka (Fig. 1).

Rhizoctonia spp. were isolated most frequently in July, less in October, and least frequently in January from both healthy and diseased plants (isolation frequencies ranged from 11.5% to 54.6%). *Fusarium* spp. made up 17% or more of the isolates from both diseased and healthy plants, except in January when almost no *Fusarium* was isolated from diseased plants. *Pythium* isolates were relatively rare in October, but they were most common from plants in January, especially healthy plants (Fig. 1).

Isolates of *Rhizoctonia* spp. were of two types, probably *R. fragariae* Husain & McKeen (11) and *R. solani* Kühn. *Rhizoctonia fragariae* made up all of 39 *Rhizoctonia* isolates from cultivar Fukuba in Kanagawa prefecture, 96.3% of 81 isolates from Donner in Saitama, and 64.0% of 331 isolates from Hokhowase in Shizuoka.

The 394 isolates of *Fusarium spp*] *included F. oxysporum* (Schl.) emend. Snyd. & Hans. (315 isolates), *F. roseum* (Lk.) emend. Snyd. & Hans. (41 isolates), *F. solani* (Mart.) Appel & Wr. emend. Snyd. & Hans. (one isolate), and *F. moniliforme* (Sheld.) emend. Snyd. & Hans. (one isolate).

Identification of Pythium spp. from the roots, and the rhizosphere.—Seventeen species of *Pythium* were identified from both roots and rhizosphere according to descriptions by previous workers (12, 13, 27, 28). Size of the reproductive cell of the respective species was of little taxonomic value (6).

Pythium afertile Kanouse & Humphrey, *P. carolinianum* Matthews, *P. inflatum* Matthews, and *P. intermedium* de Bary did not form sexual structures in culture, and thus were identified on the basis of sporangium shape. The isolates of *P. afertile* formed hyphalike sporangia, and those of *P. carolinianum* formed sphaerosporangia (20-35 μm), occasionally with internal proliferations (Fig. 2.4 to 2.7). An isolate of *P. intermedium* characteristically formed sphaerosporangia (14.0-17.5 μm) in chains that were deciduous (Fig. 2.8 to 2.10). All three species of *P. afertile*, *P. carolinianum*, and *P. intermedium* discharged zoospores in water. The isolates with lobate hyphae (Fig. 2.3) that did not discharge zoospores by the various techniques tried, were identified tentatively as *P. inflatum*, following Waterhouse (27).

Pythiaceous fungi with sexual structures were separated into two groups on the basis of presence or absence of protuberances on oogonial surfaces. Isolates identified as *P. artotrogus* (Mont.) de Bary [= *P. hydnosporum* (Mont. and Berk.) Schroet.], *P. echinulatum* Matthews, and *P. spinosum* Sawada all formed protuberances on oogonial surfaces, and were further classified on the basis of oogonial morphology, and presence or absence of conidia sensu Hendrix and Papa (8). Isolates of *P. artotrogus* formed typical oogonia (15-30 μm), even on the aerial hyphae (Fig. 2.11), but did not form sporangia or conidia. The protuberances of oogonia were cylindrical, 0.5-5.0 μm × 2.5 μm, but not sharply pointed, and were distributed regularly (Fig. 2.11, 2.12). Oogonia were granular internally with hypogynous antheridia, but oospores were not observed in five isolates examined. The isolates of *P. echinulatum* formed stout, pointed protuberances (2.5-5.0 μm × 2.0-3.7 μm) on

TABLE 2. Pathogenicity of seven different *Pythium* spp. on strawberry roots grown in soils infested, and noninfested with the respective isolates as indicated by results obtained 50 days after planting in a greenhouse at 26(20-38) C

Pythium species[a]	Roots examined[b] (no.)		Discolored roots (%)		Root rot rating[c]	
	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
Control (noninfested)	89	157	27.0	43.9	0.70 A	0.85 A
P. aphanidermatum	83	180	45.8	57.8	1.03 AB	1.00 AB
P. artotrogus	87	...	40.2	...	1.17 AB	...
P. carolinianum	92	129	69.6	66.7	1.94 BC	1.17 AB
P. echinulatum	95	143	71.6	74.8	2.15 C	1.80 BC
P. spinosum	91	167	60.4	43.1	1.69 BC	0.67 A
P. sylvaticum complex	112	174	45.5	47.3	1.19 ABC	1.16 AB
P. ultimum	108	142	70.4	56.3	1.92 BC	0.88 A

[a]One isolate of each species was used in all inoculations except three isolates of the *P. sylvaticum* complex.

[b]Data are based on two replications, three plants per treatment in the first test and five in the second.

[c]Rating system: 0 (healthy roots); 1 (1-10% root discoloration); 2 (11-25% root discoloration); 3 (26-50% root discoloration); 4 (over 51% root discoloration). Means followed by different letters in the same column are significantly different, $P = 0.05$, by Student's t-test.

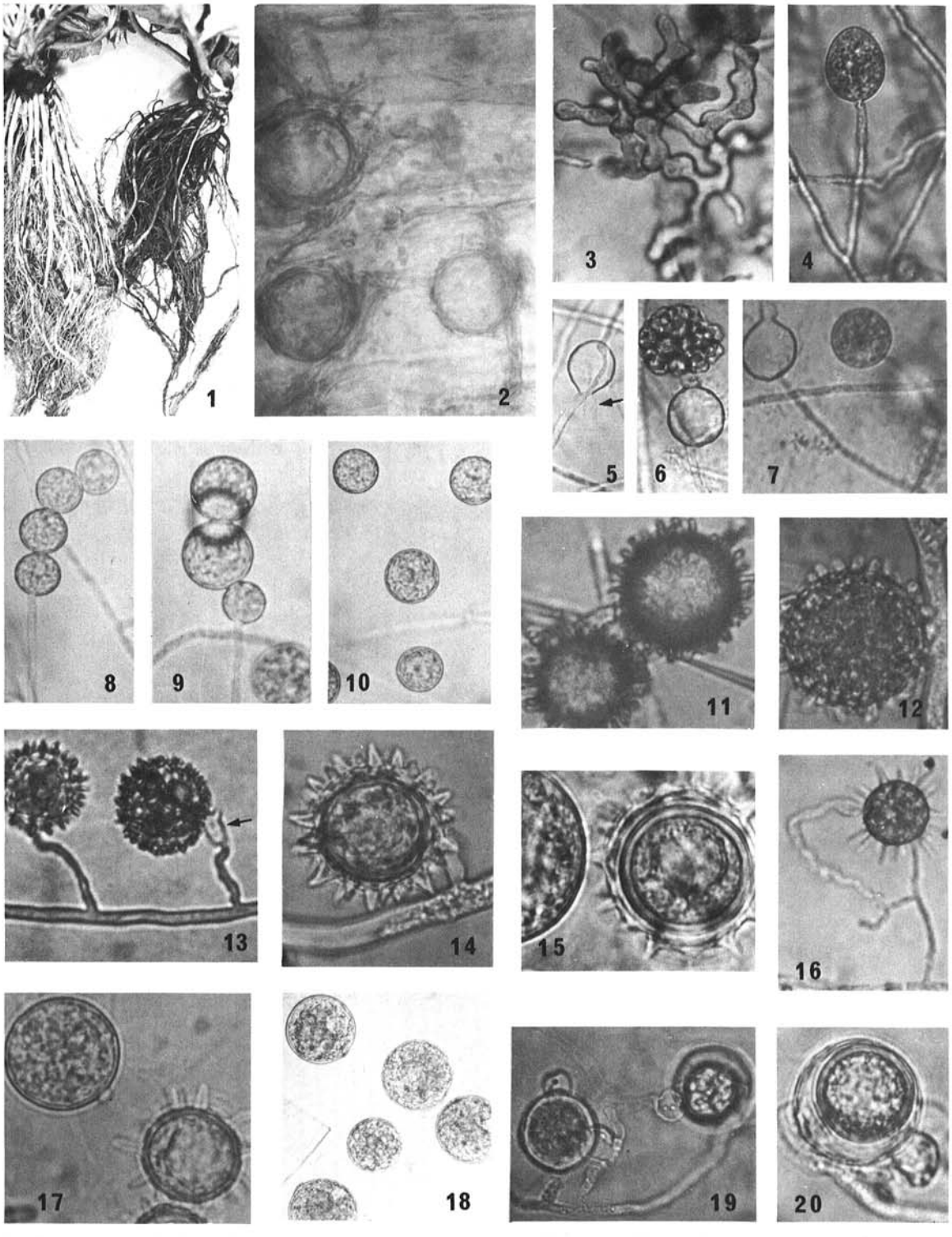
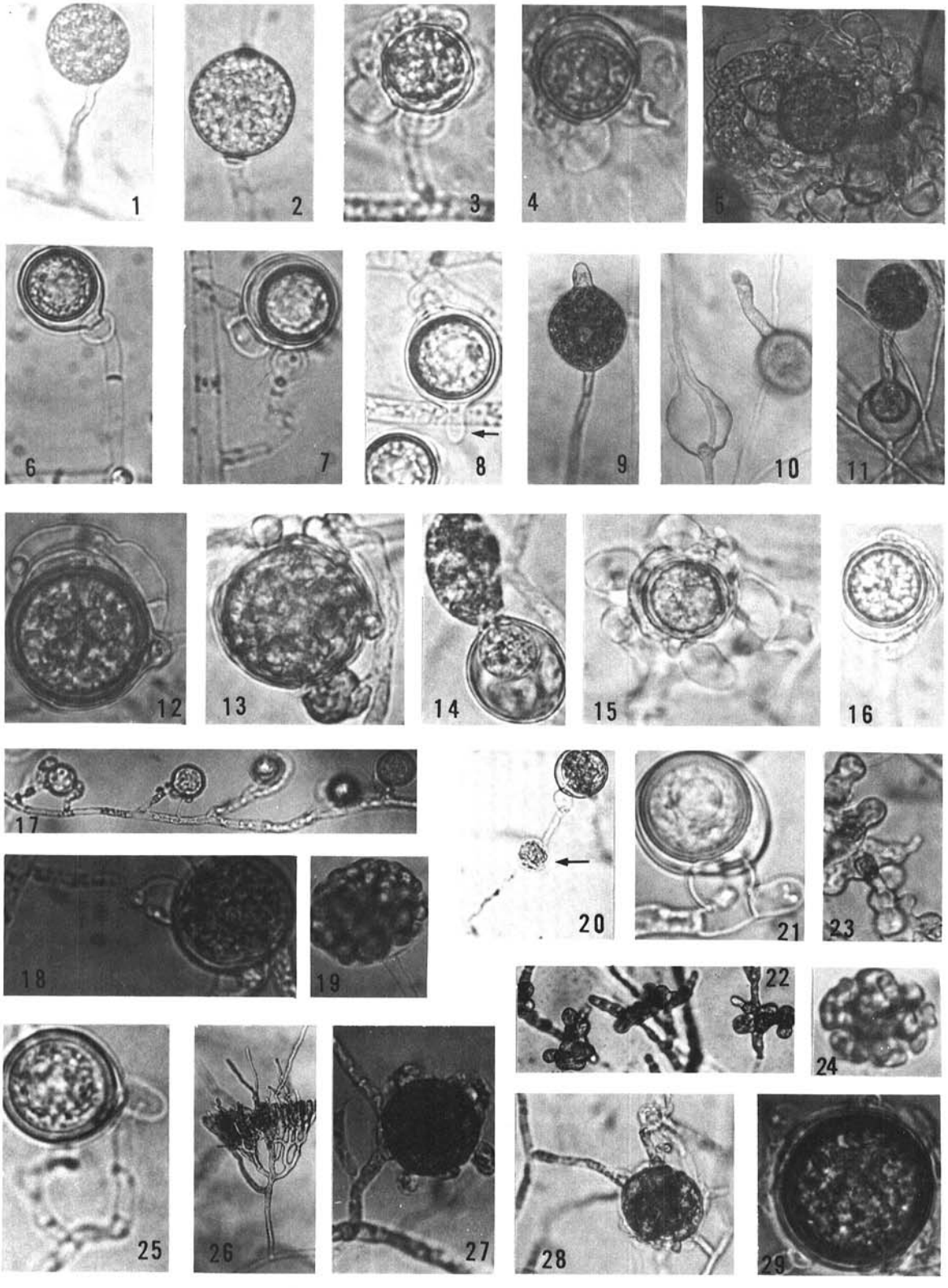


Fig. 2.1-2.20. *Pythium* spp. associated with strawberry roots in Japan. **2.1)** Strawberry roots grown in soil artificially infested with *Pythium echinulatum* (right), and noninoculated healthy roots (left). **2.2)** Oospores of *P. echinulatum* formed in the root tissue of the diseased plant in 2.1). (2.3-2.20) *Pythium* spp. isolated from strawberry roots and rhizospheres. **2.3)** *P. inflatum*; lobate hyphae. (2.4-2.7) *P. carolinianum*. **2.4)** Sporangium. **2.5)** Internally-proliferated sporangium (Note an exit papilla at an abnormal site, arrow). **2.6)** Vesicle formation. **2.7)** Vacant and nonvacant sporangia on branched sporangiophore. (2.8-2.10) *P. intermedium*. **2.8)** Catenulate sporangia on sporangiophore. **2.9)** Basipetally-developed cantenulate sporangia. **2.10)** Deciduous nature of sporangia. (2.11, 2.12) *P. artotrogus*; Echinulate oogonia on aerial hyphae (**2.11**), and on medium (**2.12**). (2.13-2.15) *P. echinulatum*. **2.13)** Echinulate oogonia and hypogynous antheridia (arrow). **2.14)** Echinulate oogonium with nearly plerotic oospore. **2.15)** Conidium and aplerotic oospore. (2.16, 2.17) *P. spinosum*. **2.16)** Oogonium with paragynous antheridium. **2.17)** Conidium and echinulate oogonium with plerotic oospore. (2.18-2.20) *P. splendens*. **2.18)** Conidia. **2.19)** Oogonia with single monoclinous, and diclinous antheridia. **2.20)** Oogonium with aplerotic oospore and antheridial cells.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oogonia (20.0-37.5 μm) (Fig. 2.14) together with hypogynous antheridia (Fig. 2.13), and usually aplerotic oospores (17.5-22.5 μm), and subspherical conidia (20-70 μm × 20.0-37.5 μm) (Fig. 2.15). The isolates of *P. spinosum* formed slender digitate protuberances (5.0-13.8 μm × 2 μm) on oogonia (15.0-27.5 μm) (Fig. 2.16), plerotic oospores (15.0-17.5 μm), and conidia (10-30 μm) (Fig. 2.17). *Pythium echinulatum* and *P. spinosum* did not discharge zoospores by the various techniques tried, although both species formed conidia (Fig. 2.15, 2.17).

Fig. 4. Healthy and stunted strawberry plants grown in autoclaved soil artificially infested with *Pythium ultimum* (right), and noninfested (left) tested at 20 C, 60 days after transplanting. Note flowering and fruiting of the healthy plant.

Pythiaceous fungi with smooth oogonia were further separated into two types, one with spherical or subspherical hyphal structures such as sporangia and conidia, and the other with simple filamentous, inflated, or lobate sporangia. Those of the former type included *P. oedochilum* Drechsler, *P. paroecandrum* Drechsler, *P. splendens* Brawn, *P. sylvaticum* complex, and *P. ultimum* Trow. Those of the latter type included *P. angustatum* Sparrow, *P. aphanidermatum* (Edson) Fitzpatrick, *P. apleroticum* Tokunaga, *P. myriotylum* Drechsler, and *P. torulosum* Coker & Patterson. All discharged zoospores except *P. splendens*, *P. sylvaticum* complex, and *P. ultimum*. The conidia of *P. splendens* were darkish (Fig. 2.18) and measured up to 45 μm in diameter; oogonia (25.0-37.5 μm) formed rarely and included aplerotic oospores (20.0-27.5 μm) together with monoclinous or diclinous antheridia (9.5-17.5 μm × 10.0-12.5 μm) (Fig. 2.19, 2.20). The isolates of *P. sylvaticum* complex formed mostly conidia, less than 35 μm in diameter, terminally or intercalarly (Fig. 3.1, 3.2), but some isolates formed conidiumlike oogonia surrounded by antheridial cells (Fig. 3.3), or elongated, branched antheridial hyphae (3.7-13.0 μm wide) (Fig. 3.5), and occasionally aplerotic oospores (12.5-23.8 μm) (Fig. 3.4). These isolates all are close to *P. sylvaticum* Campbell & Hendrix (7), or *P. heterothalicum* Campbell & Hendrix (4), and were identified as *P. sylvaticum* complex following Hendrix

TABLE 3. Fresh weights of total plants, numbers of leaves, sizes of petioles, and leaflets of strawberry plants[x] grown in soils infested, or noninfested with one isolate each of *Pythium* species as determined 60 days after planting at 9 (0-26), and 20 ± 2 C

Species	Weights (g)		Leaves per plant (no.)		Petiole[y] length (cm)		Leaflet[z] Length (cm)		Leaflet[z] Width (cm)	
	9 C	20 C	9 C	20 C	9 C	20 C	9 C	20 C	9 C	20 C
Control (noninfested)	14.1 A	24.1 A	4.8 A	6.0 AB	3.4 A	7.0 A	4.0 A	5.1 A	3.3 A	4.6 A
P. aphanidermatum	15.1 A	16.7 BC	4.8 A	5.8 AB	2.8 A	5.5 BC	4.0 A	4.8 AB	3.3 A	4.1 AB
P. myriotylum	11.5 AB	14.2 C	4.5 AB	5.3 A	3.1 A	5.1 C	4.0 A	4.3 BC	3.1 AB	3.6 BC
P. spinosum	14.7 A	19.3 B	3.8 BC	6.5 B	3.6 A	6.6 AB	4.3 A	5.1 A	3.4 A	4.3 A
P. sylvaticum complex	12.5 AB	15.0 C	4.5 AB	6.0 AB	3.4 A	5.5 BC	3.9 A	4.8 AB	3.2 AB	4.0 AB
P. ultimum	9.4 BC	10.0 D	3.8 BC	6.0 AB	3.1 A	4.4 C	3.3 B	3.7 C	2.8 B	3.1 C

[x]Data are means of two replications, four plants per replication. Means followed by different letters in the same column are significantly different, $P = 0.05$, by Student's t-test.

[y]Data are averages of whole petiole lengths of each plant.

[z]Data are averages of longest and widest portion of the three leaflets of the largest mature leaf of each plant.

Fig. 3.1-3.29. *Pythium* spp. isolated from strawberry roots and rhizospheres. (3.1-3.5) *P. sylvaticum* complex. **3.1)** Terminal conidium. **3.2)** Intercalary conidium. **3.3)** Oogonium surrounded by antheridial cells. **3.4)** Oogonium with aplerotic oospore and stout antheridial cells. **3.5)** Oogonium surrounded by branched antheridial hyphae. (3.6-3.8) *P. ultimum*. **3.6)** Oogonium containing aplerotic oospore and hypogynous antheridium. **3.7)** Oogonium with two paragynous antheridia. **3.8)** Oogonium with single protuberance (arrow), and aplerotic oospore. (3.9-3.13) *P. oedochilum*. **3.9)** Papillate sporangium. **3.10)** Internally-proliferated, and long papillate sporangia. **3.11)** Vesicle formation. **3.12)** Oogonium with plerotic oospore and wavy-contoured antheridial cell. **3.13)** Oogonium with sessile and coiled antheridia. (3.14-3.16) *P. paroecandrum*. **3.14)** Sporangium and vesicle formation. **3.15)** Oogonium with aplerotic oospore and branched stout antheridia. **3.16)** Oogonium with aplerotic oospore and sessile antheridium. (3.17-3.19) *P. angustatum*. **3.17)** Oogonia with single monoclinous antheridia. **3.18)** Intercalary oogonium with two diclinous antheridia. **3.19)** Vesicle formation from hypha-like sporangium. **3.20)** *P. apleroticum*; Oogonium with single diclinous antheridium. Note intercalary conidium (arrow). (3.21, 3.22) *P. aphanidermatum*. **3.21)** Oogonium with single aplerotic oospore and intercalarly-formed antheridial cell. **3.22)** Inflated hypha-like sporangia. (3.23-3.25) *P. torulosum*. **3.23)** Lobate sporangium. **3.24)** Vesicle. **3.25)** Oogonium with plerotic oospore and monoclinous antheridium. (3.26-3.29) *P. myriotylum*. **3.26)** Appressoria. **3.27)** Oogonium and crook-necked antheridia. **3.28)** Oogonium with diclinous antheridia. **3.29)** Plerotic oospore.

and Papa (8). The isolates of *P. ultimum* usually formed conidia (15-30 μm), and oogonia (15-25μm) including aplerotic oospores (11.2-17.5 μm) together with hypogynous or monoclinous, sessile, single or rarely two antheridia (5.0-8.8 μm × 3.7-10.0 μm) (Fig. 3.6, 3.7). Single protuberances occasionally were formed on oogonial surfaces especially in water (Fig. 3.8). The isolates of *P. oedochilum* formed sporangia (20-40 μm × 15-35 μm) (Fig. 3.9) that often were internally proliferated (Fig. 3.10), and they consistently discharged zoospores in water after vesicle formation (Fig. 3.11). This species also formed characteristic, furrowed, crescent-shaped, cylindrical or coiled antheridia (7.5-27.5 μm × 3.7-7.5 μm) of monoclinous or diclinous origin, that covered the oogonia (27.5-35.0 μm) (Fig. 3.12, 3.13) containing usually aplerotic oospores (25.0-27.5 μm). The isolates of *P. paroecandrum* formed sessile antheridia, oogonia (20-25 μm) containing aplerotic oospores (12.5-20.0 μm) (Fig. 3.15, 3.16), and the sporangia (15-25 μm) (Fig. 3.14) discharged zoospores.

In the latter group, the isolates of *P. angustatum* formed one to three typically cylindrical antheridia (10.0-15.0 μm × 5.0-7.5 μm) attached to oogonia (15.0-26.3 μm) monoclinously or diclinously, and aplerotic oospores (12.5-23.8 μm) (Fig. 3.17, 3.18). Sporangia in this species were filamentous, and discharged zoospores readily (Fig. 3.19). The isolates of *P. aphanidermatum* characteristically formed intercalary antheridia (11-25 μm × 10-25 μm), and oogonia (21.2-32.5 μm) containing aplerotic oospores (18.7-25.0 μm) (Fig. 3.21). Sporangia were dense in protoplasm, slightly inflated and lobate (Fig. 3.22), and discharged zoospores readily. An isolate of *P. apleroticum* formed single antheridia attached to oogonia (21-25 μm) mostly diclinously, aplerotic oospores (17.5-22.5 μm), and intercalary conidia (20.0-27.5 μm) (Fig. 3.20). Sporangia were filamentous, and discharged zoospores. Two isolates of *P. myriotylum* formed numerous, characteristic, clavate or knoblike appressoria (Fig. 3.26), and inflated lobate sporangia that discharged zoospores. The oogonia were 30.0-37.5 μm, with a few antheridia attached, and usually contained aplerotic oospores (25.0-32.5 μm) monoclinously or diclinously (Fig. 3.27 to 3.29). The antheridial cells of this species were often crook-necked (Fig. 3.27), and the oospores often appeared aborted. Oospore germination was observed occasionally. In the isolates of *P. torulosum*, single antheridia were attached to oogonia (15-20 μm), usually monoclinously (Fig. 3.25), but sometimes diclinously. The oospores were usually plerotic, 14.0-17.5 μm (Fig. 3.25). Sporangia were lobulate and discharged zoospores (Fig. 3.23, 3.24).

Frequency of Pythium spp. in the roots and rhizosphere soil.—Of 291 isolates of *Pythium* spp. from strawberry roots, *P. sylvaticum* complex was isolated most commonly (47.4%) followed by *P. ultimum* (15.8%), *P. spinosum* (14.4%), and *P. oedochilum* (13.7%). Rare species (frequency less than 1.7%) included *P. afertile*, *P. angustatum*, *P. apleroticum*, *P. artotrogus*, *P. echinulatum*, *P. inflatum*, *P. splendens*, *P. torulosum*, *P. myriotylum*, and *P. paroecandrum*. Of 102 isolates of *Pythium* spp. isolated from strawberry rhizosphere soil, *P. carolinianum* was most frequent (48%), followed by *P. aphanidermatum* (13.7%), *P. spinosum* (10.8%), *P. splendens* (8.8%), *P. sylvaticum* complex (8.8%), *P. artotrogus* (5.9%), *P. echinulatum* (2%), *P. angustatum* (1%), and *P. ultimum* (1%).

Pythium afertile, *P. artotrogus*, *P. echinulatum*, *P. torulosum*, *P. myriotylum*, and *P. splendens* were found only in the diseased plants, whereas *P. apleroticum* and *P. inflatum* were found only in the healthy plants.

Pythium aphanidermatum, *P. carolinianum*, and *P. intermedium* were isolated from the rhizosphere soil, but not from the roots.

Pathogenicity of Pythium spp. to strawberry plants.—Single isolates of each of six species of *Pythium* and three isolates of *P. sylvaticum* complex were pathogenic on strawberry roots on the basis of rates and degrees of root discoloration at 26 (20-38) C (Table 2). Of these seven species, *P. echinulatum* was the most pathogenic, causing severe root discoloration (Fig. 2.1). Oospores of this species were observed in about half of 40 roots of randomly-selected, inoculated plants (Fig. 2.2), but not in the roots of healthy plants. However, this species did not cause severe symptoms as observed in the field. In addition, no symptoms of the root rot of strawberry by two isolates of this species were evident at 17 ± 2 C.

Pythium ultimum was, on the other hand, the most pathogenic, typically reducing fresh weights of the total plants, and their sizes at low average temperatures of 9, 17, or 20 C (Fig. 4). *Pythium myriotylum* also showed strong pathogenicity to strawberry plants, reducing plant development. Growth of plants inoculated with other species also was poorer than in the noninoculated control, especially at 20 C. The results for 9, and 20 C are summarized in Table 3. All *Pythium* spp. used for inoculation were reisolated from all of the inoculated plants. A few oospores were found in the uninoculated healthy plants, possibly because of contamination.

DISCUSSION

Of the 58 fungus genera associated with strawberry roots in this study, 35 also were associated with strawberry roots by Nemec (16) in the USA, Gourley (3) in Canada, or both. These fungi are mostly if not entirely normal inhabitants of the rhizosphere and root of strawberry, and any involvement in the stunt disease is probably as secondary invaders, or in association with some other pathogen (30).

According to Nemec (16), of the fungi isolated from the roots with lesions, *Fusarium* spp., *Pythium* spp., and *Rhizoctonia* spp. accounted for approximately 10.0, 25.1, and 5.7%, respectively, of the isolates from cultivar Surecrop, and 11.6, 5.0, 25.9%, respectively, of the isolates from Cyclone.

In Canada, *Fusarium* was the most dominant of 38 genera associated with apparently healthy strawberry roots of nine cultivars, but no isolates of *Pythium* were obtained (3). Hildebrand (9) obtained about 20 fungus genera from 684 strawberry roots with definite lesions, and of these, *Fusarium* was the most dominant (32.7%) followed by *Pythium* (10.8%). On the other hand, Zeller (33) in the USA reported that 78.5% of 5,715 lesions of black roots examined during 1929, 1930, and 1931 yielded various strains of *Rhizoctonia*. D'Ercole and Canova (2) in Italy also found that *Rhizoctonia* and *Fusarium* were

the most dominant genera recovered from root tissues of four strawberry cultivars. Thus, the data obtained under different experimental conditions, namely, in Canada, Italy, and the USA, on the dominance of *Fusarium*, *Pythium*, and *Rhizoctonia* from strawberry roots agree very well with our results obtained in Japan. All three genera are known to be strawberry pathogens. For example, yellows caused by *F. oxysporum* f. sp. *fragariae* has been the most economically important disease of strawberry plants in Japan (19). Although isolates of *F. oxysporum* obtained in this study conceivably could include pathogenic isolates, diseased plants with yellows-type symptoms such as vein twisting, abnormal leaf development, and yellowing were excluded cautiously. *Rhizoctonia solani* and *R. fragariae* both are known pathogens of strawberry roots (11, 33), but abundant isolations of these fungi from roots of healthy plants in this study may indicate local, but not systemic, damage to strawberry plants.

There are 16 species of *Pythium* recorded from strawberry plants throughout the world, including five species from Japan (13, 18, 23). In this study, a total of 17 species including 14 from roots, and 10 from the rhizosphere soil were isolated. Of these, eight species, namely *P. afertile*, *P. angustatum*, *P. apleroticum*, *P. echinulatum*, *P. inflatum*, *P. myriotylum*, *P. paroecandrum*, and *P. torulosum*, appear to be the first reports on the associated fungi with strawberry plants throughout the world.

Identification of isolates of the *P. sylvaticum* complex was problematical in this study. *Pythium sylvaticum* Campbell & Hendrix and *P. heterothalicum* Campbell & Hendrix both are defined on the basis of globose sporangia, and heterothalism, although *P. sylvaticum* isolates with varing degrees of homothalism occur (4, 5, 7). They differ mainly in antheridial morphology; in *P. sylvaticum*, the antheridia generally branch close to the oogonia and form two prominent, inflated antheridial cells, whereas in *P. heterothalicum*, the antheridial hyphae usually divide one to several times to form a mass of inflated antheridia which often completely covers the oogonia. In addition, *P. heterothalicum* will not mate with tester strains of *P. sylvaticum* (4, 7). Although homothalic *P. heterothalicum* isolates have not been recorded to date, several isolates typified by the isolate #866 in this study appeared to be homothalic isolates of this species on the basis of antheridium morphology (Fig. 3.5). Some isolates also showed various antheridial shapes which do not fit readily into either *P. sylvaticum* or *P. heterothalicum*. Therefore, all of these isolates were identified tentatively as *P. sylvaticum* complex, following Hendrix and Papa (8). In addition, some isolates identified as *P. ultimum*, or *P. paroecandrum* formed morphological characteristics partially identical with *P. sylvaticum* complex.

Pythium sylvaticum complex was isolated most frequently from strawberry roots with the isolation frequency of 47.4% of a total of 291 isolates in this study. In the USA, this species may be one of the most dominant *Pythium* species associated with strawberry roots, because it was isolated most frequently in 22 of 29 plantings in Illinois (17, 18), and in 16 out of 49 isolates from diseased roots in Indiana (21).

According to Wilhelm (29), *Pythium ultimum* was one of the most common fungi associated with roots of strawberry in California, and its role as a root pathogen of strawberry plants was well established. In this study, *P. ultimum* also was found as the principal pathogen, significantly reducing strawberry plant development at low temperatures of 9, 17, and 20 C. However, it was not strongly pathogenic at high temperatures (26 C). At 26 C, *Pythium echinulatum* was the most pathogenic of seven species tested, and its pathogenicity to strawberry plants was first noted in this study.

Takatsu and Edo (24) reported an unidentified *Pythium* species that was the most pathogenic to strawberry plants at low temperatures of 8-15 C, and wet soil. It might be *P. ultimum* on the basis of the morphology in their drawings and pathogenicity at low temperatures.

Pythium aphanidermatum and *P. myriotylum* are regarded as noteworthy plant pathogens at high temperatures (13). *Pythium myriotylum* tested in this study showed pathogenicity to strawberry plants at low temperatures below 20 C, but *P. aphanidermatum* did not (Table 3). These two species, if tested, may become more pathogenic at high temperatures. Their pathogenicity to soybean [*Glycine max* (L.) Merr., Wase Ohsodefuriedamame], tomato (*Lycopersicon esculentum* Mill., Ponderosa), cucumber (*Cucumis sativus* L., Aofushinari K), eggplant (*Solanum melongena* L., Nakate Shinkoku), and spinach (*Spinacia oleracea* L., Nihon Ohba) is well established at temperatures over 26 C, but not at temperatures as low as 17 C (Watanabe, *unpublished*).

The concept that temperatures below the optimum for growth of the host plants favors a pathogen such as *Pythium*, and makes the host vulnerable to attack by the pathogens (1) may agree with the present case of the occurrence of the stunt disease of strawberry in Shizuoka and other prefectures in Japan, because winter temperatures are below the optimum for growth of strawberry plants (20). In addition, paddy fields, although drained, are usually wet in the growing season of strawberry plants, and this also may favor *Pythium* spp. The plants also change from the vegetative to the reproductive stage during this season (fruit set is just beginning) and thus plant growth is severely reduced (20).

It is thus possible that *P. ultimum*, one of the dominant species associated with strawberry roots, is the most responsible for the occurrence of the stunt disease of strawberry plants under low temperatures, and the prevalent forcing, cultivation practices in Japan.

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