Ecologic and Taxonomic Studies on Pythium as Pathogenic Soil Fungi : VIII. Differences in Pathogenicity of Several Species of Pythium

メタデータ	言語: English 出版者: 公開日: 2009-08-25 キーワード (Ja): キーワード (En): 作成者: TAKAHASHI, Minoru, ICHITANI, Takio, AKAJI, Kiyohiko, KAWASE, Yasuo メールアドレス: 所属:
URL	https://doi.org/10.24729/00009468

Bull. Univ. Osaka Pref., Ser. B, Vol. 22,
大阪府立大学紀要，農学・生物学， Mar., 1970.

Ecologic and Taxonomic Studies on *Pythium* as Pathogenic Soil Fungi

VIII. Differences in Pathogenicity of Several Species of *Pythium*

Minoru TAKAHASHI, Takio ICHITANI, Kiyohiko AKAJI* and Yasuo KAWASE

Laboratory of Plant Pathology, College of Agriculture

Introduction

The genus *Pythium* is usually omnivorous. It causes damping-off of different kinds of seedlings and is one of the most important pathogens in both seedbed and field. It also causes storage rots of squash and cucumber fruits, potato tuber and sweetpotato root. Furthermore, it attacks wheat and rice plant causing their root rots.

TAKAHASHI and MORIMOTO (6) reported the pathogenicity of several species of *Pythium* to different kinds of seedlings and also discussed the disease resistance of the hosts. Along with *Pythium* which was used in previous paper (6), different species of *Pythium* were compared especially for pathogenicity on sweetpotato root and potato tuber in the present paper.

Materials and Methods

Seventeen isolates representing 11 species of *Pythium* were used (Table 1). Fungal inoculum was produced by growing the test organisms on corn-meal agar (20 g corn meal, 15 g agar, and tap water to 1 liter) at 28°C for 3–4 days.

The seedlings, 3–4 cm high, grown on disinfected soil were inoculated by scattering a chopped half volume of mycelial mat in a petri dish on soil surface and kept in a moist chamber for 30 hrs. at 24°C. After taking out from the moist chamber, the inoculated seedlings were kept in glasshouse for several days and the number of diseased seedlings was recorded. There were six replicates with each inoculation. Noninoculated host tissue served as a check.

Both sweetpotato roots and potato tubers were obtained from the Osaka Agricultural Research Center. The cuttings of sweetpotato were transplanted in June and the roots were harvested in October. The roots were selected uniformly and washed with tap water. And they were disinfected with 0.1% $HgCl_2$ for 10 min., followed by washing with distilled water. The moist chambers were made by placing two pieces of wet sterilized filter papers on both top and bottom of a petri dish. The roots were cut into pieces 0.5 cm thick and put asceptically in the moist chamber. The pieces were inoculated with a disc of a fungal colony (0.7 cm in diameter) on the center of one cut surface. The chambers with the inoculated pieces were kept in incubators of different constant temperatures. The virulence of *Pythium* isolates was determined by recording the diameter of rotted areas after 2 to 3 days inoculation (7). Determinations were made on 3 to 5 replicate roots with each fungal isolate.

* Agriculture and Forestry Department, Osaka Prefectural Government.

Table 1. *Pythium* used

Organism	Stock culture no.	Source	Location (Prefecture)
P. aphanidermatum	12	*Cucumis sativus* L. (fruit)	Kyoto
〃	19	〃 (seedling)	〃
〃	26	Water	〃
〃	81	*Beta vulgaris* L. (seedling)	Kagawa
P. cucurbitacearum	23	*Cucumis sativus* L. (〃)	Kyoto
P. debaryanum	22	*Citrullus vulgaris* Schrad. (fruit)	Kochi
〃	34	*Solanum melongena* L. (〃)	Osaka
〃	38	*Apium graveolens* L. (seedling)	〃
P. hemmianum	1	*Luffa cylindrica* Roem. (〃)	Kyoto
P. monospermum	9	Water (insect cavader)	〃
P. nelumbium	55	*Nelumbo nucifera* Gaertn. (root)	Osaka
P. ostracodes	57	〃 (〃)	〃
P. spinosum	54	〃 (〃)	〃
P. ultimum	11	*Ipomoea batatas* Lam. (root)	Kyoto
〃	77	*Hibiscus manihot* L. (seedling)	〃
P. vexans	7	*Lycopersicon esculentum* Mill. (seedling)	Shiga
P. zingiberum	21	*Zingiber officinale* Rosc. (tuber)	Kyoto

The pathogenicity of *Pythium* on potato tuber was also determined by using essentially the same method as the above-described for sweetpotato root. However, since there was a considerable bacterial contamination with increasing incubation temperature, the following inoculation method was devised. The surface of tuber was disinfected by washing in tap water for 20 min., immersing in 0.2% $HgCl_2$ for 20 min., and then rinsing with water. The tubers were allowed to dry, and under asceptic conditions, plugs of tissue 1.5 cm deep and 0.7 cm in diameter were removed with a cork borer. The disc of the fungal colony (0.5 cm in diameter) was placed in each cavity, then an autoclaved moist cotton ball was added and the colony disc of the same fungus was again placed on it. The injured, inoculated area was finally covered with an adhesive tape. Inoculated tubers were incubated at different constant temperatures for 3 days. The amount of rotted tissue was determined from the tuber weight before and after removal of rot. Determinations were made on 3 replicate tubers with each fungal isolate.

Results

1. Virulence of Pythium on different kinds of plants

Pythium was compared for pathogenicity on different kinds of plants (Table 2). All of the *Pythium* used, except *P. monospermum* and *P. vexans*, was highly pathogenic on tomato, watermelon, *Hibiscus manihot* and morning-glory seedlings, potato tuber, and sweetpotato root. Although *P. monospermum* and *P. vexans* failed to infect or were considerably weakly pathogenic on the seedlings of squash (Amakuri), kidney bean and rice plant, and sweetpotato root, they were highly pathogenic on potato tuber tissue.

2. Virulence of Pythium on root tissue of sweetpotato

The root tissue of sweetpotato (Norin No. 1) was used to test the pathogenicity of *Pythium* (Table 3). All of the *Pythium* used was pathogenic and the root tissue used was highly susceptible to different species of *Pythium*.

Table 2. Inoculation experiments of *Pythium* to seedlings, tubers and roots of different plants

Organism	Stock culture no.	1[a]	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
P. aphanidermatum	12	+[b]		+++	+++	+	+++	+++	+++	+++		+	+++	+	+	+++	+++	+		++
〃	19			+++	+++	+	+++	+++	+++	+++			−	−	+	+++	+++	+		
〃	26				+++	+	+++	+++		+++		+	+++	+		+++	+++	+		
〃	81		++														+++			
P. cucurbitacearum	23			+++	+	−		+++	+++	+++		−	−	+			++	+		
P. debaryanum	22			+++	+++	+		+++	+++								+++			
〃	34							+++								++	+++			
〃	38	+++															+++			
P. hemmianum	1			++	++	+	+++	+	+	+++		+	+++	+	−	++	+	+		
P. monospermum	9		−		−	−	+	+++	−	+	−	−	+	−	−	−	+++	−	−	
P. spinosum	54			++	+			++			++				−	+	+			
P. ultimum	11			+++	+++	+		+++	+++	+++		+	+++	−	−		+++	+		
〃	77		++	+++	+++	+	+++	++	+++	+++		+	+++	+		+++	+++	+	++	
P. vexans	7		−	−	+	−	+	+++	−	++		−	++	−	−	−	+++	−	−	
P. zingiberum	21			++	+++	+	+++		++	+++		−	+++			+++		−	++	+++

[a] Plants and plant parts used: 1, *Apium graveolens* L. (Seedling); 2, *Beta vulgaris* L. (Seedling); 3, *Citrullus vulgaris* Schrad. (Seedling); 4, *Cucumis sativus* L. (Seedling); 5, *Cucurbita maxima* Duch. (Seedling); 6, *Hibiscus manihot* L. (Seedling); 7, *Ipomoea batatas* Lam. (Root); 8, *Luffa cylindrica* Roem. (Seedling); 9, *Lycopersicon esculentum* Mill. (Seedling); 10, *Nelumbo nucifera* Garrtn. (Root); 11, *Oryza sativa* L. (Seedling); 12, *Pharbitis nil* Choisy (Seedling); 13, *Phaseolus vulgaris* L. (Seedling); 14, *Pisum sativum* L. (Seedling); 15, *Solanum melongena* L. (Seedling); 16, *Solanum tuberosum* L. (Stem); 17, *Vicia faba* L. (Seedling); 18, *Zingiber officinale* Rosc. (Stem); 19, *Zinnia elegans* L. (Seedling).

[b] A rating of (+++) denotes 3–6 inoculated plants were susceptible to *Pythium*; A rating of (−) denotes resistance to *Pythium*; An intermediate number of pluses denotes an intermediate degree of susceptibility.

Table 3. Decay of the root tissue of sweetpotato (Norin No. 1) by different species of *Pythium*

Organism	Stock culture no.	No. of roots : inoculated	No. of roots : diseased[a]	Diam. (mm) of lesions[a]
P. aphanidermatum	19	5	5	11.5
P. debaryanum	34	5	5	32.4
P. ultimum	77	5	5	20.8

[a] incubated at 25°C for 2 days.

3. Virulence of Pythium on tuber tissue of potato

Virulence of *Pythium* was examined using the tuber tissue of potato (Norin No. 1) (Table 4). *Pythium* used was generally pathogenic, but the organisms such as *P. spinosum* and *P. ultimum* (Stock culture no. 11) failed to infect or were considerably weakly pathogenic on the tuber.

4. Virulence of P. ultimum on different varieties of sweetpotato roots and the relation of temperature to infection

P. ultimum (Stock culture no. 77) was compared for pathogenicity on different varieties of sweetpotato roots and the resistant reaction of the root tissues together with incubation

Table 4. Dacay of the tuber tissue of potato (Norin No. 1) by different species of *Pythium*

Organism	Stock culture no.	No. of tubers:		Amount (g) of rotting[b]
		inoculated	diseased[a]	
P. aphanidermatum	12	5	5	47.0
〃	19	5	5	97.8
〃	26	5	5	33.9
〃	81	5	5	72.6
P. cucurbitacearum	23	5	5	28.7
P. debaryanum	22	5	5	79.9
〃	34	5	5	28.3
〃	38	5	5	70.6
P. monospermum	9	5	5	23.3
P. nelumbium	55	5	5	99.4
P. ostracodes	57	5	5	59.0
P. spinosum	54	5	1	2.3
P. ultimum	11	5	3	8.0
〃	77	5	5	76.9
P. vexans	7	5	5	61.5

[a] incubated at 24°C for 3 days.
[b] Avg weight loss during the incubation.

Table 5. Decay of root tissue of different sweetpotato varieties by *P. ultimum* (Stock culture no. 77)

Variety	Diam. (mm) of lesions[a] incubated at :		
	20°C	25°C	30°C
Aichi-beniaka	34.0	29.3	12.5
Gifu No. 1	37.5	32.0	19.3
Gokoku	0.0	0.0	0.0
Hayato	0.0	11.5	0.0
Hichi-fuku	26.8	29.0	9.0
Kanto No. 23	35.3	20.5	25.5
〃 No. 31	35.3	26.8	6.0
〃 No. 32	29.3	12.3	6.0
〃 No. 33	31.8	12.3	0.0
〃 No. 34	34.8	0.0	0.0
〃 No. 35	26.5	5.5	0.0
Kokei No. 3	35.0	28.0	0.0
〃 No. 14	46.3	35.5	23.0
Norin No. 1	33.5	25.5	22.0
〃 No. 3	27.3	25.8	0.0
〃 No. 4	31.3	28.3	24.3
〃 No. 5	34.3	18.3	14.8
〃 No. 6	33.8	29.5	21.5
〃 No. 8	33.0	24.5	11.2
〃 No. 10	36.0	29.5	24.3
〃 No. 14	37.3	13.3	14.5
〃 No. 16	34.8	36.0	25.8
Okinawa No. 100	19.0	21.5	13.8
Shiro-sengan	29.8	22.0	11.5
Shizyu-nichi	28.0	23.0	16.0

[a] Avg of 3 sweetpotato roots after 3 days incubation.

temperature was discussed (Table 5). Virulence of this fungus on all varieties used was decreased as incubation temperature increased from 20° to 30°C. In some varieties white aerial mycelia with 1–2 mm high were observed on the lesion. The lesion generally turned brown and darken as the incubation proceeded. The most dark color was observed in the region of cambium. Some other varieties were soft-rotted with light yellow color. A remarkable water-soaking or soft-rotting was observed on the roots of some varieties. This fungus was weakly pathogenic on Gokoku under any given temperature, and it was also weakly pathogenic on Norin No. 3, Kokei No. 3, Kanto (Nos. 31, 32, 33, 34, 35) and Hichifuku when the incubation temperature reached up to 30°C. Apparently Gokoku was re-resistant to *Pythium*. Norin (Nos. 1, 4, 6, 10, 16), Kokei No. 14 and Kanto No. 23 were highly susceptible. Norin No. 3, Kokei No. 3, Kanto (Nos. 31, 32, 33, 34, 35) and Hichifuku became resistant near the optimum temperature for the root growth.

Further experiment was done under a broader temperature range of incubation (Table 6). The fungus was completely nonpathogenic above 36°C. Low temperature limit will be lower than 10° to 13°C, since the fungus is still parastitic in these temperature ranges.

Table 6. Decay of the root tissue of sweetpotato (Norin No. 1) by *P. ultimum* (Stock culture no. 77) under different incubation temperatures

Incubation temperature (C°)	Diam. (mm) of lesions[a] incubated for :			Symptoms
	24 hrs	48 hrs	72 hrs	
10–13	10.3	19.4	29.3	Light brown, tissue softening
16	30.3	38.8	49.0	Brown, tissue softening
20	28.5	40.7	50.7	Brown, tissue softening, aerial mycelia appeared
24	28.5	29.5	32.0	Brown, tissue softening
28	19.4	27.4	27.9	Light brown, tissue softening
32	17.7	26.0	23.4	〃
36	0.0	0.0	0.0	——

[a] Avg of 3 sweetpotato roots incubated.

Table 7. Decay of potato tuber tissue by *P. ultimum* (Stock culture no. 77) under different incubation temperatures

Variety	Weight loss (g)[a] incubated at :		
	20°C	24°C	28°C
Danshaku	51.6	65.5	75.4
Kennebec	34.4	40.6	61.4
Norin No. 1	52.3	76.4	78.8
Oziro	50.1	66.1	72.2
Shimabara	63.5	77.8	80.6
Unzen	56.4	69.2	88.1
Wheeler	66.2	79.4	81.2

[a] Avg of 3 potato tubers after 3 days incubation.

5. Virulence of P. ultimum on different varieties of potato tubers and the relation of temperature to infection

The relation of incubation temperature to the virulence of *P. ultimum* (Stock culture no. 77) on potato varieties was examined (Table 7). Virulence was increased as incubation temperature increased from 20° to 28°C. The affected part in all varieties used did not give browning, but gave soft-rotting with light yellow color. Even in Kennebec, relatively less affected variety at the beginning than the others, the decayed part showed light yellow color and did not turn brown. Finally the tuber (Kennebec) became soft throughout. All the potato varieties used were susceptible to this fungus.

Discussion

The genus *Pythium* is able to attack many kinds of plants and generally shows strong pathogenicity. But pathogenicity varies considerably with the species of *Pythium*. It remained to be proved that this pathogenic variation may be derived from the differences in susceptibility of the hosts or from the genetic characters of the species of *Pythium* or both. Although the resistant reaction of squash (Amakuri) to *P. ultimum* can be interpreted by the formation of antifungal protein-like substance(s) as a result of infection (4), there is no definite conclusion on the relationship between resistant reaction of Amakuri and pathogenicity of *P. ultimum*. In the present paper, this relationship was discussed using potato tuber and sweetpotato root.

As the incubation temperature increased, disease severity of all the tubers used was equally increased and mycelial extension was not depressed. Even in Kennebec, relatively less affected variety at the beginning than the others, the decayed part became finally soft throughout. As has been indicated in previous paper (5), the potato varieties employed can not also be used as the experimental materials for disease resistance.

On the other hand, there were distinct varietal differences of sweetpotato root in disease resistance. Gokoku was resistant to *P. ultimum* under any given incubation temperature, and Norin (Nos. 1, 4, 6, 10, 16), Kokei No. 14 and Kanto No. 23 were highly susceptible under these conditions. Other varieties tested were generally susceptible at relatively low temperature (20°C) and intermediately or highly resistant near the optimum temperature for the root growth.

To discuss the mechanisms responsible for disease resistance in plants, the hosts should be classified according to their degree of resistance and the ecological characteristics of pathogenicity of the fungus should also be determined. The sweetpotato roots were found to be useful as the experimental materials to disease resistance.

P. monospermum inhibits germination of rice plant (2), but it is nonpathogenic on cabbage head (1). *P. monospermum* as well as *P. vexans* is obviously infectious to tomato, *Hibiscus manihot* and morning-glory seedlings, but their aggressiveness is remarkably weak (6) and and these results were confirmed by this experiment. These fungi were also highly pathogenic on potato tuber, but they were not parasitic on sweetpotato root. *P. monospermum* and *P. vexans* were isolated from insect cavader in water (3) and from swampy land, respectively, and they were classified as weakly pathogenic fungi, distinguishing from such highly pathogenic fungi as *P. aphanidermatum* and *P. ultimum* (6). But *P. monospermum* and *P. vexans* showed strong virulence on potato tuber and this is naturally possible for *P. monospermum*, since it will show a clear virulence on cucumber seedlings in wet soil and it also inhibits germination of rice plant (3).

Summary

1. All of the *Pythium* used, except *P. monospermum* and *P. vexans*, was highly pathogenic

on tomato, watermelon, *Hibiscus manihot* and morning-glory seedlings. Although *P. monospermum* and *P. vexans* failed to infect or were considerably weakly pathogenic on the seedlings of squash (Amakuri), kidney bean and rice plant, they were highly pathogenic on sweetpotato root and potato tuber tissues.

2. The root tissue of sweetpotato (Norin No. 1) was highly susceptible to different species of *Pythium*.

3. Sweetpotato root (Gokoku) was resistant to *P. ultimum*, whereas the roots (Norin Nos. 1, 4, 6, 10, 16; Kokei No. 14; Kanto No. 23) were highly susceptible. Such roots as Norin No. 3, Kokei No. 3, Kanto (Nos. 31, 32, 33, 34, 35) and Hichi-fuku became resistant to the fungus near the optimum temperature for their growth. Minimum and maximum temperatures for disease development were below 10°C and above 36°C, respectively.

4. *Pythium* with the exception of *P. spinosum* and *P. ultimum* (Stock culture no. 11) was generally pathogenic on potato tuber. All the potato tuber varieties used were susceptible to *P. ultimum*.

Literature cited

1) DRECHSLER, C. (1925). *Phytopath.* **15**: 482–485.
2) ITO, S. and TOKUNAGA, Y. (1933). *J. Fac. Agr. Hokkaido Imp. Univ.* **32**: 201–233.
3) TAKAHASHI, M. (1952). *Ann. Phytopath. Soc. Japan* **16**: 19–22.
4) TAKAHASHI, M. (1958). *Bull. Univ. Osaka Pref.* Ser. B, Vol. **8**: 127–167.
5) TAKAHASHI, M. *et al.* (1970). *Kansai Byotyu Kenkyu Kaiho* **12**: 77–78.
6) TAKAHASHI, M. and MORIMOTO, T. (1954). *Forsch. Gebiet Pflanzenkrankh.*, Kyoto, **5(1)**: 18–21.
7) YAMAMOTO, M. and OZOE, M. (1956). *Ann. Phytopath. Soc. Japan* **21**: 63–67.