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Fusarium Root Rot and Wilt of Garden Peas in Taiwan[1]

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Abstract : In Taiwan, garden pea (*Pisum sativum*) is severely infected by a disease with symptoms of root rot, breakdown of vascular tissues, and wilt of the infected plants. This disease has been identified as either a wilt [caused by *Fusarium oxysporum* f. sp. *pisi* (FOP)], or a root rot [caused by *F. solani* f. sp. *pisi* (FSP)], or a disease complex in which wilt and root rot act together in the field. The major isolates of FOP isolated from field specimens in Taiwan caused 'near wilt' symptoms on inoculated pea plant in a greenhouse, and had a similar cultural characteristics to FOP race 2 when grown on PDA medium. However, the cultivar New Season which is reported by other workers to be resistant to race 1, 2, and 6 of FOP, was susceptible. Conversely, the cultivars Mini and New Era, which were reported susceptible to race 5, were resistant to this isolate. Therefore, our isolate may be a new race of FOP. Isolates of FSP with long macrospores could be obtained frequently from the vascular tissues of the upper stem of diseased plants under fairly high temperature conditions of 23°C or more. This particular group of FSP isolates caused severely root rot and vascular discoloration of the upper stem in several accessions/cultivars of peas known to be resistant to FSP or FOP race 2. The results thus indicated that this group of isolates were highly virulent ones of FSP with the ability to invade cortical and vascular tissues.

Garden pea (*Pisum sativum*) was, in the past, a minor winter crop in Taiwan and there were few records of any disease. Recently, however, the cultivation acreage has increased rapidly as a result of a new export trade in fresh pea pods, and reached about 8,000 ha. (16). Many early pea crops are sown during the hot summer months from August to October, and many formerly undiagnosed diseases have thus now become significant. Field observations from 1981 to 1983 in central and southern Taiwan revealed that pea plants were heavily damaged by a disease named 'pea likubin' (Fig. 1) by farmers . The disease bacame particularly serious after the new cultivar TC-11 had been released for commercial production since 1979. It began with rot around the cotyledon attachment area, then progressed up to the soil line and down to the tap root, and

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ended with leaf yellowing, vascular discoloration, and complete wilt of the plant. The brick red vascular discoloration occurred not only in the root, but also in the upper stem. The disease was particularly common in the early crop of peas grown in fields with no rice rotation. Diseased plants were scattered throughout the field, rather than concentrated in patches. Isolations from the many field specimens consistantly yielded both *Fusarium solani* f. sp. *pisi* (Jones) Snyd. & Hans. (FSP) and *F. oxysporum* f. sp. *pisi* (Linford) Snyd. & Hans. (FOP), from both diseased roots and stems. It seems, therefore, that both FSP and FOP were acting together in the field to produce the 'pea likubin' disease. However, the fact that FSP could be isolated so frequently from the upper stem of diseased plants was unexpected, because this fungus was thought to be a cortical decay pathogen which did not usually move up the stem above the soil line [3,4,8,9,13]. Eleven races of the wilt fungus (FOP) had been previously described [1], but only races 1, 2, 5, and 6 were considered by Kraft and Haglund to be valid [10]. In addition, the resistance of pea cultivars to races 1, 2, 5, and 6 seems to be determined by single, separate dominant genes in the host. We, therefore, studied the pathogenicity of FSP isolated from vascular tissues of upper stems, and the race identification of FOP found in peas in Taiwan.

Materials and Methods

Sources of seed.-The pea cultivars used in this investigation were : Dark Skin Perfection, Mini, New Era, New Season, WSU-23, 74SN5, 792022, P. I. 257593 (supplied by Dr. J. M. Kraft from Washington, USA), TC-9, TC-11 and Native White (supplied by Taichung District Agricultural Improvement Station, Taiwan).

Isolation of the pathogens.-Diseased plants were dug up at random from pea fields , and washed in running tap water in the laboratory. Symptoms of root rot, leaf yellowing and vascular discoloration were examined and recorded. Pieces of diseased tissue(1 cm long) of root, and the vascular tract of the upper stem from at least 6 cm above the ground, were surface sterilized in a solution of sodium hypochlorite (5.25%) and alcohol (95%) for 45 sec, then placed on PCNB [12] and potato dextrose agar (2%) and incubated at room temperature (22—30°C). Single spores were isolated on PDA and incubated at 24°C, under fluorescent light 12 hr a day. The cultural characteristics were recorded. All isolates were stored in autoclaved soil for further studies.

Pathogenicity tests.-The pathogenicity of each isolate of *F. oxysporum* was determined by inoculating the differential cultivars (Table 2) by using the root prune and dip technique [5]. When seedlings grown in perlite were 10 days old and had produced 4—5 nodes, they were pulled out of the perlite and their roots cut short approximately 4 cm below the cotyledon attachment area. The seedlings were then dipped into the spore suspension (10^5 spores/ml) so the cotyledons were covered, transplanted into sterile perlite, and watered with a Hoaglund's solution. The temperature in the growth chamber (model S10H, Conviron) was maintained at 24°C, with a 12-hr photoperiod and 15,000 lux of light per day. The number of dead plants was read 35-42 days after inoculation.

The pathogencity of each isolate of *F. solani* was determined by inoculating each

of the cultivars (Table 4) by using infested soil. A mixture of virgin soil (loam) and river sand (3 : 1, v/v) was infested with spore suspension of *F. solani* and incubated in a greenhouse for 10 days. The infested soil had an inoculum density of 10^5 propagules/g of soil assayed on PCNB medium. The inoculum density of the infested soil was then adjusted to 10^4 or 10^3 propagules/g of soil by adding clean soil. Surface-sterilized seeds were then sown, and incubated in growth chambers at different temperature (12, 20, 24, 28, or 33°C). Disease readings were taken 42 days after inocluation. The degree of root rot was rated on a scale ranging from 0 (=healthy) to 4 (=roots with severe decay extending up to the basal stem or down to the tap root) (see Table 3). Vascular discoloration in the root and upper stem was also recorded. Tissues of all diseased plants were placed on PCNB medium, in order to again isolate the pathogen.

Results

Isolation of pathogens.-Thousands of field specimens collected from central and southern Taiwan were examined, and hundreds of them were subjected to isolation of the pathogens. *F. solani* and *F. oxysporum* sensu Snyder and Hansen[15] were consistantly isolated from both diseased roots and the diseased vascular tract of upper stems. In fields where no rice rotation had been practiced, the ratio of *F. solani* to *F. oxysporum*, isolated from the vascular tract of the upper stem at least 6 cm above the ground, was 4 : 5. However, the same value in fields where rice was part of the crop rotation system was 7 : 1. According to macrospore charactreristics and pigmentation of cultures grown on PDA, the isolates of *F. oxysporum* fell into 3 categories (see Table 1). Those belonging to the first group (FO-A)were most common, and similar to *F. oxysporum* f. sp. *pisi* race 2 in terms of cultural appearance. The second group (FO-B) was much smaller, and may be a varient, since it could be obtained from old cultures of group FO-A. The last group (FO-C) seemed to comprise saprophytic fusaria. The isolates of *F. solani* definitely fell into 2 groups (Table 1). Group FS-L possessed long macrospores, while group FS-S characterized by short macrospores (Fig. 4).

Table 1. Frequency and characteristics of *Fusarium oxysporum* (FO) and *F. solani* (FS) isolated from different parts of diseased pea plants.

Group designation	Source of isolate	Frequency (%)	Type isolate	Characteristics on PDA medium				
					Macrospores			Pigmention
					Number	Size (u×u)	Septum	
FO-A	Diseased stem	54	FOP-73	Sporodochia	Many	33.0×5.0	2—3	Dark purple
FO-C	Diseased stem	8	FOP-17	Ropy	Scarce	29.5×5.0	2—3	Purplish red
FO-B	Diseased stem	38	FO-1	Sporodochia	Many	37.5×5.0	2—3	Brown
FS-L	Diseased stem & root	31	T-4	Pionnotes	Many	46.8×4.7	3—4	Blue green or reddish purple
FS-S	Diseased stem & root	69	D-4	Pionnotes	Many	32.6×4.7	3—4	Buff, cream or brown

Race identification of FOP.-Many isolates of *F. oxysporum* from pea plants were used in the inoculations. However, isolates FOP-73, FOP-17 and FO-1 were chosen to represent group FO-A, FO-B and FO-C, respectively. The results are shown in Table 2. Isolate FO-1 was not pathogenic to peas, and was not thought to be a pathogen of peas. Isolates FOP-17 and FOP-73 caused symptoms of near wilt including curling and chlorosis of leaves and stipules, lateral wilt, and a brick red discoloration of the vascular tract which symptoms extended to the top of the plant (Fig. 2). The two pathogenic isolates had a similar effect on differential cultivars (Table 2), although they varied in macrospore production and pigmentation on PDA medium (Table 1). The reactions were similar to those of FOP race 2 sensu Kraft and Haglund [10], except that cultivar New Season was susceptible to the isolates found in Taiwan.

Pathogenicity tests of FSP.-In preliminerary tests using the soil infestation method, isolates of FSP in group FS-L (long macrospores) were more virulent to peas than those in group FS-S (short macrospores). The former group caused severe root rot and stem vascular discoloration (Fig. 3) whereas the latter caused only slight or moderate root rot (Table 3) . IsolateT-4 and D-3 were chosen to represent each group in further studies.

Table 2. Percentage of dead pea plants 35—42 days after inoculation with different isolates of *Fusarium oxysporum* in a growth chamber at 24°C.

Cultivar	Isolate			Race of FOP[a]			
	FO-1	FOP-17	FOP-73	1	2	5	6
Dark Skin Perfection	0	80(S)	90(S)	R	S	S	S
Mini	0	0(R)	0(R)	S	R	S	S
New Era	0	30(R)	30(R)	R	R	S	S
New Season	0	80(S)	100(S)	R	R	S	R
WSU-23	0	20(R)	0(R)	R	R	R	S
TC-11	0	100(S)	100(S)				

a. Race concept of *F. oxysporum* f. sp. *pisi* according to W. A. Haglund and J. M. Kraft (7). S=Susceptible ; R=Resistant.

Subsequent inoculations in the greenhouse or growth chamber (model S10H, Conviron) revealed that isolate T-4 caused severe root rot in peas (cultivar TC-11) at a soil temperature of 20°C or above, but not at 12°C. It also caused vascular discoloration of the upper stem at 28°C or more, but not at 20°C or less. Isolates D-3, however, caused only slight root rot, and no vascular discoloration occurred at any of the temperatures tested (Table 3). The optimum inoculum density for disease development on peas inoculated with isolated T-4 and grown in greenhouse at a temperature of 28—33°C, was 10^3 propagules/g of soil. The inoculated plants started to show root rot symptoms 2 wk after inoculation, and reached severe decay at 4 wk, but vascular discoloration of the stem did not appear until 6 wk after inoculation.

Table 3. Effect of temperature on disease development in pea plants (cultivar TC-11) inoculated with isolates T-4, D-3, and D-4 of *Fusarium solani* (10^4 propagules/g of soil) and incubated in a growth chamber 42 days after inoculation.

Temp.[c] (°C)	Isolate	Root rot[a] index	Vascular discoloration (%)		Reisolation (%)[b]			
					Root		Stem	
			Root	Stem	FS-L	FS-S	FS-L	FS-S
33	T-4 (FS-L)	4.0	90	70	—	—	—	—
28		3.9	73	43	100	18	50	10
24		3.8	43	7	—	—	—	—
20		3.8	6	6	—	—	—	—
33	D-3 (FS-S)	1.8	0	0	—	—	—	—
28		1.3	0	0	0	89	0	33
24		1.2	0	0	—	—	—	—
20		0.4	0	0	—	—	—	—
33	D-4 (FS-S)	3.5	30	0	—	—	—	—
28		3.2	17	0	0	63	0	84
24		2.1	0	0	—	—	—	—
20		2.2	0	0	—	—	—	—
33	CK	1.4	0	0	—	—	—	—
28		1.0	0	0	0	33	0	0
24		0.6	0	0	—	—	—	—
20		0.3	0	0	—	—	—	—

a. 0=Healthy ; 1=Small lesion on cotyledon node ; 2=Large lesion on cotyledon node ; 3=Root decay, extending up to basal stem or down to tap root ; 4=Severe decay and death of plant.
b. FS-L=Long-macrospore FSP ; FS-S=Short-macrospore FSP.
c. All inoculated plants at 12°C are healthy in all treatments.

In further inoculation tests, surface-sterilized seeds of several pea cultivars grown commercially in Taiwan (TC-9, TC-11 and Native White), and several more accessions/cultivars reported either resistant (P. I. 257593), tolerant (74SN5, and 792022) or susceptible (New Season and WSU-23) to FSP, were sown in a soil which had been infested with isolate T-4 or D-3 (10^4 propergules/g of soil), and incubated at 28°C in a growth chamber for 6 wk. The results (Table 4) showed that none of the accessions/cultivars tested was resistant to isolate T-4, in that all showed symptoms of root rot. All diseased plants also showed a high level of vascular discoloration in both root and stem, except accessions P. I. 257593 and TC-9, which showed some resistance. Isolate D-3 caused only slight root rot in all tested cultivars. Reisolation of the pathogen revealed that long-macrospore FSP was obtained mainly from the stems of diseased plants inoculated with

isolate T-4. However, short-macrospore FSP was isolated mainly from roots, and in a few cases from stems, of diseased plants inoculated with isolate D-3 and T-4.

Table 4. Results of inoculation and reisolation of different cultivars of pea plants inoculated with isolates T-4 and D-3 of *Fusarium solani* f. sp. *pisi* (FSP) in growth chamber (28°C) 6 weeks after inoculation.

Isolate	Cultivar	Root rot[a] index	Vascular discoloration (%)		Reioslatation (%)[b]			
					Root		Stem	
			Root	Stem	L.	S.	L.	S.
T-4	P. I. 257593	3.8	29	7	100	0	0	9
	792022	4.0	60	60	75	75	60	20
	74SN5	4.0	100	88	100	0	12	24
	WSU-23	4.0	100	100	100	100	—	—
	New Season	4.0	100	100	100	0	100	0
	TC-9	3.2	40	40	100	25	22	0
	TC-11	3.8	92	79	100	0	50	0
	Native White	3.6	91	73	100	0	28	28
D-3	P. I. 257593	0.5	0	0	0	90	0	0
	792022	2.0	22	0	0	77	0	55
	74SN5	1.4	0	0	0	50	0	36
	WSU-23	2.4	20	0	0	100	0	0
	TC-9	0.9	0	0	0	100	0	0
	TC-11	1.0	0	0	0	55	0	84
	Native White	1.8	0	0	0	87	0	14
CK	TC-11	1.2	0	0	0	7	0	0

a. 0=Healthy ; 1=Small lesion on cotyledon node ; 2=Large lesion on cotyledon node ; 3=Root decay, extending up to basal stem or down to tap root ; 4=Severe decay and death of plant.

b. L=Long-macrospore FSP ; S=Short-macrospore FSP.

Discussion

Results from the field survey and inoculation tests indicate that 'pea likubin', the major disease of garden peas in Taiwan, is either wilt (caused by *F. oxysporum* f. sp. *pisi*) or a root rot disease (caused by *F. solani* f. sp. *pisi*), or a disease complex in which both wilt and root rot act together in the field.

Fusarium wilt of peas has been reported from many countries[9], and can be controlled by growing resistant cultivars[10]. Many physiological races of the pathgen have been described. Linford[11] first reported wilt of peas in Wisconsin in 1928. The causal

organism was reclassified as *F. oxysporum* f. sp. *pisi* race 1 by Snyder and Hansen [15]. Another race which caused 'near wilt' was described by Snyder [14] in 1933, and was designated as race 2. In 1951, Schreuder (cited in 3) reported race 3 in the Netherlands. Later, Buxton [3] found a race similar to Schreuder's race 3. In 1966, Bolton *et al.* [2] described race 4 in Canada. In 1970 and 1979, Haglund and Kraft [5,6] described races 5, and 6, respectively, in northwest U. S. A.. Howerer, in 1974, *F. oxysporum* f. sp. *pisi* was reclassified into 11 races, including those previously described as 1, 2, and 5 by Armstrong and Armstrong [1]. Later, Kraft and Haglund [10] found that these 11 races varied only in virulence, not in specific pathogenicity, and all were divided into either race 1 or 2. Kraft and Haglund [10] insisted that only race 1, 2, 5 and 6 are valid, and stated that it was essential to use pea cultivars with known genes for resistance as differentials, together with standard inoculation procedures, in evaluating pathogenic forms of *F. oxysporum* f. sp. *pisi*. According to Kraft and Haglund's race concept of pea wilt pathogens, the present study used the cultivar Mini, known resistant to race 2 and susceptible to race 1, 5, and 6 (Kraft, personal communication), and a further 4 of the 8 differential cultivars used by Haglund and Kraft [6]. The inoculation tests were conducted following the standard inoculation procedures described by Haglund and Kraft [5].

The results showed that isolate FOP-17 and FOP-73 were both pathogenic, but could not be distinguished in terms of their effect on differential cultivars. A comparison of wilt reaction in the differential cultivars to race 1, 2, 5, and 6 (based on published information) with those to our isolates (Table 2) revealed that our isolates could not be grouped into any reported race. Our isolates had similar cultural characteristics and reaction types to those of race 2, on all cultivars tested except New Season. The death of 80% and 100% of test plants of the cultivar New Season inoculated with isolates FOP-17 and FOP-73 respectively (Table 2), indicates that these isolates have specific pathogenicity to this cultivar, which, however, had been reported resistant to race 1, 2, and 6 [6]. The *F. oxysporum* f. sp. *pisi* occurring in Taiwan may, therefore, be a new race.

Since Jones [8] first described the root rot in peas, many workers [3,4,9] have agreed that the disease is characterized by cortical decay and a brilliant red discoloration of diseased vascular tissues in the root. This discoloration does not normally extend up to the stem, because FSP is not a vascular parasite. Hilderth [7] reported that he had obtained an isolate of FSP which could invade the upper stem of the pea plant far beyond its normal cotyledonal region, with a vascular penetration similar to that of near wilt caused by FOP race 2. In our studies, isolates of this pathogen with long macrospores (group FS-L) were frequently obtained from vascular tracts in the upper stems of field specimens. Most were found in stem at least 6 cm above the ground, and sometimes, at the top of the plants. The possibility of internal movement as a result of insect damage was carefully eliminated. Our inoculation results (Table 3) indicated clearly that temperatures of 28°C or more were essential for long-macrospore FSP, but not for short-macrospore FSP (group FS-S), to induce vascular discoloration in both roots and

stems. Inoculated plants incubated at 20°C showed root rot but less vascular discoloration in the root and stem. This effect of temperature on symptom expression accounts for the vascular discoloration seen in 'likubin' disease in pea crops in Taiwan, which reach the seedling stage when temperature are high. The seeding date of the early pea crop in fields which do not grow rice as part of the crop rotation is from August to October. Average daily temperatures during this period are around 24-28°C. The degree of damage naturally depends on the temperature levels which follow. Moreover, several P. I. accessions/cultivars known to be resistant to FOP race 2 or FSP, after inoculation with isolate T-4 (long-macrospore FSP), were severely attacked, and showed clear vascular discoloration in the upper stem. The two exceptions were P. I. 257593 and TC-9, which showed some degree of resistance (Table 4). The results also indicated that most pea cultivars introduced from U. S. A. could not have good growth and the resistance to FSP might be overcome at higher temperatures (above 28°C). Reisolation from the diseased upper stem again yielded long-macrospore FSP, and in a few cases short-macrospore *F. solani* (Table 3, 4). The former was the main cause, and the latter might be one of the organisms in natural soil activated by the treatment of this inoculation experiment. The results indicate that our long-macrospore FSP is not FOP race 2, but a very strongly virulent isolate of FSP with the ability to invade cortical and vascular tissues under high temperatures.

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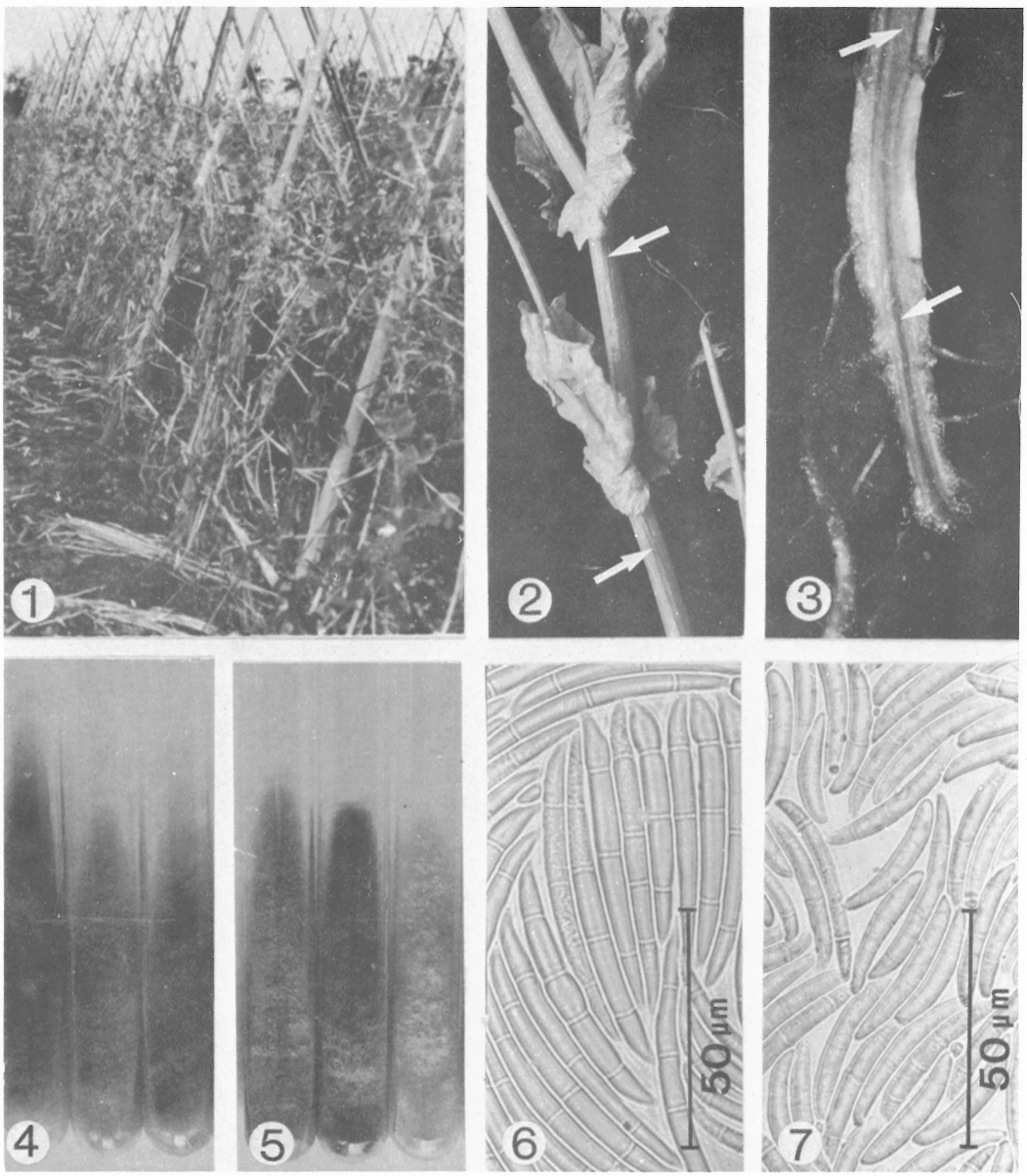


Fig. 1. Garden peas severely damaged by 'pea likubin', identified as a wilt (caused by *Fusarium oxysporum* f. sp. *pisi*) or root rot disease (caused by *F. solani* f. sp. *pisi*), or a disease complex in which wilt and root rot act together in the field.

Fig. 2. Pea wilt (cultivar TC-11) caused by *Fusarium oxysporum* f. sp. *pisi* consisting curling and chlorosis in leaves on one side of the plant and vascular discoloration (arrows).

Fig. 3. Pea root rot (cultivar TC-11) caused by *Fusarium solani* f. sp. *pisi* consisting root rot and vascular discoloration in the root and stem (arrows).

Fig. 4. Isolate T-4 of *Fusarium solani* f. sp. *pisi*.

Fig. 5. Isolate D-3 of *Fusarium solani* f. sp. *pisi*.

Fig. 6. Macrospores of isolate T-4 of *Fusarium solani* f. sp. *pisi*.

Fig. 7. Macrospores of isolate D-3 of *Fusarium solani* f. sp. *pisi*.

臺灣豌豆根腐及萎凋病[1]

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摘 要

臺灣豌豆嚴重感染一種病害，引起罹病植株根腐，維管束組織褐化以及萎凋等病徵。本病害被鑑定爲萎凋病（病原菌爲 *Fusarium oxysporum* f. sp. *pisi*）或根腐病（病原菌爲 *F. solani* f. sp. *pisi*）或者萎凋病與根腐病在田間複合感染所致。由田間標本分離所得之萎凋病菌，在溫室接種試驗中引起豌豆擬萎凋病徵，並且在馬鈴薯瓊脂培養基上表現與擬萎凋病菌（*F. oxysporum* f. sp. *pisi* race 2）相似之培養性狀。然而接種試驗顯示，被報告對萎凋病菌之 race 1, 2 及 6 具抵抗性的 'New Season' 品種却對供試菌株呈感病性。又被報告對萎凋病菌 race 5 具感病性之 'Mini' 及 'New Era' 品種却對供試菌株呈抵抗性反應。因之，我們的菌株似乎是萎凋病菌新出現的生理小種。從高溫（28°C 以上）下生長之病害標本，常可在病變的莖部維管束組織分離到具有長形大胞子的根腐病菌。接種試驗顯示這一羣的根腐病菌可在若干對根腐病及擬萎凋病呈抵抗性之品種(系)引起根腐及莖部維管束褐化等病徵。顯然這一羣特殊的根腐病菌病原性極强，並且有能力侵害皮層及維管束組織。

1.臺灣省農業試驗所 研究報告 1188 號。轉載自 'Soilborne Crop Diseases in Asia', FFTC Book Series No. 26. 本計畫承行政院農業發展委員會補助及 Dr. J. M. Kraft 提供豌豆種子與建議，謹致謝意。

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