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Control of Pythium Root Rot of Vegetable Pea Seedlings in Soilless Cultural System

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ABSTRACT

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This study was initiated to develop non-pesticide methods to control Pythium root rot of vegetable pea seedlings in a soilless cultural system. The disease was not prevalent when new seedling trays were used for growing pea seedlings. However, when the cultural trays were used repeatedly for the same purpose, mycelia and oospores of *Pythium aphanidermatum* and *P. ultimum* produced by the infected roots contaminated the trays and became an important inoculum source to cause severe root rot. Drying the used trays under sunlight for months or immersing the trays in a calcium hypochlorite solution (2,000 ppm) for 24 hours before planting was effective in preventing this disease. Incidences of the disease were drastically reduced from 60-80% to less than 10% and yields of pea seedlings averaged 772 g/tray to 212 g/tray when the used trays were pre-treated with calcium hypochlorite solution comparing to the non-treated controls during hot growing season.

Key words : *Pisum sativum*, *Pythium aphanidermatum*, *P. ultimum*, inoculum sources, disinfectant, calcium hypochlorite, control

INTRODUCTION

Pea seedlings (*Pisum sativum* L.) were produced commercially as vegetable in a soilless cultural system in recent years in Taiwan [3,5,10]. However, a severe root rot soon occurred and damaged the industry to an extent that many farms were temporarily or permanently abandoned. Infected seedlings died or were severely stunted. *Pythium aphanidermatum* (Edson) Fitzp. in summer and *P. ultimum* Trow in winter were found to be associated with the root rot [6]. Although some fungicides are available to provide a high degree of disease control [4,7,16], they are not registered for use on vegetable pea seedlings in Taiwan. Therefore, there is an urgent need to develop a suitable practice for disease management. We initiated the study to determine the main inoculum sources of pathogens in the soilless cultural system and to develop a non-pesticide control method for this disease.

MATERIALS AND METHODS

Cultivation of vegetable pea seedlings

About 500-600 g of 'Dun peas' or 'Dundale pea' seeds imported from Australia were soaked in running water for 12-18 h, and then evenly distributed into one layer over the cultural substrate, such as sawdust, in a seedling tray (60 X 30 X 3 cm). For seed germination and seedling growth, the trays with pea seeds were incubated at high humidity in dark for 4 days and then moved to a shaded plastic-sheet-house with 50-60% sunlight. Greening of young seedlings required 4 days of incubation in summer and more than 8-12 days in winter. When the seedlings had grown up to 10-12 cm, the shoots were harvested by cutting at the basal stems. After harvesting, the used cultural substrate was discarded and the cultural trays were used repeatedly for the next plantings.

Determination of inoculum sources of the root rot

Samples were taken from underground water in reservoir, pea seeds (Dun peas from Australia), cultural substrate (sawdust), root debris left over on the used cultural trays, and soil dust from the ground of a commercial farm at Wu-feng and were subjected for isolation of *Pythium aphanidermatum* and *P. ultimum*. The water samples were assayed by baiting techniques, with pea seeds, cucumber fruit or potato tuber slices as baits. Five hundred grams of pea seeds were collected randomly from 10 seed lots and were placed on water agar plates at 24℃ for 2-4 days. Then the plates were examined for growth of *Pythium* spp. under

microscope. Samples of sawdust and root debris were also plated on water agar medium. Sixteen samples of soil dust were randomly collected from the ground of the plastic-sheet-house, and dried in incubator at 28℃ for 3 days. Suspensions of the dried samples were prepared in dilution series and subjected to isolation and quantitative analysis of *P. aphanidermatum* and *P. ultimum* on the species-selective medium [2] and water agar [12], respectively. The hyphal tips were cut from the fungal colonies and transferred onto 2% water agar, potato carrot agar and V-8 agar for further identification of *P. aphanidermatum* and *P. ultimum* under microscope [17].

To determine the relative importance of the different cultural materials as sources of primary and secondary inocula, a growing-on experiment was conducted, with the following combinations of treatments: (A). Autoclaved underground water and sawdust, new cultural trays and non-treated pea seeds. (B). Surface sterilized pea seeds, autoclaved sawdust, new cultural trays and non-treated underground water. (C). Autoclaved underground water, new cultural trays, surface sterilized pea seeds and non-treated sawdust. (D). Autoclaved underground water and sawdust, surface sterilized pea seeds and non-treated used cultural trays. (E). Autoclaved underground water and sawdust, surface sterilized pea seeds and new cultural trays. (F). Non-treated underground water, pea seeds, saw dust and used cultural trays to serve as check.

There were 3 replications for each treatment combination and the experiment was conducted in greenhouse (25-35℃, RH 55-92%) at National Chung Hsing University. The data of disease incidence, frequency of pathogen isolated and yield were collected 8 days after seeding [6].

Management of root rot of pea seedlings by using air-dry sanitation on used trays

The purpose of this experiment was to determine the effect of air-drying treatment on the root rot caused by the inocula survived in the sawdust and root debris stuck in draining pores of used trays. Mycelial discs of a 72 hour old V-8 agar cultural plate of *P. aphanidermatum* (isolate P324) were put in a 250 ml flask containing 0.5 g of oat powder and 100 ml of potato dextrose broth, and incubated at 24℃ for 14 days. The mycelial mats were harvested, rinsed twice with distilled water in warning blender (National Co., Taiwan), and mixed with 150 g of sawdust as cultural substrate and put on new cultural trays for the growth of pea seedlings in the greenhouse. After harvesting, isolations were made to determine if *P. aphanidermatum* was present in the root debris left over on the infested cultural trays. The cultural trays carrying root debris and sawdust residues were piled up and air-dried in greenhouse. Ten trays were taken randomly at 0, 2, 4, 7, 15, 30, 60, 90 and 120 days after incubation and used for growing pea seedlings in growth chamber (28℃ at day and 24℃ at night; RH 85%; Fame F-860 DNH, Taiwan). Before each planting, samples of root debris on the cultural trays were also taken for measuring their water content and for determining pathogen density with the selective medium [2]. During each planting, fifty pea seedlings were collected daily for isolation of pathogens. The disease incidence and yield were recorded at harvest time, 8 days after seeding. Plants cultivated on the new trays served as control. There were 4 replications for each treatment and the experiment was repeated once.

Effect of chemicals on root rot of pea seedlings

Two disinfectants (for sanitation purpose), namely 5.25% sodium hypochlorite (Clorox, Clorox Co., USA) and 5% calcium hypochlorite (Chung Tai Chemical Co., Taiwan), and two fungicides namely etridiazole (5-Ethoxy-3-trichloromethyl-1,2,4-thiadiazole) 25% FP, Uniroyal Chemical, U. S. A. and metalaxyl MZ (1. Methyl D,L-N-(2,6-dimethyl-phenyl)-N-(2'-methoxyacetyl)-alaninate 10%; 2. Ionic Coordination of Zinc and Manganese Ethylenebisdithio-carbamate 48%) 58% WP, Novartis Crop Protection Ag, Switzerland, were tested for their efficacy to inhibit the mycelial growth of *P. aphanidermatum* and *P. ultimum* on water agar in the laboratory and to control Pythium root rot of pea seedlings under the field conditions at Wu-feng farm.

The tested chemicals were incorporated into water agar respectively, and diluted to concentrations of active ingredient ranging from 0-1,000 ppm. The plates were inoculated with a mycelial agar disc (5 mm in diameter) of *P. aphanidermatum* (P324) or *P. ultimum* (P322) taken from the colony margins of a 10 day old culture. The inoculated Petri dishes were incubated at 25℃ for 5 days. The linear mycelial growth was measured every day after inoculation. Four Petri dishes were used for each concentration of the tested chemicals.

The pathogen contaminated trays infested with plant debris were immersed in a solution of sodium hypochlorite, calcium hypochlorite, etridiazole, metalaxyl-MZ or metalaxyl-plus (concentrations ranged from 0 to 2,000 ppm) in a 500-liter plastic tank for 24 h. Then the treated trays were used for growing vegetable pea seedlings at Wu-feng farm. New trays (pathogen-free), non-treated infested trays and the ones immersed in water were also used for growing pea seedlings to serve as control. The disease incidence and yield were recorded at 8 days after seeding. There were 4 replications for each treatment and the experiment was repeated twice in summer.

Control of root rot of pea seedlings at Wu-feng farm

There were a total of about 12,000 cultural trays used for growing pea seedlings at Wu-feng farm. A solution of calcium hypochlorite at 2,000 ppm was prepared in a small pond of 8 X 5 X 1 m. All the used trays, except 30 of them to serve as control, were treated by immersing in the disinfectant solution as described before for 24 h, removed and dried for another 24 h before each use. The disease incidence, pathogen

isolation frequency and yield of pea seedlings on the treated and the 30 non-treated cultural trays (control) were surveyed and recorded during the experimental period (from July 1992 to May 1993).

Degradation of hypochlorite ion in calcium hypochloride solution

Two hundred liters of calcium hypochlorite solution at 0, 500, 1000 and 2000 ppm were prepared in big plastic tanks in greenhouse. The infested cultural trays taken from Wu-feng farm were immersed in the solutions. Another set of tanks containing tested chemical, but with no cultural trays being immersed served as control. The solutions in tanks were stirred every day and 50 ml of them were taken from each tank after stirring. The samples were subjected for detection of hypochlorite ion concentrations by iodimetric titration method [11].

RESULTS

Determination of inoculum sources of the root rot

The isolation tests on the cultural materials revealed the presence of abundant propagules of *P. aphanidermatum* and *P. ultimum* in root debris left over on the used cultural trays (12,661 p/g) and in soil dust (4,605 p/g). But, only few propagules (less than 0.5 p/g) of pathogens were found in fresh sawdust and none in underground water or pea seeds (Table 1). The inoculation tests also revealed that the root debris left over on the used trays was the only source material that could produce severe disease, yield reduction and a high pathogen recovery rate of vegetable pea seedlings. However, few pathogens could be isolated from roots of vegetable pea seedlings when sawdust was used as growing medium (Table 2).

Management of root rot of pea seedlings by using air-dry sanitation on used trays

The water content of fresh root debris contaminating the used cultural trays was as high as 83%, and numerous oospores, mixed with mycelia, of *P. aphanidermatum* were observed inside the root tissue. After air-drying in greenhouse for 30-120 days, the water content decreased to about 11-12% and only the oospores, mixed with a few sporangium-like structures, were observed in the dried root debris. The pathogen densities and germination rates of oospore in root debris determined with the selective medium [2] also decreased drastically. The used trays infested with plant debris were reused for growing pea seedlings in growth chamber. The results indicated that *P. aphanidermatum* could be easily isolated from plants 2-3 days after seeding, when the root samples were taken from trays that had been air-dried for less than 15 days in greenhouse. However, the pathogen could be only detected in plants 4 days after seeding when trays were air-dried for more than 30 days before planting. In either case, the pathogen isolation rates were over 76% at harvest time (8 days after seeding). This was true in all combinations of treatments involving air-drying of cultural trays. Moreover, the yields of pea seedlings from trays infested with fresh or

Table 1. Isolation frequency of *Pythium* spp. from cultural materials for pea seedlings in a soilless cultural system at Wu-feng farms

	Pathogen density (propagules/ml or g substrate)			
	Farm 1		Farm 2	
Material	P.a .1	P.u .	P.a.	P.u.
Underground water	0.0	0.0	0.0	0.0
Pea seed	0.0	0.0	0.0	0.0
Sawdust	0.2	0.0	1.5	0.0
Root debris	1297.0	3366.0	463.0	12667.0
Soil dust from ground	4605.0	70.0	913.0	3225.0

[1] P.a.=*P. aphanidermatum* ; P.u.=*P. ultimum*

Table 2. Effect of autoclaving and surface sterilization of cultural materials on Pythium root rot and yield of pea seedlings in a soilless cultural system

Combination of treatment	Cultural materials [1]				Disease incidence (%)	Yield (g/tray)	Isolation rate of *Pythium* spp. (%)
	Surface sterilization of pea seed	Autoclaving of					
		Water	Sawdust	Used trays			
A	-2	+	+	+	0.0	680 a [3]	0
B	+	-	+	+	0.0	697 a	0
C	+	+	-	+	0.0	711 a	4
D	+	+	+	-	20.0	550 b	86
E	-	-	-	-	35.0	450 b	94
F	+	+	+	+	0.0	670 a	0

[1] Pea seeds were surface sterilized with 0.1% sodium hypochlorite for 30 min and other cultural materials were autoclaved at 15 lb for 15 min.
[2] + =Cultural materials were autoclaved or surface sterilized; - = Cultural materials without autoclaving or surface sterilization treatment.
[3] Means within the same column followed by different letters are significantly different at 5% level according to Duncan's Multiple Range Test.

Table 3. Effect of air-drying of used cultural trays on vegetable pea seedlings root rot caused by *Pythium aphanidermatum* [1]

Length Of air-dryig (day)	Root debris			Isolation frequence (%) at seedling age (days)							Disease Incidence (%)	Yield (g/tray)
	Water content (%)	Pathogen density (p/g) [2]	Oospore Germination (%)	2	3	4	5	6	7	8		
CK [3]	-	-	-	0	0	0	2	0	0	2	0	588 a [4]
0	83	73,466	37	4	20	42	78	94	96	98	86 a	176 d
2	40	76,533	35	0	6	14	28	68	80	96	46 b	389 c
7	16	63,066	28	0	4	22	56	84	96	98	43 bc	382 c
15	14	33,000	23	0	2	10	24	42	78	92	24 cd	425 bc
30	11	15,333	17	0	0	4	18	40	68	88	18 d	502 abc
60	11	6,145	6	0	0	2	18	40	78	84	20 d	462 abc
90	12	11,333	11	0	0	4	6	26	58	76	17 d	492 ab
120	11	4,177	7	0	0	4	24	42	64	86	10 d	561 a

1. The plants were grown in growth chamber at 24-28℃.
2. p/g = Propagules per gram of substrate. Samples of root debris were taken from the cultural trays with air-drying for time as indicated and assayed on a selective medium (Burr and Stanghellini, 1973) by dilution plate method before planting.
3. Pathogen-free new trays were served as control.
4. Means within a column followed by different letters are significantly different at 5% level according to Duncan's Multiple Range Test.

semi-dried (for less than 15 days) plant debris were significantly lower than the check (new trays treatment). However, when the used trays had been air-dried for more than 30 days, the yields of pea seedlings reached 462-561 g/tray, which were not significantly different (p=0.05) from those of the control (588 g/tray) (Table 3).

Control of Pythium root rot of pea seedlings

All of the tested chemicals, including sodium hypochlorite, calcium hypochlorite, etridiazole and metalaxyl MZ were effective against the root rot of pea seedlings, when applied at the various concentrations. The yields of pea seedlings in used trays treated with tested chemicals were as high as the plant yields in new, clean trays. These yields were significantly (p=0.05) higher than those measured in the non-treated, used trays carrying infected plant debris (Table 4).

For its safety and inexpensiveness, calcium hypochlorite was chosen for use in control trials on root rot of vegetable pea seedlings at Wu-feng farm from July, 1992 to May, 1993. After the infested used trays were immersed in the solution of calcium hypochlorite at 2,000 ppm for 24 h, root debris and substrate residue were removed from the draining pores of the cultural trays. No propagule of pathogens could survive in the sloughed plant debris when detected with the selective medium [(2)] or water agar. When pea seedlings were grown in these disinfected trays, the disease incidence was at a level of 0-10%, and yields of pea seedlings were significantly (p=0.05) higher than those in the non-treated controls. The yield difference was largest in September, 1992 when the temperature was high and root rot was severe. The yields of pea seedlings reached an average of 772 g/tray when the used cultural trays were pre-treated with calcium hypochlorite solution, whereas the average yield was 212 g/tray for the non-treated control (Fig. 1).

Table 4. Effect of chemicals for treating used cultural trays on the severity of Pythium root rot of vegetable pea seedlings

Chemical [1]	Conc. (ppm)	Propagules/gram substrate		Disease incidence (%)	Yield (g/tray)
		P.a. [2]	P.u.		
None (CK1)	-	34800	210	18	535 e [4]
None (CK2)	-	0	0	0	772 ab
Sodium	1000	48	0	0	775 ab
hypochlorite	2000	0	0	0	812 a
Calcium	1000	- [3]	-	0	765 ab
hypochlorite	2000	-	-	0	816 a
Etridiazole	50	7475	0	11 a	662 d
	500	6	0	0	775 ab
Metalaxyl-MZ	50	2065	0	4 b	700 cd
	300	0	0	0	775 ab

1. CK1=Used cultural trays were immersed in tap water; CK2=New cultural trays.
2. P.a. = *P. aphanidermatum* ; P.u. = *P. ultimum*.
3. - = No data.
4. Means within the same column followed by different letters are significantly different at 5% level according to Duncan's Multiple Range Test.

Degradation of hypochlorite ion in calcium hypochlorite solution

The hypochlorite ion in the solution of calcium hypochlorite for treating used trays degraded fast. The concentration of hypochlorite ion decreased from 4,586 ppm to 2,229 ppm or from 2,548 ppm to 1,178 ppm (half of the original concentration) in the 2,000 ppm and 1,000 ppm solutions of calcium hypochlorite, respectively, after 1 day, as assayed by the iodimetric titration method [(11)]. After 6 days, the hypochlorite ion was reduced to less than 100 ppm. The decrease of hypochlorite ion in the solutions which remained

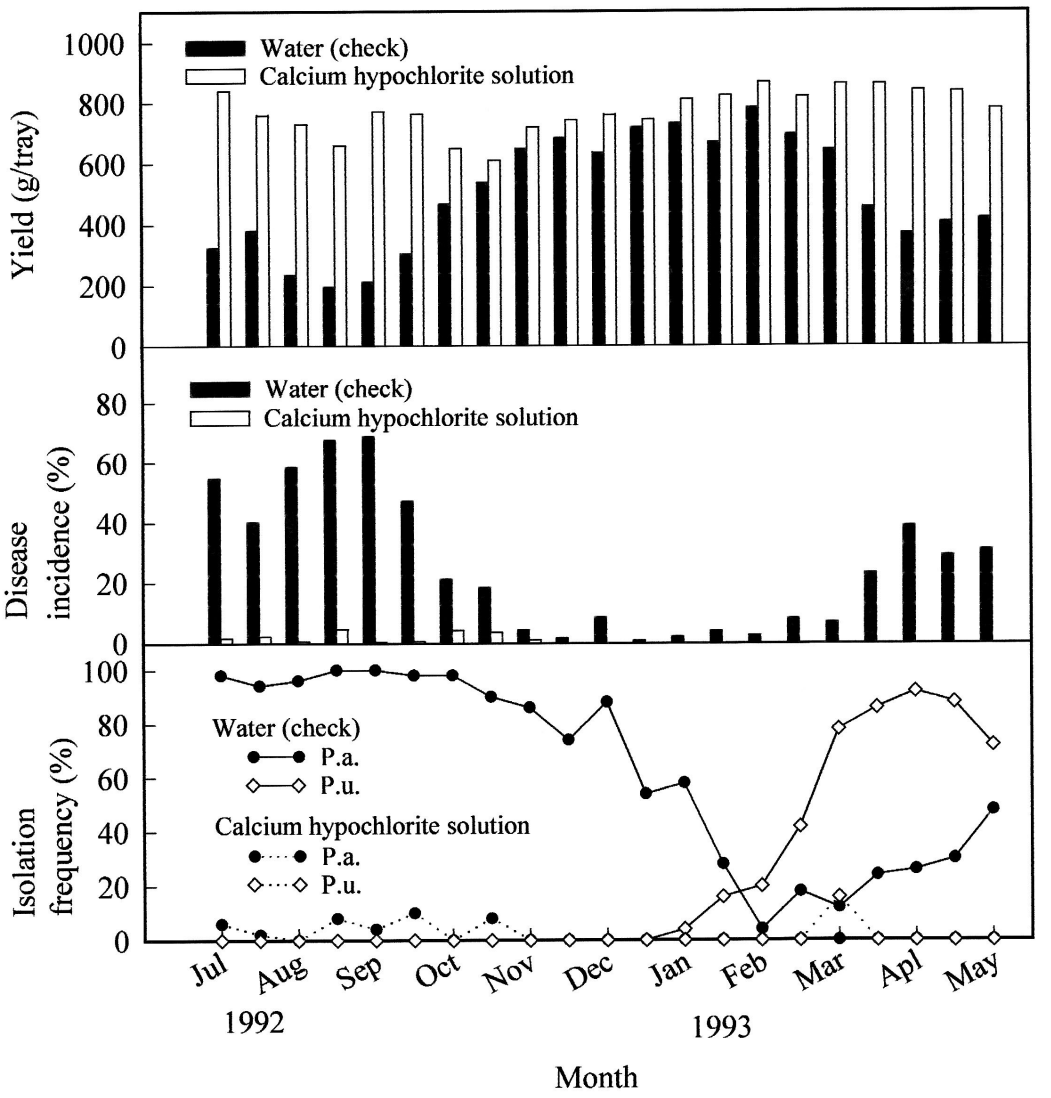

Fig. 1. Effect of calcium hypochlorite treatment of cultural trays on root rot incidence and yield of vegetable pea seedlings. The used cultural trays were immersed in water (check) or in the solution of calcium hypochlorite (2,000 ppm) for 24 hours before planting. P.a.=*Pythium aphanidermatum*; P.u.=*P. ultimum*.

unused degraded slowly, and it took 16 days for the hypochlorite ion to decrease to half of the original concentration (Fig. 2).

DISCUSSION

Species of *Pythium* have been reported as the most important pathogens of many greenhouse vegetables, especially in recirculating hydroponic cultural systems [14]. Zoospores are implied as the primary infectious propagules responsible for the spread of pathogens via the recirculating nutrient solution [15]. The seasonal occurrence of *P. aphanidermatum* in summer and *P. ultimum* in winter has been demonstrated as the cause for root rot of vegetable pea seedlings in a soilless cultural system [6]. The disease was especially severe in summer in Taiwan. How the pathogens are introduced to such a soilless cultural system and the relative importance of the various avenues are of the major concern to growers and plant pathologists. The results of this study provided evidence that *Pythium* spp. were probably introduced into this soilless cultural system through cultural substrate (sawdust). Because only small amounts of inocula of *Pythium* species could be isolated from the sawdust, with the original infection source possibly from contaminated soil dust (Table 1). These small amounts of inocula from sawdust served as the primary inoculum to infect only a few pea seedlings with no obvious symptoms when a new seedling tray was used to grow pea seedlings for the first time. *Pythium aphanidermatum* and *P. ultimum* could spread from plant to plant via hyphae and could also spread via zoospores by splashing out from the diseased plants when overhead-irrigation was applied. These zoospore inocula colonized only a few plants in the neighboring trays and produced no severe disease either. After harvesting, however, sawdust residue and

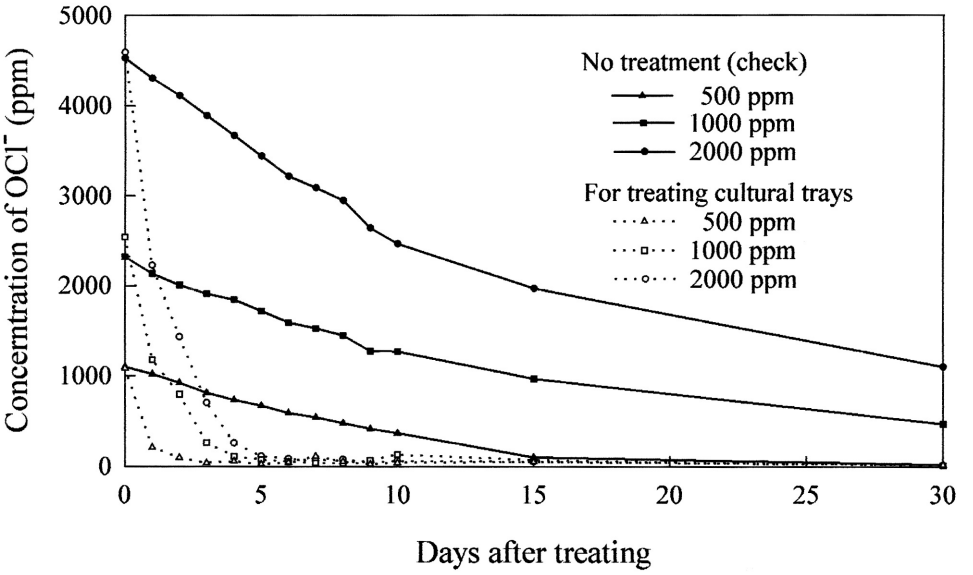

Fig. 2. The degradation of hypochlorite ion in the calcium hypochlorite solutions on treating used cultural trays.

root debris stuck in draining pores of trays had abundant active mycelia and oospores of the pathogens. When the infested trays were used repeatedly to grow vegetable pea seedlings, these infested sawdust residue and infected root debris would be the important infectious inocula responsible for causing severe root rot (Table 2,3).

The fresh root debris carrying active mycelia and oospores of *Pythium* spp. could initiate infection in pea seedlings of 2 days old and caused severe root rot at harvest time, 8 days after seeding (Table 3). However, after air-drying treatment, the mycelia were dead, and the oospores might be subjected to desiccation dormancy like some fusaria [(1)]. The oospores of *P. aphanidermatum* declined in germination rates or would not germinate under desiccation conditions but would germinate when the specimens was wetted for days [(8)]. The root debris dried for 30 days could only initiate infection to pea seedlings 4 days after seeding, and thus, the disease was not too severe at harvest time. In other words, delaying of infection would decrease the yield loss, although the pathogen isolation rates were above 76% at harvest (Table 3).

Pythium spp. have been reported to be controlled by a number of fungicides [(4,7,16)] and nonionic surfactants [(15)]. Sodium hypochlorite was considered to be toxic to *Pythium* spp. when used for surface sterilization of vegetable roots [(13)]. Chloride was considered the important factor to suppress the activity of *Pythium* spp. [(9)]. The results of our experiments conducted at Wu-feng farm for one year (Fig. 1) conclusively demonstrated that calcium hypochlorite was as good as sodium hypochlorite and the tested fungicides in controlling vegetable pea seedling root rot caused by *P. aphanidermatum* and *P. ultimum* in soilless cultural system. The hypochlorite ion in the solution of calcium hypochlorite for treating used trays degraded rapidly and had no deleterious effects on plant growth or yield.

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摘 要

林益昇[1,3]、黃晉興[2]、龔玉惠[1]. 2002. 腐霉菌引起設施豌豆苗根腐病之防治. 植病會刊11:221-228. ([1.] 國立中興大學植物病理學系，臺中市；[2.] 臺灣農業試驗所植物病理學系，霧峰鄉；[3.] 聯絡作者，電子郵件：yslin1@dragon.nchu.edu.tw；傳眞：+886-4-22870891)

本研究發展非農藥方法防治腐霉菌引起之設施豌豆苗根腐病。本病害在使用新育苗盤之農場並不嚴重，但重複使用會使罹病之豌豆根部污染育苗盤，而殘存其上病原菌 (*Pythium aphanidermatum* 和 *P. ultimum*) 之菌絲與卵孢子則成爲重要感染源，因而引起嚴重的根腐病。將污染病原菌之育苗盤曝曬於陽光下數月，或將之浸泡於次氯酸鈣溶液 (2000 ppm)，24 小時，都可有效防治本病害。在發病嚴重之炎熱夏季，使用次氯酸鈣溶液的防治方法，可使豌豆苗根腐病之發病率由 60-80% 降至 10% 以下，而且每育苗盤的平均產量由212 公克提高至772 公克。

關鍵詞：豌豆、腐霉菌、感染源來源、消毒劑、次氯酸鈣、防治