Ecology and Control of *Phytophthora melonis* in Drained Paddy Field[1)]

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摘　　要

林益昇、吳瑞香 (1985) ***Phytophthora melonis*** 在水田裡之生態及防治。（臺灣省農業試驗所）

胡瓜疫病菌 (*Phytophthora melonis*) 之游走子囊在病組織上不具脫落性，乾風無法將之吹落。但是它的游走子及游走子囊可隨灌溉水及飛濺的雨水傳播。土壤濕度強烈影響病原菌的存活，病原菌在淹水的土壤中僅能存活 4 星期，但在含水量 6 %及 20%，稍可通氣之濕土裡，也僅能存活 5～20 星期。田間試驗顯示，在經過兩期水稻輪作的病圃裡，胡瓜疫病菌不能存活。而引起胡瓜疫病之病原菌初次感染源，並非來自土壤或種子，而是來自隣田的灌溉水。在田間，殺菌劑(Ridomil MZ)和土壤添加物(雞糞和 S-H 混合物) 配合使用對胡瓜疫病之防治效果最顯着。該二種土壤添加物可提升土壤的 pH 值，但土壤 pH 值的變化與病害防治無關。雞糞和 S-H 混合物僅有促進植株生長勢的效果。在試驗中 Ridomil MZ 殺菌劑才是抑病的主要因素。

（關鍵字：胡瓜疫病，*Phytophthora melonis*，初次感染源，存活，病害傳播，病害防治，水稻田）

ABSTRACT

Dry air current failed to remove the sporangia of *Phytophthora melonis* from the disease lesions. However, the fungus was easily dispersed by its zoospores and sporangia carried in irrigation water or liberated to the air by

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wind-blown rainsplashes. The soil moisture strongly influenced the survival of *P. melonis*. The fungus persisted only for 4 wks in flooded soil, while in soil with moisture content at 6% or 20%, a condition that permitted soil aeration, the fungus could be detected from the infested soil for a period of 5~20 wks. Field experiments demonstrated that *P. melonis* did not survive, nor did it induce cucumber blight in a disease nursery after two successive crops of rice plants. The primary inoculum of *P. melonis* to cause cucumber blight in the drained paddy fields did not come from the paddy soil nor from cucumber seeds. It was mainly from irrigation water from neighbor fields. Cucumber blight was best controlled by using combinations of fungicide Ridomil MZ and soil amendments, i. e. chicken manure or S-H mixture. The soil amendments raised soil pH to alkaline range for 7 days, then it fell to the acid range again. The changing pattern of soil pH had no correspondence to the inhibition of cucumber blight. Both chicken manure and S-H mixture had nutrient effects to result in improved plant growth. However, Ridomil MZ was the main factor that inhibited disease development in our experiments.

(Key words: Phytophthora blight of cucumber, *Phytophthora melonis*, primary inoculum, survival, spread of disease, disease control, paddy field)

INTRODUCTION

Cucumber is an important vegetable in Taiwan with an annual acreage of about 5,000 ha[9]. It is mostly grown in the drained paddy fields. The population of many soilborne pathogens in soil can be considerably reduced following the cropping of paddy rice[3,11]. However, Phytophthora blight of cucumber caused by *Phytophthora melonis* Katsura was common on cucumber grown under the above conditions. The blight symptoms were found on all aerial parts of the plant. Severe occurrence of the disease was observed mostly during periods of hot wet conditions[6]. In our preliminary study, *P. melonis* could not be reisolated 4 wks after flooding the field, when the organism was grown on cucumber fruits and burried in paddy soil. How does the fungus survive in paddy soil is of great interest. In addition, the fungicide Terrazole, recommended for controlling the disease[7], was found not satisfactory in field trials. We therefore, investigated the form of primary inoculum, the survival and dispersal of *P. melonis*; the way of disease spread; and the control measures which could be adopted under conditions of growing cucumber in the paddy field between rice crops.

MATERIALS AND METHODS

Inoculation of detached cucumber leaves

Zoospores of *P. melonis* (homothallic isolate Phy-83-13) were prepared by the method of Hwang and Ko[4] into a dilution series. A drop (0.035 ml) of the diluted spore suspension containing either 1-10 or 48-56 zoospores was taken up with a microsyringe and placed onto the abaxial or adaxial surface of the detached

cucumber leaves. The inoculated leaves were kept in a moist chamber at 28 C, and symptoms were recorded 4 days after inoculation.

Dispersal of sporangia and zoospores

In the laboratory, an inoculated cucumber fruit covering with sporangia of *P. melonis* was hand-held in an upright position 10 cm from a petri dish, then a fan blew the air at various speeds over the fruit in the direction of the petri dish. In another experiment, inoculated cucumber fruits were placed on a holder 10 cm above a tray which was put in the open under heavy rain for an hour. The rain-splashes were collected in the tray. The water collected in both the petri dish and the tray was examined microscopically for the presence of sporangia and zoospores.

Spread of Phytophthora cucumber blight

Cucumber seeds (cultivar Joy) were sown in the greenhouse (28~38 C) in 3 rows (3 m long for each, 30 cm between rows). The plants of the middle row were cut off 21 days after seeding and one inoculated cucumber fruit was placed between the first and second rows at the entrance of irrigation water. No inoculum was placed between the second and third rows. The plot was irrigated carefully every other days. Water samples were collected at the end of irrigation path and passed through a 400-mesh screen. The materials collected on the screen was examined under microscope for sporangia. Attempt was also made to isolate the fungus from the water samples by baiting with cucumber leaves. The disease development was recorded during the experimental period.

Cucumber fruits were inoculated and placed at the irrigation entrance of a cucumber field (0.2 ha) during heavy raining days. Water samples were also collected from the furrows between rows and examined for the presence of the fungus zoospores and sporangia as described. The disease development in the field was recorded. When the cucumber plants were 2-month old and the disease started to develop, a home-made simple spore trap equipped with 8 petri dishes (9 cm in diam.) was installed 30-cm, 40-cm, 60-cm and 80-cm (two petri dishes at each height) above the ground in the center of the nursery. The airborne inocula were collected on fresh Phytophthora selective medium[5] in the petri dishes during a 12 hr exposure. The petri dishes were then examined under microscope for the presence of sporangia and zoospores. The collections were repeated 10 times in both sunny and raining days.

Survival of *P. melonis*

(a) Ten samples with a total of 4,500 cucumber seeds obtained from local seed stores were put on selective medium[5] for isolating *P. melonis*.

(b) Cucumber fruits were inoculated with *P. melonis* in the laboratory and, after 5 days, chopped into small pieces (3 cm long). Then, the pieces of inoculum were subjected to the following treatments: (i) being immersed in tap water; (ii) placed on soil surface; and (iii) burried under soil at 2 cm depth. The soil moisture content was maintained at 10%, 20% or 30% (flooded). All infested materials were separately put in a one-liter beaker and incubated at room temprature (24~28 C). The beaker was

sealed with a plastic sheet to prevent the loss of moisture. The inoculated tissues were examined under microscope once a week for the presence of sporangia and zoospores.

(c) Autoclaved cucumber stems (2 cm long) were inoculated with *P. melonis* and mixed in a one-liter beaker with soil. The mixture was incubated at the laboratory temperature of 18~32 C (August, 1984 to Janurary, 1985). The soil moisture content was kept at 6%, 20% or 30%. Attempts were made to reisolate the pathogen from the infested soil at weekly intervals by baiting with cucumber leaves.

(d) The survival and primary inoculum of *P. melonis* were also investigated under paddy conditions. Two successive crops of rice were grown in the blight nursery (0.2 ha) in 1984. This same field was grown with cucumber plants during March to June 1985. Occurrence of cucumber blight was recorded and attempts were made to isolate *P. melonis* during the growing period of the first season rice and cucumber from the field soil as well as from irrigation water before entering into the field.

Experiments on disease control

Field experiments were conducted on an experimental farm (0.2 ha) of Taiwan Agricultural Research Institute during May to July and September to November of 1983, and May to July of 1984. Eggplant fruits were inoculated with *P. melonis* (homothallic isolate Phy-83-13) and incubated in the laboratory (24~28 C) for 2 days. The inoculated fruits then were blended with noncropped loam soil (1 fruit/100 g soil) to serve as soil-inoculum. A disease nursery was set up by adding fresh soil inoculum into seeding rows (0.4 kg/m) at 5 cm depth from the soil surface. Three experiments were set up in this disease nursery to evaluate the effect on cucumber blight for chicken manure (2,000 Kg/ha), cow dung (3,500 Kg/ha) and S-H mixture[8] (900 Kg/ha) as soil amendments; two fungicides, Ridomil MZ (Methyl D, L-N-(2,6-dimethyl-phenyl)-N-(2'-methoxyacetyl)-alaninate, 10%; Ionic coordination of zinc and manganese ethylenebisdithiocarbamate, 48%)(1450 ppm) and Terrazole (5-Ethoxy-3-(trichloromethyl)-1, 2, 4-thiadiazole, 35%)(175 ppm), and combined applications of fungicides and soil amendments. In the first experiment (12 m × 50 m), the three soil amendments were separately added to the soil between seeding rows (20 cm away from the rows one day before seeding. The second experiment (8 m × 50 m) used no soil amendments, but each seeding hole was drenched with 100 ml of one of the test fungicides right after seeding. The same fungicide was applied as leaf sprays to cucumber plants 3 times at a 10-day interval. In the third experiment (20 m × 50 m), all possible combinations of soil amendments and fungicides were included. One plot which received neither soil amendments nor fungicides served as check in all experiments. Each treatment (plot) employed 100 plants in 2 rows. Each experiment was a complete random design with 5 replicates. The disease intensity was calculated by the following formula:

$$\text{Disease intensity} = \frac{0 \times n_0 + 1 \times n_1 + 2 \times n_2 + 3 \times n_3}{(n_0 + n_1 + n_2 + n_3) \times 4} \times 100\%$$

Where: n_0 = No. of healthy plants.

n_1=No. of plants with water-soaking lesions.

n_2=No. of plants with shriveled stems.

n_3=No. of wilted plants.

In the greenhouse (25~38 C), a noncropped loam soil was amended with chicken manure (1/4, v/v) or S-H mixture (1%, w/w) before potting. Fresh soil inoculum was then added to potted soil at a depth of 5 cm. To each 6-in clay pot, 5 cucumber seeds were sown. After seeding, each pot received 100 ml of Ridomil MZ (1450 ppm) or Terrazole (175 ppm). Disease development was recorded and the soil pH was measured during the experimental period.

RESULTS

Infection of detached cucumber leaves

The inoculation results showed that successful infection with *P. melonis* could be induced in detached cucumber leaves by as little as 1-10 zoospores in the inoculum, though a higher disease incidence resulted from an inoculum containing a higher number of zoospores.

Dispersal of sporangia and zoospores

Airborne sporangia were not detected in both the laboratory and the field when recovery experiments were carried out in windy, sunny days. By contrast, airborne sporangia and zoospores were easily obtained from rain-splashes reaching the tray and the spore trap in raining days. The collected airborne sporangia did not have 'pedicel' or the 'pedicel' was not uniform in length.

Spread of Phytophthora cucumber blight

In the greenhouse, all plants in the first row which was near the initial inoculum showed root rot symptoms and were killed in 2-3 wks, while those plants in the third row remained healthy. In the waste irrigation water, the fungus could be detected by the baiting tecnique, but no sporangium was observed under microscope. In the field, similar results were obtained. Foot rot occurred first in the plants near the inoculum, then it spread to other plants of the same row following the path of irrigation water. After several days of heavy rains, however, the disease spread all over the plots, and the diseased plants developed foot rot as well as blight symptoms on the aerial parts. In the water samples collected from the furrows between rows, the sporangia and zoospores of *P. melonis* were observed under microscope. They were also obtained on culture medium.

Survival of *P. melonis*

(a) In tests for seedborne nature of *P. melonis*, all 4,500 cucumber seeds plated were found free from the pathogen.

(b) Cucumber fruits inoculated with *P. melonis* produced abundant sporangia in water or moist soil in 7 days. The number of sporangia was reduced to a very low level by 14 days (Table 1), especially in flooded soil. No oospore was formed at 7 days on the inoculated fruits irrespective of the latter being burried in soil with a 10% or 20% moisture content or in soil flooded with water. However, numerous oospores were produced during 7-14 days under the first two moisture conditions. There was no oospore found in the flooded soil (Table 1). After 14 days, the infected cucumber fruits had decayed in the soil,

Table 1. Effect of soil moisture on the formation of sporangia and oospores of *Phytophthora melonis*[1)]

Duration (day)	Soil moisture (%)	Treatment[2)]					
		In water		On soil surface		Buried in soil	
		S.	O.	S.	O.	S.	O.
7	10			+++	−	+++	−
	20			+++	−	+++	−
	30 (flooded)			++	−	++	−
	CK (no soil)	+++	−				
14	10			+	++	+	+
	20			+	+	+	−
	30 (flooded)			−	−	−	−
	CK (no soil)	+	−				

1) The experiment was conducted in laboratory (25~28 C) using homothallic isolate Phy-83-13.

2) *Phytophthora melonis* was grown in cucumber fruits for 5 days, then the fruits were put in water (CK), on soil surface or in soil. +++, ++, + and − indicate more than 50, 10-50, 1-10 and 0 sporangia or oospores per microscopic field (100X), respectively. S=Sporangia; O=Oospores.

the examination of the fungal structures was difficult.

(c) *Phytophthora melonis* was detected from the soil up to 20, 5, and 4 wks after infestation with inoculated cucumber stems for soil moistures at 6%, 20% and 30%, respectively (Table 2). The fungus was not recovered at later times.

Table 2. *Phytophthora melonis* reisolated from infested soil of different moisture content[1)]

Duration (wk)	Soil moisture (%)		
	6	20	30
3	+	+	+
4	+	+	+
5	+	+	−
6	+	−	−
7	+	−	−
8	+	−	−
10	+	−	−
20	+	−	−
22	−	−	−

1) *Phytophthora melonis* was grown on autoclaved cucumber stems for 5 days, then mixed with soil in laboratory. The pathogen was reisolated by baiting with cucumber leaves. +, fungus obtained; −, fungus not obtained.

(d) Three weeks after flooding during the first season of rice crop, *Phytophthora melonis* could not be detected in the paddy soil, previouly established as disease nursery. However the organism was detected in soil samples taken from the border of the paddy at least for 6 wks. This field was sown with cucumber seeds on March 24, 1985 and no *P. melonis* was detected from the irrigation water or soil samples at the seeding date. The plants grew well for 2 mo after seeding. Then, on May 21, *P. melonis* was first isolated from irrigation water at its entrance into the experimental field and cucumber blight occurred on May 28. All plants in this field were killed by June 15.

Experiments on disease control

Field experiments showed that none of the soil amendments or fungicides, when applied alone, was effective in controlling cucumber blight, except that Ridomil MZ treatment slowed down the disease development to some extent (Fig. 1A, 1B). Ridomil MZ plus soil amendments, i. e., chicken manure, cow dung and S-H mixture, showed better disease control (Fig. 1C), while Ridomil MZ plus chicken manure yielded best (Fig. 2).

Greenhouse experiments gave similar results. Ridomil MZ was of value in controlling cucumber blight. Soil amended with chicken manure and S-H mixture raised the soil pH from the original 6.1 to 7.5 and 7.8 for 1 wk, respectively, then it fell to 6.3 and 6.5, respectively. The various soil amendments had a little or no economic value in controlling the disease.

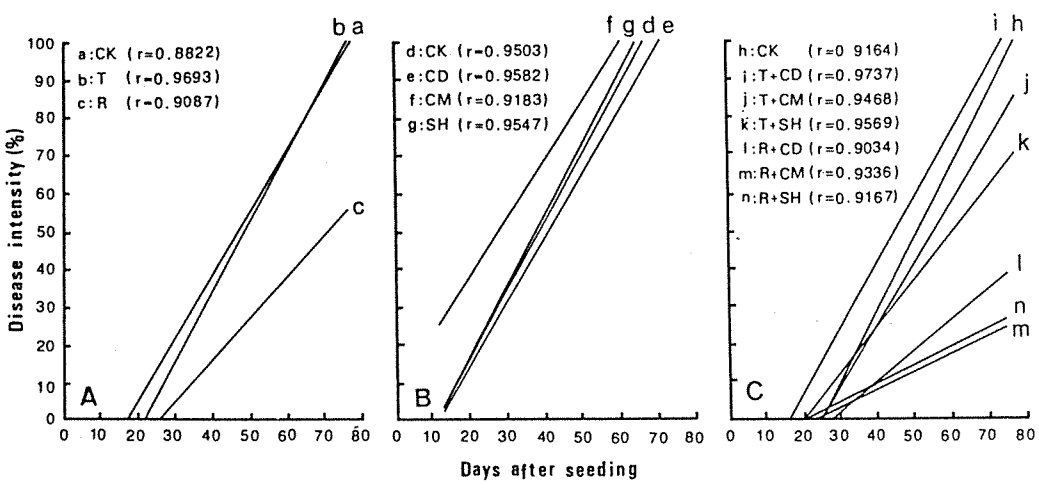

Fig. 1. Development of Phytophthora blight of cucumber in a disease nursery treated with fungicides (A) [Ridomil MZ (R) and Terrazole (T)], soil amendments (B) [chicken manure (CM), cow dung (CD) and S-H mixture (SH)], or combinations of soil amendment and fungicide (C) during March to June of 1983 in an experimental farm of Taiwan Agricultural Research Institute.

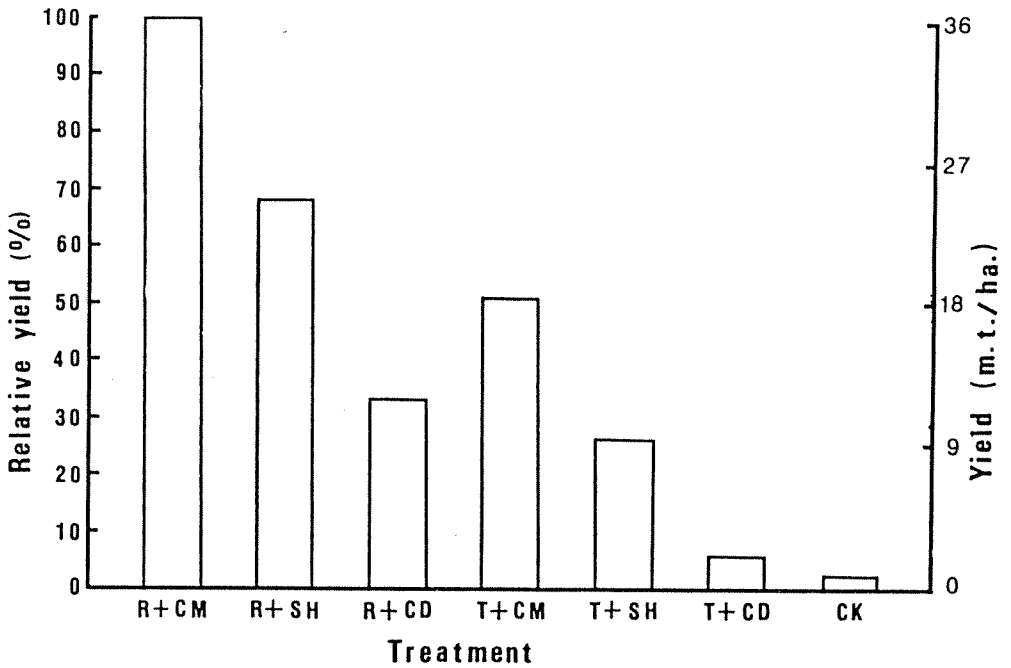

Fig. 2. Yield of cucumber in a disease nursery treated with combinations of soil amendment [chicken manure (CM), cow dung (CD) and S-H mixture (SH)] and fungicide [Ridomil MZ (R) and Terrazole (T)] during March to June of 1983 in an experimental farm of Taiwan Agricultural Research Institute.

DISCUSSION

That the sporangia of *P. melonis* could not be trapped by exposing agar plates at various heights above the ground in a disease nursery in sunny days, nor was the attempted collection of them successful in the laboratory by blowing dry air over the infected, sporangia-bearing cucumber fruits toward petri dishes provides evidence for the non-caducous nature of this fungus structure. However, they could be found in rain water passing over the surface of an infected cucumber fruit and collected in the tray situated below. The sporangia thus collected had no 'pedicel' or their 'pedicel' was not uniform in length, suggesting that they were probably broken by rain drops impeaching the disease lesions[1]. Other *Phytophthora* species[2] were also found not to release the sporangia into air current under dry condition, but they were readily dispersed by wind-blown rain-splashes. In our field experiments, *P. melonis* was found to be dispersed by zoospores and sporangia carried in irrigation water, although only the zoospores, not the sporangia, were found in greenhouse experiments. In Taiwan, rains and showers occur frequently in the spring and summer seasons, the zoospores and sporangia either carried in irrigation water or liberated to the air by wind-blown rain drops become an important means of

short distance dispersal in the field.

The soil moisture strongly influenced the survival of *P. melonis*. When the inoculum was put in water or in flooded soil, *P. melonis* would undergo vegetative growth leading to the production of sporangia and soon lysed (Table 1). By contrast, when the inoculum was burried in moist soil (10% and 20% of moisture content), the fungus would produce sporangia within 7 days, followed by the formation of many oospores during 7~14 days (Table 1). However, it is difficult to determine the survival time period of oospores of *P. melonis* in soil since their germination rate was extremely low, only 0.1% on cucumber extract agar. In our study, survival of the fungus was determined by first infesting the soil with the fungus grown in autoclaved cucumber stems and reisolation made at times by the baiting technique. In this way, the fungus could be detected over a period of 20 wks (Table 2) from the infested soil at 6% moisture content. Under the field conditions, *P. melonis* did not survive, nor induce cucumber blight in a disease nursery after two succcessive crops of rice plants. The primary inoculum to cause infection later in the experiment on cucumber plants as a succeeding crop is believed not from the soil or the cucumber seeds. It was mainly from the irrigation water which came from neighbor fields.

It has been shown previously that amendment of soil with chicken manure [10] or S-H mixture[8] raised the soil pH to alkaline range temporarily. A relatively high soil pH may either eliminate or significantly reduce *Phytophthora*[10] and *Fusarium*[8] propagules in the soil. Our greenhouse and field experiments showed that addition of chicken manure or S-H mixture raised the soil pH for a period of about 7 days, it then fell to the acid range. But this changing pattern of soil pH had no correspondance to the inhibition of cucumber blight caused by *P. melonis*. The reason may be that the fungus is not soilborne in nature and hence the soil amendments have little effect on the fungus coming from a neighbor field. However, both chicken manure and S-H mixture improved plant growth. Ridomil MZ was the main factor to inhibit the disease in our experiments.

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