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Factors affecting pycnidial production and pycnidiospore germination of *Phoma wasabiae*, the causal agent of wasabi

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ABSTRACT

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Pycnidiospores are exudated from pycnidia on infected leaves or petioles of wasabi and may play an important role for secondary infection throughout much of growing seasons. The influence of environmental factors and nutritions on the formation of pycnidia and on the subsequent release of pycnidiospores is directly related to the amount of effective secondary inoculum. In vitro study, the results indicted that PDA medium was the best for production of pycnidia among tested media. Of the carbon sources, starch resulted in the highest linear growth for 7 days incubation and higher germination percentage of pycnidiospores. However, only sorbitol and inulin were the better for pycnidial production among tested carbon sources. Nitrogen sources did not significantly affect the production of pycnidia as compared with control (without nitrogen). In environmental factors, *Phoma wasabiae* produced pycnidia on PDA medium between 12 ℃ and 32℃ with the optimum temperature at 20℃ to 28℃. Pycnidiospore germinated well between pH4.5 and pH9.5 with the optimum pH value from 6.5 to 7.5. For water potential tests, the hyphal extension rate of the fungus declined with decreasing water potential over the range -1 to -56 bars. However, Pycnidial production and releasing pycnidiospores occurred only at water potential among -1 to -10 bars. After 14 days' incubation, the optimum light intensity for pycnidial production was 150 to 200 μE m^{-2} s^{-} under a 12 hr day-length cycle and 80 to 100 μE m^{-2} s^{-} under a 24 hr day-length cycle.

Key words: nutrient and environmental factors, *Phoma wasabiae*, pycnidia, wasabi streak

INTRODUCTION

Phoma disease of wasabi caused by *Phoma wasabiae* Yokogi has been an important disease in Japan and Taiwan [4,8,16,,17,20,27]. The pathogen causes symptom of streak or black-leg on rhizomes, spot on leaves, and blight on petioles of wasabi [15,27]. Particularly, infection at rhizomes of wasabi has become a serious problem in Taiwan in the last 15 years [4,9,10,15]. It has affected approximately 30 to 70% of the total production of wasabi rhizome annually, resulting in downgrading of the rhizome and lower market prices [15].

Typical phoma disease cycles begin with the appearance of pycnidia on plant debris [3,5,6,7,11,26]. Pycnidiospores are then exudated in a gelatinous matrix during wet weather from pycnidia on infected leaves or petioles of wasabi and can be disseminated by splashing and windblown rain or sprinkler irrigation [12,24,25]. Splash- dispersed pycnidiospores land on leaves, petioles and rhizomes of wasabi plantings [25], and subsequently cause infection [23]. Final disease severity has been reported that was closely related to incidence of the phoma disease during early vegetative growth stages [15,24]. However, no predictive model has yet been developed.

Resolution of epidemics of plant diseases into infection cycles has acquired central importance in the analysis of epidemics [13,14,18]. The components of these cycles, such as infectious periods, spore production and the interaction with environmental, host and pathogen factors influence the rate of development of epidemics [20,21,22,23]. The incidence and development of phoma streak disease in wasabi fields have been correlated with the amount of rainfall in Taiwan [26]. Wang *et al* [26] reported that phoma disease of wasabi required a period of higher relative humidity (>95%) for symptom appearance. The use of infected plantings [1,2,3,15,17] has also been reported as a source of primary inoculum for phoma streak disease of wasabi. However, pycnidiospores might play an important role for secondary infection throughout much of growing season [16,21,22]. The influence of environmental factors on the formation of pycnidia and on the subsequent release of pycnidiospores is directly related to the amount of effective secondary inoculum [2,15,21,22,23]. Consequently, a more complete understanding for affecting factors of pycnidial production and pycnidiospore germination of *P. wasabiae* is necessary for preventative strategy applications because the preventive approach to control the disease has

been taken in Cha-yi, Taiwan. The objective of this investigation was to determine the effects of nutritional and environmental factors on pycnidiospore germination and pycnidial production of *P. wasabiae.*

MATERIALS AND METHODS

Inoculum preparation

In the present work, two strains, designated as Al-5-L-1 and DB-2-R-2, of *Phoma wasabiae* were isolated from wasabi plants with leaf spot and streak disease in Alishan and Ta-bang, respectively. Both isolates were selected to use in this study because of their vigorous growth and stronger virulence (Lo and Wong, unpublished). All cultures were maintained on potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI, USA) in daylight at 25℃ incubator unless stated otherwise.

Pycnidia of *P. wasabiae* were laid on PDA plates for 14-20 days incubation. Unlesss indicated otherwise, pycnidial production was counted for 14 days incubation. Pycnidiospores were obtained from mature pycnidia and suspended in sterile distilled water and adjusted to 10^5 spores/ml for all germination tests. Pycnidiospores were considered germinated when a germ tube equal in length to the largest pycnidiospore dimension developed [21]. A total of 100-150 pycnidiospores were counted for 17 hr incubation. The number germinated was divided by total number of pycnidiospores counted and multiplied by 100 to give percentage germination. Similarly, the percentage of pycnidiospore release was measured by examining the number of exudated pycnidia that was divided by total number of pycnidia counted and multiplied by 100 on each plate.

Media

A basal medium based on Czapek-dox agar was used routinely l L^{-1} distilled water: $NaNO_3$, 2.0g; KH_2PO_4, 1.0g; $MgSO_4.7H_2O$, 0.5g; KCL, 0.5g; $FeSO_4$, 0.01g; sucrose, 20g; agar 20g). This medium contained 8.42 g atoms C and 0.329 g atoms N per liter and, in initial N-utilization experiments supported growth of the two isolates. Corn meal agar medium, (Difco). Malt extract agar medium (Difco). Potato dextose agar medium (Difco). 5, 10,or 20% of V8 juice agar medium (l L^{-1} distilled water; V8 juice, 50, 100, or 200 ml; agar 20g). 5,10, or 20% of wasabi rhizome extract agar medium (l L^{-1} distilled water; wasabi rhizome extract, 50, 100, or 200 ml; agar 20g). 5,10,or 20% of wasabi leaf and stalk extract agar medium (l L^{-1} distilled water; wasabi leaf and stalk extract, 50, 100, or 200 ml; agar 20g) were used in medium requirement experiments.

Factors affecting mycelial growth, pycnidial production, and pycnidiaspore germination

Effect of temperature. Linear hyphal extension rates (mm/day) of the two isolates, Al-5-L-1 and DB-2-R-2, were determined between 4 to 40℃ on PDA (10 ml) in 9cm-diameter petri plates. For each treatment, four replicate plates were inoculated with agar discs (5mm-diameter) taken from the edge of actively growing cultures with a sterilized cork borer. The hyphal extension rates were measured after 7 days incubation. The percentage of pycnidiospore germination was determined as described above after 17 hr incubation.

Effect of pH. The effect of pH on mycelial growth and pycnidiospore germination of *P. wasabiae* strain Al-5-L-1 in Czapek-dox medium were determined in static liquid culture. Appropriate amounts of 0.1 to 1 M of HCl and NaOH were added to medium with pH ranges between 2.5 and 11.5. Medium was sterilized by autoclaving at 121℃ and 15 lbs for 15 min. Three replicate Erlenmeyer flasks (250 ml) containing 50 ml of each test medium were inoculated with 1 ml of spore suspension (1x 10^5 pycnidiospores/ml). After 10 days incubation at 24℃, the mycelial mats were harvested by vacuum filtration onto pre-weighted filter papers (Whatman No. 1) and oven-dried for 24 hr at 105℃. The dry weights of biomass were determined in the experiment to study the effect of pH value. The percentage of pycnidiospore germination was determined by counting 100-150 pycnidiospores of *P. wasabiae* after 17 hr incubation.

Effect of medium. When all media above were solidified, linear hyphal extension rates (mm/day) of the pathogen were determined on various media. For each medium, four replicate plates were inoculated with agar discs (5mm-diameter) taken from the edge of actively growing cultures of the pathogen with a sterilized cork borer. The hyphal extension rates were measured after 7 days incubation. The percentage of pycnidiospore germination was determined as described above after 17 hr incubation.

Effect of carbon and nitrogen. The effect of each carbon and nitrogen source on pycnidial production and pycnidiospore germination was determined using the petri plate technique [21]. All petri plates were inoculated at 25℃. Each carbon source (Sigma Co., MO), such as arabinose, cellulose, fructose, galactose, glucose, myo-inositol, inulin, lactose, maltose, sorbitol, starch, and sucrose was separately used to replace the sucrose of basal medium, and the total carbon weight of each carbon source was the same as 8.42 g atoms C per liter as above described. Each nitrogen source (Sigma Co., MO), as alanine, arginine, asparagines, cysteine, glycine, glutamic acid, histidine, methionine, sodium nitrate, sodium nitrite, and urea, was used to replace the sodium nitrate of basal medium, and the total N weight of each nitrogen source was 0.329 g atoms N per liter.

In C : N ratio experiments. the basal medium solution was supplemented with sucrose and sodium nitrate to give the ratio range from 0 to 100 that included C fixed (8.42 g atoms C per liter) and N-fixed (0.329 g atoms N per liter). The hyphal extension rates were measured for 7 days incubation and the percentage of pycnidiospore germination was determined after 17 hr incubation.

Effect of water potential. The water potential of corn meal agar was adjusted osmotically with the electrolyte sodium chloride (NaCl). The quantities of NaCl were added to media to give required water potentials in the range of -1 to -98 bars [19]. For this experiment, 10 ml of medium was added

to sterile plastic petri dishes. Three replicate plates of each treatment were placed in polyethylene bags to prevent water loss and incubated at 24℃. During the incubation period, hyphal extension was measured and pycnidial production was determined. In spore-germination studies, 10 mature pycnidia were removed from PDA-grown colonies and were placed to petri dish with 10 ml sterile water. The pycnidia were then pressed with a sterile glass rod and the spore suspension were disturbed by vortex for 1 min. Samples (0.1 ml) were spread over the surface of media. The percentage germination of pycnidiospores was estimated on three replicate plates after 17 hr at 24℃.

Effect of light intensity. Eight light intensities, 20,40, 60, 80, 100, 150, 200, and 300 μE m^{-2} s^{-1}, were obtained by covering plates in an illuminated growth cabinet with layers of muslin. A control was set up in the dark by covering plates with aluminum foil. The experiment was carried out with a 12 hr day-length cycle beginning with the light period and a 24 hr day-length cycle. Light-intensity measurements were monitored during the experiment using a quantum sensor (Li-Cor, Lincoln, Nebraska).

Experimental design and statistical analysis. The replicate plates were randomized within the central area of controlled- environment cabinet, where light intensities were uniform. All experiments were repeated twice. Analysis of variance was performed on the data obtained in all experiments, and Fisher's least significant difference (LSD) were used for mean separations with the Statistical Analysis System program (SAS Institute, Cary, NC)

RESULTS

Effect of media and nutrient sources on mycelial growth, pycnidial production and percentage of pycnidiospore release

In the medium tests (Table 1), the corn meal agar, malt extract agar and 20% wasabi rhizome extract agar were less for mycelial growth of *P. wasabiae* than did other tested media. PDA medium was the best for pycnidial production that produced nearly two and eighty-eight fold of pycnidia as compared with 10% wasabi rhizome extract agar and malt extract agar, respectively. However, the V-8 juice agar was the highest the percentage of pycnidiospore release from pycnidia of *P. wasabiae* on per plate.

Effect of temperature on mycelial growth, and pycnidial production

Temperature significantly affected the linear hyphal extension rates of two isolates of *P. wasabiae* (Table 2). Both isolates grew over 4 to 32℃ with a maximum extension rate of 41 to 44 mm/7 days at 24℃ and 28℃. No growth occurred only at 36℃. For pycnidial production, both isolates of *P. wasabiae* produced pycnidia on PDA medium between 12℃ and 32℃ with the optimum temperature at 20℃ to 28℃. No pycnidial production occurred at 4℃ to 8 ,and 36℃.

Table 1. Effect of different media on the mycelial growth, pycnidial production and pycnidiospore release of *Phoma wasabiae*

Medium	Mycelial [1] growth (mm)	No. of pycnidia [2] per plate (9 cm dia.)	Percentage of [3] pycnidiospore release
Corn meal agar	35.3	252	94
Czapek-dox agar	52.4	68	0
Malt extract agar	33.3	521	0
Potato dextrose agar	45.7	4437	89.3
5% V8 juice agar	44.7	302	97.8
10%V8 juice agar	47.3	510	98.3
20%V8 juice agar	50.9	703	100.0
5 % Wasabi rhizome extract agar	53.0	300	90.4
10% Wasabi rhizome extract agar	49.5	2623	43.1
20% Wasabi rhizome extract agar	28.4	1758	53.8
5% Wasabi leaf & stalk extract agar	52.0	135	91.5
10% Wasabi leaf & stalk extract agar	55.3	216	92.5
20% Wasabi leaf & stalk extract agar	54.8	426	93
LSD(p=0.05)	3.2	126	2.6

[1.] Colony diameters were measured 7 days after incubation.
[2.] Data were counted 14 days after incubation.
[3.] Data were caculated 14 days after incubation.

Effect of pH on mycelial growth, and pycnidial production

Pycnidiospore germinated between pH4.5 and pH9.5 with the optimum pH value from 6.5 to 7.5. However, mean dry weight of the fungal biomass occurred at pH values between 3.5 and 9.5 with largest biomass production taking place at pH 7.0 (Table 3).

Effect of carbon and nitrogen on mycelial growth, pycnidial production and pycnidiospore germination

The effect of various carbon and nitrogen sources on pycnidiospore germination, mycelial growth, and pycnidial production were presented in Table 4. Of the carbon sources, starch resulted in the highest linear growth for seven days incubation and higher germination percentage of pycnidiospores for 17 hr incubation at 24℃. However, only sorbitol, myo-sorbitol and inulin were the better for pycnidial production among the tested carbon sources. For nitrogen sources, cysteine and glutamic acid were less beneficial to mycelial growth of the pathogen strain than did other nitrogen sources. Adding cysteine and agrinine produced lower germination of pycnidiospores than adding without nitrogen or adding sodium nitrite and sodium nitrate produced

Table 2. Effect of temperature on mycelial growth, pycnidiospore germination and pycnidial production of *Phoma wasabiae*

Temperature	Mycelial growth (mm) [1]		Pycnidiospore germination (%) [2]		No. of pycnidiaper plate [3]	
(℃)	A[4]	B	A	B	A	B
4	3.5	3.5	0.0	0.0	0.0	0.0
8	6.7	7.7	0.0	0.0	0.0	0.0
12	15.7	13.5	8.0	9.0	527.0	464.0
16	17.5	16.9	9.0	10.0	740.0	809.0
20	38.0	37.5	75.0	73.0	5285.0	5429.0
24	44.3	42.3	85.0	87.0	5546.0	5781.0
28	41.7	43.0	90.0	89.0	5610.0	5604.0
32	30.8	34.2	73.0	72.0	1905.0	1885.0
36	0.0	0.0	0.0	0.0	0.0	0.0
LSD (p=0.05)	2.6	3.1	13.1	11.2	61.0	112.0

1. Data were measured 7 days after incubation.
2. Data were rated 17 hours after incubation.
3. Data were counted 14 days after incubation.
4. A and B represented Al-5-l-1 and DB-2-R-2 isolates of *P. wasabiae*, respectively.

Table 3. Influence of pH value on the pycnidiospore germination and mycelial growth of *Phoma wasabiae*

PH value	Pycnidiospore germination (%)[1]	Dry weight of mycelia (mg)[2]
2.5	0.0	0.0
3.5	0.0	11.4
4.5	52.1	130.2
5.5	80.8	227.1
6.5	86.1	220.2
7.0	89.4	250.8
7.5	88.1	238.6
8.5	76.4	217.7
9.5	55.3	24.5
10.5	0.0	0.0
11.5	0.0	0.0
LSD (p=0.05)	6.2	12.8

1. Data were rated 17 hours after incubation.
2. Data were measured 10 days after incubation.

germination percentage of 40 or less for 17 hr incubation at 24℃ (Table 5).

Effect of carbon (C) : nitrogen (N) ratios on mycelial growth, and pycnidial production

Significant increases in biomass production were found for the strain as the C: N ratio was increased from 10 : 1 to 80 : 1 with the carbon concentration constant at 8.42 g atoms C per liter (Table 6). In the similar results, biomass production of the strain was increased form 10 : 1 to 100 : 1 with the nitrogen concentration constant at 0.329 g atoms N per liter (Table 7). For pycnidial production, the C/N ratio was often over 70 as nitrogen concentration was constant and over 80 when carbon concentration was fixed. However, only the basal medium without nitrogen source also produced mature pycnidia for 14 days incubation at 24℃ (Table 6, 7).

Table 4. Effect of carbon sources on the mycelial growth, pycnidial production and pycnidiospore germination of *Phoma wasabiae*

Carbon sources [1]	Mycelial [2] Growth (mm)	No. of pycnidia [3] per plate (9 cm dia.)	Pycnidiospore [4] germination (%)
Starch	57.8	0	47.6
Sorbitol	43.8	1004.5	29.2
Glucose	37.0	0	29.4
Sucrose	39.5	0	34.7
Lactose	37.8	0	45.3
Fructose	48.3	0	30.3
D-Xylose	48.5	0	25.3
Cellulose	41.3	0	24.5
Galactose	33.4	0	39.8
Myo-inositol	26.8	868.6	28.1
Arabinose	37.3	0	25.7
Maltose	30.5	0	14.4
Inulin	49.3	793.5	22.3
CK	45.4	0	26.3
LSD (p=0.05)	5.6	131	4.6

1. The carbon of various carbon sources was equal in amount (8.42 g atoms C per liter).
2. The data were recorded 7 days after incubation.
3. The data were recorded 14 days after incubation.
4. The data were recorded 17 days after incubation.

Effect of water potential

The hyphal extension rate of the fungus declined with decreasing water potential over the range -1 to -56 bars. No mycelium grew on this basal media adjusted to water potential in the range -66 to -98 bars with NaCl (Table 8). Same results were obtained on pycnidiospore germination (Table 8) as mycelial growth of the fungus in the range of

Table 5. Effect of nitrogen sources on the mycelial growth, pycnidial production and pycnidiospore germination of *Phoma wasabiae*

Nitrogen sources[1]	Mycelial[2] Growth (mm)	No. of pycnidia[3] per plate (9 cm dia.)	Pycnidiospore[4] germination (%)
Histidine	47.0	0	23.2
Asparagine	53.0	0	21.6
Alanine	51.0	1416.5	27.1
Arginine	14.0	0	3.4
Methionine	39.5	0	12.0
Glutamic acid	35.5	0	36.3
Glycine	53.0	0	35.4
Sodium nitrate	50.5	0	37.0
Urea	50.2	0	28.8
Sodium nitrite	54.5	0	40.4
Cysteine	30.3	0	0.0
CK	48.5	687	40.0
LSD (p=0.05)	2.6	80	2.8

1. The nitrogen of various nitrogen sources was equal in amount (0.329 g atoms N per liter).
2. The data were recorded 7 days after incubation.
3. The data were recorded 14 days after incubation.
4. The data were recorded 17 days after incubation.

Table 6. Effect of C/N ratio (0.329 g atoms N per liter) on the mycelial growth, and pycnidial production of *Phoma wasabiae*

C/N Ratio	Mycelial[1] growth (mm)	No. of pycnidia[2] per plate
0	41.8	—
10	61.2	—
20	59.6	—
30	56.2	—
40	54.3	—
50	53.7	—
60	51.4	—
70	52.7	+
80	53.0	+
90	51.8	+
100	50.6	+
∞	49.8	649
LSD (p=0.05)	2.8	26

1. The data were recorded 7 days after incubation.
2. The data were recorded 20 days after incubation.
3. Pycnidia development:"—" = no pycnidium, "+" = only pycnidial structure and no pycnidiospore.

tested water potential.

After 14 days incubation on the basal media with NaCl, pycnidial production and pycnidiospore release had occurred only at water potential among -1 to -10 bars. No production and sporulating of pycnidia of the fungus occurred between the water potential of -24 and -98 bars (Table 8).

Table 7. Effect of C/N ratio (8.42 g atoms C per liter) on the mycelial growth and pycnidiae production of *Phoma wasabiae*

C/N Ratio	Mycelial[1] growth (mm)	No. of pycnidia[2] per plate (9-cm-dia.)
0	41.8	—
10	46.0	—
20	54.2	—
30	56.0	—
40	57.0	—
50	57.3	—
60	56.3	—
70	56.8	+
80	55.6	+
90	59.0	+
100	59.4	+
∞	49.8	649
LSD (p=0.05)	4.2	32

1. The data were recorded 7 days after incubation.
2. The data were recorded 20 days after incubation.
3. Pycnidia development:"—" = no pycnidium, "+" = only pycnidial structure and no pycnidiospore.

Effect of light intensity

For light intensity tests, there was no significant difference in the linear hyphal extension rates of *P. wasabiae* when treated with the light intensity of the range over 20 to 300 μE m^{-2} s^{-} under a 12-hr day-length cycle. The fungus grew significantly faster under all light intensity with a 12-hr day-length cycle than under the dark (Table 9). However, the fungus was significantly affected by the light intensity for hyphal extension rates at 20℃. The optimum extension rates were between 60 to 150 μE m^{-2} s^{-} under a 24 hr day-length cycle (Table 9).

After 14 days' incubation, the optimum light intensity for pycnidial production was 150 to 200 μE m^{-2} s^{-} under a 12-hr day-length cycle and 80 to 100 μE m^{-2} s^{-} under a 24 hr day-length cycle (Table 9).

DISCUSSION

This study had identified some nutritional requirements of the fungus. Although these requirements were not unusual, this information was important in designing methods for reducing the mass production of the pathogen for use of organic matters or soil amendments. In the vitro studies, Yokogi reported that PDA and cabbage extract medium were better for mycelial growth of *Phoma wasabiae* than did other media [27]. For various medium tests, we also got the same results for mycelial growth of the pathogen of wasabi on PDA medium. Moreover, our data indicated that PDA medium was the best for pycnidial production among the test media. However, V-8 juice agar had higher percentage of pycnidiaospore release from pycnidia of *P. wasabiae* than did

Table 8. Influence of water potential on pycnidiospore germination, mycelial growth, pycnidial production and pycnidiospore release of *Phoma wasabiae*

Water Potential (-bars) [1]	Pycnidiospore germination [2] (%)	Mycelial Growth[3] (mm)	No. of pycnidia Growth[4] (9-cm-dia.)	Pycnidiospore release from [4] pycnidia (%)
1	100	73.5	465	97.6
6	99.2	46.5	309	93.7
10	98.2	34.0	234	42.4
24	91.8	19.8	0	0.0
33	10.3	10.3	0	0.0
41	6.7	7.5	0	0.0
56	4.5	4.0	0	0.0
66	0.0	0.0	0	0.0
76	0.0	0.0	0	0.0
98	0.0	0.0	0	0.0
LSD(p=0.05)	4.2	6.2	42	2.3

[1.] The water potential was adjusted by adding appropriate dosage of NaCl into corn meal agar.
[2.] Data were rated 17 hours after incubation.
[3.] Data were taken 10 days after incubation.
[4.] Data were measured 14 days after incubation.

Table 9. Influence of intensity of illumination on mycelial growth and pycnidial production of *Phoma wasabiae* at 20℃

Intensity of illumination (μE m^{-2} s^{-})	Mycelial growth[1] (mm)		No. of pycnidia per plate[2] (9-cm-dia.)	
	A[3]	B[4]	A	B
0	24.3	24.3	137	568
20	37.2	33.5	124	1215
40	35.8	35.7	161	3360
60	38.5	39.2	212	4318
80	40.0	42.8	259	4954
100	38.7	45.7	338	5094
150	36.9	44.7	1059	4793
200	37.1	33.8	978	1198
300	32.3	4.9	676	0
LSD(p=0.05)	2.5	2.6	32	126

[1.] The data were recorded 10 days after incubation.
[2.] The data were recorded 14 days after incubation.
[3.] A= a 12-hr day-length cycle beginning with the light period.
[4.] B= a 24-hr day-length cycle.

on other test media. In C/N ratio requirements, the data showed that the more nitrogen concentration, the less pycnidial production. On the other hand, the carbon source could play an important role for the pycnidia development of *P. wasabiae*. Consequently, organic matters or soil amendments with lower C/N ratio could be used to reduce the pycnidial production of the pathogen.

This investigation had also identified the temperature, pH, water potential, nutrient, and light intensity optima for growth of the pathogen. For temperature tests, mycelial growth of *P. wasabiae* occurred from 4 to 32℃, with the optimum being 24-28℃. Production of pycnidia occurred from 12-32℃ and was greatest from 24-28℃. These results are similar to those obtained by Yokogi [27] and Wong *et al* [26]. Yokogi and co-workers reported that the optimum temperature for growth and pycnidial production of the fungus was 25℃ and there was no hyphal extension above 34 ℃ [27]. Wang *et al* (1992) also reported that the optimum temperature for pycnidial germination of the fungus was between 20 and 28℃ [26]. The conditions favoring the growth, germination and pycnidia production of *P. wasabiae* explained one of the reasons why phoma leaf spot of wasabi infected by the pathogen were more serious at Ta-pang and Alishan fields in summer than that of in winter [26].

The fungus produced less biomass and lower percentage of pycnidiospore germination below pH 4.5 and above pH 8.5 than between pH 5.5 and pH8.5. The results indicated that pH value might not be an important factor for affecting the disease development of wasabi infected by the pathogen because there were wide range of pH value for mycelial growth of the pathogen. In water potential requirement, hyphal extension rates declined with decreasing water potential with the minimum for growth lying between -56 and -98 bars. Particularly, the pycnidial formation and releasing pycnidiospores were only above -10 bars. The pycnidial production did not occur between -24 and -98 bars on media adjusted with NaCl. Similar results have been obtained for a range of plant pathogens including *Phoma* spp. [23,26]. For example, Wang *et al* have reported that the higher relative humility (> 98% RH) or free water was beneficial for pycnidiaospore germination, the number of pycnidial production, and pycnidiospore release of pycnidia of the pathogen [26]. The results in this paper seemed to confirm the earlier observations in field [15,17].

Vanniasingham and Gilligan (1989) reported that light intensity affected both the daily rate of production and the number of sporulating pycnidia of *Leptosphaeria maculans* Ces. & de Not. (anamorph *Phoma lingam* Desm.) [23]. Our

results indicated that there was part of interaction on mycelial growth and pycnidial production of *P. wasabiae*, when light-intensity and daylength were examined in combination. Particularly, the hyphal extension and pycnidial production of the fungus was significantly reduced in the 300 μE m^{-2} s^{-} under a 24 hr day-length cycle as compared with under a 12-hr day-length cycle. The results implied that pycnidial production might occur only under the maximum energy from light.

Phoma leaf spot caused by *P. wasabiae*, was a common fungal disease on wasabi in the central and central-southern counties of Taiwan [4,9,10,16,17]. The pycnidiospores are usually exuded during wet weather from pycnidia on infected plant tissues and can be disseminated by splashing rain or sprinkler irrigation [5,7,8,21,22,23]. Therefore, all data together, removing infected tissues, such as leaves and petioles on/in wasabi field are recommended as a fundamental control practices for the phoma disease of wasabi. On the other hand, preventing secondary infection using plastic cover can also be useful for reducing the wasabi disease in the wasabi field.

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摘　要

羅朝村[1,2]、王貴美[1]. 2000. 影響山葵黑心病菌柄子殼產生及柄孢子發芽的因子．植病會刊 9:99-106.
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在山葵黑心病菌危害山葵之過程中，柄孢子是其重要感染源；而柄孢子則來自於柄子殼。由試驗得知，影響柄子殼產生及柄孢子發芽的營養與環境因子，主要計 (1) 在測試的培養基中，以 PDA 產生柄子殼較多；至於營養成分 (碳、氮源) 方面，碳源可能是影響柄子殼產生或成熟與否的主要原因；(2) 溫度以 20-28℃ 較佳；(3) 水分潛勢在 -1 bar 至 -10 bars 間最佳；(4) 酸鹼值雖適應範圍廣，但以 pH6.5-7.5 間較佳，(5) 光照強度，在十二小時照光下以 150 至 200 μE m^{-2} s^{-} 較適合，但在二十四小時照光下則以 80 至 100 μE m^{-2} s^{-} 較適合。

關鍵詞：山葵、山葵黑心病菌、柄子殼、營養、環境因子