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Effect of Temperature, Fertilizer Level, and Fungicide Application on the Severity of Arrowhead Leaf Spot

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ABSTRACT

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Disease severity of leaf spot of arrowhead caused by *Cylindrocarpon chiayiense* was higher in a field applied with less nitrogen fertilizer than with more nitrogen fertilizer. However, the disease severity was not affect by the amount of phosphorus or potassium fertilizer applied. The optimum temperature range for the disease development was 15 to 20 C. In a potted plant trials, flusilazole and pyrifenox applied at 1 ml (*a.i.*)/20 L of water were effective, while validacin applied at the same rate was not effective in controlling the disease.

Key words: *Cylindrocarpon chiayiense*, fertilizer, chemical control, arrowhead leaf spot.

INTRODUCTION

Arrowhead (*Sagittaria trifolia* L. var. *sinensis*, Makino) is a monocotyledonous plant native in China and belongs to the Alismaceae. The plant has arrowhead-shaped leaves and is grown for corms (2,10). A leaf spot disease on arrowhead (CV: Paita) plants caused by *Cylindrocarpon chiayiense* Matsushima was found in Chiayi during the winter season, 1991 (9). Symptoms of the disease were characterized by development of small, brown foliar lesions with chlorotic outer rings, which quickly expanded and coalesced each other to cause collapse of the petioles and the leaves. The objectives of this study were to determine the effect of the fertilizer level and temperature on disease development, and to compare the efficacy of three fungicides on controlling the disease.

MATERIALS AND METHODS

Inoculum production

An isolate CCh-8 of *C. chiayiense* isolated from an infected arrowhead leaf which was collected from a field in Chiayi in 1991, was used through out this study. The spore suspension was prepared by growing *C. chiayiense* on potato-dextrose agar (PDA) plates at 25 C with diurnal light (12 hr) for 15 days, flooding the culture with sterile distilled water, and subsequently adjusting the concentration of the suspension to 10^6 conidia per milliliter. The spore suspension added with 0.02% Tween 20 (v/v) was used as the inoculum.

Influence of fertilizer level on disease severity

The experiment was conducted in a paddy field in Chiayi, from September 28, 1991 to January 28, 1992. Soil in this field was a clay loam type, with a pH of 7.02 and an organic matter content of 1.81% (Table 1). Eleven levels of fertilizer used were 0-0-0, 0-80-160, 40-80-160, 80-80-160, 120-80-160, 80-0-160, 80-40-160, 80-120-160, 80-80-0, 80-80-80, and 80-80-240 of $N:P_2O_5:K_2O$ (Kg/ha). Plots were applied with calcium superphosphate at the time of land preparation. Two other fertilizers, ammonium sulphate and potassium chloride, were applied at 15, 30, and 45 days after planting. Propagation of arrowhead (CV: Paita) was by using clean corms. They were planted in a nursery on July 20. When tillers were about 20–25 cm high, they were transplanted to the experiment field. Plant spacing was 40 cm between and 40 cm across the rows. Three plots (12 m^2 each) were used for each treatment. They were arranged in a randomized complete block design.

Disease severity was based on the number of diseased leaves counted from the base to the top on a single plant (8), and rated on 0–5 scales, where 0=no leaves showing symptoms, 1=1st leaf showing symptoms, 2=1st and 2nd leaves showing symptoms, 3=1st to 3rd

TABLE 1. Soil properties of the experimental field located in Chiayi, Taiwan

Sampling time	$N-P_2O_5-K_2O$ (Kg/ha)	pH value	Organic matter (%)	P (ppm)	K (ppm)	Ca (ppm)
Pre-planting		7.02	1.81	54.8	76.25	1062.5
Post-harvest	0- 80-160	7.19	1.73	37.5	79.25	1387.5
	40- 80-160	7.22	1.66	35.2	82.95	1568
	80- 80-160	7.26	1.84	39.4	90.5	1843.5
	120- 80-160	7.29	1.7	49.4	91.75	1478
	80- 0-160	7.3	1.74	42	109.75	1064.95
	80- 40-160	7.4	1.86	43	77.5	911.25
	80-120-160	7.27	1.81	49.4	85.5	911
	80- 80- 0	7.29	1.78	40.8	83.5	973.2
	80- 80- 80	7.41	1.83	43	89.5	938.25
	80- 80-240	7.4	1.71	36.2	83.5	939
	0- 0- 0	7.41	1.66	49.7	106	891.75

leaves showing symptoms, 4 = 1st to 4th leaves showing symptoms, and 5 = 1st to 5th or more leaves showing symptoms. The disease was recorded on December 4, 1991, 68 days after transplanting, and arrowhead corms were harvested on January 28, 1992. Data from the experiment were subjected to variance analysis and differences were presented by Duncan's multiple range test.

Influence of temperature on disease severity

Arrowhead (CV: Paita) tillers 20–25 cm in height were planted in containers (50 × 40 × 12 cm), 20 tillers per container. All plants 14 days after transplanting were inoculated by spraying with the spore suspension as described above. Inoculated tillers were enclosed with polyethylene bags to keep at 100% R.H., and placed in the growth chambers with diurnal light (12 hr) with temperatures maintained at 15, 20, 25, 30, and 35 C. After 48 hr inoculation, polyethylene bags were removed. The disease severity was rated six days after inoculation as described above. Data from the experiment was subjected to a curvilinear regression analysis.

Disease control with fungicides

Arrowhead (CV: Paita) tillers 20–25 cm in height were planted in containers (50 × 40 × 12 cm), 20 tillers per container. Three containers were used for each treatment. All containers were put on a nursery in October, 1992. Plants were sprayed onto run-off with flusilazole, pyrifenox, and validacin (Table 3). Plants sprayed with distilled water were used as control. All treated plants were then inoculated by spraying with the spore suspension. Inoculation was made in the afternoon when the air temperature ranged from 17 to 28 C, and the plants remained artificially wet for 48 hr after inoculation. Treatments were arranged in a completely randomized design. The effectiveness of treatments was based upon the reduction of the disease severity rated weekly as described above for 3 weeks. Data from the experiment were subjected to variance analysis and differences were presented by Duncan's multiple range test.

RESULTS

Influence of fertilizer level on disease severity

Soil properties of the experimental field were described on Table 1. Addition of different fertilizer levels into soil did not change soil pH, organic matter content and Mg concentration, but P, K, and Ca concentration might be changed, depending on the levels of fertilizer used. In the fertilizer experiment, disease severity of leaf spot within plots applied with less nitrogen fertilizer (0,40 kg/ha) was higher than those with more nitrogen fertilizer (80,120 kg/ha). In plots applied with 80 kg/ha of nitrogen fertilizer, P_2O_5 at the rate of 0–120 Kg/ha and K_2O at the rate of 0–240 Kg/ha did not significantly affect the disease development (Table 2). All fertilizer level treatments, except $N:P_2O_5:K_2O$ (Kg/ha) at 0-80-160, 40-80-160, and 80-0-160 reduced the disease severity.

Corm production increased in all fertilizer levels tested (Table 2). When $P_2O_5-K_2O$ was applied at 80-160 Kg/ha, corm production increased with increasing amount of nitrogen fertilizer. However, K_2O was not related to corm production. When $N-K_2O$ was applied at 80-160 Kg/ha, corm production decreased if P_2O_5 was applied.

There was a negative correlation between corm production and disease severity of leaf spot. The regression equation $Y = 16130.5 - 2659.4X$ was obtained, in which Y = yield of corms, and X = disease severity.

TABLE 2. Effect of fertilizer level on the severity of arrowhead leaf spot and yield of corms

N-P_2O_5-K_2O (Kg/ha)	Disease severity[1]	Yield of corms (Kg/ha)
0- 80-160	4.6 ab[2]	3320.20 d
40- 80-160	4.12 abc	4959.06 c
80- 80-160	3.51 c	6597.97 ab
120- 80-160	3.84 c	6577.06 ab
80- 0-160	4.1 abc	7618.44 a
80- 40-160	3.9 bc	5407.76 bc
80-120-160	3.84 c	5648.56 bc
80- 80- 0	3.87 bc	5706.22 bc
80- 80- 80	3.7 c	5925.08 bc
80- 80-240	3.66 c	5819.35 bc
0- 0- 0	4.72 a	3216.50 d

[1] Disease severity was recorded 68 days after planting based on disease ratings on a scale where 0=no leaves showing symptoms, 1=1st leaf showing symptoms, 2=1st and 2nd leaves showing symptoms, 3=1st to 3rd leaves showing symptoms, 4=1st to 4th leaves showing symptoms, and 5=1st to 5th or more leaves showing symptoms.

[2] Values in the same column followed by the same letter are not significantly different at 5% level according to Duncan's multiple range test.

The correlation coefficient, $r=-0.76$, is significant by t-test.

Influence of temperature on disease severity

The leaf spot disease was more severe on inoculated plants at 15–20 C than at 25–35 C (Fig. 1). Regression equation $Y=1.756+0.268X-0.007X^2$, $r=0.76$, was obtained, in which Y=disease severity, and X=temperature. The calculated F value for the quadratic was significant.

Disease control with fungicides

Brown lesions heavily developed on leaves and petioles in validacin and control treatments 7 days after inoculation. Spray with flusilazole and pyrifenox before inoculation significantly reduced the leaf spot disease, while spray with validacin was not effective (Table 4).

DISCUSSION

Four diseases were recorded on arrowhead in China and Japan. They were petiole base rot (caused by *Sclerotium hydrophilum* Sacc.)(5), Petiole rot (caused by *Pythium myriotylum* Drechsler)(12), leaf blight (caused by *Marssonina* sp.)(11), reddening and bulb rot (caused by *Fusarium* sp.)(4). Tsay and Tung (9) reported a new arrowhead disease, leaf spot, caused by *C. chiayiense* in Taiwan.

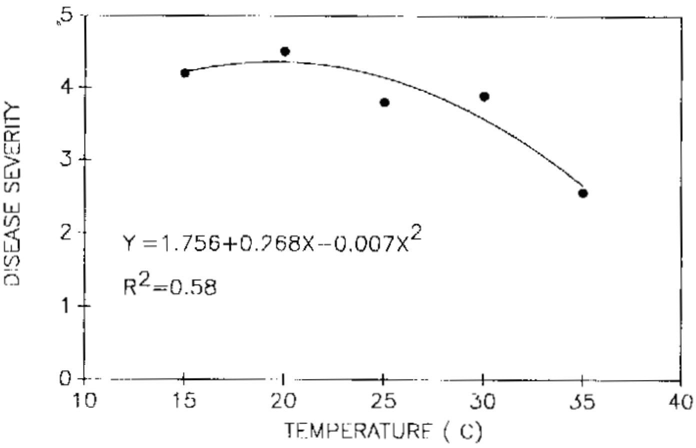

Fig. 1. Effect of temperature on the severity of arrowhead leaf spot.

In many diseases, the optimum temperature for disease development was different from that for growth of pathogen or host. For example, in the black root rot of tobacco, caused by *Thielaviopsis basicola* (Berk. & Br.) Ferr., the optimum temperature range for disease was 17 to 23 C, that for tobacco growth was 28 to 29 C, and that for the pathogen was 22 to 28 C (1). Similar results were obtained in our study on arrowhead leaf spot. The optimum temperature range for the disease was 15 to 20 C (Fig. 1), that for arrowhead growth was 18 to 25 C (3), and that for the pathogen was about 25 C (10).

Fertilizers are known to affect plant disease development (1). Reduced availability of nitrogen may increase the susceptibility of tomato to Fusarium wilt (1), and of potato to early blight (6). This was also true for arrowhead leaf spot as shown in this study (Table 2). Phosphorus has been reported to reduce the severity of take-all disease of barley and potato scab, but to increase the severity of cucumber mosaic virus on spinach and glume blotch on wheat (1). In the present study, P_2O_5 at the rate of 0-120 Kg/ha did not appear to affect the development of arrowhead leaf spot. Potassium has also been shown to reduce the severity of stem rust of wheat, early blight of tomato, and stalk rot of corn, although high amount of potassium seemed to increase the severity of rice blast and root knot which was caused by *Meloidogyne incognita* (Kofoid et White) Chitwood (1). Although K_2O at the rate of 0-240 Kg/ha did not affect the disease development in this study, corm production was increased with increasing amount of potassium applied at the rate of 0-160 Kg/ha, but decreased at 240 Kg/ha (Table 2).

Flusilazol and pyrifenox have been recommended to control powdery mildew, rusts, *Cercospora, Septoria, Molinia,* brown rot, foot rot, black rot, early and late leaf spot, scab, and others (7). In potted plant trials, flusilazole and pyrifenox were also effective in controlling leaf spot of arrowhead (Table 4). Further

TABLE 3. Fungicides used for control of leaf spot of arrowhead plant

Fungicide	Chemical name	Formulation[1]	Manufacturer
Flusilazol	1-((Bis(4-fluorophenyl)methylsilyl)methyl)-1H-1,2,4-triazole	40% E.C.	duPont de Nemours & Co., U.S.A.
Pyrifenox	2,4'-dichloro-2(3-pyridyl)acetophenone-O-methyloxime	20.8% E.C.	LA Quinoleine, France
Validacin	N-(IS-1,4,6/5)-3-hydroxymethyl-4,5,6-trihydroxy-2-cyclohexenyl(O-B-D-glucopyranosvl-(1-3)-IS-(1,2,4,3,5)-2,3,4-trihydroxy-5-hydroxymethyl-cyclohexyl) amine	5% S.	Takeda, Japan

[1] E.C.: Emulsifiable concentrates, S.: Solution.

TABLE 4. Effect of fungicides on controlling leaf spot of arrowhead plants in pots

Fungicide	Dilution fold	Disease severity[1]	
		I	II
40% Flusilazole E.C.	1 : 8000	0.23 c[2]	0.3 b
20.8% Pyrifenox E.C.	1 : 4000	0.13 c	0.34 b
5% Validacin S.	1 : 1000	1.85 b	4.15 a
Control		2.75 a	4.23 a

[1] Disease severity was determined one (I) and three (II) weeks after inoculation based on disease ratings on a scale where 0=no leaves showing symptoms, 1=1st leaf showing symptoms, 2=1st and 2nd leaves showing symptoms, 3=1st to 3rd leaves showing symptoms, 4–1st to 4th leaves showing symptoms, and 5=1st to 5th or more leaves showing symptoms.

[2] Values in the same column followed by the same letter are not significantly different at 1% level according to Duncan's multiple range test.

research should be conducted in field trials to evaluate the effectiveness of flusilazol and pyrifenox for control of arrowhead leaf spot.

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摘　　要

蔡竹固[1]、溫英源[2]、周居里[2]、童伯開[1]. 1993. 溫度、肥料用量及藥劑施用對慈姑葉斑病發生之影響. 植病會刊 2:136-140. (1. 嘉義市　國立嘉義農業專科學校植物保護科, 2. 嘉義市　國立嘉義農業專科學校農藝科)

在氮磷鉀肥料試驗田，11種肥料用量處理間慈姑葉斑病的發病度存在有差異，以不施肥組的發病最嚴重。當磷一鉀肥用量固定時，氮肥用量80或120 kg/ha較不施氮肥組發病爲低，差異達顯著。至於氮肥及鉀肥用量固定，磷肥用量0、40、120 kg/ha之間的發病度則無差異；或是氮肥及磷肥用量固定，則鉀肥用量0、80、240 kg/ha之間的發病度亦無差異。發病度(Y)與溫度(X)的關係，經二次曲線迴歸統計，所得之相關方程式爲 $Y=1.756+0.268X-0.007X^2$，$r=0.76$，迴歸係數顯著；最適發病溫度爲 15-20 C。護矽得及比芬諾(濃度50 ppm)對於慈姑葉斑病有防治效果，且與對照組不噴藥間有顯著差異，(相同濃度下)維利黴素處理的發病情形則與對照組之間沒有差異。

關鍵詞：*Cylindrocarpon chiayiense*、肥料、化學防治、慈姑葉斑病。