Baseline sensitivity of *Botrytis elliptica* to fludioxonil in Taiwan

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ABSTRACT

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Fludioxonil, the new phenylpyrrole fungicide, was introduced to control the gray mold disease of floral crops in Taiwan since 1997. To establish the baseline sensitivity of *Botrytis* spp. to fludioxonil, five hundred and thirty six isolates of *Botrytis elliptica*, 32 isolates of *B. tulipae*, 84 isolates of *B. gladiolorum* and other 20 phytopathogenic fungi were tested during past years. Log value of fludioxonil concentration and inhibition ratio in each treatment were used as variables in simple linear regression analysis. Effective concentration for 50% inhibition (EC_{50}) and minimum inhibitory concentration (MIC) of *B. elliptica* to fludioxonil were ranged from 0.002±0.001 to 0.048±0.025 mg/liter and 0.091±0.037 to 0.625±0.335 mg/liter, respectively. EC_{50} of *B. tulipae* and *B. gladiolorum* were 0.042±0.010 and 0.018±0.003 ~ 0.026±0.011 mg/liter, respectively. The results also showed that fludioxonil did not inhibit the mycelial growth of *Colletotrichum gloeosporioides*, *Fusarium oxysporum* f. sp. *lilii*, *F. solani* and Oomycetes, such as *Phytophthora* spp., *Pythium myriotylum*, and *Peronophythora litchii*. EC_{50} of *B. elliptica* to fludioxonil detected before and after application of fludioxonil for 5 times at one-week intervals in the field trial were 0.006±0.009 and 0.003±0.002 mg/liter, respectively. The result showed that there was no significant difference after intensive application of fludioxonil in the field (p=0.0799, n=60).

(Key words: *Botrytis elliptica*, *B. tulipae*, *B. gladiolorum*, fludioxonil, baseline sensitivity)

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INTRODUCTION

Lily (*Lilium* spp.) was one of popular cut flowers in Taiwan, and grown mainly in central Taiwan (http://eng.coa.gov.tw/htmlarea_file/web_articles/5846/106.pdf) from late autumn to spring next year. Most lily bulbs were imported from Netherlands and Latin America. Oriental lilies, such as Acapulco, Casa Blanca, and Marco Polo, were the major cut flowers in Taiwan floral market. Lily planted areas were 155 hectares in 1995 and expanded to 339 hectares in 2004 (http://eng.coa.gov.tw/htmlarea_file/web_articles/5846/106.pdf). Botrytis blight of lily caused by *Botrytis elliptica* Cooke occurred severely in spring season in Taiwan[5, 6]. Although chlozolinate and vinclozolin + metiram were recommended to apply continuously at 3 or 4 day intervals to control the Botrytis blight of lily in the rainy or foggy seasons, Botrytis blight still occurred and spread quickly[5, 6]. For example, 0.3-0.4 hectares of asiatic lily (*Lilium* asiatic hybrid cv. Polyanna) located at Shenkang, Taichung were destroyed within 3 weeks in spring of both 1995 and 1996[5]. In central Taiwan, flower farmers usually applied the dicarboximide fungicides, e.g. procymidone, vinclozolin and iprodione, without changing the fungicides with different mode of action through lily growing seasons and failed to control the disease in early years. No matter failure of disease control was due to the appearance of fungicide tolerant or resistant isolates, missing application time or unable to protect the infection sites of lily, the establishment of baseline sensitivity of *B. elliptica* to fungicides might be useful when the fungicide resistant isolates really appear in the field.

Fludioxonil, the analogue of phenylpyrrole antibiotic pyrrolnitrin produced by *Pseudomonas pyrociniae*[10], with mode of action different from those dicarboximide fungicides and the light-stable identity was used as a foliar and seed coating agent against *B. cinerea*, *Monilinia* spp., and *Sclerotinia* spp.[3, 7] It's been registered to control the gray mold of flowers in Taiwan since 1997. Fludioxonil induced the morphological abnormality of *B. cinerea* germ tubes such as swelling, branching or bursting, and its antifungitoxicity was reversed by alpha-tocopherol and piperonyl butoxide[8, 11]. Fludioxonil doesn't degrade in the water but by the light, and its mobility is low in soil and exists in 0-5 cm depth of soil[12]. The half life of fludioxonil was 20-23 days in vineyards' soils and 5-6 days in soils without any canopy[9]. Effective concentration for 50% inhibition (EC_{50}) of fludioxonil against *B. cinerea* has been reported[2], but there was no detail sensitivity data of *B. elliptica* to fludioxonil.

If the sensitivity of *B. elliptica* to the new introduced fludioxonil was established before its application in the field, the appearance of tolerant or resistant isolates could be monitored by comparing the sensitivity data before and after application of fludioxonil in the field. In this study, the baseline sensitivities of *B. elliptica*, *B. tulipae*, *B. gladiolorum* and selected 20 phytopathogenic fungi to fludioxonil were tested before fludioxonil registeration or application in the field. The sensitivity variation of *B. elliptica* to fludioxonil in lily growing seasons was also investigated.

MATERIALS AND METHODS

Source of isolates and inoculum preparation

Isolates of *Botrytis elliptica* were obtained from diseased tissue of lilies which were planted in Houli, Shenkang, Darlan, and Puli in central Taiwan by using Doss *et al.* method[1]. The same method was used to obtain *B. tulipae* isolates from infected leaves of gray mold of *Tulipa* spp. in 1998 and *B. gladiolorum* isolates from infected leaves of gray mold of *Gladiolus* spp. in 1999 and 2000. Besides *Botrytis* spp., twenty phytopathogenic fungi were also isolated before the fludioxonil was applied in the field since1997 (Table 1).

Mycelial discs (0.5 cm diameter) of these fungi growing on potato dextrose agar (PDA, Difco Lab.) at 24^{0}C for 3-4 days were cut from the colony margins with a sterile cork borer, and used as inocula for fungicidal efficacy tests.

Table 1. List of phytopathogenic fungi used in this study

Fungi	Disease
Phylum Zygomycota	
Choanephora cucurbitarum	Choanephora rot of cucumber
Rhizopus stolonifer	Rhizopus fruit rot
Phylum Oomycota	
Peronophythora litchii	Downy blight of litchi
Phytophthora capsici	Phytophthora rot of red pepper
Phytophthora cinnamomi	root rot of fruit trees
Phytophthora melonis	Phytophthora rot of melon
Phytophthora parasitica	Phytophthora rot of lily
Pythium myriotylum	Soft rot of zinger
Phylum Ascomycota	
Alternaria brassicola	Black spot of cabbage
A. porii	Purple leaf spot of onion
A. solani	Early blight of tomato
Botryosphaeria ribis	Grape bunch rot
Ceratocystis fimbriata	Black rot of sun hemp
Colletotrichum gloeosporioides	Anthracnose of mango
Fusarium oxysporum f. sp. *lilii*	Fusarium wilt of lily
Fusarium solani	Root rot of lily, calla lily
Pyricularia oryzae	Rice blast
Stemphylium vesicarium	Leaf blight of green onion
Phylum Basidiomycota	
Rhizoctonia solani AG4	Seedling blight of lily
Sclerotium rolfsii	Southern blight of lily

Bioassay

Fludioxonil 50WG (50% water dispersible granule), provided by Syngenta Corp., was used as the tested fungicide in this study. The efficacies of fludioxonil against *Botrytis* spp. and other phytopathogenic fungi were tested on PDA plates amended with different concentrations of fludioxonil at a range of 1000, 100, 10, 1, 0.1, 0.01, 0.001, 0 mg/liter for selected phytopathogenic fungi, and 1, 0.5, 0.1, 0.05, 0.01, 0.005, 0.001, 0 mg/liter for *Botrytis* spp. After mycelial discs were placed on PDA plates with or without fludioxonil, colony diameter of each treatment was measured 3 days after incubation at 24°C. Six replicates were used in each treatment. Inhibition percentage was obtained from the radial growth of tested fungi on fludioxonil amended PDA plates dividing with that of fungi on PDA plates without any fungicide.

Simple linear regression analysis was used to calculate the sensitivity of *Botrytis* spp. and phytopathogenic fungi to fludioxonil through the study. Log value of fludioxonil concentration and growth inhibition percentage of tested fungi in this concentration were used as variables in simple linear regression analysis. When the coefficient of determination (R^2) was larger than 0.8 and residual plot showed random pattern, the effective concentration for 50% inhibition (EC_{50}) and minimum inhibitory concentration (MIC) were calculated from the simple linear regression model and used as the sensitivity data of those tested fungi to fludioxonil.

Within the same location where *Botrytis* spp. were isolated, sensitivity data of *B. elliptica* and *B. gladiolorum* to fludioxonil were compared yearly by using analysis of variance (ANOVA).

Sensitivity variation of *Botrytis elliptica* to fludioxoinil after intensive application of fludioxonil in the field

Field trial was carried out on commercial lily farm in Hsichou, Shengan, Taichung hsien from March 11 to April 15 in 1998. Fludioxonil 50WG in 0.067% (w/v) was applied weekly in Marco Polo lily planted area 5 times. Isolates of *B. elliptica* were collected before March 11 and on April 15, and the EC_{50} and MIC were analyzed from their sensitivity variation.

RESULTS

Sensitivity of *Botrytis* isolates to fludioxonil

Five hundred and thirty six isolates of *B. elliptica*, 32 isolates of *B. tulipae* and 84 isolates of *B. gladiolorum* were obtained from Houli, Shenkang, Darlan, and Puli from 1997 to 2004 in central Taiwan (Table 2). EC_{50} of fludioxoinl against *B. elliptica* isolated from Houli, Shenkang, Darlan, and Puli were 0.002±0.001~0.013±0.012, 0.007±0.002~0.048±0.025, 0.013±0.013, and 0.015±0.009~0.019±0.012 mg/liter, respectively (Table 2). MIC of fludioxoinl against *B. elliptica* from Houli, Shenkang, Darlan, and Puli were 0.091±0.037~0.223±0.223, 0.129±0.072~0.625±0.335, 0.340±0.033, and 0.254±0.091~0.319±0.088 mg/liter, respectively (Table 2). EC_{50} of fludioxonil against *B. tulipae* and *B. gladiolorum* were 0.042±0.010 and 0.018±0.003~0.026±0.011 mg/liter, respectively (Table 2).

Table 2. Sensitivity variation of *Botrytis elliptica*, *B. gladiolorum* and *B. tulipae* to fludioxonil in central Taichung

Year	MIC[1]	EC_{50}	Sample size[2]
		Botrytis elliptica	
		Houli, Taichung	
1997	0.223±0.223 a[3]	0.011±0.005 a	33
1999	0.187±0.170 ab	0.013±0.012 a	30
2000	0.152±0.033 b	0.002±0.001 c	30
2001	0.091±0.037 c	0.006±0.001 b	52
2004	0.118±0.048 bc	0.005±0.003 bc	9
		Shenkang, Taichung	
1997	0.221±0.167 b	0.007±0.012 b	28
1998	0.219±0.202 b	0.011±0.006 b	37
1999	0.625±0.335 a	0.048±0.025 a	30
2000	0.144±0.072 c	0.010±0.007 b	88
2001	0.129±0.072 c	0.007±0.002 b	60
		Darlan, Taichung	
1998	0.340±0.033	0.013±0.013	59
		Puli, Nantou	
1999	0.319±0.088 a	0.019±0.012 a	20
2001	0.254±0.091 b	0.015±0.009 a	60
		B. tulipae	
1998	0.354±0.031	0.042±0.010	32
		B. gladiolorum	
		Houli, Taichung	
1999	0.237±0.143 a	0.019±0.011 b	27
2000	0.201±0.091 a	0.026±0.011 a	28
		Puli, Nantou	
2000	0.162±0.044	0.018±0.003	29

[1] MIC and EC_{50} values were calculated from simple linear regression model. MIC: minimum inhibitory concentration of mycelial growth. EC_{50}: Effective concentration for 50% inhibition of mycelial growth. Mean ± S.D. (Standard Deviation of the mean) were results of 6 replicates per isolate.

[2] Number of *Botrytis* spp. isolated from the infected leaves or tissue of host plants.

[3] In the same location, means within columns followed by the same letter do not significantly differ according to the Duncan's new multiple range test or t-test (p=0.05).

Sensitivity of selected phytopathogenic fungi to fludioxonil

EC_{50} of fludioxonil against *Rhizoctonia solani* T1, *Alternaria solani*, *Botryosphaeria ribis*, *A. porii*, *R. solani* R12, *Stemphylium vesicarium*, *A. brassicola*, and *Sclerotium rolfsii* were 0.0203, 0.0384, 0.0404, 0.0410, 0.0596, 0.0611, 0.0931, and 0.1038 mg/liter, respectively (Table 3). Mycelial growth of *Fusasrium oxysporum* f. sp. *lilii*, *F. solani*, Oomycetes such as *Phytophthora* spp., *Pythium myriotylum* and *Peronophythora litchii* were not affected by fludioxonil.

Sensitivity variation of *Botrytis elliptica* to fludioxoinil after intensive application of fludioxonil in field

EC_{50} values of *B. elliptica* to fludioxoinl before and after application of fludioxonil for 5 times at one-week intervals in field trial were 0.006±0.009 and 0.003±0.002 mg/liter, respectively, and there was no significant difference after intensive application of fludioxonil (p=0.0799, n=60). MIC values of these treatments were 0.200±0.368 and 0.123±0.018 mg/liter, respectively, and also showed no significant difference after intensive application of fludioxonil (p=0.2570, n=60).

DISCUSSION

Efficacy of fludioxonil against *B. cinerea* reported in Europe and its EC_{50} value was 0.0217±0.0096 mg/liter(n=14)[2]. In this study, the results showed that EC_{50} of *B. elliptica*, *B. tulipae* and *B. gladiolorum* to fludioxonil ranged from 0.002 to 0.048 mg/liter, and that only those of *B. elliptica* from Shenkang, Taichung in 1999 and *B. tulipae* from Houli, Taichung in 1998 were higher than 0.04 mg/liter (Table 2). The EC_{50} of *B. elliptica* to fludioxonil from Shenkang, Taichung in 2000 was decreased to 0.01 mg/liter and kept the similar EC_{50} value in 2001. The reasons why fludioxonil tolerant or resistant isolates did not appear next year could be due to the fact that the lily was only grown from late autumn to next year's late spring in lower altitude area, and that rotation with rice resulted in reduction of the survival ability of *B. elliptica* in hot weather and flooded soils in central Taiwan[13]. In addition, the yearly variations of EC_{50} also suggested that *B. elliptica* populations maybe come from oversea countries because the bulbs were imported from Netherlands and Latin America yearly.

Results from this study showed that EC_{50} did not shift dramatically after application of fludioxonil for 5 times at one-week intervals (p=0.0799, n=60). This result also revealed that fludioxonil could be a good protectant to control Botrytis blight of lily[6]. During the study, the lily growers were suggested to alternate the fungicides with different mode of action beside the fludioxonil to control Botrytis blight. Up to now, Botrytis blight of lily was controlled well under our recommendations. *B. elliptica* isolates were still isolated from field and their EC_{50} values were analyzed in the laboratory yearly. Results still showed no occurrence of fludioxonil tolerant or resistant isolate in the field in central Taiwan (unpublished data).

Results from this study also showed that *Alternaria*, *Botrysophaeria*, *Botrytis*, *Stemphylium*, *Rhizoctonia* and *Sclerotium* spp. were strongly inhibited by fludioxonil, but *Peronophythora, Phytophthora* spp. and *Pythium myriotylum* were not (Table 3). In addition, the result showed that fludioxonil could be a good ingredient to develop the Oomycetes selective medium since fludioxonil could suppress the mycelial growth of soilborne pathogens, such as *Rhizoctonia* and *Sclerotium* spp. (Table 3).

Problems of fungicide resistance were always a concern in the disease management. For monitoring the fungicide resistance in the field, it was better to set up the baseline sensitivity of pathogens to newly introduced fungicides before the fungicides were applied. With those baseline sensitivity data, we could clearly define whether the resistant or tolerant isolates appeared or not, and make a decision to warn the growers to alternately use fungicides for controlling the disease caused by the resistant isolates in the field. Although there was no evidence

Table 3. Baseline sensitivity of selected phytopathogenic fungi to fludioxonil in Taiwan

Fungi	Index of Fungicide efficacy[1)]		Parameters of simple linear regression[2)]		
	MIC	EC_{50}	Slope	Intercept	r^2
Alternaria brassicola	0.8660	0.0931	0.5161	1.0323	0.9984
A. porii	0.6928	0.0410	0.4071	1.0649	0.9292
A. solani	3.6674	0.0384	0.2525	0.8575	0.8673
Botryosphaeria ribis	0.5829	0.0404	0.4313	1.1011	0.8585
Ceratocystis fimbriata	$2.9x10^{2}$	3.1516	0.2546	0.3731	0.9419
Choanophora cucurbitarum	19.0261	0.1309	0.2312	0.7042	0.7826
Colletotrichum gloeosporioides	$7.4x10^{3}$	2.8775	0.1467	0.4326	0.8739
Fusarium oxysporum f. sp. *lilii*	18.4972	0.0119	0.1567	0.8014	0.6032
F. solani	$3.9x10^{-16}$	0.0710	-0.0351	0.4597	0.0322
Peronophythora litchii	$2.3x10^{8}$	$4.0x10^{3}$	0.1049	0.1221	0.9390
Phytophthora capsici	$2.2x10^{23}$	$1.7x10^{11}$	0.0413	0.0359	0.8167
Ph. cinnamoni	$1.0x10^{36}$	$2.0x10^{20}$	0.0319	-0.1473	0.0997
Ph. melonis	$1.9x10^{18}$	$2.7x10^{8}$	0.0508	0.0717	0.7963
Ph. parasitica	$1.8x10^{12}$	$3.4x10^{5}$	0.0742	0.0893	0.8889
Pyricularia oryzae	$1.1x10^{6}$	$2.4x10^{2}$	0.1367	0.1744	0.9116
Pythium myriotylum	$5.1x10^{34}$	$5.5x10^{16}$	0.0278	0.0340	0.6241
Rhizoctonia solani R12	0.9597	0.0596	0.4142	1.0074	0.9990
R. solani T1	0.5816	0.0203	0.3432	1.0808	0.9218
Sclerotium rolfsii	0.9179	0.1038	0.5283	1.0197	0.9959
Stemphylium vesicarium	1.1244	0.0611	0.3952	0.9799	0.9923

[1)] See footnote of Table 2.

[2)] Simple linear regression: Y=a+bX, where Y, inhibition percent of mycelial growth; a, intercept; b, slope of regression line; X, $\log_{10}$ (concentration of fludioxonil). r^2, coefficient of determination from regression analysis.

suggesting the occurrence of fludioxonil-tolerance or resistance isolates in Taiwan since 1997, further studies to establish the cross-resistance data among different mode of action of fungicides[4] to prevent or delay the occurrence of fungicidal resistance are needed in the future.

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摘　　要

李敏郎*　2006　臺灣百合灰黴病菌對護汰寧殺菌劑之基礎感受性分析　植保會刊 48：163– 171　(臺中縣霧峰鄉　行政院農業委員會農業藥物毒物試驗所農藥應用組)

護汰寧(fludioxonil)屬苯酚吡咯類(phenylpyrroles)殺菌劑，為 1997 年新引進用於防治灰黴病之藥劑。本試驗針對三種灰黴病菌對護汰寧殺菌劑之感受性進行分析。供試菌株包括 536 株百合灰黴病菌(*Botrytis elliptica*)、32 株鬱金香灰黴病菌(*B. tulipae*)、84 株唐菖蒲灰黴病菌(*B. gladiolorum*)及 20 株植物病原真菌。護汰寧濃度轉對數後，與該濃度下之菌絲生長抑制率同為變數，進行簡單線性回歸分析。*Botrytis elliptica* 之半數抑制濃度(effective concentration for 50% inhibtion, EC_{50})與最小抑制濃度(minimum inhibitory concentration, MIC)分別為 0.002±0.001 ~ 0.048±0.025 及 0.091±0.037 ~ 0.625±0.335 mg/liter。試驗結果亦顯示護汰寧對 *Colletotrichum gloeosporioides*、*Fusarium oxysporum* f. sp. *lilii*、*F. solani* 及 卵菌綱類之 *Phytophthora* spp.、*Pythium myriotylum* 與 *Peronophythora litchii* 之菌絲生長無抑制作用。田間百合每隔一週施藥一次，連續五次施用後調查灰黴病菌對護汰寧感受性偏移情形，結果顯示 *B. elliptica* 在施藥前後之 EC_{50} 分別為 0.006±0.009 及 0.003±0.002 mg/liter，無顯著差異 (p=0.0799, n=60)。

(關鍵詞： 百合灰黴病菌、鬱金香灰黴病菌、唐菖蒲灰黴病菌、護汰寧、基礎感受性)

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