

Relative sensitivity to the fungicides benomyl and iprodione of *Botrytis elliptica* from Taiwan and the Northwestern U.S.A.

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ABSTRACT

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Isolates of *Botrytis elliptica* were tested for growth on potato dextrose agar as well as potato dextrose agar amended with discriminatory concentrations of benomyl (1 µg a.i. ml⁻¹) or iprodione (10 µg a.i. ml⁻¹). Fourteen of these isolates originated from Washington or Oregon State in the USA, and the remaining 48 isolates were from Taiwan. Relative fungicide sensitivity was assessed by dividing the extent of growth after 96 h on amended media over the growth on unamended medium. There was no significant difference in growth rates on unamended PDA between isolates from Taiwan compared to those from the U.S.A.; however isolates from Taiwan showed higher relative growth, 4-fold or 2-fold greater, respectively, on benomyl or iprodione-amended media than isolates from the U.S.A. More research is needed to determine whether these Taiwan isolates showing less sensitivity to benomyl or iprodione also exhibit resistance in the field to normal fungicide application rates.

Key words: benomyl, *Botrytis elliptica*, fungicide sensitivity, iprodione

Blight caused by *Botrytis elliptica* (Berk.) Cooke is the most serious disease of lilies (*Lilium* spp.) wherever they are grown^(2,5,6,7). Because of the limited availability of cultivar resistance against *Botrytis* diseases, fungicides, particularly benzimidazoles and dicarboximides, have played an important role in disease control strategies. However, intensive use of these fungicides has led to the occurrence of strains resistant to one or both groups of fungicides in North America^(1,3) and in Europe⁽⁶⁾. There are, however, no reports of fungicide resistance for this pathogen from Asia, although these fungicides are commonly used. In Taiwan, dicarboximide fungicides such as procymidone, vinclozolin, and iprodione, and benzimidazole fungicides such as carbendazim and thiophanate-methyl are often used for control of *Botrytis* leaf blight of lilies. During the growing season, the growers apply fungicides at least once per week, and more often when lesions appear on the lower leaves. The purpose of this study was to compare isolates of *B. elliptica* from two important lily growing regions, Oregon and Washington States in the U.S.A. and the west-central region of Taiwan, for sensitivity to the fungicides benomyl and iprodione.

Isolates of *B. elliptica* from the USA were collected in 1986 from infected lily tissue obtained in Washington State

(Mossyrock, Mt. Vernon, Woodland) or Oregon (Aurora, Sandy). These isolates were originally obtained by visiting commercial bulb growers and collecting diseased lily foliage to determine if fungicide resistance was present in lily fields and greenhouses. The isolates have been used in previous publications^(3,4), and were maintained as stock cultures on oatmeal-sand at 4 °C⁽²⁾. Isolates from Taiwan were collected in 1996 and 1997 from Taichung, Changhua and Nantou counties in the west-central part of the island. A single large collection was made at Hsinshue, Taichung County on 23 February 1997 from several adjacent rows of lilies. In general, isolates of *B. elliptica* were obtained by surface-sterilizing diseased tissue in 1% hypochlorite for 30 s, rinsing in autoclaved distilled water, and transferring onto antibiotic-amended (100 µg ml⁻¹ streptomycin sulphate) potato dextrose agar (PDA) at 20 °C. After incubation for up to five days, the fungus was subcultured onto PDA and purified through a series of serial transfers. Stock cultures were maintained on oatmeal-sand at 4 °C.

Fungicide sensitivity was assessed by growth tests on fungicide-amended PDA. Plugs 4 mm in diameter were removed from the edges of actively growing colonies (3 to 4 days old), and placed with their mycelial surface down onto

Table 1. Fungicide sensitivity of 62 isolates of *Botrytis elliptica* from the west central region of Taiwan and the Pacific Northwest of the U.S.A.

Country	Isolates No. of	Daily growth on PDA (mm)	Relative growth on fungicide-amended medium ¹	
			Benomyl (1 µg ml ⁻¹)	Iprodione (10 µg ml ⁻¹)
Taiwan	48	8.1 ± 0.30	1.04 ± 0.006	0.089 ± 0.012
U.S.A.	14	9.2 ± 0.74	0.27 ± 0.011	0.045 ± 0.027
LSD	(p=0.05)	1.4	0.16	0.03

¹ Relative growth was calculated as growth on fungicide-amended media over growth on unamended PDA at 20 C. See text for method of calculation. Standard errors are given after each mean.

PDA or PDA containing discriminatory concentrations of iprodione (Rovral 50WP, 10 µg a.i. ml⁻¹) or benomyl (Tersan 1991 50WP, 1 µg a.i. ml⁻¹) in Petri plates. Plates were incubated at 20 °C, and radial growth of colonies was measured after 24, 48, 72 and 96 hr. Each isolate by fungicide combination was replicated three times.

Daily growth was calculated by taking the maximum growth on unamended PDA (either at 96 hr or, to avoid edge effects, at 72 hr), subtracting the growth during the first 24 hr (to minimize establishment effects), and dividing the result by the number of days of growth (either 2 or 3 days for 72 or 96 hr growth respectively). Relative growth on fungicide-amended PDA was calculated by dividing the daily growth on amended PDA (calculated as above) by the daily growth on unamended PDA. The growth data were subjected to an analysis of variance and when significant treatment effects were found (i.e. when Taiwan isolates differed in growth from U.S.A. isolates), the data were subjected to the test of least significant difference (LSD).

A total of 62 isolates was used in these studies. All isolates were obtained from separate lesions on different leaves or stems. Fourteen of the isolates originated from Washington or Oregon State in the USA, from various cultivars of Asiatic lily hybrids. The remaining 48 isolates were from Taiwan. Except for seven isolates from Easter lily (*L. longiflorum*) and one from an Asiatic lily hybrid, all the Taiwan isolates were from cultivars of Oriental lily (mostly hybrids of *L. auratum* and *L. speciosum*).

There was no significant difference in growth rates on unamended PDA between isolates from Taiwan compared to those from the U.S.A. (Table 1). However, when comparing relative growth on fungicide amended media, there were significant differences between isolates from the two countries (Table 1). Isolates from Taiwan showed higher relative growth on either fungicide-amended media than isolates from the U.S.A. (Table 1). Although the isolates from the U.S.A. had been in culture for over 10 years at the time of this study, the relative growth on fungicide-amended media reflected the previous classification of these isolates as sensitive or resistant from earlier studies (data not shown) except for one isolate which seemed to have decreased sensitivity to benomyl after storage. Perhaps this isolate was incorrectly classified initially. A much greater proportion of

isolates from the U.S.A. was totally inhibited by 1 µg ml⁻¹ benomyl or 10 µg ml⁻¹ iprodione than isolates from Taiwan (Table 2). This indicates that a random sample of Taiwan isolates had less sensitivity to either benomyl or iprodione than a random sample of U.S.A. isolates. This is the first report on fungicide sensitivity of *B. elliptica* isolates from Taiwan.

A correlation analysis for Taiwan isolates of the relative growth on benomyl-amended media compared to iprodione-amended media showed an r=0.09. This very low correlation indicates that these isolates had not been selected for multiple resistance to both benomyl and iprodione. Among the isolates from Taiwan showing the least sensitivity to benomyl (in the >1.25 relative growth category of Table 2), three isolates were from Taichung County and one from Nantou County. Two of Taichung county isolates were from Wufeng and one from Hsinshue demonstrating that decreased sensitivity to benomyl was distributed in several locations. Similarly, among the isolates showing the least sensitivity to iprodione (in the 0.2 to 0.3 relative growth category of Table 2), one isolate was from Changhua county, two from Nantou county and three from Taichung county, also demonstrating that decreased sensitivity to iprodione was distributed across the region. More research is needed to determine whether these isolates showing decreased sensitivity to benomyl or iprodione also exhibit resistance in the field to normal fungicide application rates.

Table 2. Number of isolates of *Botrytis elliptica* in each category of relative growth on PDA amended with benomyl or iprodione.

Relative growth ¹	Benomyl (1 µg ml ⁻¹)		Relative growth	Iprodione (10 µg ml ⁻¹)	
	Taiwan ²	U.S.A.		Taiwan	U.S.A.
0	0	10	0	11	10
0.01 - 0.75	6	0	0 - 0.09	20	1
0.76 - 1.25	36	4	0.1 - 0.19	11	2
> 1.25	4	0	0.20 - 0.30	6	1

¹ Relative growth was calculated as growth on fungicide-amended media over growth on unamended PDA at 20C. See text for method of calculation.

² There were two isolates missing in this test out of a total of 48 isolates from Taiwan.

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摘 要

向培健^{1,4}、謝廷芳²、Chastagner, G. A.³ 2001. 台灣和美國西北部的百合灰黴病菌對殺菌劑免賴得與依普同的相對感受性. *植病會刊* 10:93-95. (¹ Environmental Biology, University of Guelph, Guelph, Ontario, Canada; ² Department of Plant Pathology, Taiwan Agricultural Research Institute, Wu-feng, 413, Taichung, Taiwan; and ³ Puyallup Research and Extension Center, Washington State University, Puyallup, Washington, U.S.A.; ⁴ 聯絡作者, 電子郵件: thsiang@uoguelph.ca; 傳真: 519-8370442)

由 *Botrytis elliptica* 引起的百合灰黴病, 是國際上百合栽培區的重要病害之一。在缺乏優良抗病品種的情況下, 防治本病一直仰賴藥劑的使用。在密集的藥劑噴施下, 北美及歐洲地區已陸續出現抗藥性菌株的報導。本研究之目的即在於測試與比較台灣及美國的灰黴病菌菌株對免賴得 (屬 benzimidazole 藥劑) 和依普同 (屬 dicarboximide 藥劑) 兩種藥劑的感受性。由台灣中部地區罹灰黴病的百合植株上分離得 48 株灰黴病菌菌株, 並由美國華盛頓州百合罹病株上分得 14 個菌株, 分別培養在 PDA 及含藥劑的 PDA 平板上, 測試各菌株之菌絲生長情況。結果發現分離自台灣的菌株與美國的菌株間, 菌絲生長速率無明顯差異性; 然而, 比較分離自兩國之菌株在含藥劑的培養基上生長的情形, 發現台灣灰黴病菌菌株的菌絲生長勢優於美國的菌株。顯示台灣的灰黴病菌菌株對免賴得及依普同的忍受性高於美國的菌株。大部份的美國菌株在 $1 \mu\text{g ml}^{-1}$ 的免賴得或 $10 \mu\text{g ml}^{-1}$ 的依普同濃度下, 菌絲生長完全受抑制; 然而台灣的菌株在 $1 \mu\text{g ml}^{-1}$ 的免賴得濃度下菌絲生長不受抑制, 而在 $10 \mu\text{g ml}^{-1}$ 的依普同濃度下, 48 個菌株之中只有 11 個菌株的菌絲生長完全受抑制。本篇是首次發現台灣的百合灰黴病菌菌株對 benzimidazole 和 dicarboximide 兩類藥劑的感受性降低的報告。

關鍵詞: 百合、灰黴病菌 (*Botrytis elliptica*)、依普同、免賴得、殺菌劑的感受性

