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Chemical Control of Gray Blight of Tea in Korea

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Screening of effective fungicides, determination of effective fungicide application time and investigation of the emergence of resistant isolates to fungicides were conducted to establish effective chemical control strategy of gray blight of tea (*Camellia sinensis*) in Korea. Systemic fungicides, such as bitertanol, were effective for controlling gray blight when the fungicides were sprayed within 3 days after cutting tea leaves for harvest. Sprayed immediately after cutting, contact fungicides such as chlorothalonil were also effective, but control efficacy rapidly decreased with lengthening of the intervals between cutting and spraying. Korean isolates of *P. longiseta* and *P. theae* were still sensitive to bitertanol or fluazinam. However, the isolates highly insensitive to copper hydroxide or thiophanate-methyl have already emerged in Korea. Therefore, careful attention should be paid to use of fungicides and spraying programs for the effective control of gray blight of tea in Korea.

Keywords : *Camellia sinensis*, chemical control, gray blight, tea plant

Tea (*Camellia sinensis* O. Kuntze) has been cultivated commercially for the last few decades and has become a very important crop in Korea. According to a report on Korean tea culture (Chonnam Rural Development Administration, 1998), tea plantation area was approximately 1,030 ha and tea production amounted to 1,040 M/T per year in Korea. *Pestalotiopsis longiseta* and *P. theae* cause gray blight of tea plants (Ezuka and Ando, 1994; Park et al., 1996; Shin et al., 1999). The fungal pathogens infect tea leaves mainly through wounds induced by cutting for harvest or pruning. Typical blight symptoms then develop on leaves, stems and emerging shoots.

According to Horikawa (1986), 12 of 31 fungicides registered in Japan were found to be effective in controlling gray blight of tea. Spraying thiophanate-methyl or benomyl within 3 days after cutting for harvest, or chlorothalonil within 1 day, successfully controlled the disease. Although benzimidazole fungicides were very effective in controlling the disease, isolates resistant to the fungicide have emerged in most tea cultivation areas in Japan and populations of resistant isolates have increased annually (Horikawa, 1986; Oniki et al., 1986). In Korea, no fungicide is registered for the control of the disease in tea until now, but several fungicides have been currently used in farmers' fields.

In this study, screening of effective fungicides against gray blight of tea and determination of effective fungicide application time were conducted to generate fundamental data which can be used to establish effective chemical control strategy of the disease in Korea. The emergence of insensitive isolates of gray blight pathogens to fungicides was also investigated to monitor the occurrence of resistant isolates in the future.

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Materials and Methods

Screening of effective fungicides. Several fungicides including thiophanate-methyl (a.i. 70%, WP), fluazinam (a.i. 50%, EC), chlorothalonil (a.i. 75%, WP), bitertanol (a.i. 25%, WP), copper hydroxide (a.i. 58%, WP), difenoconazole (a.i. 10%, EC), mancozeb (a.i. 75%, WP) and azoxystrobin (a.i. 20%, EC) were evaluated for efficacy against gray blight of tea. The fungicides comprised overall fungicides which have been currently used in farmers' fields of tea in Korea. For the study, naturally infected 16-year-old Yabukita tea plants at Kangjin were used in 1997. The infected tea plants were harvested conventionally and then fungicides were sprayed within 3 hours after the cutting at the spray volume of 200 L per 10 a. Fungicide treatments after cutting were repeated three times at 7-day intervals from August 13, 1997. Artificially inoculated 17-year-old Yabukita tea plants were used at the same site in 1998. A conidial suspension (10^6 conidia/ml) of *P. longiseta* was prepared as inoculum. The inoculum was sprayed to drip after the cutting. The fungicides were applied as described above. Fungicide treatments after inoculation were repeated three times at 7-day intervals from August 29, 1998. Three replicates of a treatment block (10 m^2) were employed in each year. The percentage of leaves with at least one lesion was measured on the tea plants at five quadrats (20×20 cm) in each block 10 days after the final treatment of the fungicides.

Determination of effective fungicide application time. Six-year-old Yabukita tea plants grown at the Korea Tea Research Institute were used to investigate the optimum timing of fungicide application. A conidial suspension (10^6 conidia/ml) of *P. longiseta* was prepared and used as described above. Bitertanol (a.i. 25%, WP) at the level of 250 μg/ml or chlorothalonil (a.i. 75%, WP) at the level of 1,253 μg/ml were sprayed immediately, 3 days, 7 days and 10 days after the artificial inoculation after the cutting, respectively. The fungicides were sprayed at the spray volume of 200 L per 10 a. Treatment blocks (10 m^2) were replicated three times. The percentage of leaves with at least one lesion was measured on the tea plants at 5 quadrats (20 × 20 cm) in each block 20 days after the artificial inoculation.

Investigation on fungicide insensitivity of *P. longiseta* and *P. theae*. Insensitivity of *P. longiseta* and *P. theae* to thiophanate-methyl, fluazinam, chlorothalonil, bitertanol and copper hydroxide was investigated. Twenty-six isolates of *P. theae*, 14 isolates of *P. longiseta* and 5 unidentified isolates of *Pestalotiopsis* sp. were tested in this study. A mycelial disc (4 mm in diameter) of each isolate was removed from the margin of an actively growing colony and transferred to the center of a petri dish containing fresh PDA and one of the fungicides at the concentrations of 0, 1, 10, 100 or 1,000 μg/ml. Minimum inhibitory concentration (MICs) of the fungicides inhibiting mycelial growth of the pathogenic isolates were evaluated after incubation at 25°C for 5 days.

Results

Screening of effective fungicides in controlling gray blight on tea plants. Gray blight incidence in terms of the percentage of diseased leaves in the untreated control was approximately 23% for the surveyed years. No phytotoxicity of the fungicides was observed. The seven fungicides tested in 1997 were found to be effective in reducing disease incidence by 85.7% to 90%. There was no significant difference in control efficacy among the fungicides in 1997. However, significant differences were found in control efficacy among the eight fungicides tested in 1998. Fluazinam and bitertanol were the most effective and followed by azoxystrobin and chlorothalonil (Table 1).

Optimal application timing for effective fungicides. Bitertanol applied within 3 days after the cutting was found to be effective for controlling gray blight of tea (Fig. 1). Efficacy decreased with lengthening of the intervals between harvest and the first fungicide application. When chlorothalonil was sprayed immediately after harvest, it was highly effective for controlling the disease and reduced the disease incidence by 90%. However, the control efficacy rapidly declined, so that only 43% control efficacy was observed when the first spray was made 3 days after the cutting.

Emergence of isolates insensitive to fungicides. MIC was used to determine the presence of isolates insensitive to fungicides. Fungal isolates requiring more than 10 μg/ml of MIC were defined as resistant in this study. Ratios of insensitive isolates to all the tested isolates of *Pestalotiopsis* spp. demonstrated that some isolates of *Pestalotiopsis* spp. already developed resistance to several fungicides in Korea (Table 2). All the tested isolates were found to be highly insensitivity to copper hydroxide. The population of isolates also showed high insensitivity to thiophanate-methyl. For example, 22 of 26 isolates (84.6%) of *P. theae* and 13 of 14 isolates (92.9%) of *P. longiseta* were considered to be resistant to the fungicide. The population of isolates was relatively sensitive to chlorothalonil. Insensitive isolates comprised 26.9% and 21.4% of the *P. theae* and *P. long-*

Table 1. Effect of fungicide application on disease incidence of gray blight

Fungicides	Concentration (μg/ml)	Disease incidence[v]	
		1997[w]	1998[x]
Thiophanate methyl	700	2.7 b[y]	4.7 b
Fluazinam	250	2.3 b	1.6 d
Chlorothalonil	1,253	2.9 b	2.6 cd
Bitertanol	250	2.8 b	1.8 d
Copper hydroxide	1,160	3.2 b	4.6 b
Difenoconazole	50	2.5 b	3.9 bc
Mancozeb	1,500	3.3 b	3.7 bc
Azoxystrobin	100	NT[z]	2.4 cd
Untreated	–	23.0 a	23.1 a

[v]The percentage of leaves with at least one lesion was measured on the tea plants at five quadrats (20 × 20 cm) in each block 10 days after the final treatment of the fungicides.
[w]Disease incidence on naturally infected tea plants near Kangjin, Korea in 1997.
[x]Disease incidence on artificially inoculated tea plants by *Pestalotiopsis longiseta* near Kangjin, Korea in 1998.
[y]Means followed by the same letter within a column are not significantly different according to Duncan's multiple range test ($P \geq 0.05$).
[z]Not tested.

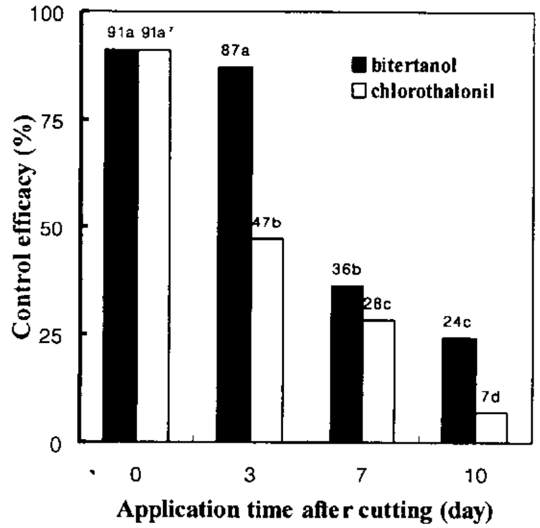

Fig. 1. Effect of application time of fungicides, bitertanol (250 μg/ml) and chlorothalonil (1,253 μg/ml), on control of gray blight caused by *Pestalotiopsis longiseta* on tea plants.

Table 2. Minimum inhibitory concentration of five fungicides against *Pestalotiopsis* spp. collected from four major tea plantation areas in Korea

Species & Fungicides[y]	Numbers of isolates with MIC[z] (μg/ml)				
	<1	1-10	10-100	100-1,000	>1,000
Pestalotiopsis theae					
Thiophanate-methyl	4	0	0	2	20
Fluazinam	26	0	0	0	0
Chlorothalonil	13	6	4	2	1
Bitertanol	8	18	0	0	0
Copper hydroxide	0	0	26	0	0
Pestalotiopsis longiseta					
Thiophanate-methyl	1	0	0	2	11
Fluazinam	14	0	0	0	0
Chlorothalonil	8	3	1	0	2
Bitertanol	3	11	0	0	0
Copper hydroxide	0	0	14	0	0
Pestalotiopsis sp.					
Thiophanate-methyl	4	0	0	0	1
Fluazinam	5	0	0	0	0
Chlorothalonil	4	1	0	0	0
Bitertanol	4	1	0	0	0
Copper hydroxide	0	0	5	0	0

[y] Twenty-six isolates of *Pestalotiopsis theae*, 14 isolates of *Pestalotiopsis longiseta* and 5 unidentified isolates of *Pestalotiopsis* sp. collected from four major tea plantation areas in Korea were tested.
[z] A mycelial disc (4 mm in diameter) of each isolate was placed on fresh potato dextrose agar amended with each fungicide at the levels of 0, 1, 10, 100 and 1,000 μg/ml, respectively. MICs were evaluated after incubation at 25°C for 5 days.

Table 3. Minimum inhibitory concentration of thiophanate-methyl against *Pestalotiopsis* spp. collected from four major tea plantation areas in Korea

Areas & Species[y]	Numbers of isolates with MIC[z] (μg/ml)				
	<1	1-10	10-100	100-1,000	>1,000
Posong					
P. theae	4	0	0	0	0
P. longiseta	1	0	0	0	0
Pestalotiopsis sp.	4	0	0	0	1
Total	9	0	0	0	1
Kangjin					
P. theae	0	0	0	2	7
P. longiseta	0	0	0	0	6
Total	0	0	0	2	13
Haenam					
P. theae	0	0	0	0	3
P. longiseta	0	0	0	2	4
Total	0	0	0	2	7
Cheju					
P. theae	0	0	0	0	10
P. longiseta	0	0	0	0	1
Total	0	0	0	0	11

[y] Twenty-six isolates of *Pestalotiopsis theae*, 14 isolates of *Pestalotiopsis longiseta* and 5 unidentified isolates of *Pestalotiopsis* sp. collected from four major tea plantation areas in Korea were tested.
[z] A mycelial disc (4 mm in diameter) of each isolate was placed on fresh potato dextrose agar amended with thiophanate-methyl at the levels of 0, 1, 10, 100 and 1,000 μg/ml, respectively. MICs were evaluated after incubation at 25°C for 5 days.

iseta population, respectively. Unlike copper hydroxide or thiophanate-methyl, all the tested isolates were highly sensitive to bitertanol and fluazinam.

Emergence of insensitive isolates to thiophanate methyl seemed to be area-dependent (Table 3). All the isolates collected at Posong where the Korean native variety has traditionally been cultivated on a small scale were highly sensitive to the fungicide. However, all the isolates at Heanam, Cheju and Kangjin where Yabukita has been grown commercially on a large scale were highly insensitive to the fungicide.

Discussion

It was already reported in Japan that resistant isolates to benzimidazole fungicides emerged and population size of the resistant isolates rapidly increase every year (Horikawa, 1986; Oniki et al., 1986). Difference in the emergence of resistant isolates to benzimidazole fungicides between of *P. theae* and *P. longiseta* was demonstrated in Japan (Oniki et al., 1986). In this study, there was no difference in the emergence between of *P. theae* and *P. longiseta* but the difference was found among the four areas surveyed. For example, isolates highly resistant to thiophanate-methyl were present at Kangjin, Haenam and Cheju areas but all the isolates collected at Posong area were still very sensitive to the fungicide. When the pathogenicity of resistant isolates to benzimidazole was compared with that of sensitive isolates, almost equal or slightly higher pathogenicity was recorded in the resistant isolates (Horikawa, 1987). Since fungicide resistance is already problematic in Japan, careful attention should be paid to the use of fungicides and spraying programs for the effective control in Korea due to the possible development of the isolates resistant to the fungicides.

Systemic fungicides such as bitertanol were effective in controlling gray blight on the condition that the fungicides were sprayed within 3 days after the cutting. Contact fungicides sprayed immediately after cutting were effective but control efficacy rapidly decreased with lengthening of the intervals between cutting and spraying. The findings were consistent with the results of Horikawa (1982). Therefore, it is strongly recommended for the successful disease management that all the fungicides should be sprayed immediately or within 3 days at the latest after harvest.

The pathogens of gray blight infect tea leaves through wounds induced by cutting for harvest or pruning and the infection is usually established within a few hours. The limited time allowed to spray contact fungicides and even systemic fungicides within 3 days after the cutting makes chemical control of the disease difficult or, in some cases, impractical. This is due to the fact that spraying and harvest should be carried out together. In that case, contamination of fungicides to the neighboring tea leaves and the resulting risk of residual fungicides seem to be inevitable. Therefore, development of systemic fungicide effective to the disease after 3 days after the cutting is needed to achieve the better performance of chemical control on tea plants in Korea in the future.

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