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Seasonal Occurrence and Development of Gray Blight of Tea Plants in Korea

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Disease occurrence and development of gray blight of tea (*Camellia sinensis*) were investigated. Higher incidences and more severe damage by gray blight were found in Japanese tea variety Yabukita than the Korean local variety. In Yabukita, *Pestalotiopsis longiseta* was more frequently observed on the diseased leaves than *P. theae* but vice versa in the Korean local variety. This indicates that there was the varietal difference in the distribution of fungal species of gray blight pathogens. Both varieties were most severely damaged during the third harvest period with weather conditions of high temperature and humidity favorable to the disease. Presence of the tea brown blight fungus *Glomerella cingulata* on the margin of gray blight lesion at the late stage suggested that the pathogenic fungi of tea gray blight were replaced by the brown blight fungus during the disease development.

Keywords **:** *Camellia sinensis, Glomerella cingulata, Pestalotiopsis longiseta, Pestalotiopsis theae,* tea plant.

The tea (*Camellia sinensis* O. Kuntze) plantation area has been increased to be more than 1,000 ha, and tea production amounted to 1,000 M/T in Korea (Shin et al., 2000). With the increase of the acreage, various tea diseases have been reported to occur on tea plants in recent years. Gray blight is one of the most destructive diseases on tea plants. *Pestalotiopsis longiseta* and *P. theae* induce typical gray blight symptoms on leaves, stems and emerging shoots (Ezuka and Ando, 1994). It was previously reported in Japan that *P. longiseta* caused more severe damage to tea plants than *P. theae*. (Ezuka and Ando, 1994; Horikawa, 1982). Damage caused by *P. longiseta* was more severe at the second and third harvest periods, while *P. theae* caused more severe damage at the second and fourth harvest periods.

In Korea, gray blight diseases on tea plants caused by *P. longiseta* and *P. theae* were reported (Park et al., 1996; Shin et al., 1999), but their occurrence and development in the field have not been studied in detail yet. Recently, effective fungicides were screened and their application time was determined to establish an effective chemical control strategy of gray blight of tea (Shin et al., 2000).

In this study, disease incidences of gray blight of tea plants were investigated in 1997 and 1998. Distribution of gray blight pathogens at 4 major tea plantation regions and susceptibility of 2 major tea varieties were examined to understand the occurrence and development of gray blight of tea plants in Korea.

Materials and Methods

Investigation on disease incidence of gray blight of tea plants. Two major tea plantation regions in Korea were selected for the survey of gray blight disease incidence in 1997 and 1998. Thirty-four-year-old tea plants of a Korean local variety have been cultivated at Posong, Chonnam, and 17-year-old tea plants of Japanese variety Yabukita have been grown at Kangjin, Chonnam. Disease incidence was recorded in four harvest periods each year (12 May, 14 July, 20 August, and 2 October in 1997; 4 May, 8 July, 17 August, and 30 September in 1998, respectively). Ratio of diseased leaves to total leaves in a quadrat (20×20 cm) was calculated. Ten quadrats were employed for each block, and three blocks were randomly selected in each region.

Distribution of gray blight pathogens in Korea. Gray blight pathogens were isolated from 114 diseased leaves collected at 4 major tea plantation regions Posong, Kangjin, Haenam and Cheju. Diseased leaves were humidified for 3 days at 25°C to induce black acervuli on the leaves. Single spores were obtained on water agar by smearing spore suspension prepared by crushing the acervuli in sterilized distilled water. Fungal isolates obtained from the single spores were transferred to fresh potato dextrose agar (PDA) in Petri dishes and incubated at 25°C for 4 weeks. Isolates were identified by the morphology of conidia formed on PDA. When two or more fungal species were isolated from the same leaf, the most popular one was counted as the major pathogen.

Susceptibility of tea varieties to gray blight pathogens. The two major tea varieties cultivated in Korea were used for investigating their relative susceptibility to the gray blight pathogens, *P. longiseta* and *P. theae*. Tea leaves on the young shoots of 4-year-old plants of the Korean local variety and Japanese variety Yabukita were excised and inoculated with conidial suspension (10^6

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conidia/ml) after wounding with a sterilized cork borer (4 mm in diameter). Cuttings of the inoculated leaves were humidified for 2 days and then incubated at 25°C in a growth chamber. Tea stems were also inoculated by soaking them in the conidial suspension after wounding with a sterilized razor. Inoculated stems were placed on filter paper fully saturated with sterilized distilled water in Petri dishes, and then incubated at 25°C in the growth chamber. Infection rate was measured by the percentage of infected leaves or stems to the total 20 leaves or stems artificially inoculated.

Results

Disease incidence of gray blight of tea plants in Korea. In 1997 and 1998, disease incidences of gray blight of tea plants were quite different between the two tea varieties examined and among tea growth stages designated as harvest periods (Fig. 1). At the first harvest period from April to May in 1997 and 1998, no disease was found on the Korean local variety at Posong and 4% was recorded as average disease incidence on Yabukita at Kangjin. At the second harvest period from May to July in both years, the average disease incidence was 4.7% in the Korean local variety and 12.5% in Yabukita. At the third harvest period from July to August, it was 8.1% and 18.1% in the Korean local variety and in Yabukita, respectively. At the fourth harvest period from August to October, 6.8% was recorded on the Korean local variety and 16.0% on Yabukita.

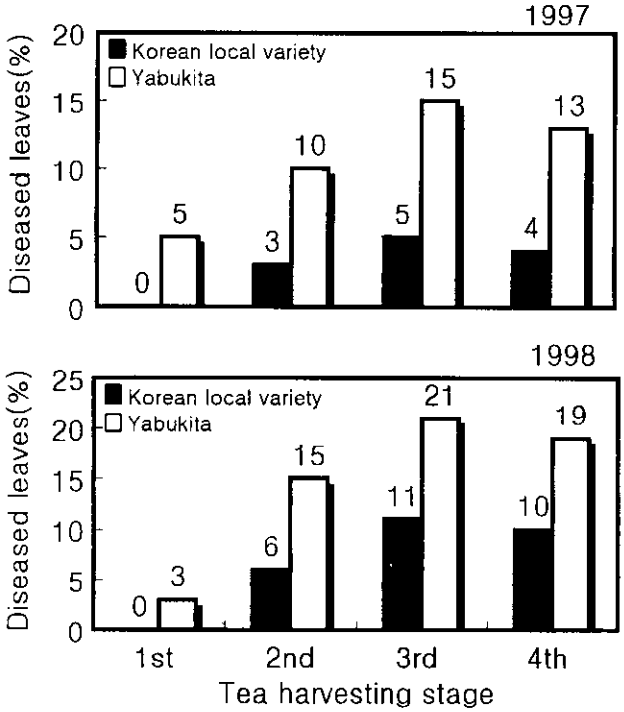

Fig. 1. Incidence of gray blight leaves in two major tea varieties in 1997 (upper) and 1998 (lower). The Korean local variety of 34-year-old tea plants has been cultivated at Posong, and Japanese variety Yabukita of 17-year-old plants has been cultivated at Kangjin, Korea.

Meteorological data showed that mean temperatures during the tea growing season from April to October were very similar in the two regions and high temperatures of approximately 26°C were maintained at the third harvest period in 1997 (Fig. 2A and B). Rainfall was more prevalent during the second harvest period in 1997 and from the second through the fourth harvest period in 1998. More rainfall was recorded in 1998 than in 1997 (388 vs. 422 mm). The Korean local variety growing at Posong was exposed to more rainfall than Yabukita cultivated at Kangjin during the two years. Relative humidity exhibited a trend similar to rainfall in both regions and in both years (Fig. 2C and D).

Distribution of gray blight pathogens in Korea. Diseased leaves were collected at 4 regions in southern Korea and the pathogenic fungal isolates causing gray blight on tea plants were identified. When diseased leaves were humidified, isolates of *Pestalotiopsis* spp. and *Glomerella cingulata* were detected on the same lesions (Table 1). On diseased leaves collected at Posong, acervuli of *G. cingulata* were more frequently observed than those of *Pestalotiopsis* spp., whereas the reverse was noted at Cheju. *Pestalotiopsis* spp. were detected at slightly lower frequency than *G. cingulata* at Kangjin and Haenam.

Ratios of *P. longiseta* to *P. theae* on the same lesion showed that relatively higher frequencies of *P. theae* were detected from the diseased leaves at the rates of 50%, 54.5% and 72.2% at Posong, Haenam and Cheju, respectively (Table 2). However, 52.9% of lesions at Kangjin displayed relatively higher frequency of *P. longiseta*. Unidentified *Pestalotiopsis* spp. were also detected on gray blight leaves collected from Posong.

Susceptibility of tea varieties to gray blight disease. Artificial inoculation using a conidial suspension of *P. longiseta* and *P. theae* on leaves or stems showed that Japanese variety Yabukita had higher disease incidence than the Korean local variety (Table 3). Yabukita was more severely affected by the disease than the Korean local variety. In an analysis of variance of the $2 \times 2 \times 2$ factorial experiment, there was a significant difference in the infection rates ($LSD_{0.01}=7.0$) between Japanese variety Yabukita and the Korean local variety. However, no significant difference in the disease incidence was shown between *Pestalotiopsis* species (*P. longiseta* and *P. theae*) and between leaves and stems inoculated.

Discussion

Gray blight on tea plants heavily occurred at the third (late July to early August) and fourth harvest periods (late August to early October) in Korea. Horikawa (1984) reported that

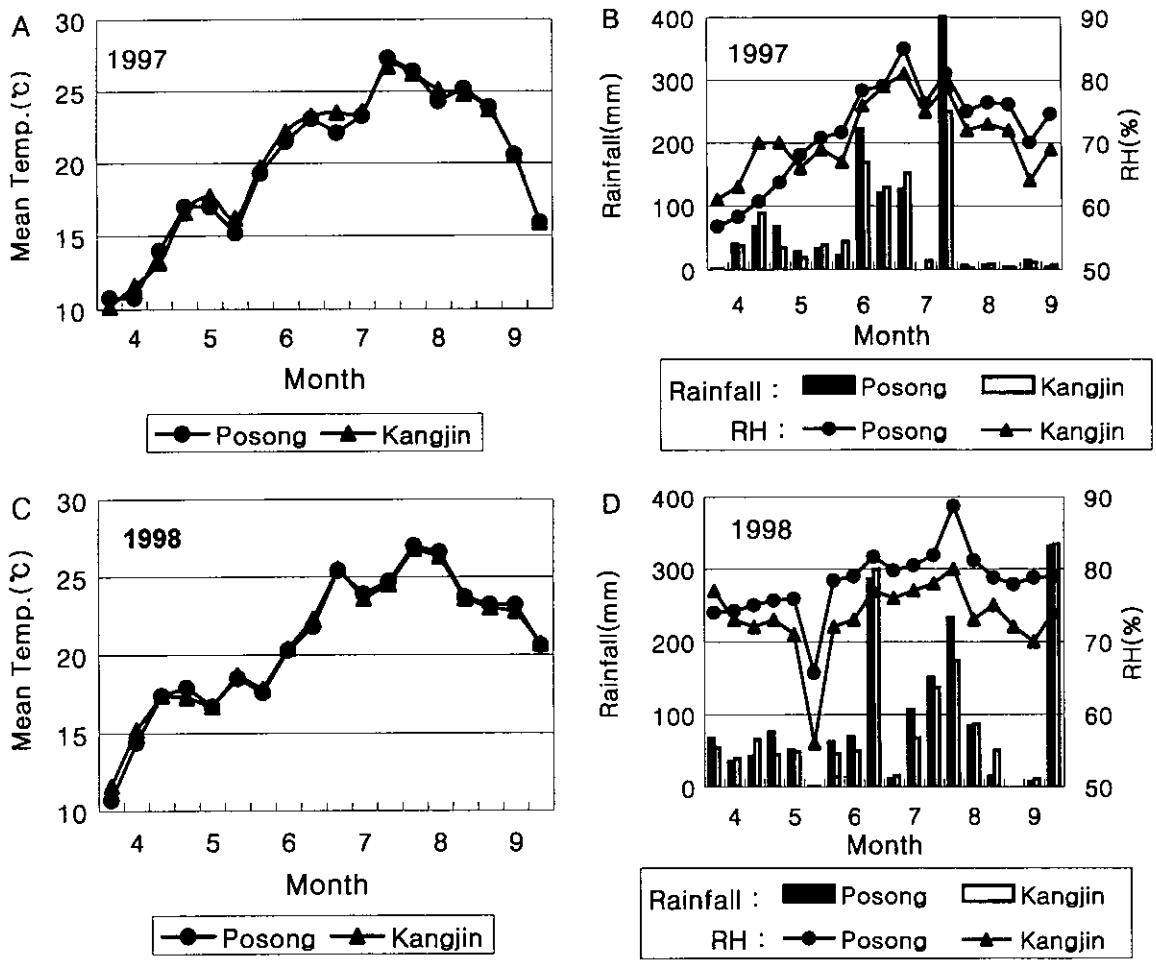

Fig. 2. Meterological data of mean temperature (A, C), and rainfall and relative humidity (B, D) during tea growth seasons at Posong and Kangjin, Korea in 1997 (A, B) and 1998 (C, D), respectively.

Table 1. Regional distribution of fungal pathogens on the diseased leaves of tea plants cultivated in four major tea plantation areas in Korea

Region	Number of tea leaves investigated	Number (percentage) of tea leaves with the major pathogen of[a]	
		Pestalotiopsis spp.	*Glomerella cingulata*
Posong	18	6 (33.3%)	12 (66.7%)
Kangjin	39	17 (43.6%)	22 (56.4%)
Haenam	25	11 (44.0%)	14 (56.0%)
Cheju	14	10 (71.4%)	4 (28.6%)

[a]The diseased leaves were humidified at 25°C for 3 days to induce black acervuli; single spore isolates were obtained on water agar and incubated on potato-dextrose agar for at 25°C 4 weeks. When two or more fungal species were isolated from the same leaf, the most popular fungal species was considered as the major pathogen.

Table 2. Regional distribution of *Pestalotiopsis* spp. causing gray blight disease on leaves of tea plants cultivated in four major tea plantation areas in Korea

Region	Number of tea leaves investigated	Number (percentage) of tea leaves with major *Pestalotiopsis* spp.[a]		
		P. theae	*P. longiseta*	Others
Posong	12	6 (50.0%)	1 (8.3%)	5 (41.7%)
Kangjin	51	24 (47.1%)	27 (52.9%)	0
Haenam	33	18 (54.5%)	15 (45.5%)	0
Cheju	18	13 (72.2%)	5 (27.8%)	0

[a]The diseased leaves were humidified for at 25°C 3 days to induce black acervuli; single spore isolates were obtained on water agar and incubated on potato-dextrose agar at 25°C for 4 weeks. When two or more *Pestalotiopsis* species were identified on the same leaf, the major species was considered as distribution.

the number of diseased leaves rapidly increased under the condition of high temperature, so that high incidence of gray blight is usually observed at the second (late June to early July) and third (late July to early August) harvest periods in Japan. Considering that the harvest periods in Japan were approximately 2 weeks earlier than in Korea, it is a similar time of harvest that the disease occurs heavily.

Table 3. Susceptibility of Japanese variety Yabukita and the Korean local variety to *Pestalotiopsis* species

Pestalotiopsis species	Infection rates (%)[b]			
	Yabukita		Korean local variety	
	Leaf	Stem	Leaf	Stem
P. theae	65.0	70.0	15.0	20.0
P. longiseta	75.0	80.0	20.0	30.0
$LSD_{0.01}$	7.0			

[a] Tea leaves on the young shoots of the 4-year-old Korean local variety and Japanese variety Yabukita were inoculated with conidial suspension (10^6 conidia/ ml) after wounding with a sterilized cork borer (4 mm in diameter). Stems were inoculated by soaking them in the conidial suspension after wounding with a sterilized razor.

[b] Infection rate was expressed by the percentage of infected leaves or stems to the total 20 leaves or stems artificially inoculated.

However, Park et al. (1996) reported that gray blight caused by *P. longiseta* was severe at the second harvest period (in July) and rarely occurred in September in Korea. Therefore, it is very likely that the occurrence of gray blight on tea plants may be dependent on the environmental conditions of each year. Meteorological data during the two years in our study showed that high temperature, intensive rainfall and high relative humidity were prevalent during the third harvest period (mid July to late August). These environmental conditions of the third period seemed to be favorable to severe occurrence of gray blight, relative to the other three harvest periods. The disease incidences were higher in 1998 than in 1997. This may be also explained by the fact that the environmental conditions of high temperature and high humidity lasted longer in 1998 than in 1997 during the third harvest period (Fig. 2).

Gray blight severity on tea plants was found to be variety-dependent. The gray blight disease occurred more severely on Japanese variety Yabukita cultivated at Kangjin than on the Korean local variety cultivated at Posong. Meteorological data during the tea growth period at the two areas showed that no significant difference in mean temperature was found but rainfall and relative humidity were higher at Posong than at Kangjin. This meant that environmental conditions were favorable to disease occurrence more at Posong than at Kangjin. Therefore, comparison of the meteorological data suggests that the difference in the gray blight severity of tea plants between the two regions may be mainly due to the different susceptibility between tea varieties rather than different environmental conditions. This was confirmed by the fact that artificial inoculation of *P. longiseta* and *P. theae* resulted in higher infection rates and more severe disease development on Yabukita than on the Korean local variety (Table 3). Variety-dependent susceptibility of tea plants to the gray blight disease was also reported in Japan (Ando et al., 1985a; Hamaya and Horikawa, 1982; Horikawa, 1987).

The Korean local variety covers approximately 77% of the total tea plantation areas in Korea, and the rest include the exotic varieties introduced mainly from Japan. Japanese variety Yabukita comprises 84% of the exotic varieties. In Korea, Yabukita is cultivated on flat land at Kangjin, Haenam and Cheju areas. Yabukita has been preferred over the Korean local variety due to good quality and high yield, but increase in damage caused by the gray blight disease is currently a main obstacle to the cultivation of Yabukita in Korea (Shin et al., 2000). Yabukita susceptible to gray blight caused by *P. longiseta* is also planted more than 60% of the total tea cultivation areas in Japan. General use of harvesting machines enhanced the occurrence and development of gray blight on tea plants (Ando et al., 1985a; Hamaya and Horikawa, 1982; Horikawa, 1984). Therefore, it is very important in tea plant cultivation that resistant varieties should be utilized to reduce the losses due to the gray blight diseases.

The pathogenic species of *Pestalotiopsis* spp. causing gray blight were variably distributed in Korea. *P. longiseta* was dominantly present at Kangjin, whereas *P. theae* was the predominant species of *Pestalotiopsis* at Posong, Haenam and Cheju. Unidentified *Pestalotiopsis* spp. were also collected at Posong. The isolates exhibited similar morphological characteristics to *P. theae*. For example, 3 median cells of conidia were equally dark olivaceous, the number of apical appendages varied 2 to 5, and no appendage was found on foot cell of conidia. In China, *P. theae* has been reported to be the major pathogenic species causing gray blight but some other species of *Pestalotiopsis* spp. also caused the disease (Chen and Chen, 1990). Oniki et al. (1986) also reported the seasonal and regional variations in the distribution of the pathogenic fungal species of *Pestalotiopsis* spp. in Japan. *P. longiseta* was found to be more virulent to Yabukita than *P. theae* based on lesion size after artificial inoculation of the two pathogens (Ando et al., 1985a). Therefore, severe occurrence and development of gray blight disease observed at Kangjin where Yabukita has been cultivated might be due to the high virulence of *P. longiseta* to the susceptible variety of Yabukita and the dominant presence of *P. longiseta* at the area, compared to Posong.

When the diseased leaves were humidified, *G. cingulata*, a pathogenic fungus causing brown blight on tea plants, was also detected on the same leaves. The pathogen usually formed acervuli on the margin of the lesions or healthy part of the leaves, whereas the acervuli of *Pestalotiopsis* spp. were formed inside the lesions. Horikawa (1987) also reported the similar finding that *Pestalotiopsis* spp. formed acervuli on the wounded parts during harvest but *G. cingu-*

lata did on the margin of lesions. The results suggest that *Pestalotiopsis* spp. infected tea leaves and developed lesions firstly, and *G. cingulata* subsequently infected the lesions (Ando et al., 1985b). The time course study on the occurrence of fungal pathogens causing gray blight on tea plants showed that detection rates of *P. longiseta* decreased on diseased leaves with increased incubation time, whereas *G. cingulata* was more frequently detected on the leaves at later stages. This result clearly demonstrates that major pathogenic fungi changed during the development of gray blight diseases on tea plants. The replacement of *Pestalotiopsis* spp. by *G. cingulata* resulted in suppression of disease development due to *Pestalotiopsis* spp. on the infected leaves (Ando, 1992; Ando and Narisawa, 1989). They suggested that subsequent infection of the less pathogenic fungus of *G. cingulata* triggered the increase in resistant ability of host plant to the pathogen and, as a result, fungal growth of *Pestalotiopsis* spp. was inhibited on the leaves. If it were true, the regional difference in disease occurrence of gray blight between Posong and Kangjin might be due partly to the difference in the occurrence of *G. cingulata* during the disease development. As presented in Table 1, detection rate of *G. cingulata* was much higher on the diseased leaves collected at Posong where the occurrence of gray blight caused by *Pestalotiopsis* spp. was also lower than at Kangjin.

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