

Incidences of Leaf Spots and Blights on Kiwifruit in Korea

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Various kinds of leaf spots and blights were found in kiwifruit (*Actinidia deliciosa*) orchards on 2006 in Korea. Disease incidences were quite variable between open-field and rain-proof shelter. Rate of diseased leaves was recorded as about 70% at open-field orchards in late season but use of rain-proof vinyl shelters alleviated the disease incidences by 20%. Angular leaf spots appeared at early infection stage on June and several other symptoms were also recognized as the disease developed afterward. On September, brown leaf blights were the most frequent, followed by grayish brown ring spots, silvery gray leaf blights, zonate leaf blights, dark brown ring spots and angular leaf spots at open-field orchards. Four fungal species were frequently isolated from the disease symptoms. *Phomopsis* sp. was the most predominant fungus associated with the leaf spot and blight symptoms on kiwifruit, followed by *Glomerella cingulata*, *Alternaria alternata* and *Pestalotiopsis* sp. *Phomopsis* sp. was commonly isolated from angular leaf spots, silvery gray leaf blights, and zonate brown leaf blights. *G. cingulata*, *A. alternata* and *Pestalotiopsis* sp. were isolated from grayish brown ring spots (anthracnose), brown ring spots and zonate dark brown leaf blights. Typical symptoms appeared on the wounded and unwounded leaves, which were inoculated by each of *Phomopsis* sp., *G. cingulata*, and *Pestalotiopsis* sp., but *A. alternata* caused symptoms only on the wounded leaves.

Keywords : *Alternaria alternata*, *Glomerella cingulata*, kiwifruit, leaf spots and blights, *Pestalotiopsis* sp., *Phomopsis* sp.

The kiwifruit [*Actinidia deliciosa* (A. Chev) Liang et Ferguson] introduced from New Zealand has been grown commercially since the early 1980s in Korea (Koh et al., 2003). It is currently cultivated on 1000 ha in southern coastal areas of Korea where winters are generally warm (Jo, 2005). Several diseases have been reported to occur on

kiwifruit as the cultivation acreages of kiwifruit have increased steadily during the past two decades (The Korean Society of Plant Pathology, 2004). Among the diseases, bacterial blossom blight, bacterial canker and postharvest fruit rots are known to be the major diseases on kiwifruits (Koh, 1995; Koh et al., 2003).

Various kinds of leaf spot and blight symptoms had been reported on the leaves of kiwifruits growing at the farmers' orchards in foreign countries (Hawthorne et al., 1982; Ushiyama et al., 1996). Leaf blight by *Pestalotiopsis menezesiana*, brown ring spot by *Alternaria alternata* and several abiotic leaf spots or blights were also reported in Korea (Jeong et al., 2008; Koh et al., 2007; Park et al., 1997). However, the incidences of the leaf spot and blight diseases and their pathogens associated with those symptoms have not been reported in detail until now in Korea. This study was undertaken to investigate the incidences of leaf spots and blights and to identify the pathogens associated with the diseases.

Materials and Methods

Survey of incidences of leaf spots and blights. Incidences of leaf spots and blights were investigated five times from June 6 to November 3 on one hundred leaves of randomly selected 10 trees at open-field and rain-proof orchards in Suncheon, Jeonnam Province, Korea during the 2006 growing season of kiwifruit.

Isolation and identification of fungi. Plant samples were collected from 100 diseased leaves from the randomly selected 10 trees at open-field and rain-proof orchards in Suncheon, Jeonnam Provinces Korea on July 25, 2006. After sterilization with 70% ethanol for 1 min., diseased leaf tissues cut into 5 mm length were placed on potato dextrose agar (PDA) plates, and incubated at 25°C for 3 days. Mycelial tips of the fungal isolates grown on the medium were cut and transferred to fresh medium for single spore isolation. Single spores isolated by dilution method on water agar were cultured on PDA plates at 25°C for identification and pathogenicity tests. Morphological

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characteristics of asexual or sexual spores produced on PDA plates were examined by light microscope to identify their genera and species.

Pathogenicity test. To confirm the pathogenicity of the four fungal species on kiwifruit leaves, 30–40 cm size of young shoots were selected, lower leaves trimmed, and planted in rock wool pots. The isolates were cultured on PDA at 25°C for 7–10 days. Their conidial suspensions of 10^6 conidia per ml were prepared by collecting the conidia filtered from the cultures using cheesecloth and diluting with the aid of a hemacytometer. Inoculation was made by spraying the conidial suspensions on the leaves unwounded or wounded 1–2 mm deep at five close points using a sterilized pin. The same quantity of sterile water was used as the control. The inoculated shoots in pots were maintained in a moist chamber at 25°C for 24 hours in the dark and then transferred to a greenhouse to check symptom developments on the inoculated leaves.

Results

Incidences of leaf spots and blights. Leaf spots and blights of kiwifruits were first observed on early June at open-field and rain-proof orchards, respectively. Incidences of the diseases at rain-proof orchards were increasing slowly during the growing season but the incidences at open-field orchards were increasingly sharply at late growing stage. The incidences rated on November 3, 2006 were 70.2% and 19.8% at open-field and rain-proof orchards,

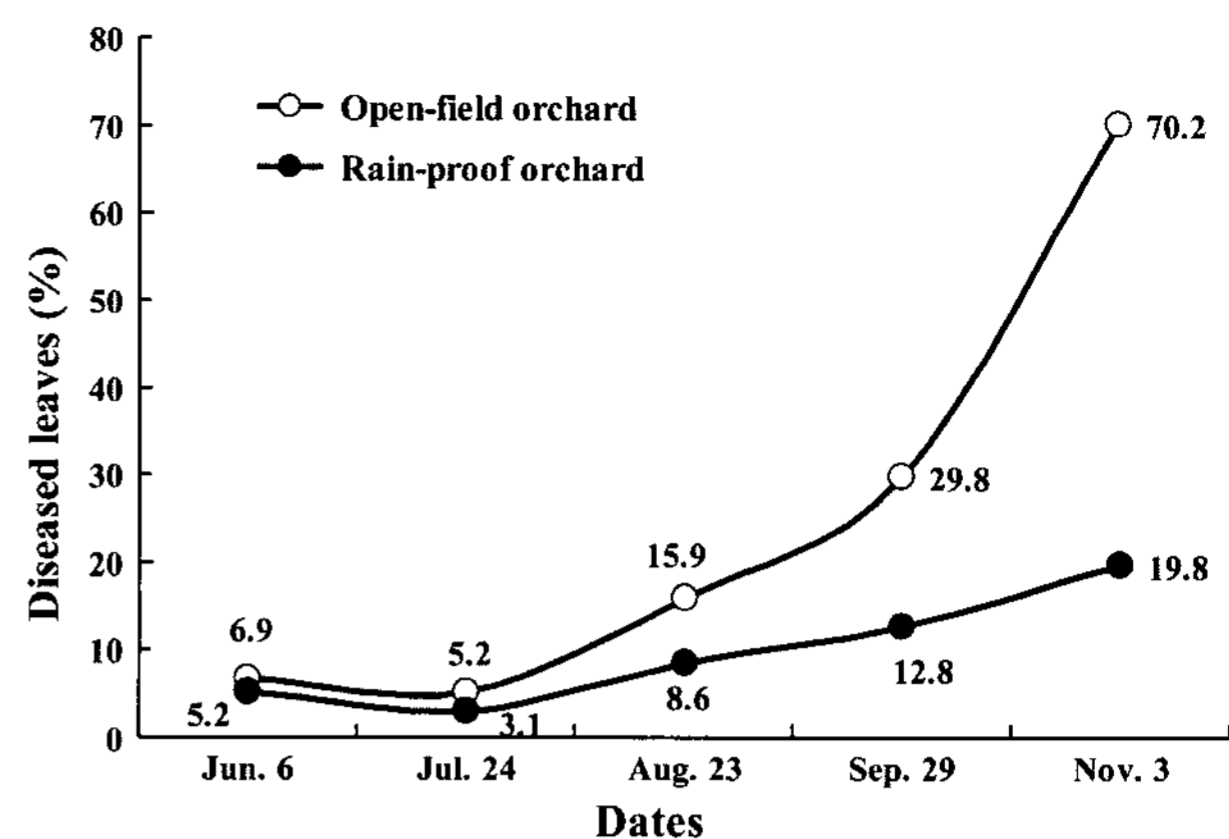


Fig. 1. Incidences of leaf spots and blights at open-field and rain-proof kiwifruit orchards in Suncheon, Jeonnam Provinces, Korea during the 2006 growing season.

respectively (Fig. 1).

Kinds of leaf spot and blight symptoms. Various kinds of leaf spot and blight symptoms were observed at open-field and rain-proof kiwifruit orchards (Fig. 2). Angular leaf spots appeared at early infection stage on June and several other symptoms were also recognized as the disease developed afterward.

When rated on September 29, 2006, brown leaf blights of which incidences were 53.5% and 61.0% among the diseased leaves samples collected at open-field and rain-proof orchards, respectively, were the most frequent and followed by grayish brown ring spots (anthracnose), zonate

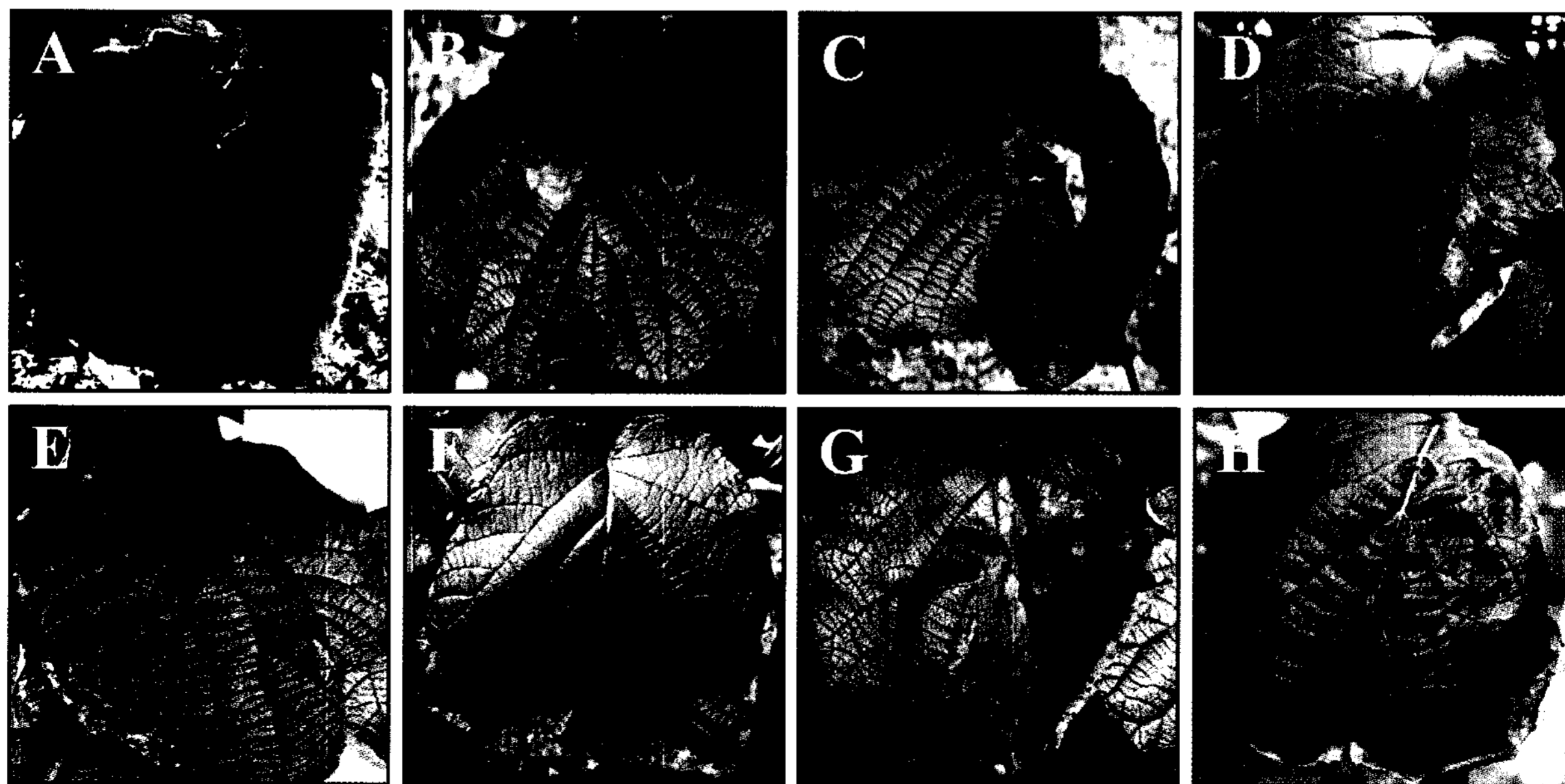


Fig. 2. Various kinds of leaf spot and blight symptoms. A: angular leaf spot, B: grayish brown ring spot, C: dark brown ring spot, D: silvering gray leaf blight, E: reddish brown zonate leaf blight, F: brown zonate leaf blight, G: dark brown leaf blight, H: grayish brown leaf blight.

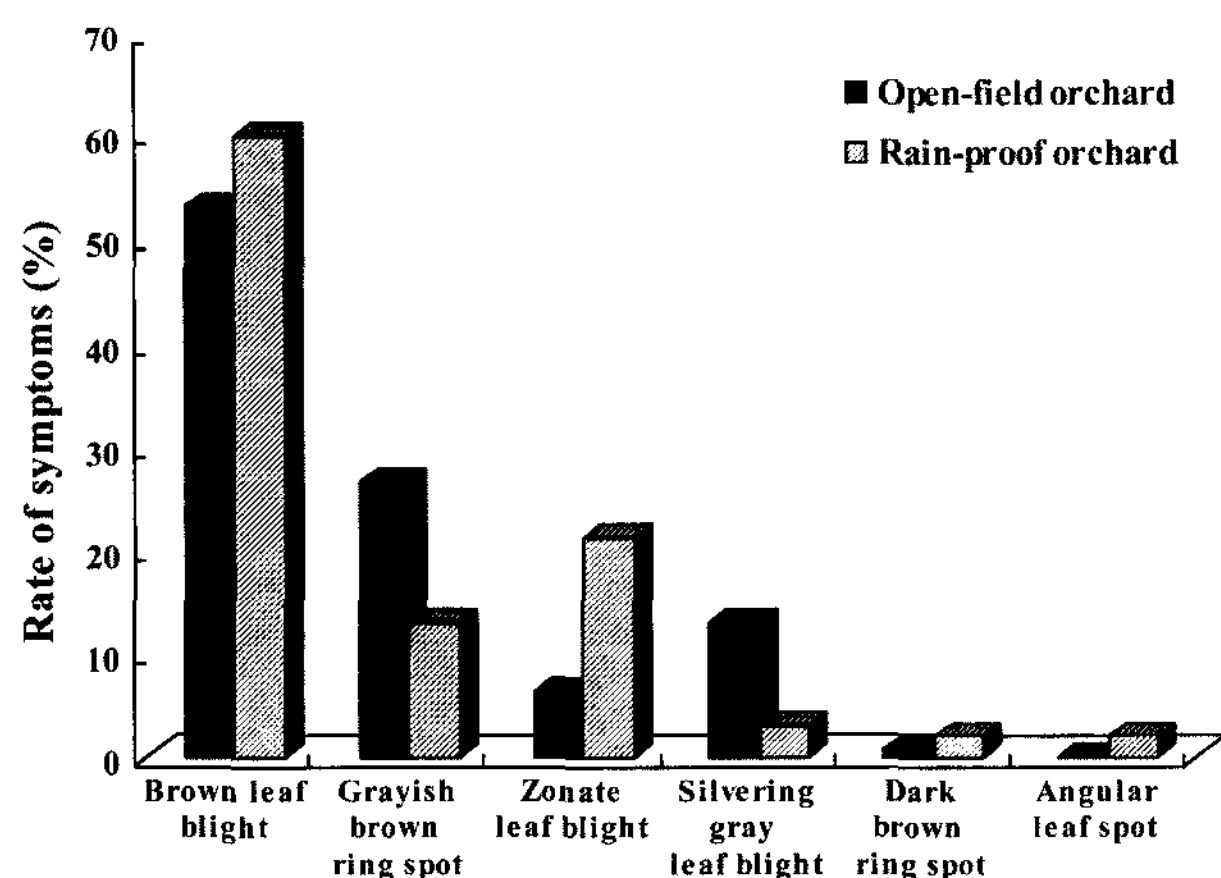


Fig. 3. Rates of various kinds of leaf spot and blight symptoms among diseased leaves samples collected at open-field and rain-proof kiwifruit orchards in Suncheon, Jeonnam Provinces, Korea on September 29, 2006.

leaf blights, silvering gray leaf blights, brown ring spots and angular leaf spots (Fig. 3). Incidences of grayish brown ring spots (anthracnose), zonate leaf blights and silvering gray leaf blights were 26.7%, 6.3% and 13.0% among the diseased leaves samples collected at open-field orchards, respectively, whereas those were 13.0%, 21.0% and 3.0% among the diseased leaves samples collected at rain-proof orchards, respectively. Incidences of brown ring spot and angular leaf spot symptoms were less than 2% among the diseased leaves samples collected at both orchards, respectively.

Kinds of the causal fungi on the symptoms. Four fungal species were consistently isolated from the leaf spot and blight symptoms of kiwifruit (Table 1). *Phomopsis* sp. was commonly isolated from angular leaf spots (Fig. 2A), silvering gray leaf blights (Fig. 2D), and brown zonate leaf blights (Fig. 2F), and *Colletotrichum* sp., *Alternaria* sp. and *Pestalotiopsis* sp. were isolated from grayish brown ring spots (anthracnose) (Fig. 2B), brown ring spots (Fig. 2C) and reddish brown zonate leaf blights (Fig. 2C), respectively. Sometimes *Phomopsis* sp. mixed with *Alternaria* sp. or *Colletotrichum* sp. was isolated from dark brown leaf blights (Fig. 2G) or grayish brown leaf blights (Fig. 2H).

Incidences of the causal fungi from the symptoms. When investigated on July 25, 2006, *Phomopsis* sp. was the most dominant fungus associated with the leaf spot and blight symptoms on kiwifruits and its incidences were 76.9% and 60.0% from open-field and rain-proof orchards, respectively (Fig. 4). Incidences of *Colletotrichum* sp., *Alternaria* sp. and *Pestalotiopsis* sp. were 11.5%, 7.7% and 3.8% from open-field orchards and those were 20.0%, 20.0% and 0% from rain-proof orchards, respectively.

Table 1. The list of fungi isolated from leaves samples of kiwifruit trees showed leaf spot and blight symptoms in Korea

Symptoms	Fungi isolated
Angular leaf spot	<i>Phomopsis</i> sp.
Anthracnose (grayish brown ring spot)	<i>Glomerella cingulata</i>
Brown ring spot	<i>Alternaria alternata</i>
Silvering gray leaf blight	<i>Phomopsis</i> sp.
Reddish brown zonate leaf blight	<i>Pestalotiopsis</i> sp.
Brown zonate leaf blight	<i>Phomopsis</i> sp.
Dark brown leaf blight	<i>Phomopsis</i> sp., <i>A. alternata</i>
Grayish brown leaf blight	<i>Phomopsis</i> sp., <i>G. cingulata</i>

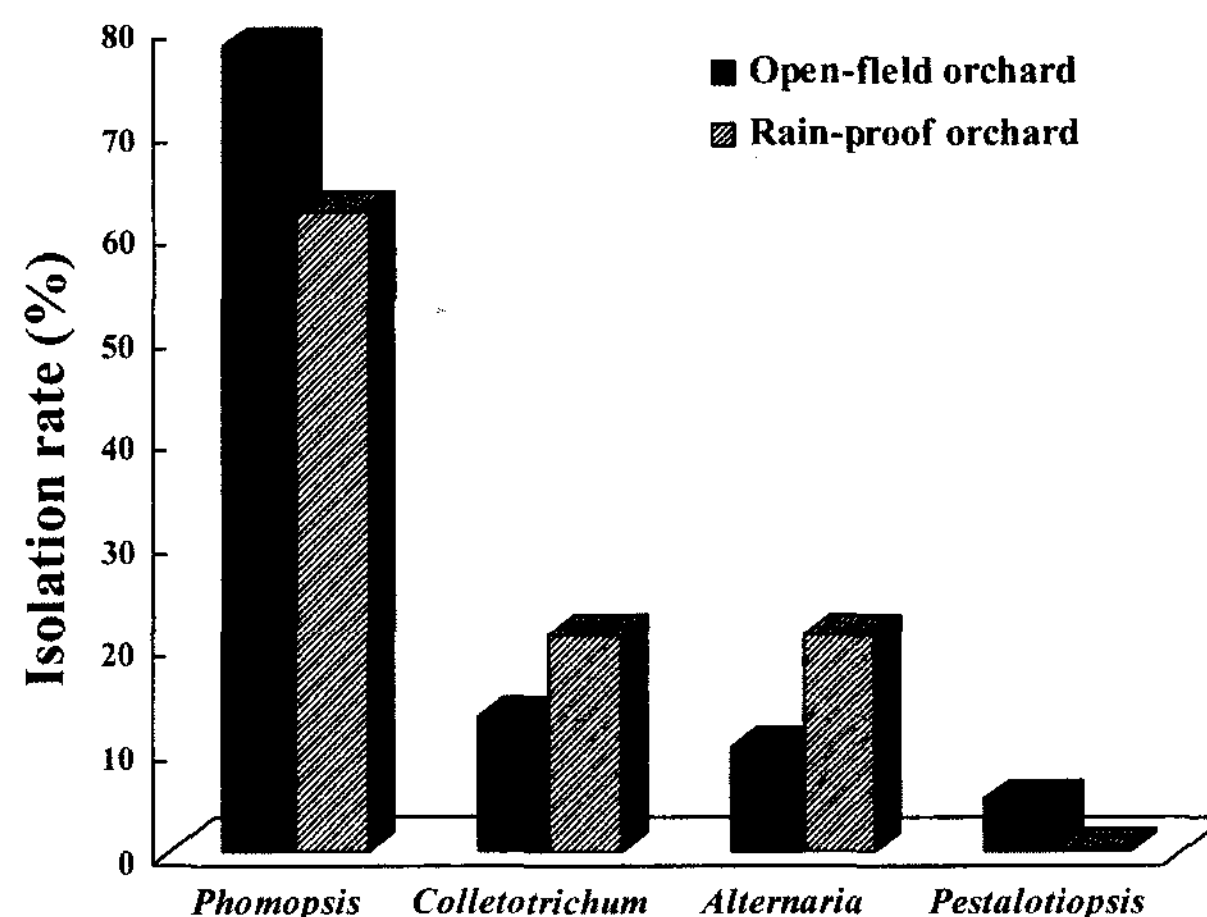


Fig. 4. Isolation rates of the fungi among leaf spot and blight symptoms of kiwifruit collected at open-field and rain-proof orchards in Suncheon, Jeonnam Provinces, Korea on July 25, 2006.

Identification of the causal fungi. *Phomopsis* sp. formed chalk white aerial mycelial mats with a circular shape on PDA plates after incubation at 25°C over 7 days (Fig. 5A). After 3 weeks of incubation at 30°C, α - and β -conidial masses formed all over the mycelial mats. The α -conidia produced on yellowish conidial masses were hyaline, unicellular, and fusiform and 1.8-2.6 μm wide \times 4.0-6.8 μm long. The β -conidia produced on milky conidial masses were hyaline, unicellular, filiform to hamate, and 1.0-2.1 μm wide \times 20.8-31.6 μm long (Fig. 5B). The characteristics were in accordance with those of *Phomopsis* sp. (Sommer and Beraha, 1975).

Colletotrichum sp. formed ash-green to dark green mycelial mats with circular shape on PDA plates after incubation at 25°C over 7 days (Fig. 5C). After 7 days of incubation at 20°C, pinkish conidia mass formed all over the mycelial mats. The conidia were unicellular, straight, cylindrical, and obtuse at the apex, and 4.1-5.8 μm wide \times 13.1-20.1 μm long (Fig. 5D). Appressoria were brown to

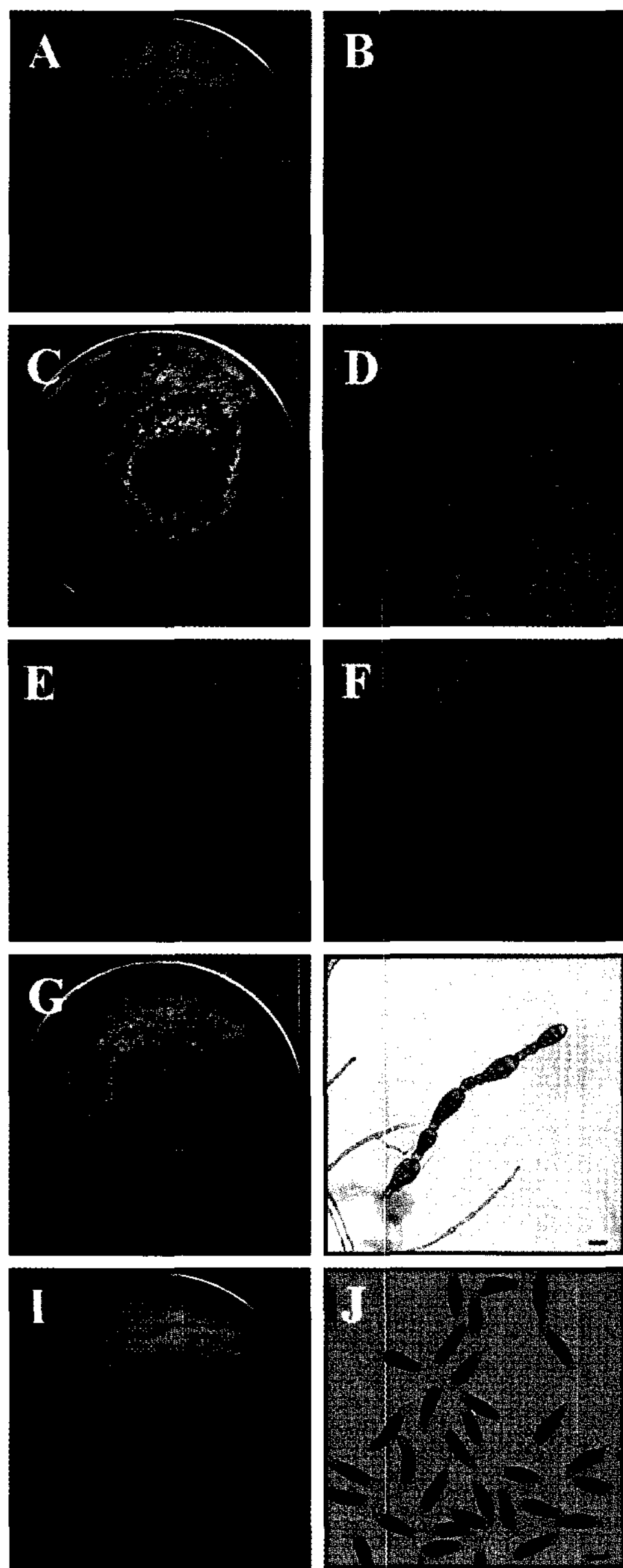


Fig. 5. Cultural and morphological characteristics of the fungi isolated from the leaf spot and blight symptoms. Colony (A) and conidia (B) of *Phomopsis* sp.; colony (C), conidia (D), appressorium (E) and perithecia with asci (F) of *Glomerella cingulata*; colony (G) and conidia (H) of *Alternaria alternata*; colony (I) and conidia (J) of *Pestalotiopsis* sp. Scale bars represent 10 μm .

dark-brown, ovate or ellipsoidal, and 4.4-6.9 μm wide \times 7.2-11.3 μm long (Fig. 5E). After 4 weeks of incubation, dark brown and globose perithecia formed on the mycelial mats, which ranged from 79.3 to 89.8 μm in diameter (Fig. 5F). Asci were hyaline, clavate to cylindrical, eight-spored, and 8.7-10.4 μm wide \times 35.8-41.4 μm long. Asospores were unicellular, hyaline, narrowly oval to cylindrical to

fusiform, and 3.1-5.7 μm wide \times 11.1-18.1 μm long. The characteristics were in accordance with those of *Glomerella cingulata* (Mordue et al., 1972).

Alternaria sp. formed grayish white mycelial mats with circular shape on PDA plates after incubation at 25°C over 7 days (Fig. 5G). After 4 days of incubation at 25°C, conidiophores formed solitary or in small fascicles all over the olive-green to black mycelial mats, and 3.1-6.1 μm wide \times 8.6-112.7 μm long (Fig. 5H). The conidia formed in chains were ovoid or obclavate, 1-4 transverse and 1-2 longitudinal or oblique septate, and 6.7-19.5 μm wide \times 16.5-42.1 μm long. The characteristics which were also reported by Jeong et al. (2008) were in accordance with those of *Alternaria alternata* (Ellis, 1977).

Pestalotiopsis sp. formed white aerial mycelial mats with circular shape on PDA plates after incubation at 25°C over 7 days (Fig. 5I). After 12 days of incubation at 20°C, dark colored acervuli formed all over the mycelial mats. The conidia were fusiform, 5-celled, 5.5-8.9 μm wide \times 14.1-27.3 μm long (Fig. 5J). The three median cells of conidia were 10.7-18.3 μm (average 14.6 μm) long. Apical appendages were 2 or 3, usually 3, broad-conic to cylindrical, 7.7-26.7 μm (average 13.5 μm) long. Basal appendages were broad and conic, 2.9-7.4 μm (average 4.6 μm) long. The characteristics were in accordance with those of *Pestalotiopsis* sp. described by Guba (1961).

Pathogenicity. Typical symptoms appeared on the wounded and unwounded leaves, which were inoculated by each of *Phomopsis* sp., *G. cingulata*, and *Pestalotiopsis* sp., respectively (Table 2). *A. alternata* caused symptoms only on the wounded leaves, but not on the unwounded leaves. Leaf blight symptoms also occurred on the wounded leaves by inoculation of *Phomopsis* sp. in combination with *A. alternata* or *G. cingulata*, respectively.

Table 2. Pathogenicity of *Alternaria alternata*, *Glomerella cingulata*, *Pestalotiopsis* sp. and *Phomopsis* sp. isolated from leaf spot and blight symptoms on kiwifruit trees

Pathogens	Disease severity ^a	
	Wounded	Unwounded
<i>Alternaria alternata</i>	++	-
<i>Glomerella cingulata</i>	++	+
<i>Pestalotiopsis</i> sp.	++	+
<i>Phomopsis</i> sp.	+	+
<i>Phomopsis</i> sp. + <i>A. alternata</i>	++	-
<i>Phomopsis</i> sp. + <i>G. cingulata</i>	++	+

^aDisease severity was evaluated at 5 days after artificial inoculation on kiwifruit leaves with or without wound. Symbols: ++, Severe symptom; +, Mild symptom; -, No symptom.

Discussion

Kiwifruit had been regarded as a disease-free fruit tree in 1980's, but 11 diseases have been reported to occur on kiwifruit until now in Korea (The Korean Society of Plant Pathology, 2004). Various leaf spot and blight symptoms have been found on leaves of kiwifruit in recent years (Jeong et al., 2008; Koh et al., 2007; Park et al., 1997). Eight kinds of leaf spot and blight symptoms were observed at open-field and rain-proof orchards in this study. The symptoms were first observed on early June at open-field and rain-proof orchards, respectively. However their incidences at open-field were not identical with those at rain-proof orchards. Incidences of the diseases at rain-proof orchards were increasing slowly during the growing season but the incidences at open-field orchards were increasingly sharply at late growing stage.

We observed lower occurrences of the diseases on the kiwifruit trees cultivated under vinyl-rain proofing than open-field conditions in this study. Although kiwifruit trees are commonly cultivated in vinyl houses to escape cold injury during winter in Korea (Koh et al., 2007), the trees are also sheltered from wind and rain during the growing season. Wind influences plant diseases by increasing the spread of fungal spores and the number of wounds on the host and rain is indispensable for the germination of fungal spores and penetration of the host by the germ tube (Agrios, 2005). Therefore, use of vinyl-rain proof shelters is considered to alleviate the occurrence and development of leaf spots and blights on the kiwifruit trees which might be predisposed by wind and rain in this study.

Eight kinds of leaf spots and blights turned out to be caused by single or mixed infection of four fungal species identified in this study. Among the causal fungi associated with the leaf spots and blights on the kiwifruit trees, *Phomopsis* sp. was the most dominant and followed by *G. cingulata*, *A. alternata*, and *Pestalotiopsis* sp. Hawthorne et al. (1982) also isolated 11 potentially pathogenic fungi including those four fungal species from diseased leaves, of which *Alternaria* sp. was the most dominant in New Zealand. Ushiyama et al. (1996) reported *Pestalotiopsis longiseta*, *Pestalotiopsis neglecta*, *Colletotrichum gloeosporioides* and *Phomopsis* sp. as the pathogens causing three types of leaf spots of kiwifruit in Japan.

Phomopsis sp. was commonly isolated from angular leaf spot, silvering gray leaf blight, and zonate brown leaf blight symptoms in this study. The fungus was also reported to cause stem-end rot of kiwifruit during storage, transportation, marketing and consumption after harvest (Beraha, 1970; Koh et al., 2005; Lee et al., 2001; Sommer and Beraha, 1975) and its teleomorph state was also identified as *Diaporthe actinidiae*. Since the characteristics of *Phomopsis*

sp. isolated from leaf spot and blight symptoms in this study are in accordance with those of anamorph state of *D. actinidiae* (Sommer and Beraha, 1975; Lee et al., 2001), *Phomopsis* sp. is considered as an alternate pathogen causing leaf and fruit diseases. Therefore, leaf spots and blights caused by *Phomopsis* sp. should be controlled in order to eliminate the potential inocula of stem-end rots of fruits disseminated from the diseased leaves.

Anthrachnose caused by *G. cingulata* was reported on kiwifruit in Korea (The Korean Society of Plant Pathology, 2004). The fungus was also found to be a major pathogen causing clear grayish brown ring spot as the typical symptom of anthracnose in this study.

Since many species of *Alternaria* are known to be saprophytic, it is not easy to decide whether an *Alternaria* fungus found on diseased leaves is the cause of the disease or a secondary contaminant (Agrios, 2005). However, *A. alternata* isolated from brown ring spots in this study reproduced clear symptoms on kiwifruit leaves by artificial wound inoculation and several diseases caused by the fungus had already been reported on various host plants including kiwifruit in Korea (Jeong et al., 2008; The Korean Society of Plant Pathology, 2004).

Sometimes *Phomopsis* sp. mixed with *A. alternata* or *G. cingulata* was isolated from severely blighted leaf symptoms compared with those associated with one of the four fungal species identified in this study. Grayish brown leaf blights caused by *Phomopsis* sp. and *G. cingulata* and dark brown leaf blights caused by *Phomopsis* sp. and *A. alternata* usually appeared on the kiwifruit trees at late growing season. Since they might play an important role of inoculum potentials for fruit rots after harvest, it is important to manage the diseases on the threshold of outbreak at early growing season.

Park et al. (1997) reported *Pestalotiopsis menezesiana* causing leaf blight on kiwifruit and Koh et al. (2006) reported *P. bicilia* from leaf spots on wild Chinese gooseberry (*Actinidia arguta*). Ushiyama et al. (1997) reported *P. longiseta* and *P. neglecta* as the pathogens causing the Pestalotia disease of kiwifruit in Japan. Similarly, a pathogenic species of *Pestalotiopsis* causing reddish brown zonate leaf blights was also isolated in this study, but its morphological characteristics were not exactly in accordance with those reported previously. Since most species in the *Pestalotiopsis* have morphological characters that overlap in many respects, the classification and delimitation of the taxon have been resolved differently by various authors (Jewon, 2004; Guba, 1961; Nag Raj, 1993; Steyaert, 1949; Sutton, 1980) and the taxonomy of *Pestalotiopsis* is still in ambiguity and confusion. Further studies are needed to identify the species of *Pestalotiopsis* causing reddish brown zonate leaf blights on the kiwifruit trees in this study.

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