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# Characterization of a Brown Rot Fungus Isolated from Dwarf Flowering Almond in Korea

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**The fruits showing brown rot symptom on dwarf flowering almond were found in Gongju, Chungchungnam-Do in Korea in July 2005. Small water-soaked lesions on the fruits were initiated, and gradually developed to soft rot covered with gray conidia. Then the diseased fruits were shrunk and became grayish-black mummies. A fungus was isolated from the diseased fruit and its morphological, cultural and molecular genetic characteristics were investigated. Typical blastospores of *Monilinia* spp. were observed under a light microscope both from tissues of the diseased fruits and from PDA-grown cultures. The fungus grew well at 25°C and on PDA. The ITS ribosomal DNA region (650 bp) of the fungus was amplified by PCR and analyzed. Comparative data on ITS sequence homology among *Monilinia* spp., ITS sequence-based phylogram and morphological characteristics showed that the fungus is *Monilinia fructicola*. This is the first report on *Monilinia fructicola* causing brown rot on fruits of dwarf flowering almond in Korea.**

**KEYWARDS: Brown rot, Dwarf flowering almond, ITS rDNA, *Monilinia fructicola***

The dwarf flowering almond (*Prunus glandulosa* Thunb.), also called as flowering cherry, is a deciduous broad-leaf shrub belonging to Rosaceae. It is widely planted on gardens because of its beautiful flowers. It blossoms white or pale pink, single or double flowers abundantly in spring. The flowers are followed by dark red fruits 1~1.3 cm in diameter. Also its fruit has been used as oriental medicine for relieving constipation and a diuretic.

Brown rot of stone fruits caused by *Monilinia* spp. is severe on stone fruits in rainy season and is important during post-harvest. The occurrence of the disease has been reported on several stone fruits such as *Prunus*, *Pyrus* and *Malus* (Biggs and Northover, 1988). The brown rot of stone fruits is mainly caused by *M. fructicola*, *M. fructigena*, and *M. laxa*. Among these species, it has been known that *M. fructicola* causes brown rot on Japanese plum (*Prunus salicina*), apricot (*Prunus armeniaca*), peach (*Prunus persica* var. *vulgaris*), and Japanese apricot (*Prunus mume*). *M. fructigena* causes the disease on pear (*Pyrus serotina*) and apple (*Malus pumila* var. *dulcissima*). *M. laxa* causes brown rot of oriental cherry (*Prunus serrulata* var. *spontanea*) and *Prunus avium*.

In July 2005, fruits showing typical brown rot symptom were found on the dwarf flowering almond in Gongju, Chungchungnam-Do. This study was carried out to isolate and identify the casual agent causing the brown rot symptom on the fruits of dwarf flowering almond. To our knowledge, the present work is the first report on the occurrence of *Monilinia* brown rot in dwarf flowering almond in Korea.

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## Materials and Methods

**Fungal isolation.** The fruits showing brown rot symptom on dwarf flowering almond (Fig. 1) were found (*Prunus glandulosa* Thunb.) in Gongju, Chungchungnam-Do, in Korea in July 2005. Small fragments were excised from the typical lesion on dwarf flowering almond and disinfected in 1% NaClO solution for 30 seconds, washed with sterile water and incubated on water agar for 24 hours at 25°C. Mycelia growing out from the fragments of diseased tissue were cut and transferred onto potato dextrose agar (PDA, Difco). Single spore isolates were obtained from the PDA grown fungal culture and used for further study.

**Cultural, morphological, and molecular characteristics.** Microstructures including conidia of the isolate (DUCC 40001) which were formed on potato dextrose agar (PDA) at 25°C for 7 days were examined under a phase-contrast microscope and a light microscope (Carl Zeiss) at 400 ×. To investigate the linear mycelial growth, agar plugs were cut from the actively growing margin of the fungal isolate grown on PDA culture with sterile cork-borer (0.5 mm diameter), transferred on new PDA, and cultured at 20°C, 25°C, 30°C, and 37°C, respectively. Also the agar plugs were transferred on PDA, oatmeal agar (OMA) and malt extract agar (MEA). Genomic DNA of

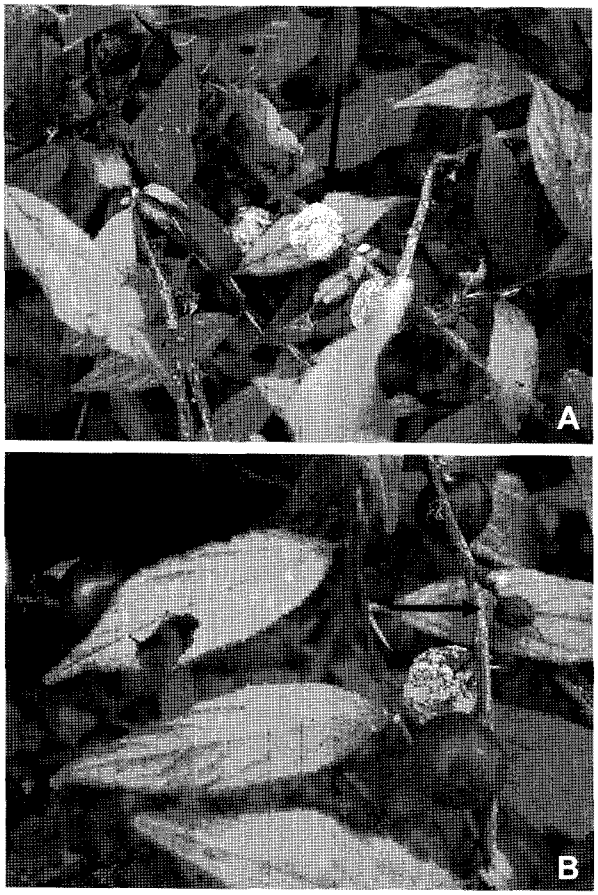


**Fig. 1.** Brown rot symptoms on dwarf flowering almond. The arrows indicates a brown rotted fruit covered with the fungal conidia (A) and dark-black mummified fruit (B).

the fungal isolate was extracted using a drilling method (Kim *et al.*, 1999). The ITS ribosomal DNA regions were amplified by PCR using universal primer pairs, ITS1-ITS4 (White *et al*, 1990). PCR reaction mixture (a total volume of 50 $\mu l$) contained 200 ng fungal genomic DNA, 40 pmol of each primer, 50 $\mu$M (each) of the four deoxynucleotide triphosphates (dNTPs), 1× PCR buffer (10 mM Tris-Cl [pH 8.0], 1.5 mM $MgCl_2$, 50 mM KCl), 1 unit Thermostable Polymerase (Solgent Corp.). Amplification was done in a Gene Amp-950 thermal cycler (ABI, USA). PCR conditions were programmed as follows: one cycle of denaturation at 94°C for 10 min, followed by 30 cycles of denaturation at 94°C for 50 s, annealing at 52°C for 50 s, and extension at 72°C for 50 s, and final one cycle of extension at 72°C for 10 min. The amplified DNA product was sequenced on Applied Biosystems ABI 373 DNA sequencer. Both strands of the PCR-amplified DNA fragments were sequenced using the PRISM Ready Reaction DyeDeoxy termination cycle sequencing kit. The obtained nucleotide sequence was searched through BLASTX at GenBank database (http://www.ncbi.nlm.nih.gov/BLAST/). Multiple DNA sequence alignment was performed using CLUSTAL W program (Thomson *et al.*, 1994). Phylogentic analysis was undertaken by use of PAUP program (Swofford, 2002).

**Pathogenicity.** Pathogenicity test was performed by inoculating the fungal mycelium plugs or spores grown on PDA onto fruits of dwarf flowering almond and other stone fruits such as *Chaenomeles sinensis*, *Prunus persica*, *Prunus salicina*, and *Prunus persica* var. *nectarine*. Before the inoculation, the fruits were disinfected with 70% ethanol, washed with sterile water twice and dried. For plug inoculation, the surface of fruits was holed (1 cm deep) using a sterile cork borer (4 mm diameter) and the holes were inoculated with mycelium plugs. For spore inoculation, the spore suspension was applied on the fruit surface using a sterile camel-haired brush. The inoculated fruits were incubated in a humid chamber at 25°C for 96 hours and rotting of fruit tissues were evaluated by observing the changes of color and softness in the fruit tissues. When the rotten area became brown color, the

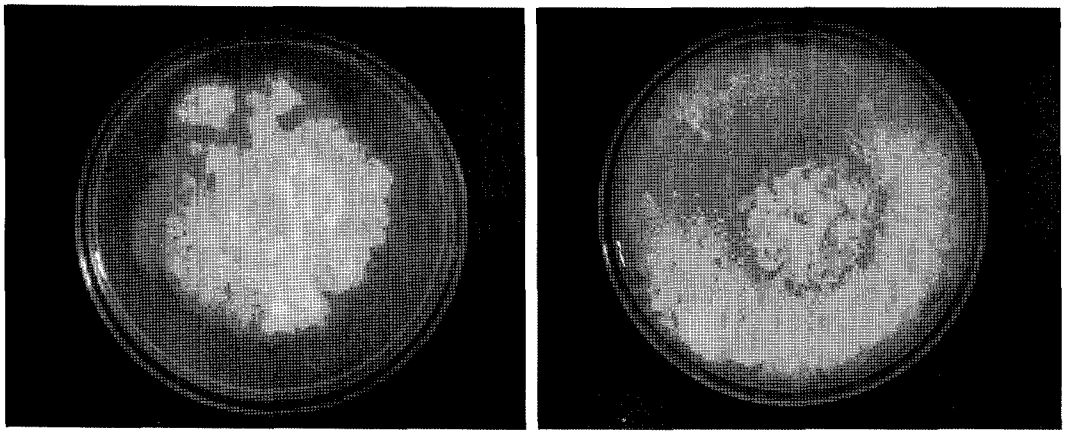

**Fig. 2.** Colony morphology of the fungal isolate from dwarf flowering almond. Culture was grown on PDA for a week. Photos were taken 3 days (left) and 7 days (right) after inoculation.

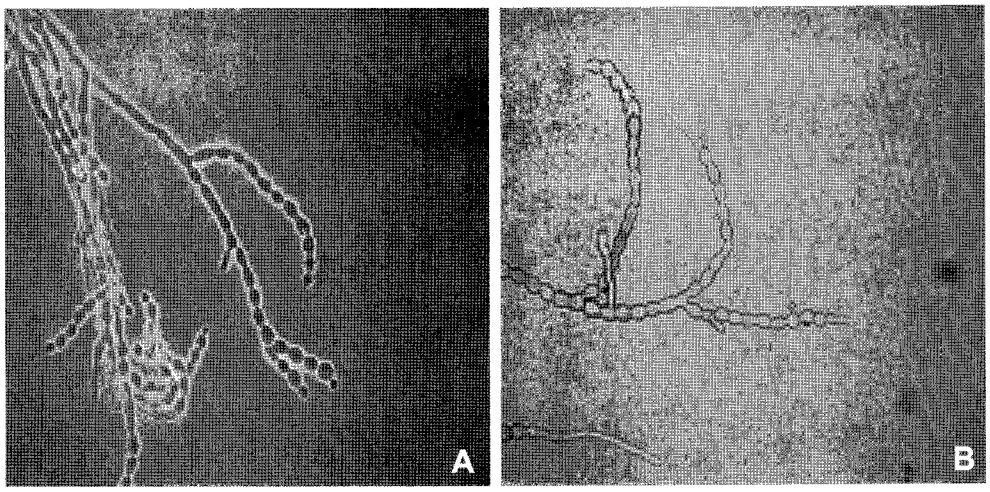


**Fig. 3.** Microscopic observation of conidia of the fungal isolate from dwarf flowering almond after lacto phenol blue staining. A: observation of rounded to short cylindric conidia in acropetalous branched chains using a phase-contrast microscope (× 400). B: observation of long and branching germ tubes from conidia using a light microscope (× 400).

inoculated fungus was reisolated from the tested rotten fruits, and its identity was confirmed by microscopic observation.

## Results and Discussion

**Symptoms.** The lesions began with small water-soaked necrosis and enlarged rapidly. Then the infected fruits became to show typical soft rot symptoms on the trees. Abundant spores of the fungus were produced on the surface of the infected fruits. Gray-colored conidial pustules were produced on the surface of rotted fruits (Fig. 1A). Eventually, the diseased fruits shrank and became grayish-black wrinkled mummies (Fig. 1B). These are some typical symptoms of brown rot on stone fruits caused by *Monilinia* spp. (EPPO/EPPO, 1988).

**Morphological characters and cultural properties.** White and gray mycelia abundantly covered the PDA plate (Fig. 2). The fungal conidial dimensions in culture were 15 × 10 mm. Conidia (blastospores) are olive to brown and some times close to, one-celled and short cylindric (Fig. 3A). Germ tubes are long and branching (Fig. 3B). These are typical morphological characters of *Monilia*, imperfect state of *Monilinia* spp., *Sclerotinia* or *Neurospora* spp. When the characters were compared based on the data sheet on quarantine pests of *Monilinia* spp. prepared by CABI and EPPO for EU (EPPO/EPPO, 1988), the characters of the fungal isolate from this study were more similar to those of *M. fructicola* and *M. fructigena* than to those of *M. laxa*. However, we did not find apothecium arising from the mummified fruit yet. The fungus grew well at temperature of 20 to 25°C with an optimum at 25°C (Fig. 4). It did not grow at 35°C. The fungus grew better on PDA than on MEA and OMA (Fig. 4).

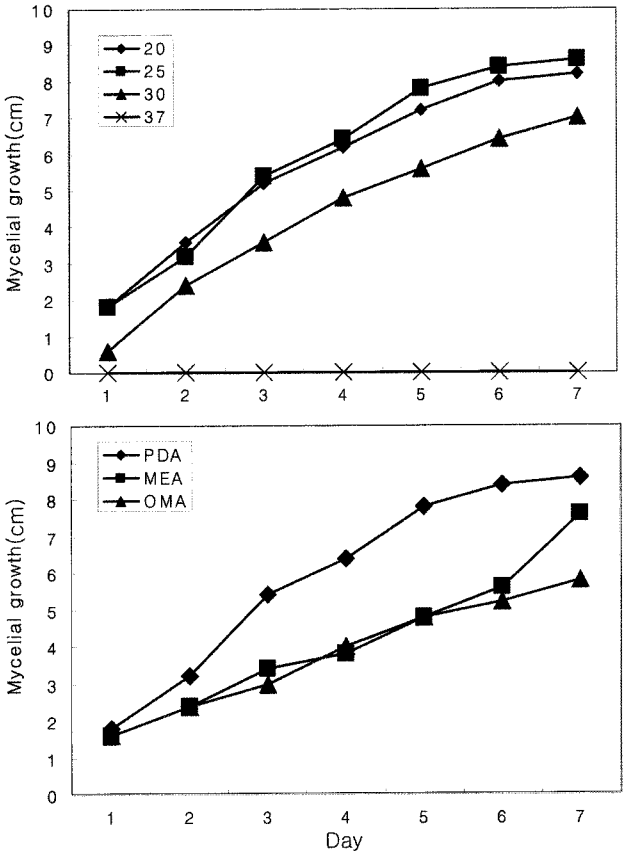


**Fig. 4.** Mycelial growth of the fungal isolate from dwarf flowering almond on PDA at different temperatures (°C) (top) and media (bottom). PDA, potato dextrose agar; MEA, malt extract agar; and OMA, oatmeal agar.

**Molecular characteristics.** Approximately 650 bp of the ITS ribosomal DNA region was amplified by PCR with the fungal isolate from dwarf flowering almond.

**Table 1.** Nucleotide sequence identity of ITS ribosomal DNA between the fungal isolate (sample) from dwarf flowering almond and other *Monilinia* and *Monilinia*-related species

| | Sample | Ss1 | Ss2 | Bf | Ml1 | Ml2 | Mf1 | Mf2 |
|---|---|---|---|---|---|---|---|---|
| Sample | 100 | 98 | 98 | 98 | 99 | 99 | 100 | 100 |
| Ss1 | | 100 | 100 | 99 | 98 | 98 | 98 | 98 |
| Ss2 | | | 100 | 98 | 98 | 98 | 98 | 98 |
| Bf | | | | 100 | 98 | 98 | 98 | 98 |
| Ml1 | | | | | 100 | 100 | 99 | 99 |
| Ml2 | | | | | | 100 | 99 | 99 |
| Mf1 | | | | | | | 100 | 100 |
| Mf2 | | | | | | | | 100 |

Ss1, *Sclerotini. sclerotiorum* (GenBank accession no. AF455526); Ss2, *S. sclerotiorum* (GenBank accession no. AF455523); Bf, *Botrytis fabae* (GenBank accession no. AY131202); Ml1, *Monilinia laxa* (GenBank accession no. AF150676); Ml2, *M. laxa* (GenBank accession no. AF150675); Mf1, *M. fructicola* (GenBank accession no. Z73777); Mf2, *M. fructicola* (GenBank accession no. AY289185).

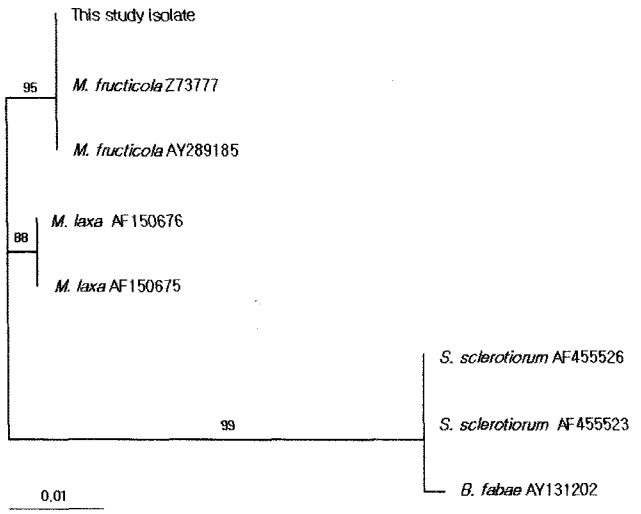


**Fig. 5.** Phylogenetic relationship of the *Monilinia fructicola* isolate DUCC40001 from dwarf flowering almond to other *Monilinia fructicola* isolates and *Monilinia* species. Phylogenetic tree was recovered from the neighbor-joining analysis of the nuclear ITS rDNA sequence data. *Sclerotinia sclerotiorum* and *Botrytis fabae* were used as outgroup taxons.

Nucleotide sequence of the PCR product was determined and searched through by Blast N program at GenBank DNA database. The determined ITS rDNA sequence showed homology with ITS sequences of *Monilinia* spp. Thus, detailed sequence comparison was performed between the ITS sequence of the fungal isolate from dwarf flowering almond and that of other *Monilinia*-related species including *Sclerotinia* species. The fungal ITS sequence of dwarf flowering almond isolate has 100% similarity with *Monilinia fructicola* isolated from an unknown vegetable crop in USA (GenBank accession number EF207423) and 98 to 99% with those of *M. laxa*, *Botrytis fabae* and *Sclerotinia* spp. (Table 1). The result of phylogenetic analysis also strongly supports the isolate is *M. fructicola* (Fig. 6). Overall, based on symptoms and morphological and molecular properties, the fungal isolate from dwarf flowering almond was identified as *M. fructicola* (G. Wint.) Honey.

**Pathogenicity.** To evaluate the pathogenic ability of the identified *Monilinia* species, the fungus was inoculated on fruits of dwarf flowering almond. Spores of the fungus produced typical brown rot symptoms on both wound-treated and non wound-treated fruits of dwarf flowering almond (Fig. 6A, 6B). The fungal isolate was re-isolated from the lesion of inoculated plants fulfilling Koch's postulation. Since *M. fructicola* has broad-range of host plants, we extended pathogenicity test to four other species of stone fruits. The *M. fructicola* isolate of dwarf flowering almond could cause rotting symptoms on three *Prunus* species, peach (*P. persica*), plum (*P. salicina*), and nectarine (*P. persica var. nectarine*) (Fig. 6D, 6E, 6F). However it did not cause disease symptom on *Chaenomeles sinensis*. It seems that the *M. fructicola* isolate of dwarf flowering almond also favors other stone fruits such as peach and nectarine as its hosts.

In conclusion, we identified *M. fructicola* as the causal agent of brown rot of dwarf flowering almond and demonstrated that it has the ability to cause brown rot symptoms on other *Prunus* species that are common in Korea. The *M. fructicola* is known to be one of the most destructive diseases of stone fruits worldwide and one of fungal species showing high resistance to benzimidazole fungicide (Zhonghua *et al.*, 2003). Recently, in Korea, the distribution of *M. fructicola* isolates that cause the brown rot on peach and are resistant to dicarboximide or to both procymidone and carbendazim has been reported (Lim and Cha, 2003). Since dwarf flowering almond is not broadly cultivated in Korea and also fungicide application is not common to this garden growing stone fruit plant, it will be worth while to investigate the sensitivity of *M. fructicola* isolate from dwarf flowering almond to diverse fungicides that are currently applied in Korea.

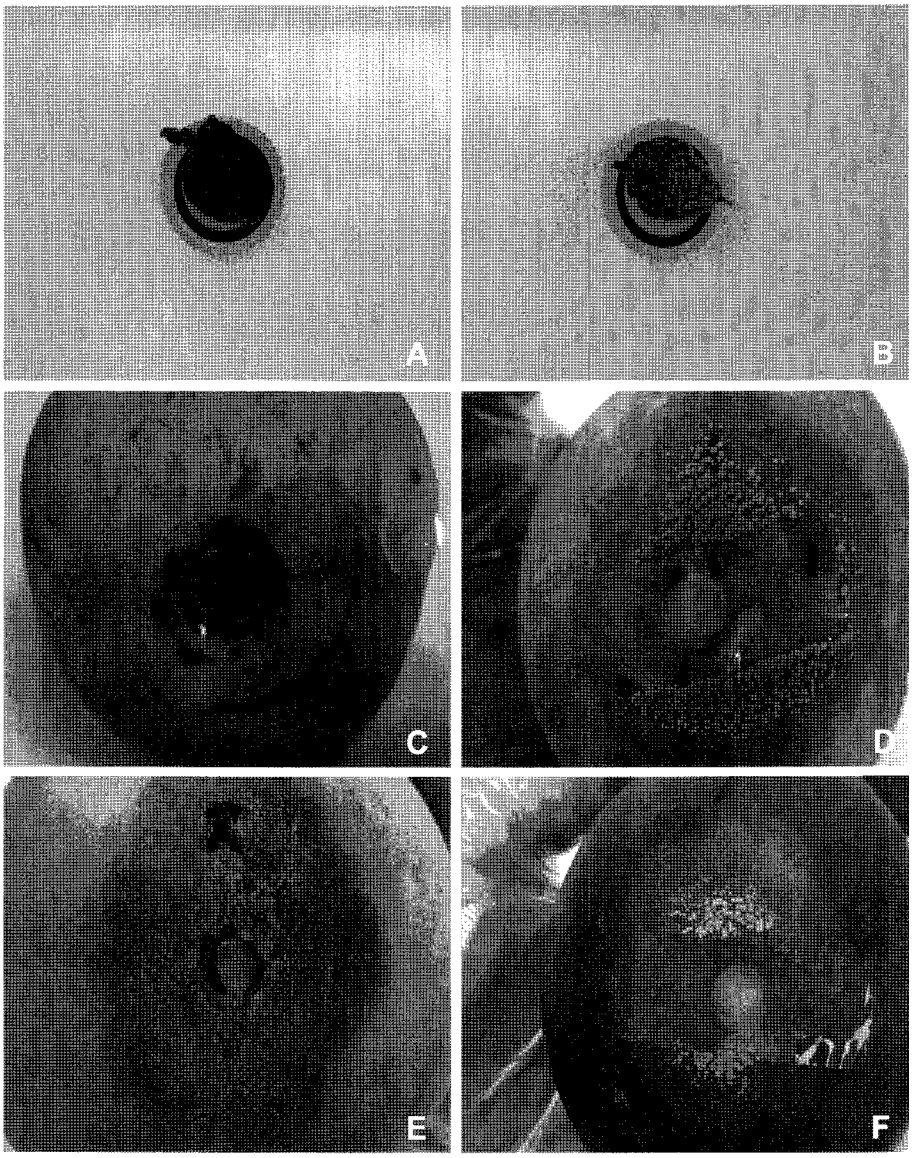


**Fig. 6.** Pathogenicity test of the *Monilinia fructicola* isolate DUCC40001 from dwarf flowering almond on different fruits. A, dwarf flowering almond inoculated without wound; B, dwarf flowering almond inoculated with wound; C, *Chaenomeles sinensis*; D, *Prunus salicina*; E, *Prunus persica*; and F, *Prunus persica* var. *nectarina*.

## References

Barnett, H. L. and Hunter, B. B. 1998. Illustrated genera of imperfect fungi. *APS*. St. Paul, Mn. USA.

Biggs, A. R. and Northover, J. 1988. Influence of temperature and wetness duration on infection of peach and sweet cherry by *M. fructicola*. *Phytopathology* **78**: 1352-1356.

Brackmann, A., Garibaldi, N. and Mauch, N. 1984. Evaluation of the efficiency of fungicides for the post-harvest control of rots in peach (*Prunus persica*). *Anais do VII Congresso Brasileiro de Fruticultura* **4**: 1080-1087.

Byrde, R. J. W. and Willetts. H. J. 1977. The brown rot fungi of fruit - their biology and control. *Pergamon Press*. New York, NY. USA.

Barta, L. B. 1991. World species of *Monilinia* (fungi): their ecology, biosystematics and control. *Mycol. Mem.* **16**: 1-246.

Cline, E. 2005. *Minilinia fructicola*. http://nt.ars-grin.gov/sbmlweb/OnlineResources/FungiOnline.cfm.

Holst-Jensen, A., Kohn M. L., Jakobsen, K. S. and Schumacher, T. 1997. Molecular phylogeny and evolution of *Monilinia* (Sclerotinaceae) based on coding and noncoding rDNA sequences. *Am. J. Bot.* **84**: 686-701.

EPPO/EPPO. 1988. Data sheets on quarantine organisms No. 153, *Monilinia fructicola*. *Bulletine OEPP/EPPO Bulletine* **18**: 509-556.

Kim, S. H., Uzunovic, A. and Breuil, C. 1999. Rapid detection of *Ophiostoma piceae* and *O. quercus* in stained wood by PCR. *Appl. Environ. Microbiol.* **95**: 287-290.

Lim, T. H. and Cha, B. 2003. Distribution of *Monilinia fructicola* isolates resistant to dicarboxyimide or to both procymidone and carbendazim in Korea. *Plant Pathol. J.* **19**: 46-50.

Ogawa, J. M., Zehr, E. I. and Biggs, A. R. 1995. Brown rot. Pages 7-10 in: Compendium of stone fruit diseases. *APS*. St. Paul, MN. USA.

Swofford, D. L. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts, USA.

The Korean Society of Plant Pathology. 2004. List of plant diseases in Korea, 4th ed. 779pp. (in Korean).

Thompson, J. D., Higgins, D. G. and Gibson, T. J. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**: 4673-4680.

White, T. J., Bruns, T., Lee, S. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp 315-322. *In* M. A. Innis, D. H. Gelfand, J.J. Sninsky, and T. J. White Eds. *PCR protocols: A guide to methods and applications*. Academic Press, San Diego, USA.

Zhounghua, M., Yoshimura, M. A. and Michailides, T. J. 2003. Identification and characterization of benzimidazole resistance in *Monilinia fructicola* from stone fruit orchards in California. *Appl. Environ. Microbiol.* **69**: 7145-7152.