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Fungi Colonizing Sapwood of Japanese Red Pine Logs in Storage

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The Korean sawmills have recently recognized the importance of prevention of fungal discoloration due to increased losses in revenue. Before establishing integrated control strategies of fungal discoloration, more complete knowledge about causal organisms is needed. As a first step, we initiated a through survey of fungi colonizing commercially important softwood (*Pinus densiflora*, *Pinus koraiensis*, and *Pinus radiata*) logs and lumber in Korea. In this paper we report results obtained from Japanese red pine (*Pinus densiflora*) log study. In summer 2000, fungi were isolated from Japanese red pine logs in storage, and identified based on their cultural and morphological characteristics. A total of 595 fungi were isolated, representing 21 genera and 30 species. Mold fungi, mostly *Trichoderma* species, were the most frequently isolating fungi, representing more than half of all isolates. Dematiaceous fungi represented approximately one fifth of the isolates, and *Rhinocladiella atorvirens* was the most abundant in all samples. *Opiostoma* species represented 7% of all isolates from cores planted on malt extract agar (MEA) and the incidence of these species doubled with the addition of streptomycin and cycloheximide to MEA. The results indicate that Japanese red pine sapwood is susceptible to colonization by a variety of fungal species. As a result, control strategies that concentrate on one fungus may have limited success because of interference from competing flora.

KEYWORDS: Dematiaceous fungi, Japanese red pine, Mold, *Ophiostoma* species, *Pinus densiflora*, Sapstain

The fungal discoloration by mold and sapstain fungi can cause serious disfigurement of log or lumber surfaces, thereby reducing their value significantly. Therefore, fungal discoloration must be controlled for the production of clean and bright logs or lumber. Fungal discoloration can be prevented by rapid kiln drying or with chemical treatments of wood surfaces (Zabel and Morrell, 1992). Because majority of softwood lumber produced in Korea is air-dried, chemical treatments are essential for preventing fungal discoloration, particularly in hot and humid season.

A variety of chemicals have been developed and tested for control of sapstain and mold, but efficacy of these compounds can vary with fungal species (Kim *et al.*, 1999; Miller and Morrell, 1989, 1990; Miller *et al.*, 1989; Roff *et al.*, 1980; Tsunoda and Nishimoto, 1985). Therefore, more complete knowledge about causal organisms is needed before establishing integrated control strategies of fungal discoloration. As a first step, we initiated a through survey of mold and stain fungi colonized commercially important softwood (*Pinus densiflora*, *Pinus koraiensis*, and *Pinus radiata*) log and lumber in Korea. In this paper, we report results obtained with Japanese red pine log study.

Materials and Methods

In summer 2000, 10 Japanese red pine logs (25 to 30 cm in diameter and 3.6 m in length) were randomly selected at a local sawmill in Bongwha, Korea. The surface and cross section of logs were heavily colonized by microfungi, and almost barks were removed. From each log, 24 cores were removed from both clear and visibly colonized areas of log surface using an increment borer, and placed in a plastic bag individually for transportation to the laboratory.

Each core was briefly flamed to kill contaminating surface microflora and was placed in plastic petri dishes on one of two media; 2% malt extract agar (MEA) and 2% MEA plus 100 ppm streptomycin and 100 ppm cycloheximide (SCMEA). Streptomycin was incorporated to inhibit bacteria, while cycloheximide was incorporated to selectively isolate *Ophiostoma* spp. The plates were incubated at room temperature (25C), and carefully observed daily for evidence of fungal growth. Any fungi growing from the cores were subcultured onto petri plates containing the same media and retained for later identification.

Fungal identification was based on cultural and morphological characteristics on suitable media in petri plates. *Penicillium* species were incubated on Czapek's agar and Czapek's yeast agar (Ramirez, 1982). *Aspergillus* species were grown on Czapek's agar (Raper and Funnell, 1973). Also, V-8 juice agar was used to increase or induce sporulation in some cultures (Wang and Zabel, 1990). The remaining isolates were grown in MEA. The isolates were identified using the appropriate literatures (Arx, 1981; Barnett and Hunter, 1987; Bissett, 1984, 1991a, b, c; Car-

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michael *et al.*, 1980; Cole and Kendrick, 1973; Ellis, 1971, 1976; Hawksworth *et al.*, 1996; Hutchison and Reid, 1988a, b; Nobles, 1965; O'Donnell, 1979; Ramirez, 1982; Raper and Fennell, 1973; Rifai, 1969; Sutton, 1980; Wang and Zabel, 1990).

Results and Discussion

A total of 595 fungi representing 21 genera and 30 species were isolated using MEA or SCMEA (Table 1). The addition of sreptomycin and cycloheximide to MEA resulted in a decrease in the number of isolates and an increase in the number of taxa isolated. The number of isolates obtained decreased from 380 to 215 while the number of taxa increased from 21 to 24. In addition, the isolating frequency of *Ophiostoma* species increased in the presence of streptomycin and cycloheximide, illustrating the potential for using SCMEA media for selectively isolating *Ophiostoma* species.

Table 1. Fungi isolated from Japanese red pine logs using malt extract agar (MEA) or streptomycin and cycloheximide-amended malt extract agar (SCMEA)

Fungus	Frequency (%)	
	MEA[a]	SCMEA[b]
Acremonium spp.	3.9	1.9
Alternaria alternata	1.1	7.9
Aspergillus fumigatus	1.1	0.9
Aureobasidium pullulans	2.9	-[c]
Bispora betulina	-	0.5
Cladosporium cladosporioides	-	1.9
Cladosporium resine	0.3	1.4
Exophiala sp.	-	0.5
Gliocladium roseum	0.3	0.9
Gliocladium virens	0.3	-
Graphium ulmi	0.3	1.9
Leptographium lundbergii	1.3	4.7
Paecilomyces variotii	1.1	0.9
Penicillium spp.	8.2	10.2
Phialocephala dimorphospora	0.8	4.7
Phialophora bubakii	2.1	1.4
Phialophora richardsiae	-	0.9
Rhinocladiella atorvirens	13.4	6.0
Sporothrix sp.	0.5	1.9
Trichoderma aureoviride	-	2.3
Trichoderma hamatum	0.3	0.5
Trichoderma harzianum	4.2	4.2
Trichoderma longibrachiatum	15.0	24.7
Trichoderma pseudokoningii	16.1	0.5
Trichoderma viride	4.5	11.2
unidentified *Trichoderma* sp.	7.9	-
Verticillium albo-atrum	-	0.5
Basidiomycetes	1.1	-
unidentified fungi	13.7	7.9

[a]Values represent percent frequency from 380 samples.
[b]Values represent percent frequency from 215 samples.
[c]Not isolated.

Ophiostoma species, which is well known as strong sapstainers of softwood species, represented 6.8% of all isolates from cores planted on MEA and 15.1% of all isolates from cores on SCMEA and included *Acremonium* spp., *Graphium ulmi*, *Leptographium lundbergii*, *Phialocephala dimorphospora*, and *Sporothrix* sp. (Fig. 1). All these fungi produced complete discoloration of wood surface and heavy stain in stain intensity in additional wood discoloration tests. Kim (2000) isolated seven *Ophiostoma* species (*Graphium putredinis*, *Graphium ulmi*, *Leptographium* sp., *Phialocephala* sp. *Ophiostoma piliferum*, *Sporothrix* sp., and *Verticicladiella abietina*, *Phialocephala* sp. *Ophiostoma piliferum*) from radiata pine logs using SCMEA media, with their isolating frequency of 44.9%. Of these, only *Graphium ulmi* and *Sporothrix* sp. were isolated from Japanese red pine logs.

In addition to the *Ophiostoma* species, dematiaceous fungi that produced either dark pigmented conidiophores or hyphae represented approximately one fifth of the isolates and included *Rhinocladiella atorvirens*, *Alternaria alternata*, *Aureobasidium pullulans*, *Phialophora bubakii*, *Cladosporium cladosporioides*, *Paecilomyces variotii*, *Cladosporium resine*, *Phialophora richardsiae*, *Bispora betulina*, and *Exophiala* sp. (Fig. 1). Except for *Rhinocladiella* and *Alternaria* species, other dematiaceous fungi were not isolated with high frequencies; however, these fungi are commonly isolated from radiata pine in New Zealand according to Butcher (1968) and Hutchison and Reid (1988b).

Mold fungi were the most abundant in all sample logs, representing more than half of all isolates, and included *Trichoderma* spp., *Penicillium* spp., *Aspergillus fumigatus*, *Gliocladium* spp., *Verticillium albo-atrum* (Fig. 2). *Trichoderma* species were the most frequently isolated fungi of all molds, and followed by *Penicillium* species. High isolating frequencies of *Trichoderma* species may be attributed to long-term soil contact of logs in storage yard at sawmill. *Trichoderma* and *Penicillum* species are primary colonizer of a variety of substrates, but the roles of these species in discoloration of wood are variable. *Aspergillus niger* was not isolated in this study although it is known as one of the most common mold fungi found in fungal colonized sapwood. Only *Aspergillus fumigatus* was isolated with very low frequencies; however, this fungus did not cause any obvious stain in additional wood discoloration tests.

Fungi of unknown biological significance lacked pigmented hyphae and spores occurred at reltively high frequencies in this study. Most isolates of these were *Mucor* species and Zygomycetes. These were reported as the dominant fungi isolated from untreated stakes of radiata pine sapwood in belowground zones (Butcher, 1968). Although fungi of unknown significance are unlikely to cause sapstain themselves or to be antagonistic towards

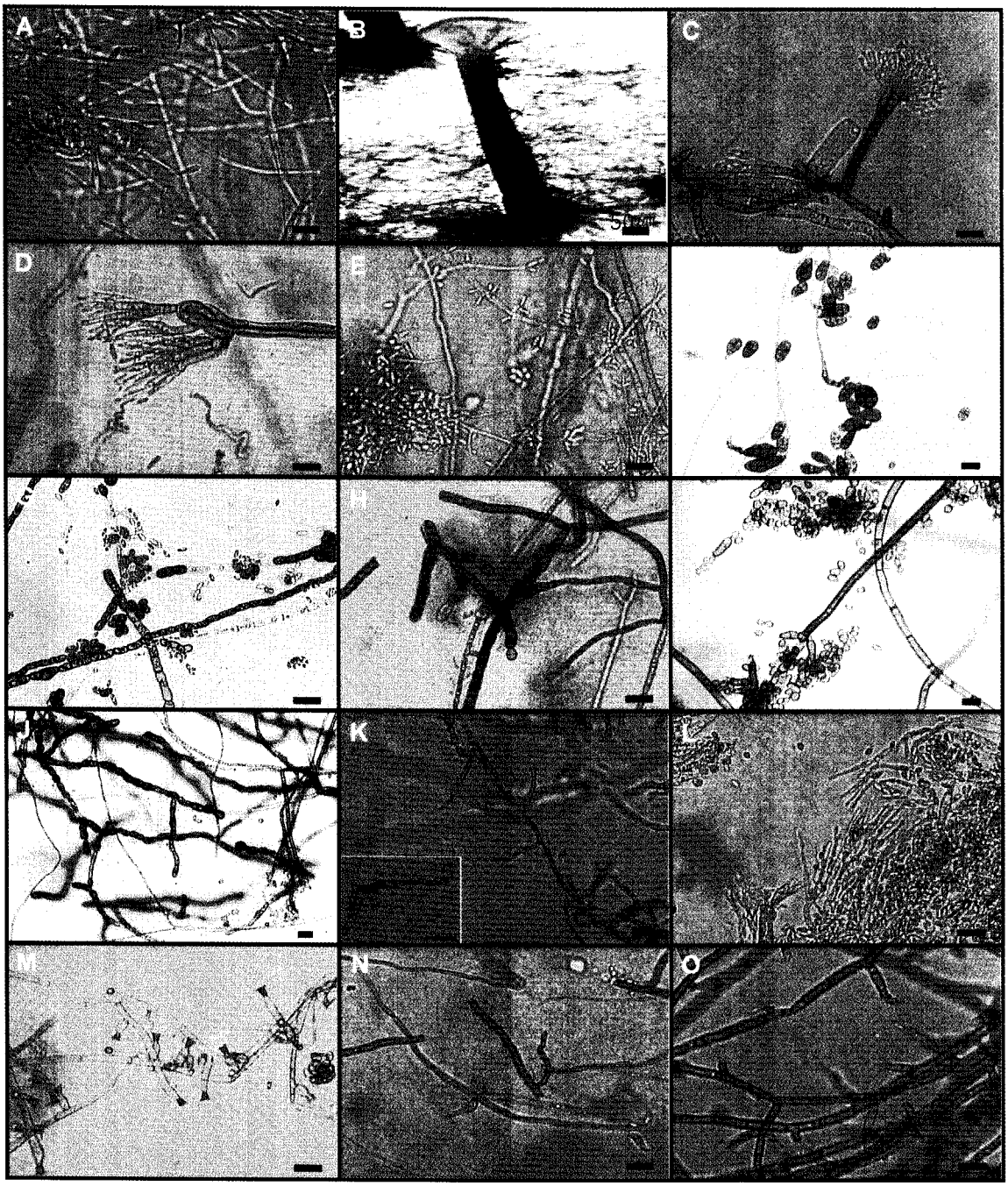

Fig. 1. Photomicrographs of *Ophiostoma* species and dematiaceous fungi isolated from Japanese red pine logs: A, *Acremonium* sp.; B, *Graphium ulmi*; C, *Leptographium lundbergii*; D, *Phialocephala dimorphospora*; E, *Sporothix* sp.; F, *Alternaria alternata*; G, *Aureobasidium pullulans*; H, *Bispora betulina*; I, *Cladosporium cladosporioides*; J, *Cladosporium resine*; K, *Exophiala* sp.;. L, *Paecilomyces variotii*; M, *Phialophora richardsiae*; N, *Phialophora bubakii*; and O, *Rhinocladiella atrovirens*. Scale bar=10 μm.

other organisms, they may have a synergistic role in the growth and the stain development of other fungi (Seifert and Grylls, 1992).

When compared with previous study conducted with radiata pine logs (Kim, 2000), the difference in fungal species between two wood species, particularly in *Ophiostoma* and dematiaceous fungi species, might be attributed to the difference in both geographical distribution of fungal flora and nutritional status of sapwood. The results indicated that current control strategies of fungal discoloration used for radiata pine would not be appropriate for Japanese red pine since efficacy of anti-stain chemicals can vary with combinations of wood and fungal species (Miller and Morrell, 1989; Miller *et al.*, 1990; Tsunoda

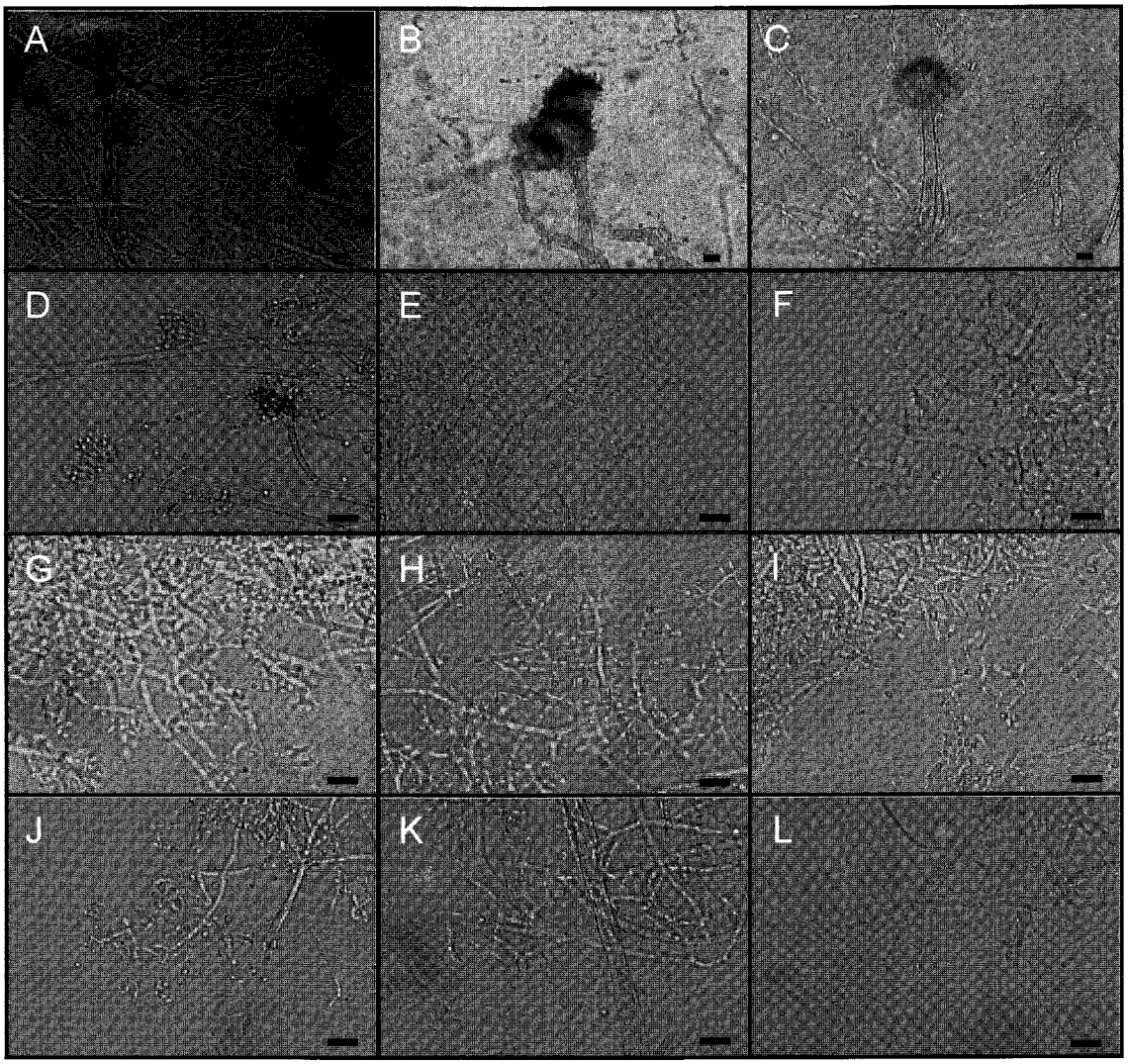

Fig. 2. Photomicrographs of mold fungi isolated from Japanese red pine logs: A, *Aspergillus fumigatus*; B, *Gliocladium roseum*; C, *Gliocladium virens*; D, *Penicillium* sp.; E, *Trichoderma aureoviride*; F, *Trichoderma hamatum*; G, *Trichoderma harzianum*; H, *Trichoderma longibrachiatum*; I, *Trichoderma pseudokoningii*; J, *Trichoderma viride*; K, unidentified *Trichoderma* sp.; and L, *Verticillium albo-atrum*. Scale bar=10 μm.

and Nishimoto, 1985).

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