Ophiostomatoid fungi isolated from *Pinus radiata* logs imported from New Zealand to Korea

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Abstract: Ophiostomatoid fungi discolor lumber, logs, and tree sapwood. Stained wood has a lower market value and can be refused by importing customers because such products can potentially carry pathogenic fungi. Little information is available on the ophiostomatoid fungi that colonize *Pinus radiata* D. Don (radiata pine) logs imported from New Zealand into Korea. In this work, we attempted to identify the native and non-native fungi colonizing wood imported into Korea. At least 12 species of ophiostomatoid fungi, including an unknown *Ophiostoma* sp. A, were identified among the fungi-staining radiata pine. They were *Leptographium procerum* (Kendr.) Wingf., *Leptographium bistatum* Kim & Kim, *Ophiostoma floccosum* Mathiesen, *Ophiostoma huntii* (Rob.) Hoog & Scheff., *Ophiostoma ips* (Rumbold) Nannf., *Ophiostoma nigrocarpum* (David.) Hoog, *Ophiostoma piceae* (Münch) H. & P. Sydow, *Ophiostoma piliferum* (Fries) H. & P. Sydow, *Ophiostoma quercus* (Georgév.) Nannf., *Ophiostoma radiaticola* Kim et al., and *Ophiostoma setosum* Uzunovic et al. Of these species, *O. floccosum* was the dominant species in both logs and boards. We confirmed that many of the sapstain species that we isolated have been previously reported in Korea. However, four species, *O. radiatcola*, *O. setosum*, *O. huntii*, and *O. nigrocarpum* have not been reported previously in Korea. We also found the new species, *L. bistatum*, along with an unknown *Ophiostoma* sp. A.

Key words: radiata pine, ophiostomatoid fungi, New Zealand, non-native organisms, β-tubulin gene.

Résumé : Les champignons ophiostomatoïdes décolorent les grumes et les billes de bois ainsi que l'aubier des arbres. Le bois coloré à une moindre valeur au marché et peut être refusé par les clients à l'importation, puisque certains de ces produits peuvent transporter des champignons pathogènes. Il existe peu d'information sur les champignons ophiostomatoïdes qui colonisent le *Pinus radiata* D. Don (pin de Monterey) importé de la Nouvelle-Zéllande, en Corée. Les auteurs ont cherché à identifier les champignons indigènes et non indigènes qui colonisent le bois importé en Corée. Ils ont identifié au moins douze espèces de champignons ophiosmatoïdes incluant une espèce inconnue, l'*Ophiostoma* sp. A, parmi les champignons qui décolorent le pin de Monterey. Il s'agit des *Leptographium procerum*, (Kendr.) Wingf., *Leptographium bistatum* Kim & Kim, *Ophiostoma floccosum* Mathiesen, *Ophiostoma huntii* (Rob.) Hoog & Scheff., *Ophiostoma ips* (Rumbold) Nannf., *Ophiostoma nigrocarpum* (David.) Hoog, *Ophiostoma piceae* (Münch) H. & P. Sydow, *Ophiostoma piliferum* (Fries) H. & P. Sydow, *Ophiostoma quercus* (Georgév.) Nannf., *Ophiostoma radiaticola* Kim et al. et *Ophiostoma setosum* Uzunovic et al. Les auteurs confirment que plusieurs des champignons colorant l'aubier, qu'ils ont isolés, ont déjà été rapportés en Corée. Cependant quatre espèces, soient les *O. radiaticola*, *O. setosum*, *O. huntii*, et *O. nigrocarpum*, n'ont jamais été rapportées en Corée auparavant. Ils ont également trouvé le *L. bistatum* ainsi que l'espèce inconnue *Ophiostoma* sp. A.

Mots clés : pin de Monterey, champignons ophiostomatoïdes, Nouvelle-Zélande, organismes non-indigènes, gène de la β-tubuline.

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Introduction

Radiata pine (*Pinus radiata* D. Don) is of great economic importance to New Zealand, accounting for approximately 95% of all exotic forestry plantations (Kay et al. 1998). In Korea, radiata pine logs are also the most important lumber source, representing approximately 70% of the nation's lumber production. The logs are currently being imported into Korea from Australia, Chile, and New Zealand.

Like other fast-growing pines, radiata pine produces a high proportion of sapwood that is usually highly susceptible to the dark discoloration caused by sapstaining fungi (Held et al. 2003). Wood discoloration is often exacerbated by the warm, moist conditions that often prevail prior to, during, and following shipping, and by the amount of time that it takes for the wood products to reach their destinations. For these reasons, several researchers have studied the sapstain organisms found in New Zealand (Butcher 1968; Hutchison and Reid 1988*a*, 1988*b*; Farrell et al. 1997; Kay et al. 1998; Thwaites et al. 2003). They reported that *Leptographium procerum* (Kendr.) Wingf., *Ophiostoma floccosum* Mathiesen, *Ophiostoma huntii* (Rob.) Hoog & Scheff., *Ophiostoma ips* (Rumbold) Nannf., *Ophiostoma*

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piceae (Münch) H. & P. Sydow, *Ophiostoma piliferum* (Fries) H. & P. Sydow, *Ophiostoma pluriannulatum* (Hedg.) H. & P. Sydow, *Ophiostoma quercus* (Georgév.) Nannf., *Ophiostoma setosum* Uzunovic et al., and *Sphaeropsis sapinea* (Fr.) Dyko & Sutton were the major causes of sapstain in exported logs, and that these fungi were present in New Zealand forests.

The sapstaining fungi listed above belong to the ophiostomatoid group and they are responsible for considerable economic losses in the forest products industry worldwide. They are associated with root diseases and blue-stain, and they are often vectored by bark beetles (Coleoptera: Scolytidae) that infest pine trees (Harrington 1988; Wingfield et al. 1988; Wingfield 1993). Most ophiostomtoids are weak pathogens; however, a few species are able to kill trees without the contribution of their beetle vector (Harrington and Cobb 1983; Wingfield et al. 1988). Although the international trade of untreated logs and wood products is increasing throughout the world, many countries now understand that such material can harbour non-indigenous pests that could become invasive in a new ecosystem (Allen and Humble 2002; Mireku and Simpson 2002; Tkacz 2002). Given this, an increasing number of countries have developed regulatory legislation and quarantine strategies for non-manufactured wood, especially raw logs.

Several fungal pathogens of radiata pine have already been introduced into Australia, South Africa, and North America. To develop appropriate quarantine strategies to prevent the introduction of exotic fungi, Korea needs to more systematically survey the fungi that are potentially present on imported wood.

The aim of the work described here was to identify native and non-native organisms colonizing radiata pine logs imported from New Zealand into Korea. Fungal identification was carried out by morphological and molecular methods. DNA data included the internal transcribed spacer (ITS) 2 and the partial large subunit (LSU) regions of the ribosomal DNA (rDNA) genes or part of the β-tubulin gene. These data were compared with the data of reference cultures from culture collections.

Materials and methods

Fungal isolation

Imported radiata pine logs (25–30 cm in diameter and 3.6 m in length) from New Zealand were randomly selected at the imported log yard in Incheon, Korea. In this mill yard warehouse most of the wood imported is from New Zealand. The facility does not handle wood from Korea. Ten logs were selected during the winter of 2000. From each log, 28 small pieces were removed from both exposed cross sections and log surfaces. During the summer of 2001, five New Zealand logs were processed at a local sawmill at Incheon. The logs were then cut and 50 freshly sawn sapwood boards were stored in a mill yard warehouse at the sawmill. After 1 month, four small pieces were removed from each board. The wood samples (logs or boards) were placed onto 2% malt extract agar (20 g Difco malt extract, 15 g Difco agar, and 1000 mL distilled water) and on malt extract agar amended with cycloheximide (100 ppm) to select for the *Ophiostoma* species (Harrington 1981). Inoculated plate media were then incubated at room temperature.

For identification, the cultural characteristics described by Upadhyay (1981), Hutchison and Reid (1988*a*, 1988*b*), Jacobs and Wingfield (2001), and Harrington et al. (2001) were used to group the different isolates. Two or three representatives of each group were then single-spore isolated (Uzunovic et al. 2000) for mating experiments and DNA analyses. Fungal isolates were stored in both sterile water at 4 °C and 20% (*v*/*v*) glycerol solution at –80 °C for further studies. They were deposited in the Korean University Culture Collection, Seoul, Korea, and in Breuil's culture collection, University of British Columbia, Vancouver, British Columbia, Canada. We have also deposited the newly described species at CMW (Culture Collection of the Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa) or DAOM (Canadian Collection of Fungal Cultures, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada) (Kim et al. 2004, 2005).

Fungal identification

Morphological studies

Morphological features were observed on fungi grown on 2% malt extract agar and on sterile lodgepole pine (*Pinus contorta* var. *latifolia* Engelm.) sapwood wafers. For light microscopy, fungal structures were mounted in water and observed with a Zeiss Axioplan light microscope.

Mating experiments

Mating tests were carried out on sterile lodgepole pine sapwood wafers with known *Ophiostoma* tester species, including species from the *O. piceae* complex and *O. piliferum* (Uzunovic et al. 2000). The known mating testers were obtained from the Breuil's culture collection (Table 2). Mating tests were also attempted with *Ophiostoma radiaticola* Kim et al. and *Leptographium bistatum* Kim & Kim, two new species that have been recently identified. Single spore isolates of each species were mated in all possible combinations at room temperature for 4–5 weeks.

Molecular studies

DNA extraction was carried out using the method described by Kim et al. (1999). The rDNA, including the ITS2 region and partial LSU rDNA genes, were amplified using the primers ITS3 (White et al. 1990) and LR3 (Vilgalys and Hester 1990). The β-tubulin gene was amplified using the primer T10 (O'Donnell and Cigelnik 1997) and BT12 (Kim et al. 2003). Polymerase chain reaction (PCR) amplification was performed as described by Lee et al. (2003). The PCR products were purified using a Qiaquick PCR purification Kit (Qiagen Inc., Mississauga, Ontario, Canada). Sequencing was performed on an ABI 3700 automated sequencer (Perkin-Elmer Inc., Foster City, California, USA) at the DNA Synthesis and Sequencing Facility, MACROGEN (Seoul, Korea). Fungal DNA sequences were then compared with datasets from GenBank or other reference cultures that we sequenced (Table 3). It is important to note that the β-tubulin of *L. procerum* could not be amplified. Consequently, we only used the rDNA for this species.

Table 1. Ophiostomatoid fungi isolated from *Pinus radiata* logs and boards using 2% malt extract agar media with cycloheximide.

Species	No. of isolates (% frequency)[a]			References[b]
	Log	Board	Total	
Ophiostoma floccosum	58 (43.9)[c]	10 (25.6)[c]	68 (39.8)[c]	A, B
Ophiostoma piceae	24 (18.2)[c]	—[d]	24 (14.0)[c]	A, B, C
Ophiostoma piliferum	15 (11.4)[c]	3 (7.7)	18 (10.5)[c]	A, B, C
Ophiostoma radiaticola	10 (7.6)	—	10 (5.8)	D
Leptographium procerum	8 (6.1)	—	8 (4.7)	A, B, E
Ophiostoma setosum	5 (3.8)	—	5 (2.9)	A, B
Ophiostoma huntii	4 (3.0)	3 (7.7)	7 (4.1)	A, B, E
Ophiostoma ips	4 (3.0)	—	4 (2.3)	A, B, C
Ophiostoma quercus	4 (3.0)	2 (5.1)	6 (3.5)	A, B
Leptographium bistatum	—	8 (20.5)[c]	8 (4.7)	Not reported
Ophiostoma nigrocarpum	—	10 (25.6)[c]	10 (5.8)	F
Ophiostoma sp. A	—	3 (7.7)	3 (1.8)	Not reported
Total isolates	132	39	171	
No. of sampling points	280	200	480	
Species richness (*S*)	9	7	12	
Simpson's index of diversity (*C*)	0.760	0.812	0.795	

Note: Logs were sampled on 1 March 2000 and boards were sampled on 31 July 2001.

[a]Frequency = (No. of isolates / Total isolates) × 100.

[b]References reporting the isolation of the species from radiata pine in New Zealand. A, Thwaites et al. (2003); B, Farrell et al. (1997), however, *O. setosum* was reported as a *Graphium* species with black-veined synnema; C, Hutchison and Reid (1988*a*); D, Hutchison and Reid (1988*b*), *O. radiaticola* was reported as *Hyalopesotum pini*, but the generic name was recently changed to *Pesotum* by Okada et al. (1998); E, Jacobs and Wingfield (2001); F, De Beer et al. (2003).

[c]Dominant species. A species was considered dominant if $P_i > 1/S$, where P_i is the proportion of the total sample represented by species *i* and *S* (species richness) is the number of competing species present in the community (Camargo 1993).

[d]Not isolated.

Statistical analyses

We utilized the Simpson diversity index to compare fungal diversity on the logs and boards. The Simpson index (*C*) places more importance on abundant species (Simpson 1949). This index is defined as follows:

$$C = 1 - \sum_{i=1}^{i=S} P_i^2$$

where P_i is the probability of sampling species *i*, *i* is the frequency of species *i* / total frequency for all species, *S* is the species richness (i.e., the number of species per sample). The value of *C* ranges from 0 to 1 and denotes the probability that two randomly selected individuals in a community belong to different species. A value close to 0 suggests that dominant species may exist in a population, and conversely, a value close to 1 indicates species equitability, that is, that the species are evenly distributed.

Dominance or subordinance in fungal communities was determined with Camargo's index (1/*S*) (Camargo 1993), where *S* represents species richness; the number of competing species in a community. A dominant species is present if $P_i > 1/S$.

Results

In this work, only the results obtained on malt extract agar amended with cycloheximide are reported. Without antibiotics, we found a high proportion (90%) of moulds, including mainly *Trichoderma*, *Mucor, Alternaria, Phialophora, Penicillium*, and *Cladosporium* species (Kim and Kim 2000*a*). This is not surprising since the sampling and isolation were carried out in the spring and during a hot summer. A total of 171 isolates associated with blue-stained radiata pine were obtained from logs and boards. They were identified using morphological and molecular methods (Tables 1 and 3). Among all the identified isolates, about 60% were from the *O. piceae* complex that is included in the pyrenomycetes. This complex consists of morphologically and phylogenetically related species that have a *Pesotum* anamorph and a *Sporothrix* synanamorph. These fungal species are often dispersed by insects (Harrington et al. 2001).

From the logs, nine species of ophiostomatoid fungi were identified as the fungi staining radiata pine: *O. floccosum*, *O. piceae*, *O. piliferum*, *O. radiaticola, L. procerum*, *O. setosum*, *O. huntii*, *O. ips*, and *O. quercus*. Among these species, *O. floccosum* was the most frequently isolated, representing 44% of the isolates. For the other fungi, the frequencies of occurrence were 18.2%, 11.4%, 7.6%, and 6.1% for *O. piceae*, *O. piliferum*, *O. radiaticola*, and *L. procerum*, respectively.

From the boards, at least six species of ophiostomatoid fungi were obtained. They were *O. floccosum*, *Ophiostoma nigrocarpum* (David.) Hoog, *L. bistatum*, *O. piliferum*, *O. huntii*, *O. quercus*, and an unknown *Ophiostoma* sp. A. As in logs, *O. floccosum* was commonly isolated; however,

Table 2. Type cultures used in mating tests.

Species	Isolate No.	Mating type	Host	Origin	Collector
Ophiostoma floccosum	AU55.1	A	*Pinus contorta*	Canada	A. Uzunovic
	AU56.2	B	*Pinus contorta*	Canada	A. Uzunovic
Ophiostoma piceae	AU40	A	*Picea glauca*	Canada	A. Uzunovic
	AU40.2	B	*Picea glauca*	Canada	A. Uzunovic
Ophiostoma quercus	H1039	A	*Quercus* sp.	UK	J.F. Webber
	H1042	B	*Quercus* sp.	UK	J.F. Webber
Ophiostoma setosum	AU160-27	A	*Tsuga heterophylla*	Canada	A. Uzunovic
	AU160-25	B	*Tsuga heterophylla*	Canada	A. Uzunovic
Ophiostoma piliferum	AU42.1	A	*Picea glauca*	Canada	A. Uzunovic
	AU41.3.1	B	*Picea glauca*	Canada	A. Uzunovic

Note: All isolates were from the Breuil culture collection, University of British Columbia, Vancouver, British Columbia, Canada.

Table 3. Cultures used in this study and the GenBank accession numbers for the sequences.

Species	Isolate No.[a]	Host	Origin	Collector or supplier	Accession No.[b]	%[c]
Leptographium procerum	KUC 2038	*Pinus radiata*	Korea	J.-J. Kim, G.-H. Kim	*AY789161*	
	NZFS 168	*Pinus radiata*	New Zealand	M. Dick	*AY789162*	100
	C 83	*Pseudotsuga menziesii*	USA	T.C. Harrington	*AY789163*	100
Ophiostoma floccosum	KUC 2014	*Pinus radiata*	Korea	J.-J. Kim, G.-H. Kim	AY789140	
	NZFS 637	*Pinus radiata*	New Zealand	M. Dick	AY789141	99.9
	387N	Unknown (softwood)	Canada	Forintek Co.	AY789142	99.9
Ophiostoma huntii	KUC 2042	*Pinus radiata*	Korea	J.-J. Kim, G.-H. Kim	AY789143	
	NZFS 170F	*Pinus radiata*	New Zealand	M. Dick	AY789144	99.8
	UAMH 4997	*Pinus contorta*	Canada	R.C.R. Jeffrey	**AY349023**	97.9
Ophiostoma ips	KUC 2016	*Pinus radiata*	Korea	J.-J. Kim, G.-H. Kim	AY789145	
	UAMH 9719	*Pinus radiata*	New Zealand	J. Reid	AY789146	100
	CBS 151.54	*Pinus sylvestris*	Sweden	A.M. Käärik	**AY194950**	99.9
Ophiostoma nigrocarpum	KUC 2761	*Pinus radiata*	Korea	J.-J. Kim, G.-H. Kim	AY789147	
	NZFS 1556	*Pinus radiata*	New Zealand	R. Farrell	AY789148	100
	C 201	*Dendroctonus* sp.	USA	R.W. Davidson	AY789149	97.4
Ophiostoma piceae	KUC 2015	*Pinus radiata*	Korea	J.-J. Kim, G.-H. Kim	AY789150	
	NZFS 332.01	*Pinus radiata*	New Zealand	M. Dick	AY789151	100
	H 2181	*Picea* sp.	UK	J. Webber	AY789152	99.9
Ophiostoma piliferum	KUC 2039	*Pinus radiata*	Korea	J.-J. Kim, G.-H. Kim	AY789153	
	NZFS 20	*Pinus radiata*	New Zealand	M. Dick	AY789154	95.1
	CBS 129.32	*Pinus sylvestris*	Unknown	H. Diddens	**AF221628**	97.0
Ophiostoma quercus	KUC 2210	*Pinus radiata*	Korea	J.-J. Kim, G.-H. Kim	AY789155	
	NZFS 3182	*Pinus radiata*	New Zealand	M. Dick	AY789156	99.9
	H 1039	*Quercus* sp.	UK	J. Webber	AY789157	97.1
Ophiostoma setosum	KUC 2035	*Pinus radiata*	Korea	J.-J. Kim, G.-H. Kim	AY789158	
	NZFS 3652/1	*Pinus radiata*	New Zealand	M. Dick	AY789159	99.9
	AU 160-27	*Tsuga heterophylla*	Canada	A. Uzunovic	AY789160	99.8

[a]KUC, Korea University Culture Collection, Seoul, Korea; NZFS, Forest Research Culture Collection, Rotorua, New Zealand; UAMH, the University of Alberta Microfungus Collection and Herbarium, Devonian Botanic Garden, Edmonton, Alberta, Canada; C, Culture Collection of T.C. Harrington, Iowa State University, Ames, Iowa, USA; CBS, the Centraalbureau voor Schimmelcultures, Institute of the Royal Netherlands Academy of Arts and Sciences, Utrecht, Netherlands; H and AU, Breuil culture collection, University of British Columbia, Vancouver, British Columbia, Canada. Isolates in italics were provided by Dr. A. Uzunovic.

[b]Italics indicate rDNA sequences. The other sequences are for the β-tubulin gene. Boldface type indicates accessions from the GenBank database.

[c]Similarity scores from pairwise alignments of sample sequences (strains isolated from radiata pine logs imported to Korea) with reference strains or closest BLAST match.

O. nigrocarpum was commonly isolated as well. The frequency of occurrence of each fungus was 26%.

In general, the occurrence frequency of the ophisotomatoid isolates was approximately two times higher in logs than in boards. Four species, *O. floccosum*, *O. piliferum*, *O. huntii*, and *O. quercus* were found on both logs and boards. *Ophiostoma piceae*, *O. radiaticola*, *L. procerum*, *O. setosum*, and *O. ips* were isolated only from logs, while

O. nigrocarpum, *L. bistatum*, and *Ophiostoma* sp. A were isolated only from boards.

The fungal species frequencies for logs and boards are summarized in Table 1. We also included figures for species richness and values determined by the Simpson's diversity index. When we compared communities in logs with those in boards, the species richness was higher in logs (nine), while the diversity was higher in boards. The data showed that *O. floccosum* was the dominant species in both logs and boards. In logs, the dominant species, according to Camargo's index, were *O. floccosum*, *O. piceae*, and *O. piliferum*, while in boards they were *O. floccosum*, *O. nigrocarpum*, and *L. bistatum*.

Both mating test and molecular data were helpful in identifying the ophiostomatoid fungi. Within a month, *O. piliferum* isolates and the isolates from the *O. piceae* complex, except *O. setosum*, mated successfully with the mating testers. Although *O. setosum* failed to produce ascocarps with the two *O. setosum* testers, it was easily identified by comparing its DNA profile with those of known species. Two other species, *O. radiaticola* and *L. bistatum*, were mated with each other in numerous combinations. *Ophiostoma radiaticola* produced ascomata on the surface of sterile lodgepole pine wafers only when two mating types were paired, indicating that this species is heterothallic. However, for *L. bistatum*, no fruiting bodies were obtained.

Discussion

This is the first comprehensive survey of sapstaining fungi associated with radiata pine imported from New Zealand to Korea. In this work, nine species of ophiostomatoid fungi were recognized as the organisms responsible for the discoloration of radiata pine logs imported to Korea.

Three species of the *O. piceae* complex isolated in this study, *O. floccosum*, *O. piceae*, and *O. setosum*, are not considered pathogens and are commonly found on conifer timber (Seifert and Grylls 1993; Schirp et al. 1999; Uzunovic et al. 2000; De Beer et al. 2003). *Ophiostoma quercus* occurs primarily on hardwoods in Europe, but it is also commonly found on conifer sapwood in North America, Korea, and in the Southern Hemisphere (Australia, Chile, New Zealand, South Africa, and Uruguay) (Brasier and Kirk 1993; Halmschlager et al. 1994; Harrington et al. 2001; De Beer et al. 2003; Zhou et al. 2004). The dominant species in our work, *O. floccosum,* is frequently isolated from logs and lumber of *Pinus koraiensis* and *Pinus densiflora* growing in Korea. In 1951, Mathiesen from Sweden described the original species. Since then, it has been found in the United Kingdom and North America, as well as New Zealand and Korea (Kay et al. 1998; Harrington et al. 2001; Kim et al. 2002*a*, 2002*b*; Thwaites et al. 2003). In Korea, *O. piceae* and *O. quercus* have also been isolated from pines while *O. setosum* has only been encountered in New Zealand and in the western regions of North America (Oh 1999; Uzunovic et al. 2000; Harrington et al. 2001; Kim et al. 2002*a*, 2002*b*).

Another economically important fungal species associated with bark beetles, *O. ips*, causes sapstain in coniferous trees, logs, and lumber (Seifert 1993). *Ophiostoma ips* appears to be vectored by a broad range of insects, including *Ips* and *Dendroctonus* species, and it has been reported in North America, Europe, Japan, New Zealand, and South Africa (Rumbold 1931; Benade et al. 1995). Although the fungus is commonly isolated in New Zealand and raises serious forest health issues, it is not known when this fungal pathogen was introduced to this country and no insect vector has been reported (Hutchison and Reid 1988*a*; Zhou et al. 2001). Although *O. ips* has been frequently isolated from reddish-brown stained logs and lumber of *P. koraiensis* and *P. densiflora* in Korea, especially when ophiostomatoid fungi were collected in spring (April to June), it was not commonly isolated in hot summers (July to August) (unpublished data). These results are in agreement with Thwaites et al.'s (2003) data, indicating that *O. ips* was not present in radiata pine logs cut at Austral winter.

From the three *Ophiostoma* species with a *Sporothrix* anamorph, *O. piliferum*, *O. nigrocarpum*, and an unknown *Ophiostoma* sp. A., only *O. piliferum* has been commonly isolated from pines in Korea (Oh 1999; Son et al. 2001: Kim et al. 2002*a*). This species is present worldwide and causes a blue to grey-black discoloration of sapwood. *Ophiostoma nigrocarpum* is also broadly distributed in pines in Austria, Canada, Japan, New Zealand, South Africa, and the United States (De Beer et al. 2003). It has not, however, been found in Korea until now. This is the first report of its presence in Korea.

Other *Ophiostoma* and *Leptographium* species, *O. huntii*, *Leptographium lundbergii* Lagerberg & Melin, and *L.* procerum have been reported from conifers that have been infested with bark beetles (*Hylastes ater* and *Hylurgus ligniperda*) in New Zealand (Farrell et al. 1997; Jacobs and Wingfield 2001). These insects are native to Europe and have been introduced into New Zealand with their fungal associates (Bain 1977; Milligan 1978; Harrington 1988; Wingfield et al. 1988). In Korea, a new species, *Leptographium koreanum* Kim & Kim, has been recently isolated from *P. koraiensis* and *P. densiflora* logs infested with *Tomicus piniperda* (Kim et al. 2005*b*). *Ophiostoma huntii* has not been isolated from any of the tree species in Korea; however, it was found in logs imported from New Zealand. This species is a strong sapstainer of radiata pine (Kim and Kim 2000*b*). *Leptographium procerum* has also been found in *P. koraiensis* infested with various bark beetles in Korea (Oh 1999). However, it has not been reported in the recent surveys published by Son et al. (2001) and Kim et al. (2002*a*, 2002*b*). This fungal species is spread by weevils and is implicated in white pine root decline in the eastern United States, Europe, and New Zealand (Shaw and Dick 1980; Farrell et al. 1997; Jacobs and Wingfield 2001; Thwaites et al. 2003).

Two new species, *L. bistatum* and *O. radiaticola*, that cause sapstain were isolated from boards and logs (Kim et al. 2004*a*, 2005*a*). Kim et al. (2004) reported that none of the *Leptographium* spp. associated with bark beetles from New Zealand resemble *L. bistatum* and further studies are required to resolve this question. *Ophiostoma radiaticola* produces dark perithecia with a long neck and hyaline reniform ascospores with a hat-shaped sheath (Kim et al. 2005*a*). The anamorph form of this fungus, *Pesotum pini* (Hutchison & Reid) Okada & Seifert, has been commonly isolated from radiata pine logs stored in New Zealand where

it causes pale yellowish to brown stain in wood (Hutchison and Reid 1988*b*; Okada et al. 1998). The strains isolated in New Zealand mated with the ones isolated from radiata pine logs in Korea. Since this fungus has not been reported in Korea previously, it is likely that this species was introduced to Korea in radiata pine logs from New Zealand.

Thwaites et al. (2003) examined which sapstain fungi colonized wood between the time of harvesting and processing of the trees in New Zealand and the date of the arrival of the logs in Japan. They also concluded that sapstain development was more of a concern when the trees were harvested during the New Zealand summer than during the winter. Given this, to minimize fungal discoloration of wood or prevent introducing non-native organisms into trading partners' ecosystems, it would be preferable to import radiata pine logs that are cut during the New Zealand winter rather than it's summer.

Non-manufactured wood, especially in its raw, log form, provides an optimal path for the movement of forest insects and pathogens into new environments (Tkacz 2002). In 1999, Simpson reported that, in Australia, several non-native stain fungi (e.g., *Leptographium abietinum* (Peck) Wingfield, *Ophiostoma piceaperdum* (Rumbold) von Arx, and *O. setosum* isolated from green Douglas-fir lumber imported from North America) are a threat to commercial conifer plantations. *Ophiostoma huntii* is a strong sapstainer of radiata pine and it is associated with various bark beetles (Kim and Kim 2000*b*; Zhou et al. 2004). This fungal species might have been transferred to native bark beetles infesting pines in Korea. However, more work and careful monitoring is required to resolve this association with native Korean bark beetles. Furthermore, the pathogenicity of the fungal species to native pines in Korea should be determined to assess their importance in Korea.

In conclusion, we confirmed that many of the sapstain species that we isolated have been previously reported in Korea. However, four species, *O. radiaticola*, *O. setosum*, *O. huntii*, and *O. nigrocarpum*, isolated from New Zealand radiata pine imports have not been reported previously in Korea. We also found the new species, *L. bistatum*, along with an unknown *Ophiostoma* sp. A. Fortunately, two pathogens isolated from pines in New Zealand, *L. lundbergii* and *S. sapinea* were not found in our sampling trial (Wingfield and Marras 1983).

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