Mycoscience (2009) 50:212–223
DOI 10.1007/s10267-008-0474-9

FULL PAPER

Hayato Masuya · Yuichi Yamaoka · Shigeru Kaneko
Yuichi Yamaura

Ophiostomatoid fungi isolated from Japanese red pine and their relationships with bark beetles

Received: June 23, 2008 / Accepted: December 18, 2008

Abstract We isolated ophiostomatoid fungi from bark beetles infesting *Pinus densiflora* and their galleries at 24 sites in Japan. Twenty-one ophiostomatoid fungi, including species of *Ophiostoma*, *Grosmannia*, *Ceratocystiopsis*, *Leptographium*, and *Pesotum*, were identified. Among these, 11 species were either newly recorded in Japan or were previously undescribed species. Some of these fungal species were isolated from several bark beetles, but other species were isolated from only a particular beetle species. Thus, it is suggested that some ophiostomatoid fungi have specific relationships with particular beetle species. In addition, fungus–beetle biplots from redundancy analysis (RDA) summarizing the effects of beetle ecological characteristics suggested that the association patterns between bark beetles and the associated fungi seemed to be related to the niches occupied by the beetles.

Key words Bark beetle · *Grosmannia* · Insect–fungus associations · *Leptographium* · *Ophiostoma* · *Pinus densiflora*

Introduction

Ophiostomatoid fungi (*Ophiostoma*, *Ceratocystis*, *Grosmannia*, and some other related genera in Ascomycotina) are a morphologically similar but phylogenetically unrelated group of fungi (Hausner et al. 1992, 1993; Wingfield 1993; Blackwell and Spatafora 1994; Spatafora and Blackwell 1994; Kirk et al. 2001; Zipfil et al. 2006). They are characterized by perithecia with a long neck, hyaline mucilaginous ascospores, and evanescent asci. These morphological characteristics are thought to be related to their dispersal biology; indeed, the dispersal of many species of ophiostomatoid fungi depends heavily on arthropods (Malloch and Blackwell 1993).

In ophiostomatoid fungi, *Ophiostoma*, *Ceratocystis*, and their anamorphic species (*Pesotum*, *Leptographium* Largerberg & Melin, *Hyalorhinocladiella*, and *Sporothrix*), which are known to be the causal agents of sap-stain and tree wilt diseases (Largerberg et al. 1927; Käärik 1980; Brasier 1991), are mainly vectored by bark beetles (Coleoptera: Scolytidae). *Ophiostoma ulmi* and *O. novo-ulmi*, which are known as the causal agents of Dutch elm disease (Strobel and Lanier 1981; Brasier 1991), are vectored by *Scolytus* spp. and *Hylurgopinus rufipes* Eichh (Lanier and Peacock 1981). *Ceratocystis polonica*, together with the bark beetle species *Ips typographus*, causes widespread mortality of Norway spruce (Horntvedt et al. 1983; Christiansen 1985; Solheim 1992). In addition, *O. minus*, *O. ips*, several other *Ophiostoma* species, and the species of their anamorphic genus *Leptographium*, which are the causal agents of sap-stain (Lagerberg et al. 1927; Melin and Nannfeldt 1934; Seifert 1993), are also associated with many bark beetles (Francke-Grosmann 1967; Whitney 1982; Harrington 1988, 1993).

The association of ophiostomatoid fungi and bark beetles is very complex, and there are multiple interactions among fungi and beetles (Whitney 1982). Although the fungi are simply vectored by the beetles, as already mentioned, close associations of the fungi and the beetles are also found (Francke-Grosmann 1967; Whitney 1982; Harrington 1988, 1993). In this case, the bark beetles have mycangia, which are cuticular structures, to carry fungal spores and mycelia (Francke-Grosmann 1967), and the fungi seem to serve as food for the larvae. The association of beetles and mycangial fungi is known to be species specific (Francke-Grosmann 1967; Harrington 1988). Furthermore, vectored fungi may influence the distribution and abundance of bark beetles by altering host plant quality and availability (Whitney 1982).

Thus, the fungi have multiple interactions with the bark beetles and are thought to exert important effects on the beetles and host trees in the forest ecosystem. However, basic information on the relationships of fungi with beetles

H. Masuya (✉) · S. Kaneko · Y. Yamaura
Forestry & Forest Products Research Institute, Matsunosato 1, Tsukuba, Ibaraki 305-8687, Japan
Tel. +81-298-73-3211, ext. 404; Fax +81-298-73-1543
e-mail: massw@ffpri.affrc.go.jp

Y. Yamaoka
Graduate School of Life and Environmental Sciences, University of Tsukuba, Ibaraki, Japan

is restricted to North America and Europe (Kirisits 2004). There are only a few reports on critical comparisons of fungal species associated with beetle species distributed in Asia, including Japan. The available information from Japan is derived from the studies of Aoshima (1965) and Yamaoka et al. (1997, 1998, 2004).

Although a comprehensive work was conducted by Aoshima (1965), there was considerable confusion in the taxonomy of each isolated fungus at that time; as a result, many ophiostomatoid fungal species assigned by this author are now of uncertain nomenclatural statute. Yamaoka et al. (1997, 1998, 2004) clearly assigned isolated fungal species, and their results were in good agreement with the results obtained by researchers in other countries; however, they targeted the fungi from only 7 species of bark beetles. Thus, we do not have adequate knowledge about the patterns of association of fungi with bark beetles in Japan. In this study, therefore, we attempted to address this deficit in information by comparing the results of our study with those obtained in other areas and to clarify the pattern of association of fungi with bark beetles. Additionally, we discuss the factors that influence the patterns of fungi–beetle associations. To achieve our aims, we isolated the fungal species from many bark beetle species in Japan. *Pinus densiflora* Sieb. & Zucc. was selected as the host tree in this study because of its wide distribution in Japan, its susceptibility to many types of bark beetle species, the accumulation of knowledge of similar beetle species infesting pines from other countries, and its economic importance.

Materials and methods

Sampling

Samples were collected from the bark of *P. densiflora* Sieb. & Zucc. deteriorating or dead within a year at 24 sites during 1996–2001 in Japan. The vegetation of each site varied, but *P. densiflorae* was the dominant species at these sites.

When entrance holes of bark beetles were found at the sites, we cut the bark or logs with a hatchet and saw and brought them to the laboratory. The collection date and species of beetles obtained were recorded at each site (Table 1). Although each beetle species has various life stages, adult beetles and their galleries at the breeding stage were used in the isolation experiments. Breeding sites of the beetles also varied but sometimes overlapped within several beetle species. In this case, the overlapping breeding sites may influence the results of isolation. However, we analyzed the isolation data without separating the data of the overlapped breeding sites of the beetles because the beetle species were potentially associated with the fungi vectored by other beetle species.

A total of 1335 beetles were used in this study, comprising 13 species of bark beetles, including an unidentified species (Table 2). Almost all the collected beetles were in the breeding stage, but some were in the overwintered stage. Some of these beetle species co-occurred under the bark of the host trees in the same site. In contrast, some beetle species were found in quite a different season or site in the host trees. These field observations, together with information from the literature (Francke-Grosmann 1952; Inoue 1953; Bakke 1968; Nobuchi 1966), are summarized in Table 2.

Isolation

Bark beetles in the wood samples were picked up with sterilized tweezers and placed in Petri dishes containing 1% malt agar (MA: malt extract, 10 g; agar, 15 g; distilled water, 1000 ml) and kept at 15°C in the dark. The beetles were allowed to crawl on the malt extract agar for 2 days, and they were then removed from the plates. Additionally, two small pieces of wood were taken from each beetle's gallery with a sterilized scalpel and placed on 1% MA plates. The plates were then incubated at 15°C in the dark. After 2 months, fungi that had grown on the plates were isolated by picking up hyphae or conidial masses with a sterilized tungsten needle and transferring these structures to Petri dishes containing 2% malt agar (malt extract, 20 g; agar, 15 g; distilled water, 1000 ml). The Petri dishes were incubated at 15°C in the dark for 2 weeks. After 2 weeks, small pieces of sterilized pine twig or bark were added to the plates to stimulate sporulation.

Fungal structures that were produced on the medium were mounted on glass slides in 1% lacto-fuchsin, and observed as well as measured by using a light microscope. *Ophiostoma* and its related species were selected from all identified fungi.

The representative isolates of each species have been deposited in the Ministry of Agriculture, Forestry, and Fisheries (MAFF), Genetic Resource Center, Culture Collection of National Institute of Agrobiological Resources, Japan; JCM, Japan Collection of Microorganisms, RIKEN BioResource Center; and MCC, private culture collection of the first author.

Data analysis

The frequencies of occurrence of each fungus isolated from adult beetles and their galleries were calculated using the following formula (Yamaoka et al. 1997): $F = (NF/NT) \times 100$, where F represents the frequency of occurrence (%) of a fungus, NT is the total number of beetles or their galleries from which isolations were carried out, and NF is the number of beetles or their galleries from which a particular fungus was isolated.

Before the analysis, we logarithmically transformed the frequency data of the fungi isolated from the beetles. The frequency data from the galleries were not analyzed because we did not obtain data from the galleries of some beetle species. In addition, the frequency data of the overwintered stage were not included in multivariate analysis for critical comparisons between beetle species. To assess the associa-

Table 1. Localities and date that bark beetle species and their egg galleries were collected

Collecting site	Collecting date	Collected species of bark beetle[a]												
		Tomicus piniperda	*Tomicus minor*	*Hylurgops interstitialis*	*Hylastes paralleus*	*Hylastes plumbeus*	*Cryhalus fulvus*	*Cryphalus joholensis*	*Cryptourgus pusillus*	*Ips acuminatus*	*Orthtomicus angulatus*	*Orhtomicus tosaensis*	*Orhtomicus sutualis*	*Dryocoetes* sp.
Takizawa, Iwate	25 Sep. 1997			+*			+*							
	28 Nov. 1996							+*						
Morioka, Iwate	25 Nov. 1997						+*							
	24 May 2002									+			+	
Ichinoseki, Iwate	19 Jun. 1996	+*					+*							
Kisakata, Akita	26 Sep. 1997						+*							
Iwaki, Fukushima	18 Jun. 1996	+					+		+	+				
Katsurao, Fukushima	30 May 1998									+*				
Amasakae, Fukushima	13 Jul. 1996	+												
Naruko, Miyagi	22 Sep. 1997			+*			+*		+*					
Motegi, Tochigi	13 Jul. 1996	+												
Tsukuba, Ibaraki	29 Mar. 1995	+*												
	7 Jun. 1995													
	31 May 1997	+*												
Makabe, Ibaraki	13 Feb. 1998										+*			+*
	16 Apr. 1998			+*										
Kukizaki, Ibaraki	16 May 1996	+*			+*	+*								
	20 Jan. 1998											+*		
	6 Feb. 1998						+*							
Takahagi, Ibaraki	28 Apr. 1998	+				+*								
Chichibu, Saitama	28 Aug. 1996	+												
Masuho, Yamanashi	15 May 1996	+*	+*				+*							
	12 May 1998		+*										+*	
Kofu, Yamanashi	16 May 1996	+												
Kawakami, Nagano	24 Jul. 1996					+								
Tateyama, Toyama	27 Aug. 1996													
Nakajima, Ishikawa	24 Aug. 1996						+							
Takasu, Gifu	26 Aug. 1996	+							+					
Himeji, Hyogo	24 Sep. 1996													
Hiroshima, Hiroshima	25 May 1997	+												

[a] +, egg gallery; *, adult beetles

Table 2. Ecological characteristics of each bark beetle collected in this study

Beetle species	Tribe	Host	Aggressiveness[a]	Type of marriages	Breeding period	Breeding habitat	Breeding gallery type	Body size (average), mm	Sampling days from 1 April
Tomicus piniperda	Hylurgini	*Pinus* spp.	FP	Monogamy	Spring	Lower	Simple longitudinal	4.5	57 ± 6.2
Tomicus minor	Hylurgini	*Pinus* spp.	FP	Monogamy	Spring	Upper	Multiple transverse	3.75	43 ± 1.5
Hylurgops interstitialis	Hylastini	*Pinus* spp. and other genus	S	Monogamy	Spring to summer	Root, lower	Simple longitudinal	4.7	120 ± 52.5
Hylastes paralleus	Hylastini	*Pinus* spp. and other genus	S	Monogamy	Spring to summer	Root, lower	Simple longitudinal	4.5	45 ± 0
Hylastes plumbeus	Hylastini	*Pinus* spp. and other genus	S	Monogamy	Spring to summer	Root, lower	Simple longitudinal	2.8	36 ± 9
Cryhalus fulvus	Cryphalini	*Pinus* spp.	S	Monogamy	Spring to autumn	Lower to upper, twigs	Multiple transverse	1.5	169 ± 33.8
Cryphalus sp.	Cryphalini	Unknown	S	Monogamy	Unknown	Upper, twigs	Star shaped	1.6	237 ± 0
Cryptourgus pusillus	Crypturgini	*Pinus* spp. and other genus	S	Monogamy	Spring to summer	Lower	Simple longitudinal	1.15	171 ± 0
Ips acuminatus	Ipini	*Pinus* spp. and other genus	FP	Polygamy	Summer	Upper, twigs	Multiple longitudinal	3.1	59 ± 0
Orthtomicus angulatus	Ipini	*Pinus* spp. and other genus	S	Polygamy	Summer	Upper, twigs	Multiple longitudinal	3.4	317 ± 0
Orthtomicus tosaensis	Ipini	*Pinus* spp.	S	Polygamy	Summer	Upper, twigs	Multiple longitudinal	3	294 ± 0
Orthtomicus sutualis	Ipini	*Pinus* spp. and other genus	S	Polygamy	Summer	Upper	Multiple longitudinal	2.1	41 ± 0
Dryocoetes sp.	Xyleborini	Unknown	S	Polygamy	Unknown	Upper	Star shaped	3	317 ± 0

[a] FP, facultative parasite; S, saprophyte

Table 3. Ecological characteristics of beetles considered in redundancy analysis (RDA)

Explanatory variable	Min	25%	50%	75%	Max	Coding
Body size (mm)	1.2	2.5	3.1	4.1	4.7	
Sampling day[a]	36	44	59	170	317	From 1 April
SD (day)	0.0	0.0	0.0	2.1	4.0	Logarithmically transformed
Pinus	Only *Pinus* = 4 sp., *Pinus* and others = 7 sp.					Coded as a dummy variable
Trunk[b]	Trunk = 6 sp., non-trunk = 5 sp.					Coded as a dummy variable
Upper trunk[b]	Upper trunk = 6 sp., non-upper trunk = 5 sp.					Coded as a dummy variable
Twig[b]	Twig = 4 sp., non-twig = 7 sp.					Coded as a dummy variable
Galley pattern	Longitude = 9 sp., non-longitude = 2 sp.					Coded as a dummy variable
Polygamy	Polygamy = 4 sp., non-polygamy = 7 sp.					Coded as a dummy variable
Saprophy	Saprophy = 8 sp., non-saprophy = 3 sp.					Coded as a dummy variable

[a] Sampling day was represented as lapsed days from 1 April; categorical variables were coded as dummy variables (1/0)
[b] Breeding habitats of beetles consisted of five categories (basal part of trunk, trunk, upper part of trunk, and twigs); basal part of trunk was treated as reference category, and not treated directly (see Legendre and Legendre 1998); basal part of trunk was breeding habitat of three beetles

tion between the fungi and bark beetles, 10 ecological characteristics of bark beetles were considered: host tree, breeding habitats (upper trunk, trunk, and twig), body size, galley type, marriage type, aggressiveness, sampling day, and standard deviation of the day of sampling (Table 2). The ecological characteristics of bark beetles were obtained from field observation and previous reports (Table 2). We examined the association by redundancy analysis (RDA) by using CANOCO (Ter Braak and Similauer 2002). We used logarithmically transformed frequencies of 17 fungal species as response variables and the 10 ecological characteristics of 11 bark beetle species as explanatory variables (Table 3). Categorical variables were coded as dummy variables. Standard deviation of sampling days was logarithmically transformed. The 10 explanatory variables were selected by a forward variable selection with 999 permutations ($\alpha = 0.10$). In the RDA analysis, the species were standardized and centered. All other CANOCO defaults were followed.

Results

Fungal isolation and identification

Approximately 9000 isolates of ophiostomatoid fungi were obtained from the bark beetles and their galleries. This collection included 12 *Ophiostoma*, 1 *Ceratocystiopsis*, 6 *Leptographium*, and 2 *Pesotum* species. Among these species, 10 species are hitherto known in Japan, but the rest are either newly recorded in Japan or are undescribed species (Table 4). Some of these species have already been reported as new species or were newly recorded species in Japan in previous studies (Masuya et al. 2000, Masuya et al. 2005). Various fungal species belonging to the genera *Ambrosiella*, *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, *Sphaeropsis*, and *Trichoderma* and different sterile mycelia and yeasts were also frequently isolated, but these were not characterized.

Comparison of fungal isolation frequencies between localities

Tomicus piniperda, *Hylurgops interstitialis*, *Cryphalus fulvus*, *Ips acuminatus*, and *Orthotomicus suturalis* were collected from multiple localities. Some fungi were commonly isolated from the beetles even if the latter were collected at different localities, whereas others were not.

In *T. piniperda*, a total of 12 ophiostomatoid fungi were isolated from the beetle from three localities (Ichinoseki, Iwate; Masuho, Yamanashi; Tsukuba, Ibaraki) (Table 5). Four species, namely *O. ips*, *O. minus*, *L. koreanum*, and *L. pini-densiflorae*, were commonly detected from each locality. *Leptographium koreanum* had a high frequency of isolation, and *O. minus* had a relatively low frequency of isolation although it was consistently isolated. *Ophiostoma ips* and *L. pini-densiflora* were isolated less frequently and mainly from galleries. Other fungal species were not consistently isolated, and their frequencies of occurrences varied. Moreover, their frequencies of occurrence were very low even when they were isolated.

In *H. interstitialis*, a total of five fungal species were obtained from three localities (Table 6). Two species, namely, *L. koreanum* and *Pesotum* sp. HY, were consistently isolated with a high frequency from each locality.

A total of nine fungal species were isolated from *C. fulvus* from seven localities (Table 7). *Ophiostoma* cf. *stenoceras* was consistently isolated but with a very low frequency. *Ophiostoma ips* and *L. koreanum* were frequently detected from five localities. The frequencies of occurrences and localities of other fungi varied.

Four fungal species were isolated from *I. acuminatus* from three localities, but only *O. ips* commonly occurred at a high frequency (Table 8). *Leptographium koreanum* was only isolated from one locality.

Seven fungal species were detected from *O. suturalis* from two localities (Table 9). *Leptographium koreanum* and *Grosmannia olivacea* were isolated from both localities, but *G. olivacea* in particular had a high frequency of occurrence.

Table 4. Frequencies of occurrences (%) of Ophiostoma and related species isolated from pine bark beetles and their galleries[a]

Beetle species	*Tomicus piniperda*		*Tomicus minor*		*Hylurgops interstitialis*		*Hylastes paralleus*		*Hylastes plumbeus*		*Cryphalus fulvus*		*Cryphalus joholensis*		*Cryptourgus pusillus*		*Ips accuminatus*		*Orthotomicus anglatus*		*Orthotomicus suturalis*		*Orthotomicus tosaensis*		*Dryocoetes* sp.		unidentified sp.	
Isolation source[b]	B	G	B	G	B	G	B	G	B	G	B	G	B	G	B	G	B	G	B	G	B	G	B	G	B	G	B	G
Total of sample (*n*)	471	206	261	60	55	13	5	3	48	20	229	48	25		39		97	19	18		41		44	16	30		2	41
Ceratocystiopsis minuta											4		24															
Leptographium koreanum	51	73	3	13	47	93	100	100	46	65	39	90	8		90		41		5.6		8							
Ophiostoma cf *stenoceras*	9	8	16	10	9	23					8	8			28		1		21		4		2		17			20
O. pusillum	5										1																	
O. botsuliforme													60															
O. nigrogranum	1																										100	24
O. nigrocarpum		1																										
O. canum	1	2	56	87																								
O. ips	3	5									18	6					71	79	56		5				20		100	73
O. minus	15	15									2	6																
Grosmannia olivacea																					34							
O. piliferum		1																										
O. quercus									23																			
L. alethinum									4	5																		
L. serpens									2	10																		
L. procerum		2	1		2	8	40	33	8	5																		
L. wingfieldii	3	2																										
L. pini-densiflorae	4	3	1	7							4								28						17			5
L. yunnanense	3	12			2	15					1	6					1											
Pesotum fragrans	1	3	1	7															39		5				30			
Pesotum sp. HY	1	1			55	93			73	65	1				18						2							

[a] Each frequency data point was half-adjusted after the decimal point
[b] B, beetles; G, galleries

Table 5. Frequencies of occurrences of ophiostomatoid fungi isolated from *Tomicus piniperda* and its galleries in different sites (%)

Species	Sampling site					
	Ichinoseki, Iwate		Masuho, Yamanashi		Tsukuba, Ibaraki	
	Breeding adult (n = 27)	Egg gallery (n = 61)	Breeding adult (n = 30)	Egg gallery (n = 67)	Breeding adult (n = 87)	Egg gallery (n = 72)
Leptographium koreanum	51.9	78.7	46.7	86.6	36.8	56.9
Ophiostoma cf. *stenoceras*	7.4	11.5	10.0	25.6		
O. canum			6.7	6.0		
O. ips		3.3		1.5	6.9	11.1
O. minus	7.4	4.9	10.0	25.4	19.5	15.3
O. piliferum				1.5		
L. procerum				7.5		
L. pini-densiflorae		1.6	3.3	7.5	1.1	
L. wingfieldii		4.9				1.4
L. yunnanese		1.6			4.6	31.9
Pesotum fragrans		9.8	3.3	20.9		
Pesotum sp. HY	7.4	3.3				
Sample date	19 Jun. 1996		15 May 1996		31 Mar. 1996	7 Jun. 1996

Table 6. Frequencies of occurrences of ophiostomatoid fungi isolated from *Hylurgops interstitialis* and its galleries in different sites (%)

Species	Sampling site		
	Takizawa, Iwate	Naruko, Miyagi	Tsukuba, Ibaraki
	New adult (n = 15)	New adult (n = 21)	Breeding adult (n = 19)
Leptographium koreanum	53.3	42.9	89.5
Ophiostoma cf. *stenoceras*		14.3	10.5
Leptographium procerum			5.3
L. yunnanense			5.3
Pesotum sp. HY	33.3	66.7	84.2
Sample date	21 Sep. 1997	22 Sep. 1997	16 Apr. 1998

Table 7. Frequencies of occurrences of ophiostomatoid fungi isolated from *Cryphalus fulvus* and its galleries in different sites (%)

Species	Sampling site (n = sampling no.)						
	Takizawa, Iwate	Morioka, Iwate	Ichinoseki, Iwate	Kisakata, Akita	Naruko, Miyagi	Kukizaki, Ibaraki	Masuho, Yamanashi
	Breeding adult[a] (n = 20)	Breeding adult (n = 42)	Breeding adult (n = 82)	Breeding adult (n = 21)	Breeding adult (n = 11)	New adult[b] (n = 26)	Breeding adult (n = 22)
Ceratocystiopsis minuta	3					9	
Leptographium koreanum	6	10	56	14	5		
Ophiostoma cf. *stenoceras*	2	6	2	2	1	8	4
Ophiostoma pusillum		2					
O. ips		7	11	11		14	3
O. minus		1	4				
L. pini-densiflorae					2		
L. yunnanense		2					
Pesotum sp. HY					2		
Sample date	25 Sep. 1997	25 Nov. 1997	19 Jun. 1996	26 Sep. 1997	22 Sep. 1997	6 Feb. 1998	15 May 1996

[a] Adult beetles in egg galleries for breeding
[b] Adult beetles in pupal chambers

Table 8. Frequencies of occurrences of ophiostomatoid fungi isolated from *Ips acuminatus* in different sites (%)

Species	Sampling site		
	Iwaki, Fukushima	Katsurao, Fukushima	Morioka, Iwate
	Breeding adult (n = 6)	Breeding adult (n = 76)	Breeding adult (n = 15)
Leptographium koreanum		52.6	
Ophiostoma ips	50	68.4	53.3
Ophiostoma cf. *stenoceras*		1.3	20
L. yunnanense	16.7		
Sample date	18 Jun. 1996	30 May 1998	24 May 2002

Table 9. Frequencies of occurrences of ophiostomatoid fungi isolated from *Orthotomicus sutualis* in different sites

Species	Sampling site	
	Masuho, Yamanashi	Morioka, Iwate
	Breeding adult (n = 11)	Breeding adult (n = 30)
Leptographium koreanum	36.4	13.3
Grosmannia olivacea	63.6	90
Ophiostoma ips	45.5	
Ophiostoma cf. *stenoceras*		13.3
Leptographium wingfieldii		13.3
Pesotum fragrans		6.7
Pesotum sp. HY		20
Sample date	12 May 1998	3 Jun. 2002

Comparison of fungal isolation frequencies among beetle species

The species and frequencies of isolated fungi differed between the beetle species (see Table 4). Among the isolated fungi, *L. koreanum* was the fungal species most commonly detected from the beetles at a high frequency. In contrast, *O*. cf. *stenoceras* was often isolated from various beetle species but at low frequencies. On the other hand, *O. canum* was only frequently isolated from *T. minor*. *Ophiostoma ips* was frequently isolated from *C. fulvus*, *I. acuminatus*, and *Orthotomicus angulatus*. *Ceratocystiopsis minuta* was isolated from two *Cryphalus* species, but at a low frequency as compared to the other fungal species. *Ophiostoma pusillum* was isolated from *T. piniperda* and *C. fulvus* at a low frequency. *Grosmannia olivacea* was only obtained from *Orthotomicus suturalis*. *Ophiostoma nigrogranum* and *O. botsuliforme* were relatively often isolated from an unidentified beetle species and *Cryphalus* sp., respectively. *Ophiostoma nigrocarpum*, *O. piliferum*, and *O. piceae* were only rarely detected, from one beetle species each. *Leptographium procerum* was mainly isolated from *Tomicus* spp., *Hylastes* spp., and *Hylurgops interstitialis*. *Leptographium serpens*, *L. alethinum*, and *Pesotum* sp. HY were isolated mostly from *Hylurgops* sp. and *Hylastes* spp. *L. pini-densiflorae* was isolated from six beetle species.

Associations of fungal frequencies of occurrence with the ecological characteristics of beetles

The RDA revealed that the frequencies of occurrence of 17 fungal species were significantly associated with three ecological characteristics of beetles: breeding habitat (the upper part of the trunk and the trunk) and host tree (*Pinus*). These ecological characteristics explained 46% of the variation in fungal frequencies of occurrence. The biplot showed specific associations of the 17 fungal species with three ecological characteristics of beetles (Fig. 1A). We also show the biplot summarizing ecological characteristics of the beetles (Fig. 1B). As shown in Fig. 1A, the following species frequently occurred in beetles whose breeding habitats were the upper trunk and trunk: *O. ips*, *G. olivacea*, *Pesotum fragrans*, *O.* cf. *stenoceras*, *L. pini-densiflorae*, and *C. minuta*. These beetles included *Orthotomicus suturalis*, *O. angulatus*, *I. acuminatus*, and *C. fulvus* (Fig. 1B). On the other hand, the following fungal species frequently occurred in beetles whose breeding habitats were not the upper trunk and trunk: *Pesotum* sp. HY, *L. procerum*, *O. quercus*, *L. alethinum*, and *L. serpens*. These beetles included *Hylurgops interstitialis*, *H. paralleus*, and *H. plumbeus* (Fig. 1B). Figure 1A also showed that the fungal species *O. pusillum*, *O. minus*, *L. wingfieldii*, *L. yunnanense*, and *C. minuta* frequently occurred in beetles whose host trees were *Pinus* (*T. piniperda* and *C. fulvus*). The occurrence of *O. canum* was not associated with the ecological characteristics of the beetles.

Discussion

In this study, we isolated 21 species of *Ophiostoma* and its related genera; this is approximately twice the number of species that have ever been reported in Japanese red pine in Japan (Nisikado and Yamauti 1933, 1934, 1935; Aoshima 1965; Kaneko and Harrington 1990). Because our sampling sites and the number of beetle species collected were limited, it is suggested that there are many more ophiostomatoid species that potentially inhabit Japanese red pine in Japan.

Our collection of ophiostomatoid fungi in this study included *Ophiostoma minus*, *O. ips*, *O. piliferum*, and *O. piceae* (Table 4), which are known as the causal agents of

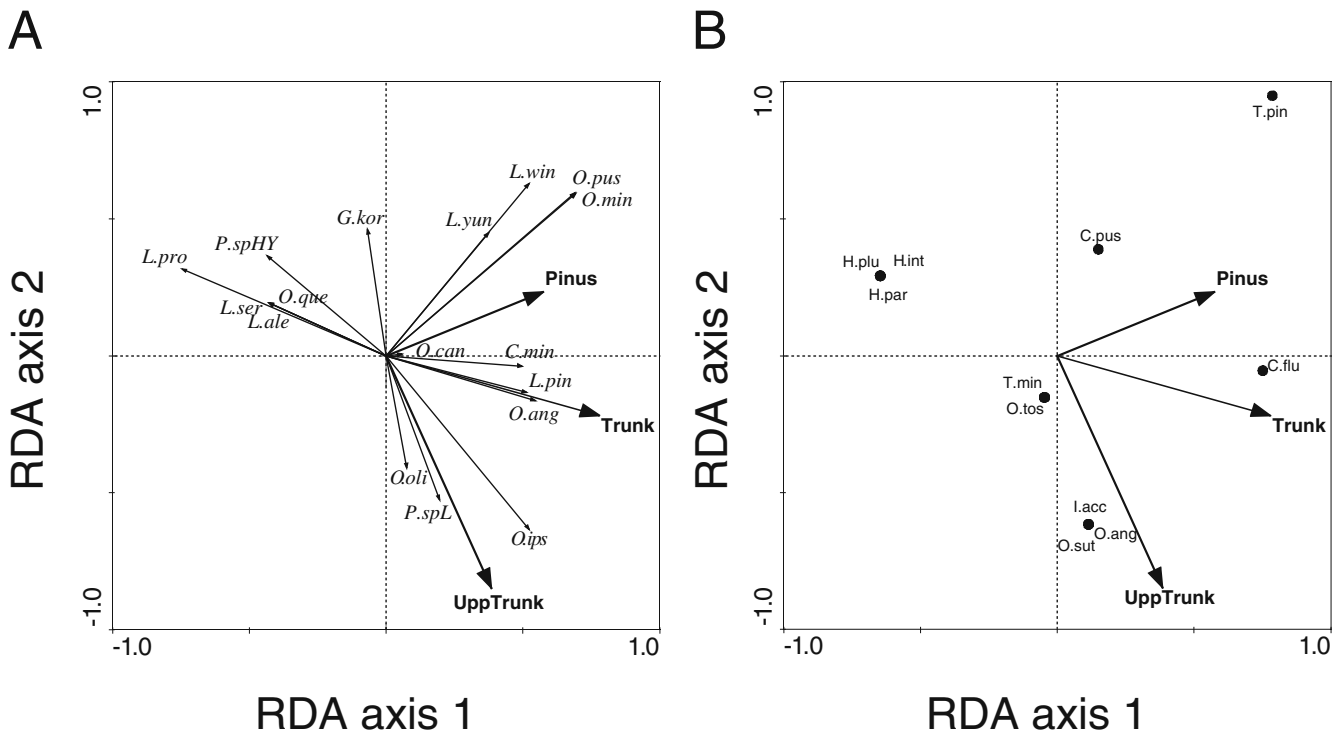

Fig. 1. Fungus–beetle biplots from redundancy analysis (RDA) summarizing the effects of beetle ecological characteristics. **A** Fungus–beetle trait relationships revealed by RDA by using fungal frequencies of occurrence as response variables and beetle traits as explanatory variables. See Table 3 for abbreviations and details of explanatory variables. Abbreviations of fungal species names are as follows: *C. min* = *Ceratocystiopsis minuta*, *L. pro* = *Leptographium procerum*, *L. ser* = *L. serpens*, *L. ale* = *L. alethinum*, *P. spHY* = *Pesotum* sp. HY, *P. spL* = *Pesotum fragrans*, *L. win* = *L. wingfieldii*, *L. yun* = *L. yunnanense*, *L. pin* = *L. pini-densiflorae*, *O. ips* = *Ophiostoma ips*, *O. ang* = *O*. cf. *stenoceras*, *O. que* = *O. quercus*, *G. kor* = *L. koreanum*, *O. pus* = *O. pusillus*, *O. min* = *O. minus*, *O. can* = *O. canum*, *O.oli* = *Grosmannia olivacea*. Three of the 10 explanatory variables were selected by forward variable selection ($\alpha = 0.10$). *Thick bold arrows* and *thin arrows* represent explanatory variables (beetle traits) and fungal species, respectively. The angle between the arrow of beetle traits and that of fungal species indicates the correlation between them. For example, the arrow of the trunk and that of *O.* cf. *stenoceras* have similar directions, which implies that *Ophiostoma* cf. *stenoceras* frequently occurred in beetles whose breeding habitats were the trunk. In contrast, the *arrow* of *L. procerum* is in the opposite direction to that of the trunk, which implies that *L. procerum* frequently occurred in beetles whose breeding habitats were not the trunk. The first axis explains 22% variation in fungal frequencies of occurrence, whereas the second shows 16% variation. *UppTrunk*, upper trunk. **B** Beetle–beetle trait relationships in the redundancy analysis (RDA). *Circles* represent the beetles. T. pin = *Tomicus piniperda*, T. min = *T. minor*, C. pus = *Cryptourgus pusillus*, C. flu = *Cryphalus fulvus*, H. int = *Hylurgops interstitialis*, H. plu = *Hylastes plumbeus*, H. par = *Hylastes paralleus*, O. tos = *Orthotomicus tosaensis*, O. ang = *O. angulatus*, O. sut = *O. suturalis*, I. acc = *Ips accuminatus*

blue-stain in lumber. *O. piliferum* and *O. piceae* in particular were isolated from beetles at a low frequency of occurrence. Both species are well known as blue-stain fungi in lumber and are reported to occur in lumber at a high frequency (Seifert 1993; Uzunovic et al. 1999), but in our study they were rarely detected. Therefore, these species are not considered to be mainly vectored by bark beetles but may be vectored by other methods, e.g., water splash, mites, other arthropods, or wind, as suggested by Dowing (1973). In contrast, *O. minus* and *O. ips* were relatively often detected from bark beetles. These findings suggest that the blue-staining *Ophiostoma* species have different strategies for their dispersal.

Ophiostoma ips was isolated from many bark beetle species but particularly at a high frequency from *I. acuminatus* and *Orthotomicus angulatus*. Although *Ophiostoma ips* was already reported by Nisikado and Yamauti (1933), they did not describe its associated beetles. Aoshima (1965) reported that *O. ips* was isolated from *C. fulvus* and *I. acuminatus* at high frequencies. Our study results are in accordance with their results. In addition, the association of the beetle *Ips* spp. with *O. ips* is reported to be a common pattern in the United States (Rumbold 1931; Mathre 1964) and Europe (Siemaszko 1939; Mathiesen-Käärik 1953). However, Mathiesen-Käärik (1953) reported that *O. ips* was isolated from *I. acuminatus* at a very low frequency but that *O. clavatum* was isolated at a high frequency. This association pattern is different in Japan, and additional studies are required on the fungal associates of *I. acuminatus*.

Ophiostoma canum was isolated at a high frequency from *T. minor*. This association was previously reported by several authors from different countries (e.g., Mathiesen-Käärik 1953), and the close association between *O. canum* and *T. minor* appears to be a common pattern. This close association has also been previously reported in Japan (Masuya et al. 1999). However, these authors could not fully explain the existence of the very specific association

between *T. minor* and *O. canum*. Because the presence of mycangia was not confirmed in *T. minor* (Francke-Grosmann 1952), Mathiesen-Käärik (1960) noted that some other hitherto unknown factors must be decisive for the association, which most likely pertain to the dispersal biology of the fungal species.

In this study, the most commonly isolated fungus was *Leptographium koreanum*. The teleomorph of this species was recently described by Masuya et al. (2005), and it is considerably similar to *G. piceaperda* sensu Jacobs et al. and *L. yunnanense* in terms of their morphology and the partial rDNA and beta-tubulin gene sequences. The species can be differentiated on the basis of their mating behavior. In addition, both *G. piceaperda* and *L. yunnanense* are not known to be dominant species in pines. In this study, *L. koreanum* was frequently isolated from *T. piniperda* in particular. This association pattern is not known to occur in other countries, except in Japan (Masuya et al. 1998, 1999); moreover, this observation differs from the reports of Aoshima (1965) in Japan. This difference may be explained by the results of environmental changes that have occurred in the Japanese pine forest in the past several years.

Ophiostoma minus, a well-known blue-stain fungus, was isolated in this study. This fungus has been reported previously by several authors in Japan (Nisikado and Yamauti 1933; Aoshima 1965; Masuya et al. 1998, 1999); it was isolated from beetles and is often detected in *T. piniperda*. This association has also been reported in other countries, mainly in Europe (Gibbs and Inman 1991; Solheim and Långström 1991). On the other hand, Mathiesen-Käärik (1960) noted that this fungus was very common even in logs without insect infestations in lumberyards and pine stumps. *Ophiostoma minus* is known to have two distinct populations with different mating systems (Gorton et al. 2004). We did not conduct the critical mating experiments; thus, additional studies on the taxonomy of this fungus are required. In the present study, however, this fungus could only rarely produce perithecia on the medium and appears to possess a heterothallic mating-type system.

In this study, *L. pini-densiflorae*, which is found only in Japan and Thailand (Yamaoka et al. 2007) at present, was isolated from *T. piniperda*, *T. minor*, *C. fulvus*, and *O. angulatus*, which are not root-feeding beetles. Generally, *Leptographium* species are known to be associated with root-feeding beetles or bark beetles that infest the lower part of trunks (Harrington 1988; Wingfield and Gibbs 1991). In our study, three species of *Leptographium* were isolated from the root-feeding beetles *Hylastes* and *Hylurgops* spp. and *T. piniperda*, which infested the lower part of the trunk. However, only one *Leptographium* species, namely, *L. pini-densiflorae*, was not detected in *Hylastes* and *Hylurgops* species, but was detected in *Cryphalus*, *Orthotomicus*, and *Tomicus* beetle species, which are not root-feeding beetles (see Table 4). In addition, this fungal species showed little growth at a high temperature of 35°C (Masuya et al. 2000), whereas other fungal species in our study showed no growth at 35°C. This result implies that *L. pini-densiflorae* may have different ecological characteristics from other *Leptographium* species collected in our study.

In this study, we compared the fungal species obtained from five beetle species among the localities. No difference was observed among the localities with regard to each main fungal associate of the beetle species *T. piniperda*, *H. intersutitialis*, *C. fulvus*, *I. acuminatus*, and *O. suturalis*. This common association between the fungi and the beetles may be explained by the presence of the mycangia; however, these beetles, except for *I. acuminatus*, were not known to have mycangia. Moreover, the mycangia of *I. acuminatus* are known to contain the fungus *Ambrosiella macrospora* (Francke-Grosmann) Batra and not *O. ips*, which was commonly isolated from this beetle in the present study. Thus, some other factors, excluding the presence of the mycangia, might contribute to the aforementioned common association. This common association, together with the difference in fungal species among beetle species, is discussed later. On the other hand, the fungal species isolated from *C. fulvus* varied among localities. Generally, the breeding habitat of *C. fulvus* often overlaps with that of other bark beetle species (Inoue 1953). This ecological characteristic may affect the isolation of fungal species. With regard to the relationship between fungal and beetle species (Table 4), some fungal species were isolated from many beetle species whereas other species were isolated from only a single beetle species. Furthermore, *Leptographium* and *Pesotum* species were isolated mostly from *Hylurgops* sp. and *Hylastes* spp., while *O. ips* and *L. pini-densiflorae* were frequently isolated from *C. fulvus*, *I. accuminatus*, and *O. angulatus*. These results suggest that some ophiostomatoid fungi are commonly associated with more than one beetle species but that others have specific relationships with particular beetle species.

The fungal frequencies of occurrence were mainly determined by the breeding habitats of the beetles because the upper trunk and trunk were two of the three explanatory variables that significantly explained these frequencies. The fungal species were clearly divided into two groups in terms of the habitats of the beetles. One group consisted of fungi occurring in beetles whose breeding habitats were the upper trunk and trunk (e.g., *O. ips*). The other group consisted of fungi occurring in beetles whose breeding habitats were the lower part of the trunk and not the upper trunk and trunk (e.g., *L. procerum*). Romón et al. (2007) also reported that the differences in common fungal associates among bark beetle species on *Pinus radiata* in Spain could be linked to the different niches that these beetles occupy. Thus, our results together with those of Romón et al. (2007) suggest that, regardless of the country and host tree, the association patterns between bark beetles and the associated fungi are related to the niches occupied by the beetles.

A possible reason why the breeding habitats of the beetles (the upper trunk and trunk) determined the fungal frequencies of occurrence is the differences in the physiological characteristics of the fungal species. Mathiesen-Käärik (1960) showed that each blue-stain fungus species has its special nutritional and moisture requirements and growth time; the author also suggested that these physiological differences might explain the specific association between the fungi and the beetles. This hypothesis can fully

explain the consistent isolation of the same fungi from a particular beetle species among the localities and may explain the difference in fungal species depending on a beetle's niche. As predicted by the niche theory (Austin 1999, 2002), the environmental conditions of the upper trunk and trunk (e.g., temperature and moisture) may enhance the frequencies of occurrence of one species group whose physiological requirements are fitted to these conditions. The physiological characteristics of each fungus species isolated in this study should be investigated in detail.

Another determinant of the fungal frequencies of occurrence was the host tree of the beetles. Several fungi were associated with beetles whose host tree was restricted to *Pinus* (*T. piniperda* and *C. fulvus*). These fungi formed a third distinct species group in terms of responses to the ecological characteristics of beetles. A possible reason for this is that several beetle species, e.g., *O. suturalis*, are known to infest *Larix* species.

Acknowledgments We are grateful to Dr. M.J. Wingfield from the Tree Pathology Co-operative Programme, Forestry and Agricultural Biotechnology Institute, University of Pretoria, and Dr. T.C. Harrington from the Department of Plant Pathology, Iowa State University, for their help in the identification of *Leptographium* and *Ophiostoma*; to Dr. H. Goto and Dr. M. Isono from the Forestry and Forest Products Research Institute, for their help in identification of bark beetles; to Dr. M. Osawa from the Yamanashi Forestry and Forest Products Research Institute for sampling the beetles; to the Yamanashi Forestry and Forest Products Research Institute, for providing experimental trees; and to Dr. K. Katsuya and Dr. M. Kakishima from the Institute of Agriculture and Forestry, University of Tsukuba, for their encouragement during the course of this study.

References

Aoshima K (1965) Studies on wood-staining fungi of Japan (in Japanese with English summary). Doctoral thesis, University of Tokyo, Tokyo

Austin MP (1999) A silent clash of paradigms: some inconsistencies in community ecology. Oikos 86:170–178

Austin MP (2002) Spatial prediction of species distribution: an interface between ecological theory and statistical modelling. Ecol Model 157:101–111

Bakke A (1968) Ecological studies on bark beetles (Coleoptera: Scolytidae) associated with Scots pine (*Pinus sylvestris* L.) in Norway with particular reference to the influence of temperature. Det Nor Skogforsoksvesen Vollebekk Norge 83:443–602

Blackwell M, Spatafora JW (1994) Molecular data sets and broad taxon sampling in detecting morphological convergence. In: Hawksworth DL (ed) Ascomycete systematics: problems and perspectives in the nineties. Plenum, New York, pp 243–248

Brasier CM (1991) *Ophiostoma novo-ulmi* sp. nov., causative agent of the current Dutch elm disease pandemics. Mycopathologia 115: 151–161

Christiansen E (1985) *Ceratocystis polonica* inoculated in Norway spruce: blue-staining in relation to inoculum density, resinosis and tree growth. Eur J For Pathol 15:160–167

Dowing P (1973) Effects of felling time and insecticide treatment on the interrelationships of fungi and arthropods in pine logs. Oikos 24:422–429

Francke-Grosmann H (1952) Über die Ambrosiazucht der beiden Kiefernborkenkafer *Myelophilus minor* Htg. und *Ips acuminatus* Gyll. Medd Statens Skogsforsoksanst 41:1–13

Francke-Grosmann H (1967) Ectosymbiosis in wood-inhabiting insects. In: Henry SM (ed) Symbiosis, vol 2. Academic Press, New York, pp 141–205

Gibbs JN, Inman A (1991) The pine shoot beetle *Tomicus piniperda* as a vector of blue stain fungi to windblown pine. Forestry 64: 239–249

Gorton C, Kim SH, Henricot B, Webber J, Breuil C (2004) Phylogenetic analysis of the blue stain fungus *Ophiostoma minus* based on partial ITS rDNA and β-tubulin gene sequences. Mycol Res 108:759–765

Harrington TC (1988) *Leptographium* species, their distributions, hosts and insect vectors. In: Harrington TC, Cobb Jr FW (eds) *Leptographium* root disease in conifers. APS Press, St. Paul, pp 1–39

Harrington TC (1993) Disease of conifers caused by species of *Ophiostoma* and *Leptographium*. In: Wingfield MJ, Seifert KA, Webber JF (eds) *Ceratocystis* and *Ophiostoma*: taxonomy, ecology, and pathogenicity. APS Press, St. Paul, pp 161–172

Hausner G, Reid J, Klassen GR (1992) Do galeate–ascospore members of the Cephaloascaceae, Endomycetacea and Ophiostomataceae share a common phylogeny? Mycologia 84:870–881

Hausner G, Reid J, Klassen GR (1993) On the subdivision of *Ceratocystis* s. l., based on partial ribosomal DNA sequences. Can J Bot 71:52–63

Horntvedt R, Christiansen E, Solheim H, Wang S (1983) Artificial inoculation with *Ips typographus*-associated blue-stain fungi can kill healthy Norway spruce trees. Medd Nor Inst Skogforsk 38:1–20

Inoue M (1953) Ringyou gaichu boujo-ron (in Japanese). Chikyu Shuppan, Tokyo

Käärik A (1980) Fungi causing sap stain in wood. Document IRG/WP/199. The Swedish University Agricultural Science Inter Research Group on Wood Preservation, Stockholm

Kaneko S, Harrington TC (1990) *Leptographium truncatum* isolated from Japanese red and black pines. Rept Tottori Mycol Inst 28:171–174

Kirisits T (2004) Fungal associates of European bark beetles with special emphasis on the ophiostomatoid fungi. In: Lieutier F, Day K, Battisti A, Grégoire JC, Evans H (eds) Bark and wood boring insects in living trees in Europe: a synthesis. Kluwer, Berlin, pp 181–235

Kirk PM, Cannon PF, David JC, Stalpers JA (2001) Ainsworth and Bisby's dictionary of fungi, 9th edn. CABI, Wallingford

Lagerberg T, Lundberg G, Melin E (1927) Biological and practical researches into blueing in pine and spruce. Svenska Skogsfor Tidskr 25:145–272

Lainer GN, Peacock JW (1981) Vectors of the pathogen. In: Stipes RJ, Campana RJ (eds) Compendium of elm diseases. APS Press, St. Paul, pp 14–16

Legendre P, Legendre L (1998) Numerical ecology, 2nd English edn. Elsevier, Amsterdam

Malloch D, Blackwell M (1993) Dispersal biology of ophiostomatoid fungi. In: Wingfield MJ, Seifert KA, Webber JF (eds) *Ceratocystis* and *Ophiostoma*: taxonomy, ecology and pathology. APS Press, St. Paul, pp 195–206

Masuya H, Kaneko S, Yamaoka Y (1998) Blue stain fungi associated with *Tomicus piniperda* (Coleoptera: Scolytidae) on Japanese red pine. J For Res 3:213–219

Masuya H, Kaneko S, Yamaoka Y, Osawa M (1999) Comparisons of ophiostomatoid fungi associated with *Tomicus piniperda* and *T. minor* in Japanese red pine. J For Res 4:131–135

Masuya H, Wingfield MJ, Kaneko S, Yamaoka Y (2000) *Leptographium pini-densiflorae* sp. nov. from Japanese red pine. Mycoscience 41:425–430

Masuya H, Kim JJ, Wingfield MJ, Yamaoka Y, Kaneko S, Breuil C, Kim GH (2005) Discovery and description of a teleomorph for *Leptographium koreanum*. Mycotaxon 94:159–173

Mathiesen-Käärik A (1953) Eine Übersicht die gewöhnlichsten mit Borkenkäfern assoziierten Bläuepilze in Schweden. Medd Från Stat Skogsfor 43:1–74

Mathiesen-Käärik A (1960) Studies on the ecology, taxonomy and physiology of Swedish insect-associated blue stain fungi, especially the genus *Ceratocystis*. Oikos 11:1–25

Mathre DE (1964) Survey of *Ceratocystis* spp. associated with bark beetles in California. Contrib Boyce Thompson Inst 22:353–362

Melin E, Nannfeldt JA (1934) Researches into the blueing of ground wood pulp. Svenska Skogsfor Tidskr 32:397–616

Nisikado Y, Yamauti K (1933) Contributions to the knowledge of the sap stains of wood in Japan. 1. Studies on *Ceratostomella ips*

Rumbold, the cause of a blue stain in pine tree in Western Japan. Ohara Inst Landw Forsch Ber 5:501–538
Nisikado Y, Yamauti K (1934) Contributions to the knowledge of the sap stains of wood in Japan. 2. Studies on *Ceratostomella pini* Münch, the cause of a blue stain in pine tree in western Japan. Ohara Inst Landw Forsch Ber 6:467–490
Nisikado Y, Yamauti K (1935) Contributions to the knowledge of the sap stains of wood in Japan. 3. Studies on *Ceratostomella piceae* Münch, the cause of a blue stain in pine tree in western Japan. Ohara Inst Landw Forsch Ber 6:539–560
Nobuchi A (1966) Bark-beetles injurious to pine in Japan (in Japanese). Bull Gov For Exp Sta 185:1–49
Romón P, Zhou XD, Iturrondobeitia JC, Wingfield MJ, Goldarazena A (2007) *Ophiostoma* species (Ascomycetes: Ophiostomatales) associated with bark beetles (Coleoptera: Scolytinae) colonizing *Pinus radiata* in northern Spain. Can J Microbiol 53:756–767
Rumbold CT (1931) Two blue-stain fungi associated with bark-beetle infestation of pines. J Agric Res 43:847–873
Seifert KA (1993) Sapstain of commercial lumber by species of *Ophiostoma* and *Ceratocystis*. In: Wingfield MJ, Seifert KA, Webber JF (eds) *Ceratocystis* and *Ophiostoma*: taxonomy, ecology, and pathogenicity. APS Press, St. Paul, pp 141–151
Siemaszko W (1939) Zespoty grzybow towarzysacych kornikom. Polskim (fungi associated with bark-beetle in Poland). Planta Pol 7:1–54
Solheim H (1992) The early stages of fungal invasion in Norway spruce infested by the bark beetle *Ips typographus*. Can J Bot 70:1–5
Solheim H, Långström B (1991) Blue-stain fungi associated with *Tomicus piniperda* in Sweden and preliminary observation on their pathogenicity. Ann Sci For 48:149–156
Spatafora JW, Blackwell M (1994) The polyphyletic origins of ophiostomatoid fungi. Mycol Res 98:1–9
Strobel GA, Lanier GN (1981) Dutch elm disease. Sci Am 245:40–50
Ter Braak CJF, Similauer P (2002) Canoco reference manual and CanoDraw for Windows user's guide: software for canonical community ordination, ver. 4.5. Microcomputer Power, Ithaca, NY
Uzunovic A, Yang DQ, Gagné A, Breuil C, Bernier L, Byrne A, Gignac M, Kim SH (1999) Fungi that cause sapstain in Canadian softwoods. Can J Microbiol 45:914–922
Whitney HS (1982) Relationships between bark beetles and symbiotic organisms. In: Mitton JB, Sturgeon KB (eds) Bark beetles in North American conifers. A system for the study of evolutionary biology. University of Texas Press, Austin, pp 183–211
Wingfield MJ (1993) *Leptographium* species as anamorph of *Ophiostoma*: progress in establishing acceptable generic and species concepts. In: Wingfield MJ, Seifert KA, Webber JF (eds) *Ceratocystis* and *Ophiostoma*: taxonomy, ecology, and pathogenicity, APS Press, St. Paul, pp 40–48
Wingfield MJ, Gibbs JN (1991) *Leptographium* and *Graphium* species associated with pine-infesting bark beetles in England. Mycol Res 95:1257–1260
Yamaoka Y, Wingfield MJ, Takahashi I, Solheim H (1997) Ophiostomatoid fungi associated with the spruce bark beetle *Ips typographus* f. *japonicus* in Japan. Mycol Res 101:1215–1227
Yamaoka Y, Wingfield MJ, Ohsawa M, Kuroda Y (1998) Ophiostomatoid fungi associated with *Ips cembrae* in Japan and their pathogenicity to Japanese larch. Mycoscience 39:367–378
Yamaoka Y, Masuya H, Ohtaka N, Goto H, Kaneko S, Kuroda Y (2004) *Ophiostoma* species associated with bark beetles infesting three *Abies* species in Nikko, Japan. J For Res 9:67–74
Yamaoka Y, Masuya H, Chung WH, Goto H, To-Anun C, Tokumasu S (2007) Two species of *Leptographium* isolated from blue-stained sapwood of *Pinus khasya* and bark beetles in Thailand. Mycoscience 48:100–103
Zipfil RD, de Beer ZW, Jacobs K, Wingfield BD, Wingfield MJ (2006) Multi-gene phylogenies define *Ceratocystiopsis* and *Grosmannia* distinct from *Ophiostoma*. Stud Mycol 55:75–97