NOTE / NOTE

Fungi associated with the North American spruce beetle, *Dendroctonus rufipennis*

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Abstract: Fungi were isolated from individual *Dendroctonus rufipennis* (Kirby) collected from six populations in Alaska, Colorado, Utah, and Minnesota, U.S.A. In all populations, *Leptographium abietinum* (Peck) Wingfield was the most commonly isolated mycelial fungus (91–100% of beetles). All beetles in all populations were associated with yeasts and some with only yeasts (0–5%). In one population, *Ophiostoma ips* (Rumbold) Nannf. was also present on 5% of the beetles but always in combination with *L. abietinum* and yeasts. *Ophiostoma piceae* (Munch) H. & P. Sydow was found on 2% of beetles in another population. *Ceratocystis rufipenni* Wingfield, Harrington & Solheim, previously reported as an associate of *D. rufipennis*, was not isolated from beetles in this study. *Ceratocystis rufipenni* is a virulent pathogen of host *Picea*, which has led to speculation that *C. rufipenni* aids the beetle in overcoming tree defenses and therefore contributes positively to the overall success of the beetle during colonization. However, our results, considered along with those of others, indicate that *C. rufipenni* may be absent from many populations of *D. rufipennis* and may be relatively rare in those populations in which it is found. If this is true, *C. rufipenni* may be only a minor or incidental associate of *D. rufipennis* and, as such, not likely to have significant impacts on beetle success or population dynamics. Alternatively, the rarity of *C. rufipenni* in our and others isolations may be due to difficulties in isolating this fungus in the presence of other faster growing fungi such as *L. abietinum*.

Résumé : Des champignons ont été isolés sur des dendroctones (*Dendroctonus rufipennis* Kirby) capturés dans six populations provenant de l'Alaska, du Colorado, de l'Utah et du Minnesota aux États-Unis. Dans toutes les populations, *Leptographium abietinum* (Peck) Wingfield était le champignon le plus communément isolé (91–100 % des dendroctones). Tous les dendroctones dans toutes les populations étaient associés à des levures et certains seulement à des levures (0–5 %). Dans une population, *Ophiostoma ips* (Rumbold) Nannf. était également présent sur 5 % des dendroctones mais toujours en association avec *L. abietinum* et des levures. *Ophiostoma piceae* (Munch) H. & P. Sydow a été isolé sur 2 % des dendroctones dans une autre population. *Ceratocystis rufipenni* Wingfield, Harrington & Solheim, déjà mentionné comme étant associé à *D. rufipennis*, n'a pas été isolé des dendroctones dans cette étude. *Ceratocystis rufipenni* est un pathogène virulent du genre *Picea*, ce qui a donné naissance à l'idée que *C. rufipenni* pouvait aider le dendroctone à vaincre les défenses de l'arbre et par conséquent contribuer positivement au succès global du dendroctone lors de la colonisation. Cependant, nos résultats considérés avec ceux d'autres chercheurs indiquent que *C. rufipenni* est probablement absent dans plusieurs populations de *D. rufipennis* et relativement rare dans les populations où il est retrouvé. Si cela est vrai, *C. rufipenni* pourrait être associé à *D. rufipennis* de façon marginale et accessoire et comme tel n'aurait vraisemblablement pas d'impact majeur sur la dynamique de population et le succès du dendroctone. Par contre, la rareté de *C. rufipenni* dans nos isolations et les isolations d'autres chercheurs pourrait être due à la difficulté d'isoler ce champignon en présence d'autres champignons qui croissent rapidement comme *L. abietinum*.

[Traduit par la Rédaction]

Introduction

Fungi are ubiquitous associates of bark beetles (Coleoptera: Scolytidae (alt. Scolytinae)). Most bark beetle species are associated with two, and sometimes more, specific mycelial fungi as well as several yeasts (Whitney 1982; Six and Paine 1999; Six 2003). The most common mycelial fungi associated with bark beetles are in the genera *Ophiostoma*, *Leptographium*, and *Ceratocystiopsis* (Harrington 1993*a*, 1993*b*). However, the Eurasian spruce beetle, *Ips typographus* L., the North American spruce beetle, *Dendroctonus rufipennis* (Kirby), and the larch bark beetle, *Ips cembrae* Heer, are also associated with *Ceratocystis* species (Wingfield et al. 1997; Harrington and Wingfield 1998).

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Table 1. Location of collections and population status of *D. rufipennis* used for isolations of fungi.

Location	Host tree species	Population status	*N*
Centennial, Kenai Wildlife Refuge, Alaska	*Picea* ×*lutzii* Little	Postepidemic	66
Primrose, Chugach National Forest, Alaska	*Picea sitchensis* (Bong.) Carrière	Building	112
Hayden Pass, Uinta National Forest, Utah	*Picea engelmannii* Parry ex Engelm.	Endemic	102
Floyd, Routt National Forest, Co.	*P. engelmannii*	Epidemic	55
North Lake, Routt National Forest, Co.	*P. engelmannii*	Epidemic	196
Cascade River State Park, Cook Ct., Minn.	*Picea glauca* (Moench) Voss	Endemic	8

Ceratocystis are often virulent pathogens of plants. The *Ceratocystis* species carried by *D. rufipennis* and *I. typographus* are capable of killing mature trees (Hornvedt et al. 1983; Christiansen 1985; Solheim 1988; Christiansen and Solheim 1990; Solheim and Safranyik 1997). In contrast, most *Ophiostoma* and *Leptographium* species are either not pathogenic or only weak pathogens, and few species are capable of killing trees under natural conditions (Harrington 1993*a*).

The various fungi associated with a bark beetle species can have markedly different effects on the fitness of their host, with some fungi conferring strong positive effects and others conferring only weak positive effects, no effects, or even negative effects (Barras 1970, 1973; Yearian et al. 1972; Goldhammer et al. 1990; Coppedge et al. 1995; Six and Paine 1998). Interpopulational differences in the frequency of fungal associates have been noted for several species of bark beetles (Bridges 1983; Viiri 1997; D.L. Six and B.J. Bentz, unpublished data). Because fungal associates differ in their effects on host beetles, the relative frequency of each fungal associate within a population may potentially affect population status (outbreak versus nonoutbreak) as well as the ability of a population to respond to increases in favorable environmental conditions (aggressiveness).

Dendroctonus rufipennis is associated with several fungi including yeasts. Fungal spores and yeast cells are transported to host trees on the outer surface of adult beetles in pits in the pronotum and elytra (Solheim 1995). Once in the tree, the spores germinate and the fungi colonize the phloem and sapwood. The relatively few records of isolations of fungi associated with *D. rufipennis* indicate the existence of among-population differences in the presence and relative abundance of fungal associates (Safranyik et al. 1983; Reynolds 1992; Solheim 1995). *Leptographium abietinum* (Peck) Wingfield is a common associate of *D. rufipennis* in many populations (Davidson 1955; Hinds and Buffam 1971; Solheim 1995) and, in some, may be the only associate other than yeasts (Reynolds 1992). *Ceratocystis rufipenni* Wingfield, Harrington & Solheim is associated with some populations but not others (Reynolds 1992; Solheim 1995). *Ophiostoma piceaperdum* (Rumb.) von Arx (Rumbold 1936), *Ophiostoma penicillatum* (Grosm.) Siemaszko (Davidson 1955), *Ophiostoma olivaceum* Mathiesen (Hinds and Buffam 1971), *Ophiostoma piliferum* (Fries) H & P. Sydow, and *Ophiostoma piceae* (Munch) H. & P. Sydow (Safranyik et al. 1983) have also been found in association with *D. rufipennis* but appear to be minor or incidental associates.

Unfortunately, in most studies of fungal associates of *D. rufipennis*, isolations were conducted from only one or a few beetles per population. Such small samples may not have captured the full range of fungal associates. In other studies, isolations were made from wood, making an explicit connection between vector and fungus tenuous. Furthermore, most isolation cultures were held at room temperature for growth of fungi prior to identification, a practice that may have affected which species were recovered. For example, the relative rarity of isolations of *C. rufipenni* in some studies may be due to the use of incorrect incubation temperatures (Solheim 1995). *Ceratocystis rufipenni* has a low optimal growth temperature (18 °C with no growth at 28 °C) (Solheim 1995) relative to other bark beetle associated fungi and isolations held at higher temperatures (room temperature, for example) may not yield this species.

To better understand how associated fungi affect *D. rufipennis* populations, a basic understanding of the species composition, frequency, and distribution of fungal associates of this beetle is needed. Therefore, our objectives with this research were to survey the fungi associated with several populations of *D. rufipennis* and, using our results and a review of previous research, develop a list of fungal associates for this beetle.

Materials and methods

Adult *D. rufipennis* were collected by removing adult beetles from under the bark of infested *Picea* at six sites in Alaska, Colorado, Utah, and Minnesota, U.S.A. (Table 1). The Centennial site was located on the Kenai Peninsula in southwest Alaska (60.373°N, 151.150°W) at an elevation of 60 m. The Primrose site was also located on the Kenai Peninsula (60.500°N, 149.333°W) at an elevation of 210 m. Hayden Pass is located in the Uinta National Forest, Utah (40.700°N, 110.883°W), at an elevation of 3139 m. Floyd (40.667°N, 106.667°W) and North Lake (40.750°N, 106.600°W) are in the Routt National Forest, Colorado, at elevations of 2750 and 2530 m, respectively. The Cascade River site is located in Cook County, Minnesota, at an elevation of 710 m. All sites comprised stands of mature spruce.

New adults (brood adults just prior to emergence) were collected at all sites. At the North Lake site, parental adults (parent beetles that had overwintered in the tree after producing brood the previous year) were also collected. After collection, beetles were placed into Petri dishes containing moistened filter paper and stored live on ice or in a refrigerator until isolations could be made. Isolations of fungi from individual beetles were made within 2 weeks of collection.

Isolations of fungi were conducted by grasping individual beetles with forceps and, with firm pressure, streaking both dorsal and ventral surfaces of the beetle (where spore-carrying pits and setae are located) across the surface of 2%

Table 2. Numbers (%) of various fungi carried by *D. rufipennis* collected from five populations.

Population	*N*	*L. abietinum* plus yeasts	*O. ips* plus *L. abietinum* plus yeasts	*O. piceae* plus yeasts	Yeasts only
Kenai	66	57 (86)	3 (5)	0 (0)	6 (9)
Primrose	114	106 (95)	0 (0)	2 (2)	6 (5)
Hayden Pass	102	95 (93)	0 (0)	0 (0)	7 (7)
Floyd	55	53 (96)	0 (0)	0 (0)	2 (4)
North Lake	196	192 (98)	0 (0)	0 (0)	4 (2)
Cascade River	8	8 (100)	0 (0)	0 (0)	0 (0)
Total	541	511 (95)	3 (<1)	2 (<1)	25 (5)

malt extract agar. Each beetle was streaked across the surface of two malt extract agar filled dishes. One dish from each beetle was held at 15–18 °C (to select for *C. rufipenni*), and the other was held at room temperature (21–25 °C). After allowing at least 2–4 weeks of growth, fungi in initial isolation dishes were identified using morphological characteristics (primarily conidia and conidiophores for *L. abietinum* and most *Ophiostoma ips* (Rumbold) Nannf. isolates and perithecia for one *O. ips* culture) (Rumbold 1936; Upadhyay 1981; Harrington and Wingfield 1998; Jacobs et al. 1998). Comparisons of isolates with known cultures from the first author's culture collection were also made. When identification of fungi from initial isolation plates was ambiguous, subcultures were made onto malt extract agar and (or) pine twig agar (2% agar with short longitudinally split sections of autoclaved pine twigs) and subsequent growth of fungi in these cultures used for identifications.

Results

Leptographium abietinum was isolated from beetles in all populations and was the most commonly isolated mycelial fungus (isolated from 91–100% of beetles) (Table 2). All beetles in all populations were associated with yeasts and some with only yeasts (0–5%) (Table 2). Several yeast species were present but were not identified to species. In one population, *O. ips* was also present on 5% of the beetles, but always in combination with *L. abietinum* and yeasts (Table 2). Frequency of individuals associated with *L. abietinum* and yeasts, or yeasts only, did not differ between new adults and parental adults in the North Lake population where both types of adults were collected (all beetles except two parents and two new adults that carried only yeasts carried *L. abietinum*). Therefore, results for both adult types were pooled for the North Lake population (Table 2). *Ceratocystis rufipenni* was not isolated from beetles from any of the five populations.

Discussion

Leptographium abietinum was the most common mycelial fungus associated with *D. rufipennis* in this study, as was true in most past studies (Davidson 1955; Reynolds 1992; Solheim 1995). In the one exception, Safranyik et al. (1983) reported *O. piceae* to be the most common associate of *D. rufipennis*; however, the authors provided no information on location where beetles or colonized wood was collected, method of isolation (wood or beetles), number of isolates obtained, or frequency of various fungal associates within the population(s) sampled, and therefore, it is difficult to evaluate their findings.

Ophiostoma ips was isolated from 5% of the beetles in the Kenai, Alaska, sample. *Ophiostoma ips* is a common associate of several *Ips* species. Its presence on *D. rufipennis* may have resulted from cross-contamination with fungi inoculated into the host tree by cohabiting scolytids associated with this fungus. *Dryoceotes affaber* Mann., *Polygraphus rufipennis* (Kirby), and *Ips pini* (Say) collected from *Picea glauca* (Moench) Voss in the Great Lakes region were found to carry *O. ips* (Haberkern et al. 2002), and R.W. Davidson isolated *O. ips* from *Ips*-infested *P. glauca* in Alaska (Upadhyay 1981). These beetles often cohabit *Picea* with *D. rufipennis* (Werner and Holsten 1984; Gara et al. 1995), and the fungi that they vector to the host tree may occasionally colonize tissues of the tree colonized by *D. rufipennis*. Likewise, *D. affaber* and *P. rufipennis* have been found to carry *L. abietinum* at low frequencies (Haberkern et al. 2002), indicating that these beetles, in turn, may sometimes acquire the fungus associated with *D. rufipennis*. Cross-contamination of fungal associates in trees containing cohabiting scolytids is probably not uncommon and has been found with *Dendroctonus ponderosae* Hopkins, *I. pini*, and *Ips emarginatus* (LeConte) cohabiting *Pinus* (D.L. Six, unpublished data).

Ophiostoma piceae, *O. piceaperdum*, *Ophiostoma bicolor* Davidson & Wells, *O. olivaceum*, *O. penicilliatum*, and *O. piliferum* have also periodically been isolated from *D. rufipennis* (Table 3) but appear to be minor or incidental associates. We isolated *O. piceae* from only two beetles in our study (Table 2). The presence of at least some of these fungi with *D. rufipennis* may also be due to cross-contamination with fungi associated with other cohabiting scolytid species. For example, *O. piceaperdum* is carried at relatively high frequencies by *D. affaber* and *Dryoceotes autographus* (Ratzeburg), and *O. bicolor* is carried, at lower frequencies, by *I. pini*, *D. affaber*, and *P. rufipennis* (Haberkern et al. 2002).

Unlike some other species of bark beetles that have two or more common associates, *D. rufipennis* appears to be associated with only one predominant fungus, *L. abietinum*. The effect of this fungus on the beetle is unknown. It is only weakly pathogenic and is not likely to contribute substantially to the killing of host trees. It may be mutualistic through positive nutritional effects on beetle brood, or conversely, it may be antagonistic, lowering brood survival.

Table 3. Ophiostomatoid fungi associated with *D. rufipennis* colonizing various *Picea* species.

Species	Substrate	Location	Host tree	Reference
C. rufipenni	Wood[a]	British Columbia	*P. glauca*	Solheim 1995
	Wood	British Columbia	*P. glauca*	Wingfield et al. 1997
	Wood	British Columbia	*P. engelmannii*	Wingfield et al. 1997
	Wood	Colorado	*P. engelmanni*	Davidson 1955
	Wood	Colorado	*P. engelmannii*	Hinds and Buffam 1971
	Unknown	Unknown	Unknown	Safranyik et al. 1983
L. abietinum	Beetles	Alaska	*P. sitchensis*	This study
	Beetles	Alaska	*P. ×lutzii*	This study
	Beetles	Utah	*P. engelmannii*	This study
	Beetles	Colorado	*P. engelmannii*	This study
	Beetles	Minnestota	*P. glauca*	This study
	Beetles	British Colombia	*P. glauca*	Solheim 1995
	Wood	British Columbia	*P. glauca*	Solheim 1995
	Wood	Alaska	*P. ×lutzii*	Reynolds 1992
	Wood	Colorado	*P. engelmannii*	Davidson 1955
	Wood	Colorado	*P. engelmannii*	Hinds and Buffam 1971
O. olivaceum	Wood	Colorado	*P. engelmannii*	Hinds and Buffam 1971
O. bicolor	Beetles	Colorado	*P. engelmannii*	Davidson 1955
O. penicillatum	Wood	Colorado	*P. engelmannii*	Davidson 1955
O. piceae	Unknown	Unknown	Unknown	Safranyik et al. 1983
	Beetles	Alaska	*P. sitchensis*	This study
O. piceaperdum	Wood	Nova Scotia	*P. glauca*	Rumbold 1936
	Wood	Quebec	*P. glauca*	Rumbold 1936
	Beetle	Nova Scotia	*P. glauca*	Rumbold 1936
	Unknown	Unknown	Unknown	Safranyik et al. 1983
O. piliferum	Unknown	Unknown	Unknown	Safranyik et al. 1983

[a]Wood colonized by *D. rufipennis*.

Both types of effects have been found in other bark beetle – fungus associations (Barras 1970, 1973; Coppedge et al. 1995; Goldhammer et al. 1990; Six and Paine 1998; Ayres et al. 2000); however, neither of these possibilities has been investigated for the *D. rufipennis – L. abietinum* association.

Ceratocystis rufipenni, which has been hypothesized to be an important associate of *D. rufipennis* because of its virulence to host trees, was not found to be associated with *D. rufipennis* in this study or in several other studies. It is possible that the fungus is more widespread than the results of isolation studies have indicated due to inherent difficulties in isolating this fungus. The fungus has a low optimal growth temperature and most strains will not grow at temperatures commonly used to grow isolations of fungi associated with bark beetles. In this study, we were unable to isolate this fungus despite careful efforts to separate and identify all fungi that occurred in mixed cultures and to incubate a set of cultures at optimal temperatures for the growth of *C. rufipenni*. However, the method of isolation and artificial medium used in this study may not have been appropriate for isolating all fungi present on the exokeleton of the individual beetles, especially the slow-growing, more fastidious species.

Interestingly, no isolations of *C. rufipenni* have been made directly from beetles, only from wood colonized by *D. rufipennis*. The beetle is capable of vectoring the fungus, as fresh green bolts inoculated with beetles collected from naturally colonized material later yielded isolations of *C. rufipenni* from the inner bark and sapwood (Solheim 1995). This indicates that isolations may need to be made in freshly cut wood and that artificial media, or at least those used in studies to date, may not be appropriate for detection of this fungus. Therefore, in future studies, it will be desirable to employ a variety of insect treatments (grinding, vortexing) as well as both artificial and natural media (fresh logs) held under various conditions.

Ceratocystis rufipenni has only been found in association with *D. rufipennis* (Wingfield et al. 1997), indicating that the beetle is a likely vector of the fungus. However, if the incidence of this fungus with *D. rufipennis* is as low as results from this and other studies indicate, then vector efficiency may be poor. Vector efficiency in this system is probably closely tied to timing of fungal succession, and timing and location of production of dispersal propagules, within a colonized tree. As a pathogen, *C. rufipenni* grows quickly in newly attacked trees and is found at the leading edge of fungal growth toward the sapwood (Solheim 1995). However, as tree tissues die and decline over the 1- to 2-year period from attack to emergence of brood adult beetles, saprophytic fungi such as *L. abietinum* that predominate in the region near the cambium where beetles develop (Solheim 1995) may become dominant, decreasing the likelihood of contact between beetles and *C. rufipenni*. Until more is known regarding the distribution and consistency of association of *C. rufipenni* with *D. rufipenni*, it will be difficult to assess what role, if any, this fungus may have with the beetle.

Solheim (1995) observed ascospores of *C. rufipenni* in pits on the pronotum and elytra of *D. rufipenni*. However, because perithecia of many ophiostomatoid fungi associated with bark beetles tend to form in old galleries (Rumbold

1936; Davidson 1955; Six 2003) where contact with brood adults is unlikely, it is unclear how ascospores are acquired by the beetle. Further investigation into where and when perithecia of *C. rufipenni* form, how spores may be acquired by brood adults, and the strength of vector efficiency in this system is needed if we are to understand the relationship between the beetle and this fungus.

In this study, as in studies with many bark beetles, yeasts were the most frequently isolated fungi. There is some evidence that yeasts may be required for, or at least have positive effects on, normal development of *D. rufipennis* brood. Safranyik et al. (1983) found that time of development from egg to adult was halved by adding yeast to their diet. Similar but more dramatic effects of yeasts have also been seen with the mountain pine beetle *D. ponderosae*, where brood developing with yeast developed to adulthood but those developing without yeast died as larvae (Safranyik et al. 1983). Relatively little is known about bark beetle associated yeasts, and studies on their taxonomy, distribution, relative abundance, effects on the host, and interactions with mycelial associates will be necessary to develop a comprehensive perspective of bark beetle – fungus interactions.

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