Two species in the *Ceratocystis coerulescens* complex from conifers in western North America

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Abstract: Two species of *Ceratocystis* are described from western North America. *Ceratocystis rufipenni* Wingfield, Harrington, & Solheim is associated with the North American spruce bark beetle *Dendroctonus rufipennis* infesting both Engelmann spruce (*Picea engelmannii* Parry) and white spruce (*Picea glauca* (Moench) Voss) in British Columbia. This fungus is a primary component of the bark beetle mycota and has a relatively high degree of virulence to Engelmann spruce. *Ceratocystis douglasii* (Davidson) Wingfield & Harrington was previously described as *Endoconidiophora coerulescens* f. *douglasii*. It is common on Douglas-fir lumber in western North America where it causes blue stain. Both fungi grow optimally at low temperatures and produce perithecia only after extended incubation under refrigeration. *Ceratocystis rufipenni* produces conidiophores mostly in association with perithecia, whereas conidia and conidiophores of *C. douglasii* are produced abundantly on wood and agar.

Key words: *Ceratocystis*, *Chalara*, *Dendroctonus*, *Ophiostoma*, bark beetle – fungus interactions, symbiosis.

Résumé : Les auteurs décrivent deux espèces de *Ceratocystis* de l'ouest de l'Amérique du Nord. Le *Ceratocystis rufipenni* Wingfield, Harrington & Solheim est associé à l'insecte de l'écorce du pin, le *Dendroctonus rufipennis*, qui infeste l'épinette d'Engelmann (*Picea engelmannii* Parry) aussi bien que l'épinette blanche (*Picea glauca* (Moench) Voss), en Colombie Canadienne. Ce champignon est une composante principale du mycota à dendroctones et possède un degré relativement élevé de virulence sur l'épinette d'Engelmann. Le *Ceratocystis douglasii* Wingfield & Harrington a été précédemment décrit comme le *Endoconidiophora coerulescens* f. *douglasii*. Il est commun sur les billes de sapin douglas dans l'ouest de l'Amérique du Nord, où il cause la tâche bleue. La croissance optimale des deux champignons se situe aux basses températures et ils produisent des périthèces seulement après de longues périodes d'incubation sous des conditions de refrigération. Le *Ceratocystis rufipenni* produit des conidiophores surtout en association avec les périthèces, alors que les conidies et conidiophores du *C. douglasii* se forment en abondance sur le bois et sur l'agar.

Mots clés : *Ceratocystis*, *Chalara*, *Dendroctonus*, *Ophiostoma*, interactions insectes des écorces et champignons, symbiose.
[Traduit par la rédaction]

Introduction

For many years, the genus *Ceratocystis* Ell. & Halst. has been considered part of a group of genera that has become broadly known as the Ophiostomatoid fungi in the order Ophiostomatales. This group includes *Ceratocystis* sensu stricto, Ophiostoma H.& P. Sydow and *Ceratocystiopsis* Upadhyay & Kendrick. Species within these genera share various morphological characters that facilitate insect dispersal. For example, most species have elongate ascomatal necks from which masses of sticky ascospores exude. In many cases, their conidial states are also typified by sticky conidial masses that are acquired by insects (Wingfield et al. 1993).

A contemporary view of the Ophiostomatoid fungi is that they represent at least two distinct phylogenetic entities. These are typified by *Ceratocystis* sensu stricto and *Ophiostoma*. *Ceratocystis* species have anamorphs in *Chalara* Rabenhorst, with conidia produced in chains by ring wall building (Minter et al. 1982). This is in contrast to *Ophiostoma*, in which conidia are produced by apical wall building and the most common anamorphs are treated in genera such as *Graphium* Corda, *Leptographium* Lagerberg & Melin and *Sporothrix* Hektoen & Perkins (Upadhyay 1981; Mouton et al. 1994). Species of *Ophiostoma* are also unusual among true fungi and unlike *Ceratocystis* sensu stricto in that they contain cellulose and rhamnose in their cell walls (Rosinski and Campana 1964; Jewell 1974; Weijman and De Hoog 1975). In culture, the two groups can easily be distinguished by the fact that *Ophiostoma* spp. tolerate high concentrations of cycloheximide, whereas *Ceratocystis* spp. are extremely sensitive to this antibiotic (Harrington 1981). Recent studies based on rDNA sequence data have also confirmed that these two groups are distinct and phylogenetically unrelated (Hausner et al. 1992, 1993; Blackwell 1994; Spatafora and Blackwell 1994).

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Figs. 1–6. Perithecia, ascospores, and conidiophores of *Ceratocystis rufipenni* (holotype). Fig. 1. Perithecium. Scale bar = 200 μm. Fig. 2. Base of perithecium showing hyphal ornamentation. Scale bar = 200 μm. Fig. 3. Ostiolar hypae at apex of perithecial neck. Scale bar = 30 μm. Fig. 4. Sheathed ascospores with distinct guttules at either end. Scale bar = 14 μm. Fig. 5. Elongate tubular conidiogenous cell. Scale bar = 40 μm. Fig. 6. Short conidiogenous cell showing flared apex. Scale bar = 20 μm.

One of the best known species of *Ceratocystis* is *C. coerulescens* (Münch) Bakshi, first described as *Endoconidiophora coerulescens* Münch from blue stained spruce (*Picea* spp.) and pine (*Pinus* spp.) in Germany (Münch 1907). It was subsequently recorded in northern Europe, Great Britain and the U.S.A. (Lagerberg et al. 1927; Davidson 1935, 1944; Siemaszko 1939; Bakshi 1950, 1951; Roll-Hansen and Roll-Hansen 1980) as a saprophyte or wound colonizer of sapwood. Ascomata have long necks terminating in ostiolar hyphae and distinct spines ornamenting the bases. Ascospores are elongate and are sheathed (with an outer and inner wall). The species has a *Chalara* anamorph and tends to produce a strong fruity aroma, presumably for insect attraction.

Considerable confusion has surrounded the taxonomy of *C. coerulescens*. For example, isolates resembling this species from hardwoods were described as *Endoconidiophora virescens* Davidson (Davidson 1944). This fungus, which was later transferred to *Ceratocystis* as *C. virescens* (Davidson) Moreau (Moreau 1952), is the causal agent of an important disease of maple known as sapstreak (Kile 1993). Subsequent authors, however, treated *C. virescens* as a synonym of *C. coerulescens* (Hunt 1956; Griffin 1968; Upadhyay 1981). Another species in the *C. coerulescens* complex, considered to be unique by Davidson (1953), was found to occur specifically on Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) in the western United States. It was designated *Endoconidiophora coerulescens* f. *douglasii*. This fungus was also treated as a synonym of *C. coerulescens* by Hunt (1956) and subsequently by Upadhyay (1981). Unfortunately, the re-description of *C. coerulescens* by Upadhyay (1981), which is the most recent treatment of this fungus, is confused by the fact that data were taken from an amalgamated group of isolates that probably represent a number of distinct taxa. Davidson (1955) reported the presence of an *Endoconidiophora* sp. similar to *E. coerulescens* (including *E. coerulescens* f. *douglasii*) among fungi associated with Engelmann spruce (*Picea engelmannii* Parry), infested with the bark beetle *Dendroctonus rufipennis* (Kirby) (*D. engelmannii* Hopk.) in the Rocky Mountain area of the United States. In a more recent study of the fungi associated with *D. rufipennis* in British Columbia, Solheim (1995) commonly isolated a species of *Ceratocystis*.

A recent examination of isozyme variation in the *C. coerulescens* complex identified many distinct taxa, including *E. coerulescens* f. *douglasii* and the *D. rufipennis* associate (Harrington et al. 1996). The aim of the present study was to describe the latter fungus as a new taxon and to elevate the status of the former to that of a distinct species.

Materials and methods

Engelmann spruce trees at Caribou Creek, East Nelson, B.C., Canada, and white spruce (*Picea glauca* (Moench) Voss) at McGregor River, Prince George, B.C., Canada, infested with *D. rufipennis*, were examined during October and November 1992. Isolations were made from the sapwood of beetle-infested Englemann spruce trees. In the case of white spruce, isolates originated from ascospore masses on perithecia produced on wood that had been stored in a cold room.

A single isolate (CBS 142.53) of *E. coerulescens* f. *douglasii* from the holotype specimen (FP 70703) collected by Dr. R.W. Davidson from Douglas fir lumber shipped from Oregon was available for study. A second strain (CBS 269.53) proved to be of another unidentified *Chalara* species. Various herbarium specimens of the fungus collected by Davidson and later examined by Hunt (1956), including the type, were obtained from the Herbarium of the State University of New York, College of Forestry, Syracuse, New York and the Commonwealth Agricultural Bureau, International Mycological Institute (IMI). Six isolates of the *Ceratocystis* sp. from *D. rufipennis* were examined in detail, one of which was induced to produce perithecia by storing cultures at 10°C. These perithecia were then used for morphological comparisons.

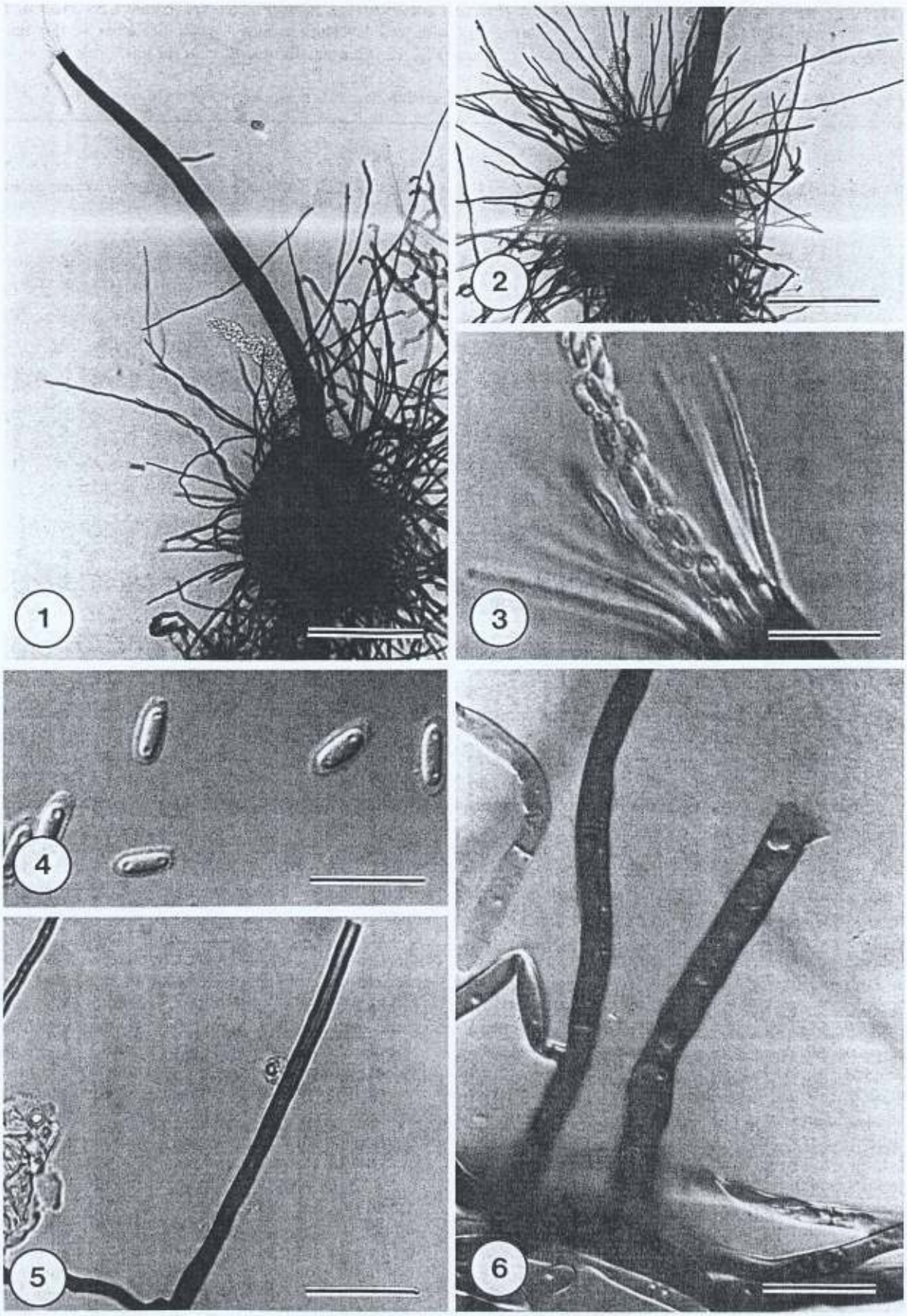
Temperature requirements for growth in culture were compared in two isolates of the *Ceratocystis* sp. from *D. rufipennis*, the one isolate of *E. coerulescens* f. *douglasii* and an isolate considered to be typical of *C. coerulescens* from Scots pine (*Pinus sylvestris* L.) in England (Harrington et al. 1996). The latter isolate (C 488) was collected by J. Gibbs on Windsor Estate, Berkshire, England in 1988. Mycelial plugs of each isolate were placed at the centre of three Petri dishes containing 20 mL malt extract agar (MEA; 20 g Difco malt extract; 20 g Difco Bacto agar/L water). The dishes were then placed upside down in incubators ranging in temperature from 10 to 35°C at 5° intervals and the fungi allowed to grow in the dark for 5 days. Two diameter measurements of each colony were taken at right angles, and averages of the six measurements for each fungus and temperature were computed.

Results

Detailed examination of the *Ceratocystis* sp. from *D. rufipennis* and *E. coerulescens* f. *douglasii* have led us to conclude that both fungi represent distinct taxa. These taxa appear to grow best at low temperatures and are morphologically similar, but occur in distinct niches. They can be distinguished from each other as well as from other *Ceratocystis* spp. occurring on conifers on the basis of a number of morphological characteristics. Isozymes also distinguish these two new taxa (Harrington et al. 1996). We provide the following description for the new *Ceratocystis* sp. associated with *D. rufipennis* on spruce in western North America:

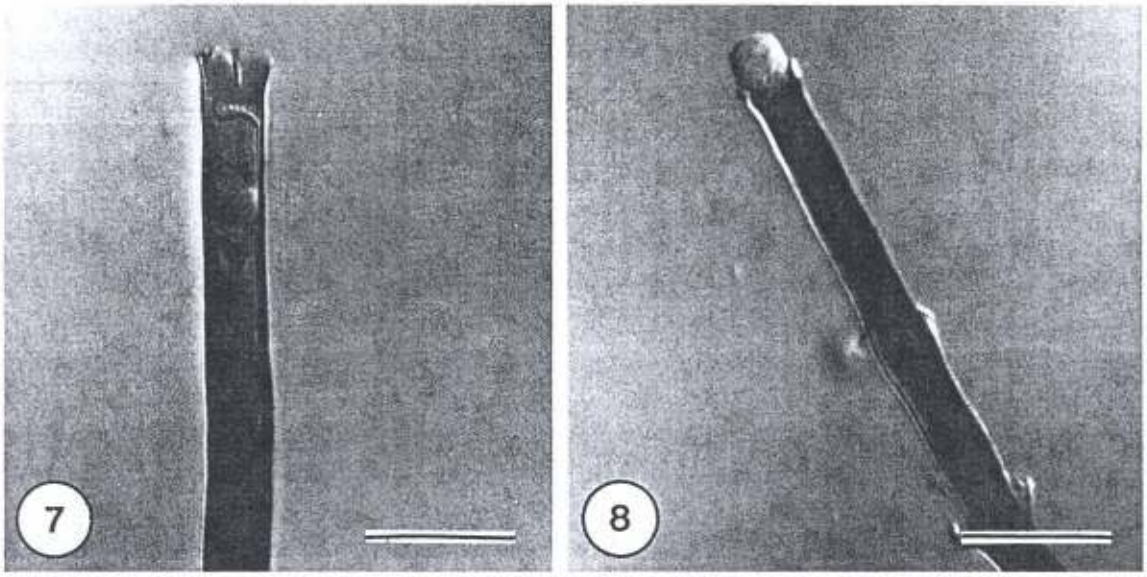
Ceratocystis rufipenni Wingfield, Harrington, & Solheim sp.nov. Figs. 1–8

Segregata in malti extracti agaro variabilia ratione incrementi, ratione optima ad 20°C attingente 30 usque 60 mm diam. quinque diebus. Incrementum ad 25°C multum restrictum, nullo incremento eveniente ad 30°C. Coloniae atroviridule olivaceae (Ridgeway 1912; imago XXX) colore, initiopallide coloratae et atricolorescentes dum senescunt nullo paene aerio mycelio. Perithecia formantur in porticibus scarabei Dendoctroni rufipennis, et aliquando in segregatis coacervatis demissis temperaturis (15–20°C) et praesertim



Figs. 9–15. Perithecium, ascospores, conidiophores, and conidia of *Ceratocystis douglasii* (ex holotype culture CBS 142.53). Fig. 9. Perithecium. Scale bar = 80 μm. Fig. 10. Apex of perithecial neck with well developed ostiolar hyphae. Scale bar = 180 μm. Fig. 11. Base of perithecium with hyphal ornamentation. Scale bar = 50 μm. Fig. 12. Elongate ascospores with sheaths. Scale bar = 10 μm. Fig 13. Long flexuous, slightly tapering conidiophore formed on wood. Scale bar = 90 μm. Fig. 14. Short brush-like conidiophores. Scale bar = 90 μm. Fig. 15. Young, cylindrical, hyaline conidia of variable size. Scale bar = 20 μm.

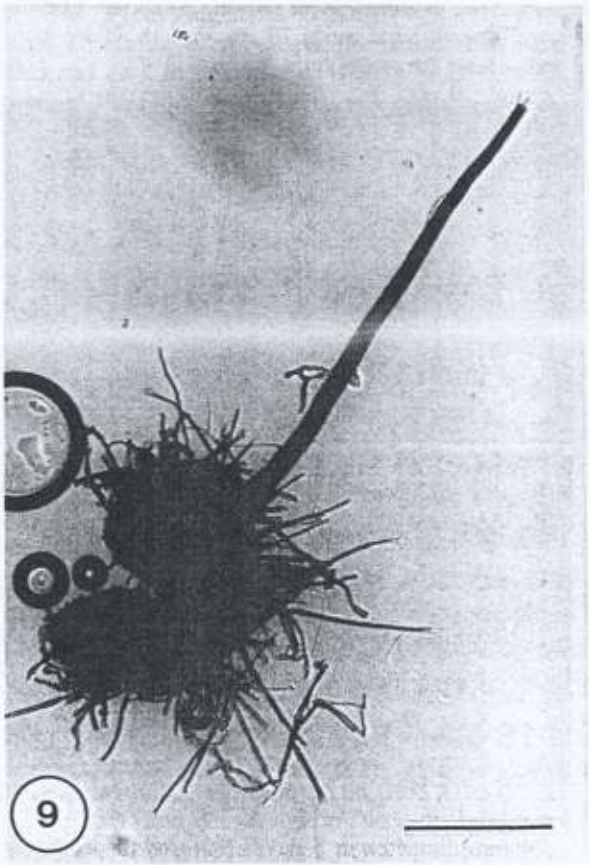
Figs. 7 and 8. Conidiogenous cells of *Ceratocystis rufipenni* (holotype). Fig. 7. Apex of conidiogenous cell slightly flared with torn outer wall tissue and cylindrical conidium. Scale bar = 20 μm. Fig. 8. Conidiogenous cell that has undergone percurrent proliferation. Scale bar = 20 μm.

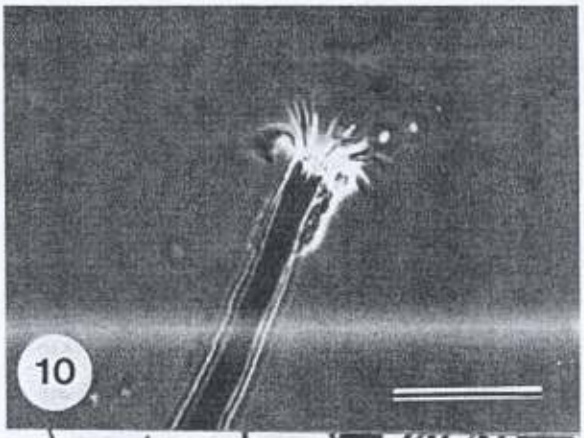


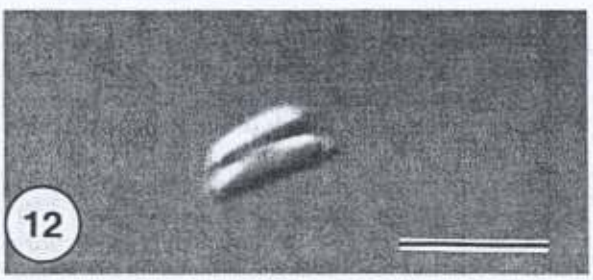
in vitro in summis penariis ampullis ad 10°C diutius conservatis. Ascosporae solae gignentes colonias et autofertiles et autosteriles proportionibus quasi aequalibus. Perithecia robusta atris basibus 224–480 μm (286 μm) diametro et ornata brevibus pigmentiferis angustatis hyphis et collis exigue pallescentibus colore ad apicem versus, 640–1800 μm (926 μm) longis, 14–18 μm (16 μm) latis ad apicem et 24–38 μm (30 μm) latis in medio. Colla terminantia in cristam ostiolarium hypharum 23–44 μm (34 μm) longarum et 2–3 μm (2 μm) latarum. Ascosporae productae vel exigue curvatae finibus rotundatis et cinctae distincto et comparate lato translucido strato externo vel vagina 6–8 μm (7 μm) longae et 2–3 μm (2 μm) latae vagina exclusa, quae variat ab 1 μm usque 2 μm longitudine ad extremum utrumque sporarum. Conidiophora typica speciei *Chalarae*, brunnea, erecta, et quasi tubularia sed aliquot eorum sunt exigue attenuata ad apices apertos versus qui aliquando ex texto lacerato constituti sunt et quoque aliquando prolificant gignentes novum locum conidiogenum 64–190 μm (133 μm) longum et 5–6 μm (6 μm) latum. Conidia hyalina, rectangula vel exique doliiformia et valde variabilia longitudine, 7–17 μm (10 μm) longa et 5–10 μm (7 μm) lata, manifestius doliiformescentia dum maturescunt et saepe melaizascentia.

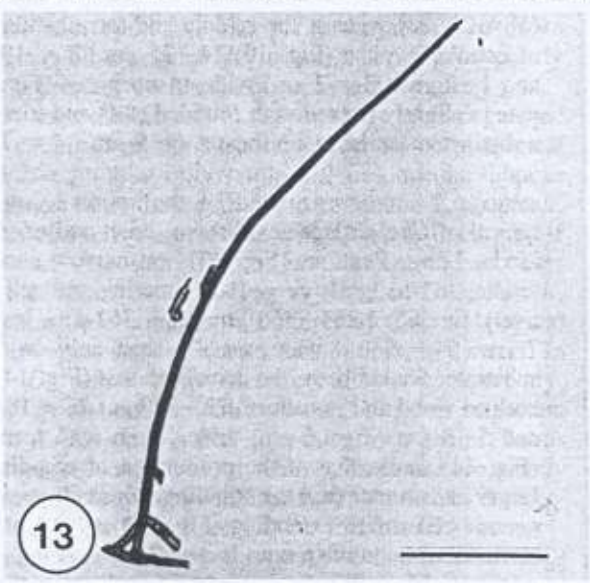
HOLOTYPUS: Caribou Creek, East Nelson, Columbia Britannica, Canada, ex *Picea engelmannii* infesta *D. rufipenni*, Novembris, 1992. Coll. H. Solheim, C 608, 252/8, DAOM 222349.

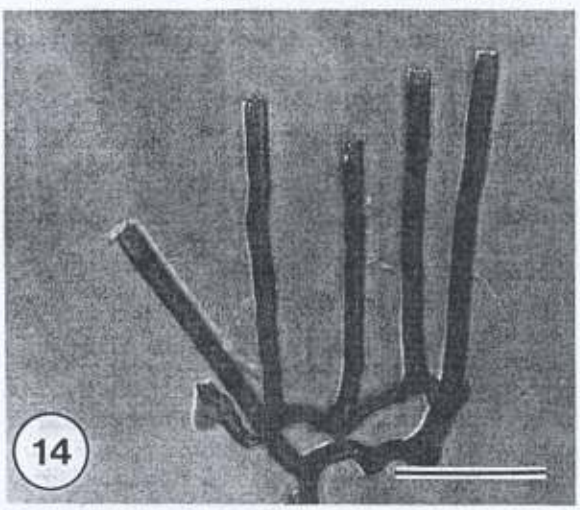
Isolates on malt extract agar variable in growth rate with an optimum at 20°C, 30–60 mm diam. in 5 days. Growth at 25°C slow, no growth at 30°C. Colonies dark greenish olive (Ridgeway 1912, plate XXX), light coloured at first and becoming darker with age, with virtually no aerial mycelium on MEA. Perithecia forming in galleries of the bark beetle *Dendroctonus rufipennis*, occasionally in isolates stored at low temperatures (15–20°C), and especially on the glass near the edge of MEA in vials maintained at 10°C. Single ascospores giving rise to both self-fertile and self-sterile colonies in approximately equal proportions. Perithecia (Fig. 1) robust with black (olive green) bases 224–480 μm ($\bar{x}$ = 286 μm) in diameter, ornamented (Fig. 2) with short, pigmented and tapered hyphae, and with necks that become slightly lighter in color towards the apex, 640–1140 μm ($\bar{x}$ = 926 μm) long, 14–18 μm ($\bar{x}$ = 16 μm) wide at the apex and 24–38 μm ($\bar{x}$ = 30 μm) wide at the middle. Necks terminating in ostiolar hyphae 23–44 μm ($\bar{x}$ = 34 μm) long and 2–3 μm ($\bar{x}$ = 2 μm) wide (Fig. 3). Ascospores (Fig. 4) elongate to slightly curved, with rounded ends and surrounded by a distinct and relatively broad translucent outer layer or sheath 6–8 μm ($\bar{x}$ = 7 μm) long and 2–3 μm ($\bar{x}$ = 2 μm) wide excluding sheaths that vary from 1 to 2 μm in length at each end of the spores. Conidiophores typical of *Chalara* species, brown, erect and approximately tubular (Figs. 5 and 6) but in some cases slightly tapered towards the open apices, which are sometimes composed of torn tissue (Figs. 6 and 7) and also occasionally proliferate (Fig. 8) to give rise to a new conidiogenous locus, 64–190 μm ($\bar{x}$ = 133 μm) long and 5–6 μm ($\bar{x}$ = 6 μm) wide. Conidia at first hyaline, rectangular to slightly barrel-shaped

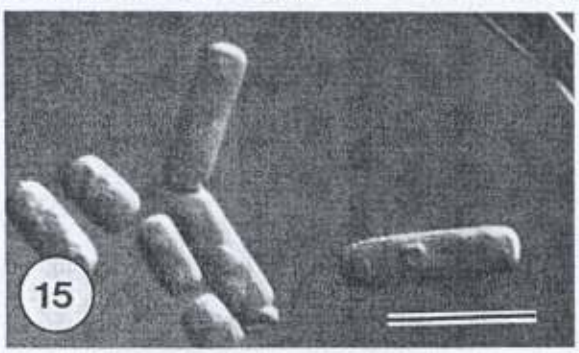




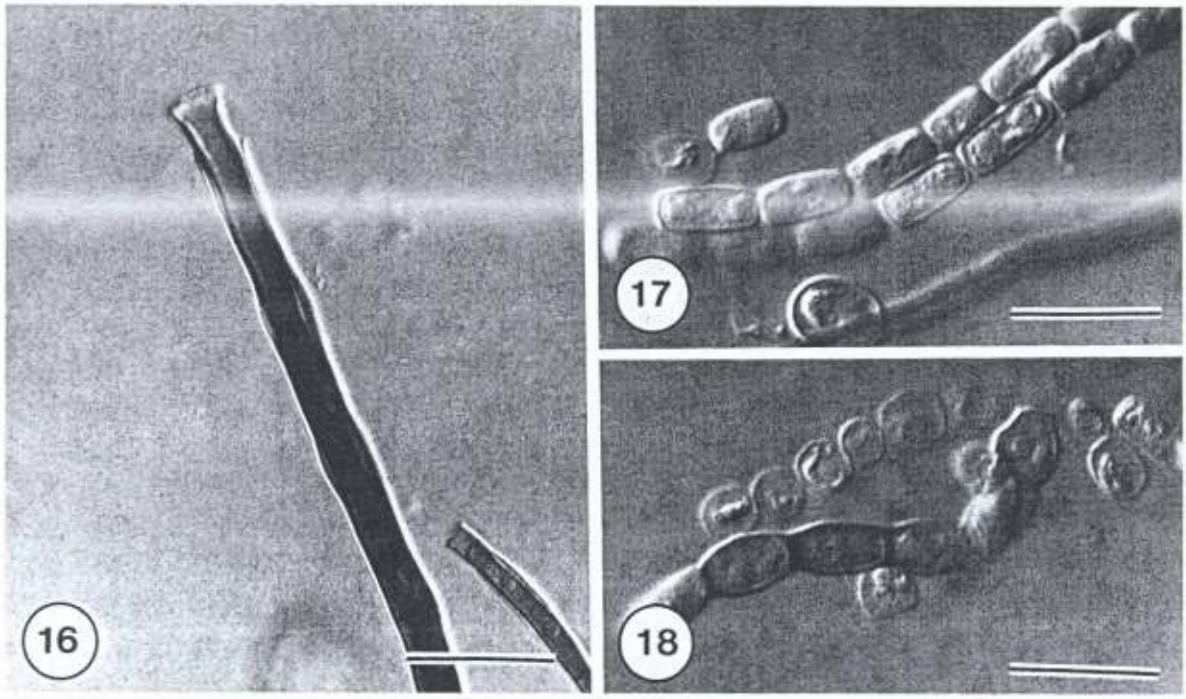








Figs. 16–18. Conidiophore and conidia of *Ceratocystis douglasii*. Fig. 16. Apex of conidiophore showing percurrent extensions. Scale bar = 20 μm. Fig. 17. Chains of elongate conidia becoming slightly barrel shaped. Scale bar = 20 μm. Fig. 18. Older, barrel-shaped to round conidia, in some cases with thickened and darkly pigmented walls. Scale bar = 20 μm.



and very variable in length, 7–17 μm ($\bar{x}$ = 10 μm) long and 5–10 μm ($\bar{x}$ = 7 μm) wide, becoming more obviously barrel-shaped with maturity and often developing pigmented walls.

MATERIAL EXAMINED: HOLOTYPE: Caribou Creek, East Nelson, British Columbia, Canada, from *Picea engelmannii* infested with *D. rufipennis*, November 1992. Coll. H. Solheim, C 608, 252/8, DAOM 222349. PARATYPE. Caribou Creek, East Nelson, British Columbia, Canada, from *Picea engelmannii* infested with *D. rufipennis*, November 1992. Coll. H. Solheim, C 609, 252/8, DAOM 222350; Caribou Creek, East Nelson, British Columbia, Canada, from *Picea engelmannii* infested with *D. rufipennis*, November 1992. Coll. H. Solheim, C 610, 280/7, DAOM 222351; Caribou Creek, East Nelson, British Columbia, Canada, from *Picea engelmannii* infested with *D. rufipennis*, November 1992. Coll. H. Solheim, C 611, 258/8, DAOM 222352; McGregor River, Prince George, British Columbia, Canada, from *Picea glauca* infested with *D. rufipennis*, February 1993. Coll. H. Solheim, C 613, 404/2, DAOM 222353.

Morphological characters as well as isozyme data (Harrington et al. 1996) for the fungus known as *E. coerulescens* f. *douglasii* are quite distinct. It appears to occur commonly on Douglas-fir lumber in western North America. We elect to raise the status of this fungus from form to species level and redescribe it as follows:

Ceratocystis douglasii (Davidson) Wingfield & Harrington comb. et stat.nov. Figs. 9–18

≡ *Endoconidiophora coerulescens* Munch f. *douglasii*, Mycologia 45: 584. 1953. (Basionym)

Emended description: Relatively slow growing on MEA, with a growth optimum between 20 and 25°C reaching 24 mm diameter in 5 days. No growth occurring at 30°C. Colony light coloured initially and becoming dark greenish olive (Ridgeway 1912; plate XXX) with age, with virtually no aerial mycelium. Perithecia (Fig. 9) only observed in culture after extended incubation at 10°C but abundant on wood, robust, with black (olive green) bases 160–320 μm ($\bar{x}$ = 245 μm) wide and ornamented (Fig. 11) with distinct, short, pigmented and tapered hyphae 60–140 μm ($\bar{x}$ = 138 μm) long. Necks black at the base, 610–1080 μm ($\bar{x}$ = 788 μm) long, tapering slightly to a somewhat lighter apex, 10–13 μm ($\bar{x}$ = 12 μm) wide at the apex, 17–29 μm ($\bar{x}$ = 23 μm) wide at the middle and terminating in a crest of ostiolar hyphae (Fig. 10) 14–25 μm ($\bar{x}$ = 19 μm) long and 1–2 μm ($\bar{x}$ = 2 μm) wide. Ascospores (Fig. 12) elongate to slightly curved with rounded ends and surrounded by a distinct outer layer or sheath, 5–9 μm ($\bar{x}$ = 7 μm) long and 4–6 μm ($\bar{x}$ = 3 μm) wide excluding sheaths, which average 1 μm at each end of the spores. Conidiophores typical of *Chalara* species, brown, erect and of two distinct kinds. Long, flexuous (Fig. 13) and narrow conidiophores tending to be slightly curved and tapering gradually to a relatively fine apex 264–560 μm ($\bar{x}$ = 362 μm) long and 5–7 μm ($\bar{x}$ = 6 μm) wide, usually seen only on the natural substrate. Short brush-like conidiophores (Fig. 14) abundant both on wood and in culture, 72–320 μm ($\bar{x}$ = 183 μm) long and 5–8 μm ($\bar{x}$ = 6 μm) wide, often with torn apertures (Fig. 14), indicative of the production of conidia (Fig. 15) larger in diameter than the conidiogenous cell apex. Conidiogenous cells often extending (Fig. 16) at least once to give rise to a new conidiogenous locus. Conidia (Figs. 15 and 17) very variable in length, probably correlated with the length of conidiophores from which they originate, cylindrical, hyaline, with two attachment points and produced in chains;

6–21 μm ($\bar{x}$ = 13 μm) long and 5–7 μm ($\bar{x}$ = 6 μm). With age, conidia becoming barrel-shaped to round and often thick-walled and dark in colour (Fig. 18).

HOLOTYPE: BPI 59527, also known as F.P. 70703, Ft. Collins, Colorado, USA, from lumber of *Pseudotsuga menziesii* shipped from Oregon, collected by R.W. Davidson, 1953. PARATYPES: IMI 56862 and J. Hunt 150 (Herbarium of the State University of New York, College of Forestry, Syracuse, New York, USA). OTHER MATERIAL EXAMINED: BPI 595726, also known as J. Hunt 144, Bellingham, Washington, USA, from wood of *Pseudotsuga menziesii*, collected by R.W. Davidson, 1953; IMI 56851, also known as J. Hunt 25 (Herbarium of the State University of New York), from wood of *Pseudotsuga menziesii*; IMI 47571, on wood of *Pseudotsuga menziesii*, collected by D. Wells, 13.10, 1949. Culture from the holotype deposited at the Centraalbureau voor Schimmelcultures as CBS 142.53 and in the collection of T.C. Harrington as C 324; DAOM 222362 is a dried culture of CBS 142.53.

Discussion

In this study, we have characterized two species of *Ceratocystis* belonging to a group of fungi that we consider to represent a complex of morphologically similar but distinct taxa, the *Ceratocystis coerulescens* complex. We recognise that other taxa in the group have yet to be studied and described (Harrington et al. 1996). Davidson (1953) was aware of the fact that *C. douglasii* was distinct from *C. coerulescens* and described it as a new form. Similarly, Davidson (1955) noted unique features of the form of *C. coerulescens* associated with *D. rufipennis*. With better understanding of the ecology of *Ceratocystis* and with the isozyme analyses of the *C. coerulescens* complex (Harrington et al. 1996), we can now recognize Davidson's fungi as distinct species.

The two species described here have various features in common that distinguish them from other members of *Ceratocystis* on conifers. Both species have very robust ascomata with bases wider than those found in other isolates of the *C. coerulescens* complex. The perithecia also have necks longer than in other species and the new species grow poorly and fail to produce perithecia at room temperature.

Despite their similarities, *C. rufipenni* can be distinguished from *C. douglasii* on the basis of numerous morphological characteristics. *Ceratocystis rufipenni* has ostiolar hyphae that are almost twice the length of those found in *C. douglasii*. It also has distinctive ascospores with a well defined guttule at either end. Ascospore sheaths in *C. rufipenni* are better defined and about twice as long as those in *C. douglasii*. Conidiophores are common in cultures of *C. douglasii* and also develop profusely on the surface of colonised wood. In contrast, *C. rufipenni* produces conidiophores sparingly, often only in association with ascomata. Another important feature of *C. douglasii* is that it produces two distinct forms of conidiophore, long flexuous conidiophores that are usually only seen on colonized wood and short, brush-like conidiophores found both in culture and on the natural substrate.

Although neither species is well studied, *Ceratocystis rufipenni* and *C. douglasii* appear to differ in their ecology. *Ceratocystis douglasii* is reported as a common saprophyte, specifically on Douglas-fir timber. In contrast, *C. rufipenni* is a pathogen on spruce that is closely associated with an important bark beetle (Davidson 1955; Solheim 1995). *Ceratocystis douglasii* produces a strong, fruity aroma in culture, which may be important in attracting insects vectors in nature. This feature is absent in our cultures of *C. rufipenni*, and it would probably be unnecessary for a *Ceratocystis* species that sporulates in bark beetle galleries (Harrington et al. 1996). Abundant conidiophores appear to be a well developed feature in *C. douglasii*, and conidia dispersed by insects are probably important for spermatization. Development of ascomata in *C. rufipenni* appears to be triggered by wounding which may be another derived feature of bark beetle associates (Harrington et al. 1996).

The description of *C. rufipenni* has provided us with a third example of a *Ceratocystis* sp. that is closely associated with a specific bark beetle vector. The overwhelming majority of Ophiostomatoid fungi that are carried by Scolytid bark beetles are species of *Ophiostoma* and related anamorphs (Upadhyay 1981; Harrington and Cobb 1988; Wingfield et al. 1993). Until recently, the only recognised example of a *Ceratocystis* carried by a bark beetle was *C. laricicola*, which is associated with *Ips cembrae* Heer (Redfern et al. 1987). More recently, it has been discovered that *O. polonicum*, which is closely associated with *Ips typographus* (Furniss et al. 1990; Solheim 1992*a*), is a species of *Ceratocystis* (Visser et al. 1995) and also occurs together with a number of other *Ophiostoma* spp. (Solheim 1986; Solheim 1992*b*). This fungus is the only *Ceratocystis* sp., and also the most virulent pathogen among the fungi carried by *I. typographus* (Horntvedt et al. 1993; Solheim 1988, 1993). *Ceratocystis laricicola*, which is very similar to *C. polonica* (Visser et al. 1995; Harrington et al. 1996), also appears to be a highly virulent pathogen (Redfern et al. 1987). Similarly, *C. rufipenni* appears to be considerably more virulent than other Ophiostomatoid fungi occurring in association with *D. rufipennis* (Solheim and Safranyik 1997). We therefore believe that these bark beetle associated *Ceratocystis* spp. and the wood-staining *Ceratocystis* sp. on conifers are important from the ecological, biogeographical, and evolutionary biology standpoints.

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