The *Ceratocystis* species on conifers[1]

Thomas C. Harrington and Michael J. Wingfield

Abstract: Seven *Ceratocystis* species are recognized as having conifers as their primary hosts, and these species comprise a monophyletic group. Despite being morphologically similar, these taxa are distinguished by intersterility and isozyme electromorphs. The first described species in the group, *Ceratocystis coerulescens* (Münch) Bakshi, is neotypified; it causes bluestain of *Picea* and *Pinus* in Europe and North America. A similar species, *Ceratocystis pinicola* sp.nov., also causes bluestain but has thus far been recorded only on *Pinus* in Britain. In contrast, *Ceratocystis resinifera* sp.nov. has been recorded from *Picea* on continental Europe and North America and invades wounds of living trees. *Ceratocystis polonica* (Siemaszko) C. Moreau is neotypified and distinguished ecologically from *Ceratocystis laricicola* Redfern & Minter; both species occur across Eurasia and are associated with *Ips* bark beetles on *Picea* and *Larix*, respectively. A key is presented for these species as well as the recently recognized species from North America, *Ceratocystis rufipenni* Wingfield, Harrington, & Solheim and *Ceratocystis douglasii* (Davidson) Wingfield & Harrington, which occur on *Picea* and *Pseudotsuga*, respectively.

Key words: *Ceratocystis*, Pinaceae, bluestain, Scolytidae.

Résumé : On reconnait sept espèces de *Ceratocystis* ayant des conifères comme hôte primaire, et ces espèces constituent un groupe monophylétique. Bien qu'ils soient morphologiquement similaires, on peut distinguer ces taxons par leur interstérilité et par leurs électromorphes isozymiques. Les auteurs proposent un néotype pour la première espèce décrite dans ce groupe, le *Ceratocystis coerulescens* (Münch) Bakshi, causant le bleuissement chez des *Picea* et *Pinus* en Europe et en Amérique du Nord. Une espèce similaire, le *Ceratocystis pinicola* sp.nov., cause également le bleuissement mais n'a été jusqu'ici observée que sur des *Pinus* en Grande Bretagne. Au contraire, le *Ceratocystis resinifera* sp.nov. a été observé sur des *Picea* en Europe continentale et en Amérique du Nord et infecte les blessures d'arbres vivants. Les auteurs proposent un néotype pour le *Ceratocystis polonica* (Siemaszko) C. Moreau qui se distinguent écologiquement du *Ceratocystis laricicola* Redfern & Minter, ces deux espèces se retrouvant dans l'ensemble de l'Eurasie et étant associées avec des *Ips* corticoles venant sur *Picea* et *Larix*, respectivement. Les auteurs présentent une clé pour ces espèces ainsi que pour les espèces récemment reconnues en Amérique du Nord, le *Ceratocystis rufipenni* Wingfield, Harrington, & Solheim et le *Ceratocystis douglasii* (Davidson) Wingfield & Harrington, qui se retrouvent sur les *Picea* et les *Pseudotsuga*, respectivement.

Mots clés : *Ceratocystis*, Pinaceae, bleuissement, Scolytidae.

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Introduction

As currently recognized, the genus *Ceratocystis* Ellis & Halsted sensu stricto represents a relatively small group of plant parasites, occurring primarily on angiosperms (Kile 1993). Excluding the morphologically similar but phylogenetically unrelated *Ophiostoma* (Harrington 1987; Hausner et al. 1993; Spatafora and Blackwell 1994), we recognize seven species of *Ceratocystis* that occur primarily on conifers, all on members of the Pinaceae. These seven species are economically important in that they all appear capable of staining sapwood blue to gray, at least some of the species can colonize the living sapwood of wounded trees, and three of the species are important symbionts of bark beetles that aid the beetles in killing trees (Harrington et al. 1996; Paine et al. 1997). These seven species are morphologically similar and share a number of common isozyme electromorphs (Harrington et al. 1996). They also appear to be closely related to four species that occur on hardwoods, namely, *Ceratocystis virescens* (Davidson) C. Moreau, which is found on *Acer* and other tree species in eastern North America (Davidson 1944) and three Australasian species: *Ceratocystis eucalypti* Kile et al. (Kile et al. 1996) and the anamorphic species *Chalara australis* Kile & Walker and *Chalara neocalidoniae* Kiffer & Delon (Harrington et al. 1996). The hardwood and conifer species can be separated based on conidiophore morphology (Harrington et al. 1996). Analysis of the DNA sequences of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (Witthuhn et al. 1998) grouped the seven conifer species into a single clade, and the hardwood species were basal to the conifer species. From these data, it appears that adaptation to coni-

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fers as primary hosts occurred once in the genus *Ceratocystis*.

The first described *Ceratocystis* species on conifers was *Cerstocystis coerulescens* (Münch) Bakshi (Münch 1907), and we have referred to the seven conifer species and the four related hardwood species as the *C. coerulescens* complex (Harrington et al. 1996). The *MAT-2* strains of the seven conifer species and of *C. virescens* are capable of selfing because of unidirectional mating type switching (Harrington and McNew 1997), so tests for interfertility have proven difficult, but these species do appear to be intersterile (Harrington and McNew 1998). The conifer species also appear to occupy distinct ecological niches (Harrington et al. 1996). Isozyme analysis (Harrington et al. 1996) and DNA sequence analysis (Witthuhn et al. 1998) clearly separate all but *Ceratocystis laricicola* Redfern & Minter and *Ceratocystis polonica* (Siemaszko) C. Moreau, which have been shown to differ at a single isozyme locus and appear to be specialized to larch (*Larix*) and spruce (*Picea*), respectively. Thus, species limits among the seven conifer species are quite clear based on ecology and phylogeny, but they remain difficult to distinguish based on morphology.

Two species previously referred to as *C. coerulescens* were recently recognized (Wingfield et al. 1997), and the data above support the distinction of two additional new species. We herein designate a specimen of *C. coerulescens* sp. B (Harrington et al. 1996) as the neotype of *C. coerulescens* and describe *C. coerulescens* sp. A and *C. coerulescens* sp. C as new. *Ceratocystis polonica* is also neotypified. We provide a key to the seven conifer species based on morphology and substrate.

Materials and methods

Isolates studied

The origins of isolates were listed in Harrington et al. (1996). *Ceratocystis coerulescens* sp. A in that publication corresponds to *Ceratocystis pinicola* sp.nov. described here; a specimen of *C. coerulescens* sp. B is used as the neotype of *C. coerulescens*; *C. coerulescens* sp. C is described here as *Ceratocystis resinifera* sp.nov.; *C. coerulescens* sp. D is now *Ceratocystis rufipenni* Wingfield, Harrington, & Solheim and *C. coerulescens* f. *douglasii* is now *Ceratocystis douglasii* (Davidson) Wingfield & Harrington. All isolates studied are in the collection of the senior author, and their three-digit accession number is preceded with a "C." Representative isolates of all species, including those from holotypes, have been deposited in the Centraalbureau voor Schimmelcultures (CBS).

Selected isolates of *C. coerulescens*, *C. resinifera*, *C. pinicola*, and *C. polonica* were grown on 2% malt extract agar (MEA; 20 g agar and 20 g malt extract in 1000 mL water) with or without sections of pine twigs and incubated at room temperature (20–25°C) in the dark until the onset of sporulation. Fruiting structures were mounted in lactophenol. Neotype and holotype materials have been deposited in DAOM as microscope slides.

Growth rate

Growth studies of selected isolates were also conducted on 2% MEA. Isolates were first grown on MEA for approximately 1 week. Discs 3 mm in diameter were cut from the actively growing colony margins and placed at the center of 60-mm Petri dishes, with three replicates per isolate, and incubated for 5 days in darkness at temperatures ranging from 10 to 35°C. Colony diameters were measured at right angles and the average growth rate calculated.

Key to conifer species of *Ceratocystis*

1. Ascospores surrounded by an uneven sheath, in top view wider at the sides than at the ends
   2. Associated with bark beetles on *Picea* . . . . . *C. polonica*
   2. Associated with bark beetles on *Larix* . . . . . *C. laricicola*
1. Ascospore sheaths even around the spore, in top or side view about the same width or wider at the ends than at the sides
   3. Ascospores averaging more than 7.0 µm long excluding sheath, perithecial necks greater than 600 µm long, on *Picea* or *Pseudotsuga* in North America
      4. Conidiophores abundant in culture, on *Pseudotsuga* . . . . . *C. douglasii*
      4. Conidiophores rare in culture, on *Picea* . . . . . *C. rufipenni*
   3. Ascospores averaging less than 7.0 µm long excluding sheath, perithecial necks less than 600 µm long, or if greater than 600 µm then on *Pinus*, in North America or Europe
      5. Perithecial bases less than 120 µm in width, rarely up to 150 µm . . . . . *C. coerulescens*
      5. Perithecial bases 120 µm in width or more
         6. Perithecial necks greater than 550 µm in length, averaging over 700 µm, ascospores averaging 5.0 µm (4.0–6.4 µm) in length excluding sheath, on *Pinus* . . . . . *C. pinicola*
         6. Perithecial necks less than 550 µm, rarely up to 750 µm, ascospores averaging 6.2 µm (5.2–7.2 µm) in length excluding sheath, on *Picea* . . . . . *C. resinifera*

Description of species

Ceratocystis coerulescens (Münch) Bakshi, Trans. Br. Mycol. Soc. 33: 114. 1950. (Figs. 1–8)

≡*Endoconidiophora coerulescens* Münch, Naturwiss. Z. Forst. Landw. 5: 564. 1907.

≡*Ophiostoma coerulescens* (Münch) Nannf., Sv. Skogsvardsf. Tidskr. 32: 408, 1934.

EMENDED DESCRIPTION:: Colonies on 2% MEA relatively slow growing with an optimum growth at 20°C in the dark. Colonies attaining a diameter of 36 mm at 20°C and 31 mm at 25°C after incubation for 5 days in the dark. Colonies varying in color but mostly deep olive (Ridgeway 1912, plate XL) to grayish olive (plate XLVI), growing appressed with little or no aerial mycelium. Single ascospores giving rise to both self-sterile and self-fertile colonies in equal proportions, with perithecia being produced sparsely in culture in approximately 3 weeks, particularly in association with sterile pine wood in the medium. Perithecia dark with bases composed of a limited layer of cells making them lighter in color than the bases of the necks. Bases round to ovoid, 96–128 µm (mean 98 µm) in diameter, giving rise to distinct basal spines 70–220 µm (mean 121 µm) long. Necks dark and tapering distinctly to the apex, 290–600 µm (mean 490 µm) long, 12–30 µm (mean 19 µm) wide at the middle, and 10–14 µm (mean 11 µm) wide at the apex, terminating in a crest of ostiolar hyphae 9–20 µm (mean 18 µm) long and 1–2 µm (mean 2 µm) wide. Ascospores accumulating at the apices of mature perithecia, elongate to slightly curved with round ends and surrounded by a distinct translucent sheath layer, 5–7 µm (mean 6 µm) long and 2–3 µm (mean 2 µm) wide excluding sheaths that are 0.4–1.0 µm long at either end of the ascospores. Conidiophores tubular, typical of *Chalara* species, rectangular, terminating in a cylindrical and sometimes slightly flared collarette, 110–240 µm (mean 178 µm) long and 3–5 µm (mean 5 µm) wide. Conidia rectangular with two attachment points and produced by ring wall building development in distinct chains, variable in length 6–16 µm (mean 10 µm) long and 3–5 µm (mean 4 µm) wide.

NEOTYPE: Itasca County, Minnesota, from *Pinus banksiana* Lamb., collected by R.N. Campbell, 1956, DAOM 225445, from isolate C301 (= ATCC 12859 and CBS 100198).

OTHER MATERIAL EXAMINED: DAOM 225446, a dried specimen of a self-fertile strain (DM173–17) derived from a cross of isolate C301 and C693 (C693 = CBS 489.80, as *Chalara ungeri* Sacc., from Helsinki, Finland, isolated from discolored pine and spruce logs by A. Lilja).

CULTURES EXAMINED: Rotterdam, Netherlands, from moist timber, collected by Kneteman, isolate C320 (= CBS 137.34, West Germany, on *Picea abies* (L.) Karst. wood, determined by D.T. Rhode, isolate C313 (= C695 and CBS 140.37).

Münch (1907) described and illustrated *C. coerulescens* from darkly stained pine (*Pinus*) and spruce (*Picea*) in Germany, but no dried material from his examinations is available to use as a holotype (Hunt 1956). His description could fit either of two species that we (Harrington et al. 1996) referred to as *C. coerulescens* sp. B or sp. C, respectively, which are clearly distinct species based on mating studies (Harrington and McNew 1998), DNA sequence data (Witthuhn et al. 1998), and isozymes (Harrington et al. 1996). Morphologically, however, it is difficult to separate these two species. We have relied primarily on the width of the perithecium base to separate *C. coerulescens* sp. B and sp. C, but Münch (1907) did not give that dimension for his fungus. It appears that *C. coerulescens* sp. C is more common than *C. coerulescens* sp. B in colonizing wounds of living spruce, and *C. coerulescens* sp. B may be more common as a bluestain fungus on dead wood or cut timber (Harrington et al. 1996). Münch's (1907) report of *C. coerulescens* as a bluestain fungus on spruce and pine in central Europe leads us to believe that *C. coerulescens* sp. B of Harrington et al. (1996) is Münch's species, and his description of *C. coerulescens* has thus been emended. *Ceratocystis coerulescens* sp. C is described as new, *C. resinifera*.

Thus defined, *Ceratocystis coerulescens* is morphologically similar to *C. resinifera* but differs in having perithecial bases that are smaller and lighter in color. The perithecial necks, from the perithecial base to the ostiole, tend to taper more in *C. coerulescens* than in *C. resinifera*. Also, isolate ATCC 12859 (C301) of *C. coerulescens* grew only half as fast at 25°C as did isolate C662 of *C. resinifera* (colony diameters at 5 days were 30 and 66 mm, respectively). Both *C. coerulescens* and *C. resinifera* have been found in North America and Europe, but the former has been found on both *Picea* and *Pinus*, while the latter has thus far been found only on *Picea*.

We have no teleomorph material of *C. coerulescens* from central Europe, and the only isolate (ATCC 12859) that produces perithecia and ascospores in culture originated in the United States (Campbell 1957). The dried specimen designated as the neotype of *C. coerulescens* has perithecia with narrower bases and necks that taper more than in *C. resinifera*. However, perithecial sizes are often highly variable in *Ceratocystis* species, and these differences may not be apparent when a wider range of material is examined. Indeed, Campbell (1957), who deposited ATCC 12859, reported perithecial bases 130–210 µm (mean 175 µm) wide for *C. coerulescens*, but this range may have been based on more than just isolate ATCC 12859. Our measurements of perithecia produced by isolate ATCC 12859 are 96–128 µm (mean 98 µm) wide. We also examined perithecia produced from a cross between ATCC 12859 and C693, and this, too, had perithecia with narrower bases (less than 150 µm) than those described by Campbell (1957) and those found in *C. resinifera*.

Other dimensions of perithecia and ascospores reported for *C. coerulescens* may have been based on observations of both *C. coerulescens* and *C. resinifera*. Other European reports of *C. coerulescens* were as a bluestain fungus on pine and spruce (Siemaszko 1939) and primarily as a wound colonizer of living spruce (Lagerberg et al. 1927). Siemaszko's (1939) description may have been of *C. coerulescens*, with perithecial bases reported at 126–182 µm wide. Lagerberg et al. (1927) reported bases of 168–208 µm in width, slightly larger than those of Siemaszko's (1939) and closer to those we found in *C. resinifera*. Reports of *C. coerulescens* from Britain (Bakshi 1951) are believed to be of *C. pinicola*,

Figs. 1–8. *Ceratocystis coerulescens* (neotype). Fig. 1. Perithecium. Scale bar = 200 µm. Fig. 2. Ostiolar hyphae at apex of perithecial neck. Scale bar = 20 µm. Fig. 3. Base of perithecium showing hyphal ornamentation. Scale bar = 100 µm. Fig. 4. Peridium of the perithecial base. Scale bar = 20 µm. Fig. 5. Ascospores. Scale bar = 8 µm. Fig. 6. Conidia. Scale bar = 20 µm. Fig. 7. Conidiophore with conidium emerging from collarette. Scale bar = 20 µm. Fig. 8. Older, barrel-shaped conidia that have become darkened and thick-walled. Scale bar = 20 µm.

Figs. 9–15. *Ceratocystis resinifera* (holotype). Fig. 9. Perithecium. Scale bar = 200 µm. Fig. 10. Base of perithecium showing hyphal ornamentation. Scale bar = 100 µm. Fig. 11. Ostiolar hyphae at apex of perithecial neck. Scale bar = 50 µm. Fig. 12. Ascospores. Scale bar = 13 µm. Figs. 13–14. Conidiophores with conidia. Scale bars = 50 and 20 µm, respectively. Fig. 15. Conidia. Scale bar = 20 µm.

which has smaller ascospores than *C. coerulescens* and *C. resinifera*. Other reported dimensions of perithecia and ascospores of *C. coerulescens* (Hunt 1956; Griffin 1968; Upadhyay 1981) are clearly from measurements of two or more taxa, including the more distantly related *C. virescens*.

Münch (1907) referred to the anamorph of *E. coerulescens* as *Ch. ungeri* based on a single collection (Unger 1847). Nag Raj and Kendrick (1975), in their monographic work on *Chalara*, determined that type material of this species no longer exists, and they designated a neotype (IMI 20164) for *Ch. ungeri* based on a collection from blued pine wood in Scotland. Unfortunately, the anamorphs of the species treated here are very similar, and in the absence of a reasonable sample of teleomorph material or molecular comparisons, we are unable to confidently separate *C. coerulescens* from *C. pinicola*, and *C. resinifera*. However, the neotype (Nag Raj and Kendrick 1975) of the anamorph *Ch. ungeri* also has perithecia, and these appear to be those of *C. pinicola*, which may not occur in central Europe. Thus, we do not believe that the neotype of *Ch. ungeri* represents the anamorph of *C. coerulescens* sensu Münch (1907).

Ceratocystis resinifera Harrington & Wingfield sp.nov. (Figs. 9–15)

Coloniae in malti agaro, comparate celeriter crescentes, optimo incremento ad 20°C in tenebris. Coloniae attingentes diametrum medium 69 mm ad 20°C et 66 mm ad 25°C post quinque dierum incubationem. Perithecia atris basibus, 120–240 µm (res media 175 µm) lata gignentiaque distinctas basilares spinas, 84–160 µm (res media 128 µm) longas. Perithecialia colla atra, 420–540 µm (res media 490 µm) longa, exique attenuata versus apicem, 14–22 µm (res media 18 µm) lata in medio et 10–14 µm (res media 11µm) lata ad apicem, terminantia in cristam divergenter dispositarum ostiolarium hypharum, 10–28 µm (res media 17 µm) longarum et 1–2 µm (res media 1.5 µm) latarum. Ascosporae 5–7 µm (res media 6.2 µm) longae et 2–3 µm (res media 2.0 µm) latae vaginis exclusis. Longitudo vaginae ad alterutum extremum sporarum 0.8 µm.

HOLOTYPUS: Aasa, Norvegia, ex vulnerata arbore *Picea abiete*, 1986, lectus ab H. Solheim, DAOM 225449, ex segregato C662, quod quoque appellatur CBS 100202.

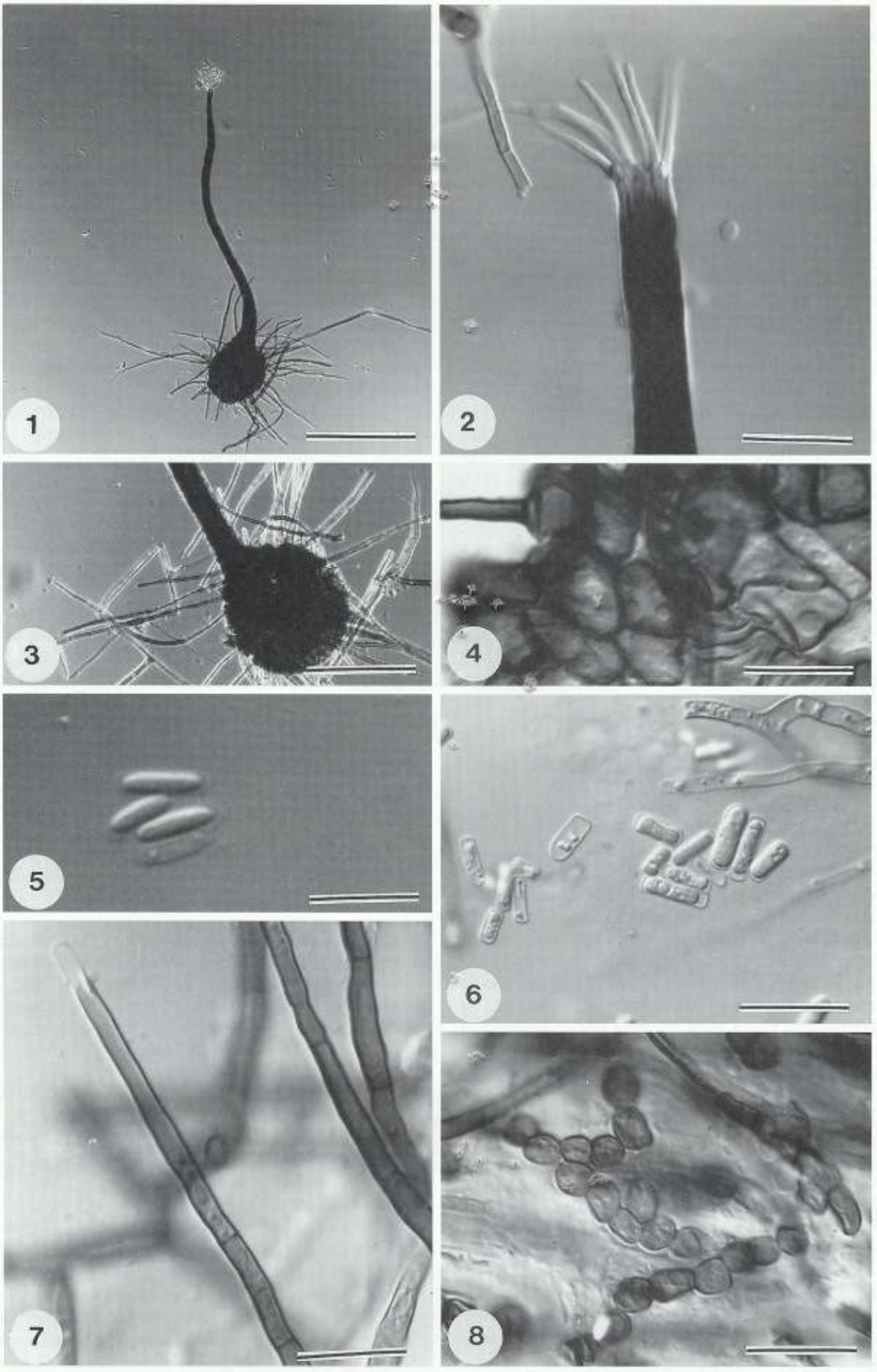
Colonies on MEA relatively fast growing, with an optimum growth at 20°C in the dark. Colonies attaining an average diameter of 69 mm at 20°C and 66 mm at 25°C after 5 days of incubation. Colonies varying in color but mostly deep olive (Ridgeway 1912, plate XL) to grayish olive (plate XLVI), with little or no aerial mycelium, except around perithecia. Single ascospores giving rise to both self-sterile and self-fertile colonies in equal proportions, but perithecia produced in culture develop slowly over approximately 1 month, particularly when sterile pine wood is added to the culture medium. Perithecia with black bases, 120–240 µm (mean 175 µm) wide and giving rise to distinct basal spines, 84–160 µm (mean 128 µm) long. Perithecial necks black, 420–540 µm (mean 490 µm), tapering slightly towards the apex, 14–22 µm (mean 18 µm) wide in the middle and 10–14 µm (mean 11 µm) wide at the apex, terminating in a crest of divergently arranged ostiolar hyphae 10–28 µm (mean 17 µm) in length and 1–2 µm (mean 1.5 µm) wide. Ascospores accumulating at the apex of necks of perithecia, elongate to ellipsoid with distinct outer sheaths. Ascospores 5–7 µm (mean 6.2 µm) long and 2–3 µm (mean 2.0 µm) wide excluding sheaths. Sheath length at either end of spores 0.8 µm. Conidiophores typical of the genus *Chalara*, single, septate, terminating in discrete tubular and sometimes slightly flared collarette, produced profusely on the agar surface and of variable length, 80–232 µm (mean 162 µm) long and 3–6 µm (mean 4.3 µm) wide. Conidia produced by ring wall building, rectangular and of very variable size, 4–22 µm (mean 11.9 µm) long and 5–6 µm (mean 5.6 µm) wide and becoming swollen and barrel shaped to ovoid with age.

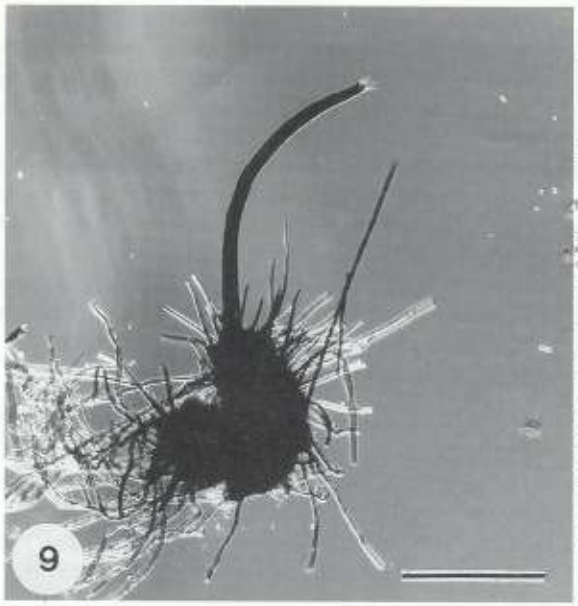
MATERIAL EXAMINED: HOLOTYPE: Ås, Norway, from wounded *Picea abies* tree, 1986, collected by H. Solheim, DAOM 225449, from isolate C662 (= CBS 100202).

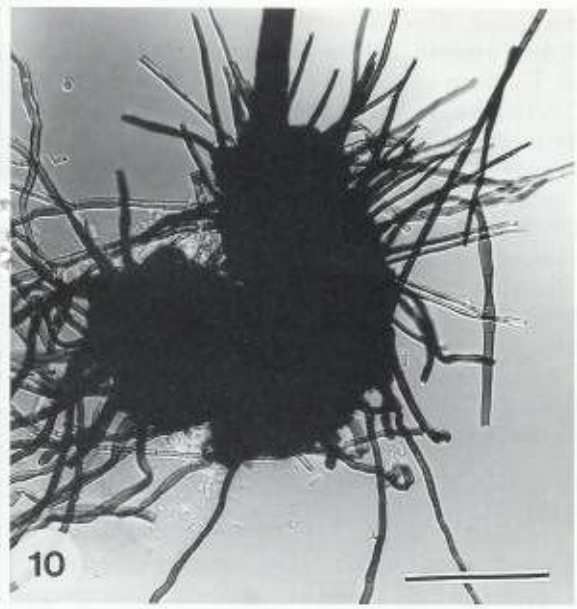
OTHER MATERIAL EXAMINED: DAOM 225450, a dried specimen of a self-fertile strain (DM150–9) derived from a cross between self-sterile progeny of C662 and C665, the latter collected at Hurdal, Akerhus, Norway, from wounded stem of *Picea abies*, 17 July 1966, by H. Roll-Hansen (= C276, NFRI 66–157/21, and ATCC 44993).

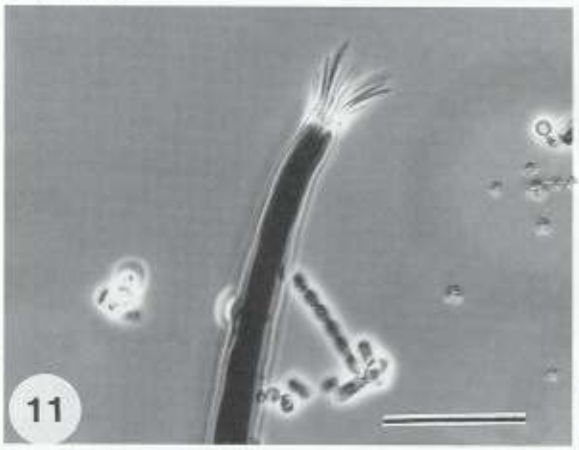
CULTURES EXAMINED: Nannestad, Akerhus, Norway, from *Picea abies* log, 27 August 1958, collected by H. Roll-Hansen, C666 (= C277 and NFRI 1750/2); Norway, from *Picea abies* log, 1986, collected by H. Solheim, isolate C278 (= NFRI 86–434/9); New Mexico, U.S.A., from bluestained *Picea engelmannii* Parry, 1971, collected by T. Hinds, isolate C50 (= CO381, CMW 451, and CBS 100204).

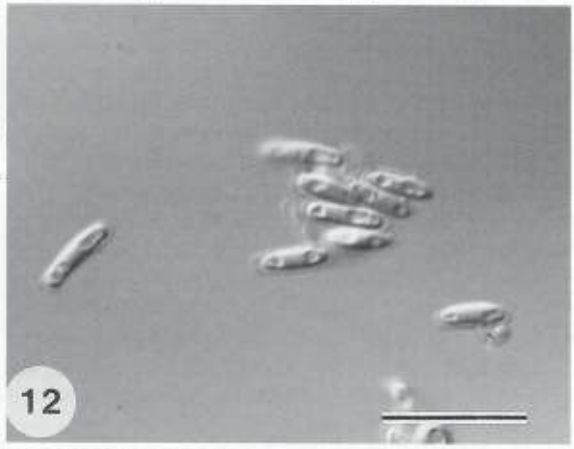
Ceratocystis resinifera, like *C. coerulescens*, has been found within continental Europe and North America but, thus far, only on *Picea*. At least two of the isolates examined were from sapwood near wounds on living trees, and *C. resinifera* may be more pathogenic than *C. coerulescens*, though, to our knowledge, the pathogenicity of these species

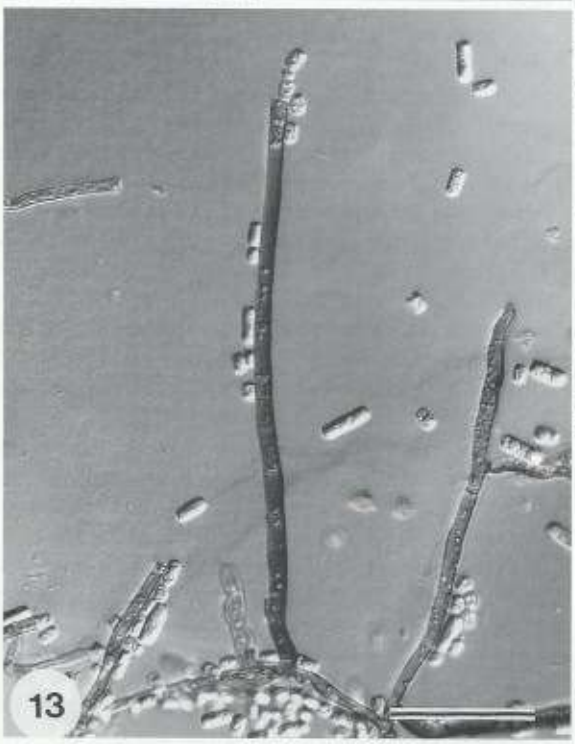


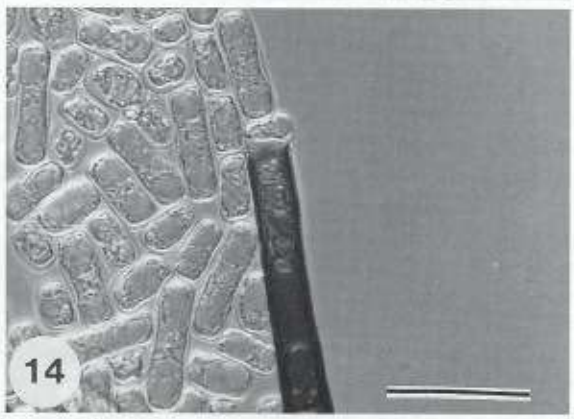












has not been tested. Reports from Scandinavia (Lagerberg et al. 1927; Roll-Hansen and Roll-Hansen 1980) of wound colonization in *Picea abies* by *C. coerulescens* are most likely of *C. resinifera*. As stated above, *C. resinifera* is morphologically most similar to *C. coerulescens* but generally has wider and rounder perithecial bases, less tapering perithecial necks, and grows twice as fast as *C. coerulescens* at 25°C. It differs from *C. pinicola* in its shorter perithecial necks and longer ascospores, though there is considerable overlap in these dimensions. Mating studies (Harrington and McNew 1998) show *C. resinifera* to be intersterile with the other *Ceratocystis* species, and DNA sequence analysis (Witthuhn et al. 1998) and isozymes markers (Harrington et al. 1996) show *C. resinifera* to be more closely related to *C. rufipenni* than to *C. coerulescens* or *C. pinicola*.

Ceratocystis pinicola Harrington & Wingfield, sp.nov. (Figs. 16–22)

Coloniae in 2% malti extracti agaro celeriter crescentes, incremento optimo ad 25°C. Coloniae attingentes diametrum medium 54 mm ad 20°C et 66 mm ad 25°C post quinque dierum incubationem in tenebris. Perithecia atra, basibus variantibus ab 120–240 µm (res media 195 µm), constituta ex stratis nonnullis cellarum fuscarum vel nigrarum et gignentes distinctas basilares spinas, 70–300 µm (res media 162 µm) longas. Colla 560–880 µm (res media 745 µm) longa, 10–18 µm (res media 14 µm) lata ad medium et exique attenuata versus apicem 8–12 µm (res media 10 µm) latum. Colla terminant in cristam 6–12 divergente dispositarum ostiolarium hypharum, 8–13 µm (res media 12 µm) longarum et 1.6–2.0 µm (res media 1.7 µm) latarum. Ascosporae aggregantes in massas gloeoideas ad apices peritheciorum maturorum, productae vel ellipsoideae et exigue curvatae, extremis distincte rotundatis, cinctae vagina distincta visa translucida per microscopicum opticum, 4–6 µm (res media 5.0 µm) longae et 1.6–2.4 µm (res media 1.7 µm) latae vagina exclusa, quae 0.8–1.2 µm (res media 0.8 µm) lata est ad alterutrum ascosporae extremum.

HOLOTYPUS: DAOM 225447, specimen siccatum ex hybrida inter duo segregata: C488, quoque appellatum CBS 100199, ex Latifundio Vindesoriensi, Barcheria, Anglia, *Pinus sylvestris* L., lectum a J. Gibbs 17 Mai 1988, et C490, quoque appellatum CBS 100200, Silva Lynfordiensis, Anglia, ex *Pinus* sp., lectum a J. Gibbs, 12 Aprilis 1988.

Colonies on 2% MEA fast growing, with an optimum growth at 25°C. Colonies attain an average diameter of 54 mm at 20°C and 66 mm at 25°C after 5 days of incubation in the dark. Colonies dark greenish olive (Ridgeway 1912, plate XXX), growing appressed with relatively little aerial mycelium. Single ascospores give rise to both self-sterile and self-fertile isolates. Perithecia black, with bases ranging from 120–240 µm (mean 195 µm), composed of several layers of dark brown to black cells and giving rise to distinct basal spines 70–300 µm (mean 162 µm) long. Necks 560–880 µm (mean 745 µm) in length, 10–18 µm (mean 14 µm) wide at the middle and tapering slightly to the apex, 8–12 µm (mean 10 µm) wide. Necks terminate in a crest of 6–12 divergently arranged ostiolar hyphae, 8–13 µm (12 µm) long and 1.6–2.0 µm (mean 1.7 µm) wide. Ascospores accumulating in gloeoid masses at the apices of mature perithecia, elongate to ellipsoidal and slightly curved in shape, with distinctly rounded ends, surrounded by a distinct sheath appearing to be translucent using light microscopy; 4–6 µm (mean 5.0 µm) long and 1.6–2.4 µm (mean 1.7 µm) excluding sheath, which is 0.8–1.2 µm (mean 0.8 µm) wide at either end of the ascospore. Conidiophores produced profusely on the surface of the medium, highly variable in length, 140–400 µm (mean 290 µm) long, and 4–6 µm (mean 5.1 µm) wide. Conidia rectangular, with two points of attachment and produced by ring wall building development in distinct chains, 6–31 µm (mean 14 µm) long and 3–6 µm (mean 4 µm) wide. With maturity, conidia may become dark colored and barrel shaped to round.

MATERIAL EXAMINED: HOLOTYPE: DAOM 225447, a dried specimen from a cross between two isolates: C488 (= CBS 100199), from Windsor Estate, Berkshire, England, *Pinus sylvestris*, collected by J. Gibbs, 17 May 1988, and C490 (= CBS 100200), Lynford Forest, Thetford, England, from *Pinus* sp., collected by J. Gibbs, 12 April 1988.

OTHER MATERIAL EXAMINED: Watford, Herts, England, from *Pinus* sp., collected W.O. Harper, 25 November 1948, IMI 32291; Scotland, board of *Pinus* sp., collected by Wilson, designated as neotype of *Ch. ungeri* by Nag Raj and Kendrick, IMI 20164; Thetford Forest, England, *Pinus nigra* Arnold, collected by A. Uzunovic, September 1993, DAOM 225448, from isolate C795 (= CBS 100201 and 126/1e1).

CULTURES EXAMINED: Murdford, Thetford Forest District, Norfolk, England, from *Pinus* sp., collected by J. Gibbs April 1992, isolate C487 (= CMW 1383); Alice Holt Forest, Surrey, England, from *Pinus* sp., collected by J. Gibbs, April 1992, isolate C 489 (= CMW 1312).

This species fruits readily in culture and was used (as *C. coerulescens* sp. A) to develop a genetic model for unidirectional mating type switching in *Ceratocystis* (Harrington and McNew 1997). *Ceratocystis pinicola* can be distinguished from *C. coerulescens* and *C. resinifera* by its longer perithecial necks, shorter ostiolar hyphae, and shorter ascospores, though there is some overlap in these characters among the three species. All specimens and cultures examined thus far have been from *Pinus* in Britain, where it appears to be an important agent of bluestain but not generally colonizing living pines (Gibbs 1993). *Picea* species are not indigenous to Britain, and *C. pinicola* may have evolved from a more generally distributed species that occurred on *Pinus* and *Picea* on continental Europe and North America. DNA sequence data (Witthuhn et al. 1998) and isozyme analysis (Harrington et al. 1996) places *C. pinicola* closest to *C. coerulescens* sensu stricto.

Bakshi's (1951) detailed description of *C. coerulescens* appears to be the fungus we now recognize as *C. pinicola*, as is the specimen used to neotypify the anamorph name, *Ch. ungeri* (Nag Raj and Kendrick 1975). It is more likely that Münch's (1907) concept of *Ch. ungeri* matches the anamorph of *C. coerulescens* sensu stricto, as emended here, or *C. resinifera*, but uncertainty precludes us from connecting *Ch. ungeri* with a teleomorph at this time.

Ceratocystis polonica (Siemaszko) C. Moreau, Rev. Mycol. Suppl. Col. 17:22. 1952. (Figs. 23–28)

Figs. 16–22. *Ceratocystis pinicola* (holotype). Fig. 16. Perithecium. Scale bar = 200 μm. Fig. 17. Ostiolar hyphae at apex of perithecial neck. Scale bar = 20 μm. Fig. 18. Ascospores. Scale bar = 13 μm. Fig. 19. Conidiophore with conidium emerging from collarette. Scale bar = 20 μm. Fig. 20–21. Conidia. Scale bar = 20 μm. Fig. 22. Older, barrel-shaped conidia that have become darkened and thick-walled. Scale bar = 20 μm.

Figs. 23–28. *Ceratocystis polonica* (neotype). Fig. 23. Perithecium. Scale bar = 200 μm. Fig. 24. Base of perithecium showing hyphal ornamentation. Scale bar = 200 μm. Fig. 25. Ostiolar hyphae at apex of perithecial neck. Scale bar = 20 μm. Fig. 26. Ascospores in top view showing thick sheath, wider at the sides than on the ends of the ascospores. Scale bar = 13 μm. Fig. 27. Ascospores in side view, showing flattened, slightly curved bottom of spore and sheath. Scale bar = 13 μm. Fig. 28. Conidiophore near base of perithecium, with emerging conidium. Scale bar = 40 μm.

≡*Ophiostoma polonicum* Siemaszko, Plant Pol. 7:32. 1939.

EMENDED DESCRIPTION: Colonies dark greyish olive (Ridgeway 1912, plate XLVI). Single ascospores giving rise to both self-sterile and self-fertile colonies, with perithecia being produced sparsely in culture in approximately three weeks. Perithecia dark, bases round to slightly ovoid, 192–360 μm (mean 277 μm) in diameter giving rise to distinct basal spines 70–220 μm (mean 121 μm) long. Necks dark and tapering to the apex, 560–1200 μm (mean 920 μm) long, 20–42 μm (mean 37 μm) wide at the middle, and 20–34 μm (mean 25 μm) wide at the apex, terminating in a crest of ostiolar hyphae 20–40 μm (mean 29 μm) long and 2–3 μm (mean 2.2 μm) wide. Ascospores accumulating at the apices of mature perithecia, oblong, and slightly flattened at the bottom, 5–6 μm (mean 5.5 μm) long and 2–3 μm (mean 2.5 μm) wide excluding sheaths when viewed from the top, with a translucent outer sheath, negligible in size at either end, flattened at the bottom, the ascospore in top view 4–5 μm (mean 4.6 μm) wide including the sheath. Conidiophores rare, usually associated with the bases of perithecia, typical of the genus *Chalara*. One conidiophore seen in the neotype, 120 μm long, 5 μm wide at the middle, 4 μm wide at the apex. Conidium cylindrical, produced by ring wall building development, 6 μm long and 4 μm wide.

MATERIAL EXAMINED: NEOTYPE: Namdalsaid, Nord Tronidelag, Norway, from *Picea abies*, collected by H. Solheim, 1990, DAOM 225451, derived from culture C791 (= CMW 2224, NCC 90-120/181, and CBS 100205).

OTHER MATERIAL EXAMINED: Japan, from *Picea jezoensis* (Sieb. & Zuec.) Carr. infested by *Ips typographus* L., collected by Y. Yamaoka, 1989, DAOM 225452, from isolate C755 (= CMW 2273, YCC 067, and CBS 100206).

OTHER CULTURES EXAMINED: Poland, gallery of *I. typographus* in *Picea abies*, collected by W. Siemaszko, deposited in CBS in 1938 as the type, CBS 133.38 (= C322); Norway, collected by H. Solheim, ATCC 62335 (= C731 and CMW1164); Ås, Norway, from *Picea abies* infested with *I. typographus*, collected by H. Solheim, 1980, CBS 228.83 (= NCC 80-69/34 and C320).

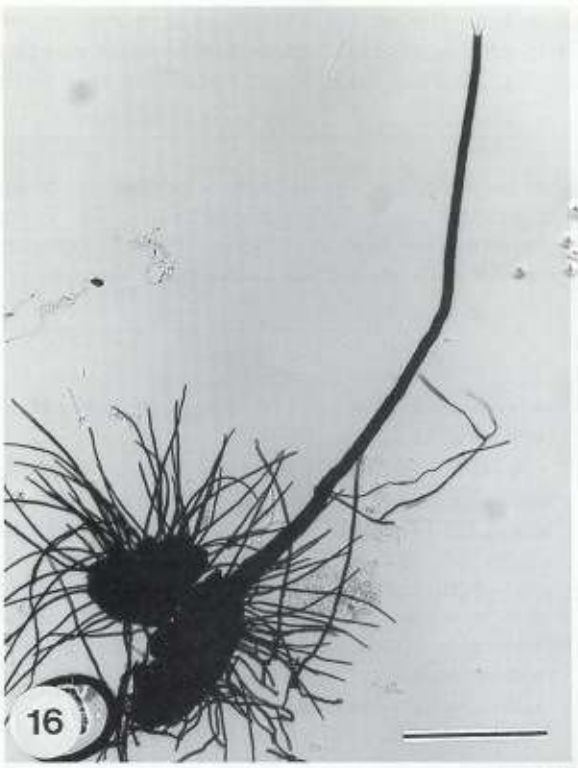
This species was not recognized as a true *Ceratocystis* until recently (Harrington et al. 1996; Visser et al. 1995). Siemaszko's (1939) original description states that *C. polonica* has a *Leptographium* anamorph, but this is in error; the fungus produces a *Chalara* anamorph typical of true *Ceratocystis* species (Visser et al. 1995), although the conidiophores are rare and are often found only attached to the perithecial base.

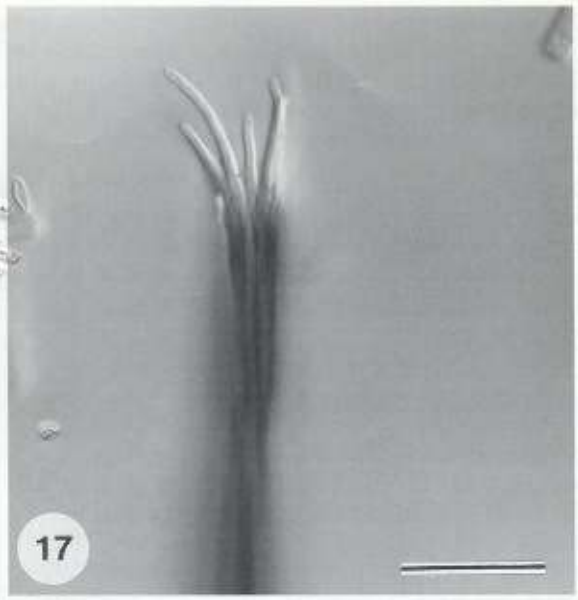
No dried material linked to Siemaszko's (1939) examinations appears to be available, although a culture deposited by him (CBS 133.38) is viable and has the isozyme profile typical for this species. Unfortunately, this culture does not produce perithecia or conidiophores. The only perithecial material we have available is from Norway and Japan, and Norwegian material was selected for the neotype. All examined isolates are from *Picea*, most of which had been attacked by the bark beetle *I. typographus* (Solheim 1993; Yamaoka et al. 1997). The ascospores of *C. polonica* and *C. laricicola* are unique among the *Ceratocystis* species that we have examined in that the sheaths are considerably thicker at the sides than at the end of the ascospores, and they have flattened bases.

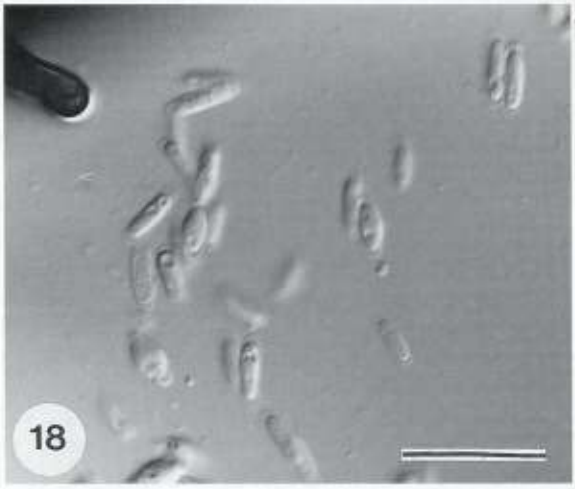
Ceratocystis laricicola Redfern & Minter, Plant Pathol. 36: 468. 1987.

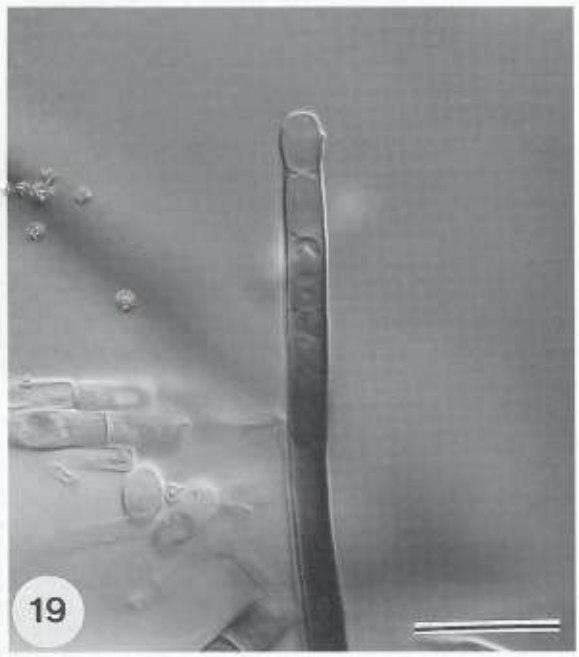
MATERIAL EXAMINED: Scotland, *Larix* sp., collected by D. Redfern, DAOM 225453, from isolate C181 (= 56–10 and CBS 100207); Scotland, *Larix* sp., collected by D. Redfern, isolate C179 (= 56–2); Mount Fuji, Japan, from *Larix* sp., collected by Y. Yamaoka, DAOM 225454, from isolate C746 (= CMW1955, CMW1785, L20A-A1, and CBS 100208); Mount Fuji, Japan, from *Larix* sp., collected by Y. Yamaoka, isolate C745 (= CMW1954 and L30A-A2).

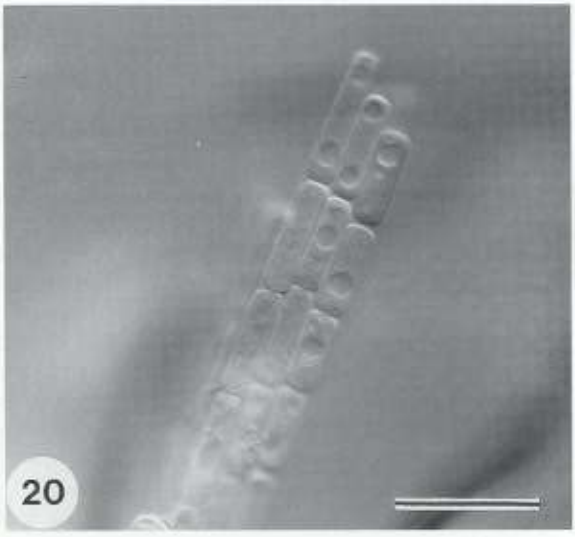
It appears that *C. laricicola* is restricted to *Larix* attacked by *Ips cembrae* (Heer). We have examined cultures of *C. laricicola* from Scotland and Japan (Harrington et al. 1996). This species was well described (Redfern et al. 1987) but was not compared with *C. polonica*. *Ceratocystis polonica* and *C. laricicola* are associated with bark beetles in the genus *Ips* across Eurasia (Harrington et al. 1996). Their ascospores, particularly their sheaths, are distinct from other *Ceratocystis* species, but we are not able to separate these two species based on morphology at this time. The former species appears to be adapted to *Picea*, and the latter, to *Larix*. Isolates from these respective hosts differ electrophoretically at an enzyme locus (Harrington et al. 1996), and crosses between the two species give rise to no perithecia or perithecia with aborted ascospores and very low germination rates (Harrington and McNew 1998). We, thus, recognize these species as distinct. The sequences of the ITS region are identical for the two species (Witthuhn et al. 1998), so we believe that *C. polonica* and *C. laricicola* have recently diverged.

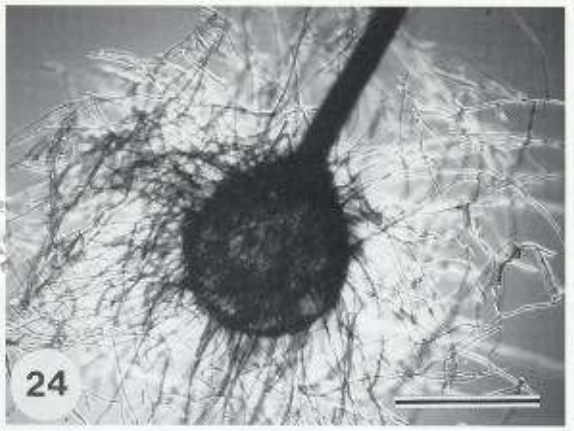


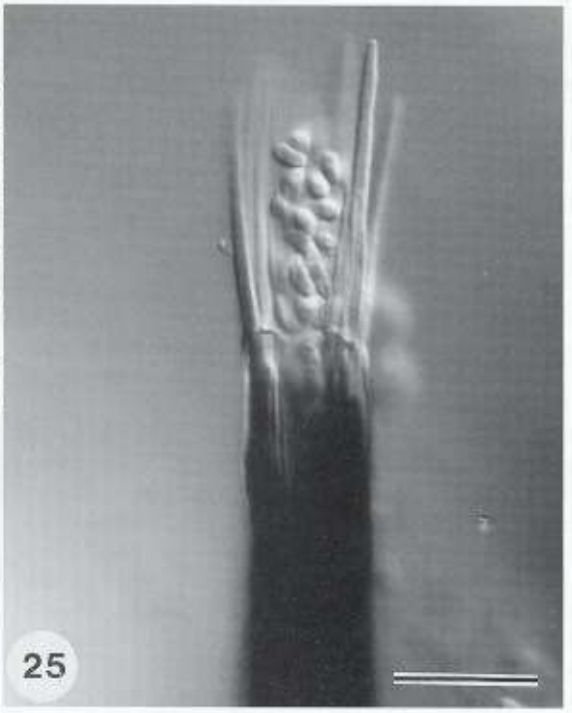


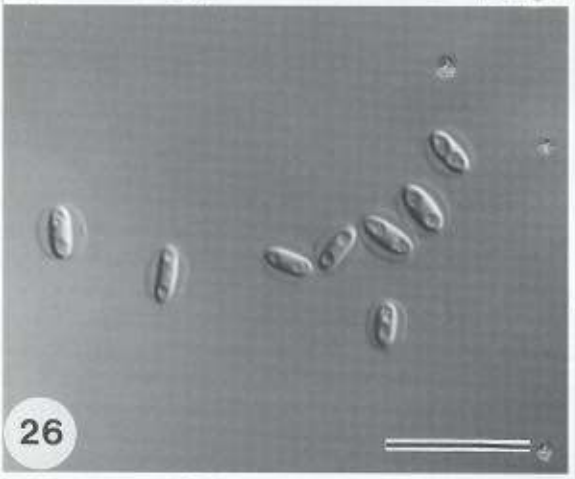


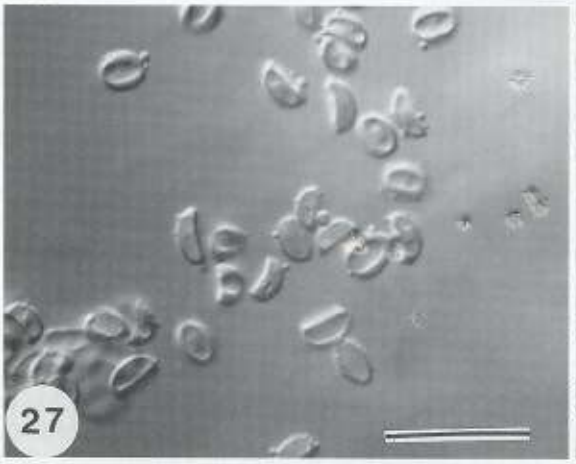


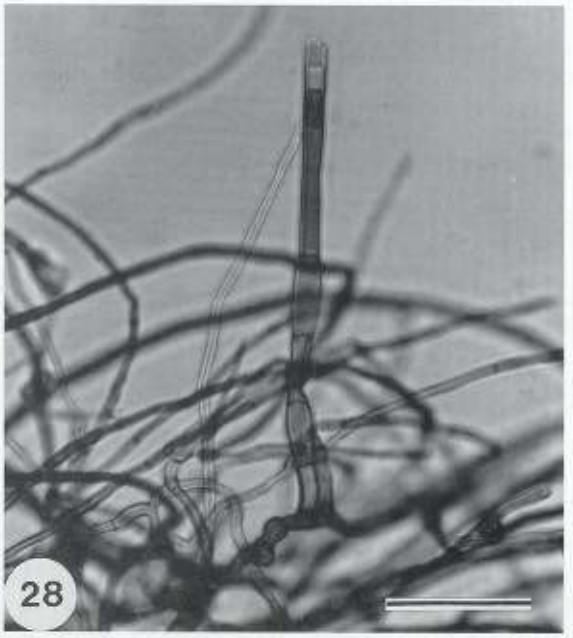












Ceratocystis rufipenni Wingfield, Harrington, & Solheim, Can. J. Bot. 75: 828. 1997.

HOLOTYPE: Caribou Creek, East Nelson, British Columbia, Canada, from *Picea engelmannii* infested with *Dendroctonus rufipennis* (Kirby), November 1992, collected by H. Solheim, DAOM 222349, from isolate C608 (= NFRI 252/8 and CBS 100209).

To date, all examined material has been from British Columbia, Canada, but Davidson's (1955) report of *C. coerulescens* from spruce in Colorado, U.S.A., is also of *C. rufipenni*. This species may be associated with *D. rufipennis* throughout its range in North America. A morphological description and illustrations are found in Wingfield et al. (1997).

Like the other species associated with bark beetles, *C. polonica* and *C. laricicola*, *C. rufipenni* rarely produces conidiophores and generally lacks the fruity odor common in species of *Ceratocystis*. Loss of conidiophore production and fruity odors, as well as production of perithecia in response to wounding of mycelium, are believed to be adaptations to bark beetle vectors, rather than to the more general insect vectors (primarily fungal feeding insects) known for other members of *Ceratocystis* (Harrington et al. 1996). Because the isozyme electromorphs and ITS sequences of *C. rufipenni* differ substantially from those of *C. laricicola* and *C. polonica*, we believe that these adaptations to bark beetles arose independently, at least twice in the evolution of *Ceratocystis* species.

DNA sequence analysis (Witthuhn et al. 1998) indicates that *C. rufipenni* is most closely related to *C. resinifera*. Both species occur on *Picea*, though *C. rufipenni* is known only in North America and only in association with the bark beetle *D. rufipenni*. It is pathogenic to *Picea* in inoculations (Solheim and Safranyik 1997) and may play an important role in killing living tissue around the vicinity of bark beetle attacks. The capacity of *C. resinifera* to colonize wounds of *Picea* was discussed earlier.

Morphologically, *C. rufipenni* is easily distinguished from the other *Ceratocystis* species on conifers by its large perithecia, though they do not form abundantly in culture at room temperature. Both *C. rufipenni* and *C. douglasii* have temperature optima for growth at 20°C and typically produce perithecia at temperatures below this, often only after many months under refrigeration (about 5°C).

Ceratocystis douglasii (Davidson) Wingfield & Harrington, Can. J. Bot. 75: 832. 1997.

≡*Endoconidiophora coerulescens* Münch f. *douglasii* Davidson, Mycologia, 45: 584. 1953.

HOLOTYPE: Fort Collins, Colorado, U.S.A., from lumber of *Pseudotsuga menziesii* (Mirb.) Franco shipped from Oregon, collected by R.W. Davidson, 1953, BPI 59527 (= F.P. 70703).

CULTURE DERIVED FROM HOLOTYPE: C324 (= CBS 556.97, formerly known as CBS 142.53); DAOM 222362 is a dried specimen of CBS 556.97.

This fungus appears to be restricted to wood of *Pseudotsuga menziesii* in western North America, where it causes bluestain (Davidson 1953). An emended description and illustrations are found in Wingfield et al. (1997). A typographic error was made, however, in the dimensions of the ascospores, which are 2–4 µm (mean 3 µm) wide excluding sheath rather than 4–6 µm (mean 3 µm) wide excluding sheath

Ceratocystis douglasii appears to have a biology similar to *C. coerulescens* and *C. pinicola* except that the host is *Pseudotsuga* rather than *Pinus* or *Picea*, and the geographic distribution of the fungus may be restricted to the natural geographic range of its host, western North America (Wingfield et al. 1997). Of all the species treated here, *C. douglasii* has the most distinctive isozyme profile (Harrington et al. 1997) and ITS sequence (Witthuhn et al. 1998). It produces long conidiophores on wood and relatively large perithecia and ascospores.

Discussion

Of the recognized *Ceratocystis* species, only seven occur primarily on conifer species, all on members of the Pinaceae. Partial interfertility (Harrington and McNew 1998) and similar isozyme patterns (Harrington et al. 1996) suggest that these seven species are closely related, and phylogenetic analysis of the ITS region of rDNA shows this group to be monophyletic (Witthuhn et al. 1998). *Ceratocystis virescens* and *C. eucalypti* occur on angiosperms, and, along with the asexual *Chalara australis* and *Chalara neocaledoniae*, are apparently the nearest relatives to the conifer clade (Witthuhn et al. 1998). It appears that a single evolutionary event led to the *Ceratocystis* lineage that occurs on the Pinaceae, and this seems likely to account for the fact that the seven species of *Ceratocystis* on conifers are morphologically quite similar and are difficult to distinguish on this basis.

These fungi have similar *Chalara* anamorphs and typical *Ceratocystis* ascomata, with spines at their bases and with necks terminating in distinct ostiolar hyphae. They also have ascospores of similar size and shape. One of the most characteristic features of the species discussed here is the elongate to slightly curved ascospores with distinct sheaths. These protrude particularly from the long ends of the ascospores in all but *C. laricicola* and *C. polonica*. In the latter two species, the sheaths are noticeably thicker at the sides than at the ends of the ascospores in top view, and in side view tend to be flattened on the bottom. This feature easily separates these two species from the other five conifer species of *Ceratocystis*. From ultrastructural studies of sheathed ascospores in species of *Ceratocystis* and *Ophiostoma* (van Wyk et al. 1991) we believe that these sheaths represent rigid walls. It is important to note that measurements of ascospores made by early authors (Münch 1907; Hunt 1956; Davidson 1953) probably did not include these sheaths. Uncertainty regarding this question has also made interpretation of previous descriptions difficult.

The seven conifer species of *Ceratocystis* discussed here well illustrate the complexity of the genus *Ceratocystis* and the limitation of morphology in defining species. Based solely on morphology, only two species in this group might be clearly distinguished: *C. coerulescens* and *C. polonica*. The morphological features of *Ceratocystis* used in taxonomy are adaptations for insect dispersal. With the exception

of the three species associated with bark beetles, the vectors of *Ceratocystis* species are a diverse collection of fungal feeders that are attracted to the fruity odor of the mycelium. These unspecialized insect associates may serve to carry conidia to other mycelial mats for spermatization of protoperithecia or may disperse ascospores to fresh plant wounds or bare wood. We believe that host adaptation has been more important than vector adaptation for speciation within *Ceratocystis*, and substrate relationships are valuable phenotypic characters in defining these species. Other physiological characters, such as isozyme electromorphs and growth response to temperature, have proven invaluable in delineating these morphologically cryptic species. Further, mating responses and DNA sequence analysis have aided in identifying lineages within the group. It is likely that similar studies in other major clades of *Ceratocystis* will also identify many new taxa.

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