The pathogenicity of *Ceratocystis montia* to lodgepole pine

Gary A. Strobel and Fumio Sugawara
Department of Plant Pathology, Montana State University, Bozeman, MT, U.S.A. 59717

Received April 9, 1985

Strobel, G. A., and F. Sugawara. 1986. The pathogenicity of *Ceratocystis montia* to lodgepole pine. Can. J. Bot. **64:** 113–116.

Ceratocystis montia (Rumb.) Hunt, an ascomycetous fungus, is associated with bark beetle infested lodgepole pine in the intermountain region of United States and portions of western Canada. The organism, when inoculated into lodgepole pine (20 years old) caused necrosis of the inner bark, a blue-stained appearance of the sapwood, and chlorosis and necrosis of the foliage. Koch's postulates were fulfilled in these experiments. Particles of inner bark provided the best support for fungal growth and inhibitors of fungal growth may develop in sapwood during the process of drying.

Strobel, G. A., et F. Sugawara. 1986. The pathogenicity of *Ceratocystis montia* to lodgepole pine. Can. J. Bot. **64:** 113–116.

Le *Ceratocystis montia* (Rumb.) Hunt, un champignon ascomycète, est associé au *Pinus contorta* infesté par l'insecte de l'écorce dans la région montagneuse de l'ouest canadien et américain. L'organisme, lorsqu'inoculé dans le *Pinus contorta* 20 ans) provoque une nécrose de l'écorce interne, une coloration bleue de l'aubier, et une chlorose ainsi qu'une nécrose du feuillage. Le postulat de Koch a été vérifié dans ces expériences. Des particules de l'écorce interne se sont avérées le meilleur support pour la croissance fongique et des inhibiteurs de cette croissance peuvent se développer dans l'aubier au cours du séchage.

[Traduit par le journal]

Introduction

Invariably, lodgepole pine (*Pinus contorta* Dougl.) attacked by the mountain pine beetle (*Dendroctonus ponderosae* Hoph.) becomes host to a complex of fungi which grow in beetle frass and galleries. Eventually, some penetrate the sapwood of the tree (Whitney 1971; Rumbold 1941; Ballard *et al.* 1984). In midsummer adult beetles attack the tree. In the following spring to midsummer the foliage turns yellowish green and finally bright orange (Amman *et al.* 1977). Dead trees typically show a "blue-stained" appearance in radial patterns in the sapwood. The fungi associated with the blue-stained wood are inoculated into the tree by bark beetles (Robinson 1962). Habitat, maturity, and physiological factors of the lodgepole pine influence the development of beetle infestations (Cole and Amman 1980). Presently, much of the natural range of lodgepole pine in the United States and Canada is experiencing a severe epidemic with the accompanying loss of billions of board feet of timber per year.

Although the blue-stain fungi have been implicated in the decline and death of beetle-infested lodgepole pine, no definitive studies on the pathology of this phenomenon have appeared. Thus, the purpose of this report is to show that *Ceratocystis montia* is capable of causing decline and death of lodgepole pine. Furthermore, factors in the tree governing growth of *C. montia* are described.

Materials and methods

Ceratocystis montia was originally isolated from lodgepole pines (40–60 years old) in the later stages of decline from several locations in the Gallatin National Forest, Montana. It was obtained as a pure culture from infected wood samples by placement on the *Ceratocystis* selective medium of Miller *et al.* (1981). Cultures obtained from colonies developed from single conidia were placed on small lodgepole pine logs and incubated until the perithecial stage of the fungus developed. The sexual structures of the fungus were examined and the organism was keyed to *C. montia* (Hunt 1956). The inoculum for trees was grown on sawdust made from multiple chain-saw cuts into the sapwood of a freshly cut tree. It was mixed 1:5 w/v with distilled H_2O. The slurry was autoclaved and inoculated with plugs of the fungus from an agar culture and incubated while standing at 23°C for 3–4 weeks.

For field inoculation studies, a plot was picked in the Hyalite drainage of the Gallatin National Forest, Montana, located in a 20-year-old lodgepole pine stand growing in an *Abies lasiocarpa* – *Carex geyeri* habitat type (Pfister *et al.* 1977). The trees selected for inoculation were all 13.3 ± 3.8 cm in diameter at breast height. The trees were inoculated by two methods at ca. 45 cm from ground level. (*i*) The alternating flap technique (AFT) involved pulling down of flaps of bark 10–12 cm wide (horizontal measurement) and 4–6 cm long (longitudinal measurement) in an alternating pattern around the circumference of the tree, allowing a continuation of the cambial integrity of the tree. Beneath each flap 8–10 g of sawdust colonized by *C. montia* was placed and all of the flaps were sealed back to the trunk with several wrappings of duct tape. (*ii*) In the quadrant flap technique (QFT) only four flaps 4 × 4 cm were made in the tree at 90° angles and 2–4 g of fungal-colonized sawdust was placed under each flap and then sealed with duct tape. The QFT did not result in girdling of the tree. There were six to eight trees in each group. A set of trees inoculated with microbe-free sawdust served as controls for each group. Also, a set of trees was completely girdled (1.5 cm) to allow comparison of symptoms that would develop in such trees with those that developed in trees inoculated with *C. montia.* All treatments were done in mid-June and the trees were observed monthly during the course of two complete growing seasons (June–September). Evaluations were made on the basis of the number of trees of each group showing symptoms beyond the normal 0–10% needle death that is commonly observed in lodgepole pine in the study area. A tree was evaluated as having symptoms if it had 30% or more of needles in its crown showing chlorosis or necrosis. The evaluations were made by mutual agreement of at least two experienced observers.

Since growth of the fungus in the tree may be related to its ability to produce disease, we examined several tree materials for their ability to support fungal growth. For standard fungal growth studies, the sapwood sawdust from a freshly cut, ca. 20-year-old healthy lodgepole pine was mixed 1:4 w/v with distilled H_2O and the sawdust pieces were made into smaller pieces by homogenizing in a Waring blender for a few minutes. In some experiments a standard 9-cm Petri plate was used, in which case 4 g of the wet plant material – H_2O mixture was placed in the bottom dish to give a uniform layer. When the deep-well Petri plates (9 × 2 cm) were used, 7 g of plant material was placed in the dish. The loaded plates were autoclaved for 20 min. Under these experimental conditions there was, after autoclaving, free water surrounding the plant material in each plate. A plug of agar containing *C. montia* was placed in the center of each plate. Small

Printed in Canada / Imprimé au Canada

TABLE 1. Incidence of disease in lodgepole pine at the end of the second growing season after inoculation with *C. montia*

Inoculation technique	Trees having symptoms	Trees dead	Trees with blue stain	Total no. trees
Alternating flap technique	7/8*	3/8*	7/8*	8
Control	0/7	0/7	0/7	7
Quadrant flap technique	1/6	0/6	1/6	6
Control	0/7	0/7	0/7	7
Girdled trees	7/7*	7/7*	0/7	7

NOTE: Numbers followed by * in a column are significantly different from the others at the 0.05 level by χ^2 contingency tests.

pieces (3 × 5 mm) of freshly prepared inner bark and sawdust of dried sapwood (2–4 weeks drying at 23°C with 20–30% relative humidity) from the same lodgepole pine tree were also tested for their ability to support growth of *C. montia*. Measurements of radial growth (four made at 90° angles) were made at 5 and 9 days. Results are the average of three replications. Comparable studies were conducted on fresh sapwood sawdust and dried sawdust that had been extracted with cold ethanol (1:10 w/v for 24 h) and placed, once the ethanol was removed, in Petri plates for the standard assay. Also, growth of *C. montia* was measured on sawdust extracted with H_2O (1:10 w/v for 24 h), the excess H_2O removed to give an appropriate 1:4 w/v and the standard growth test performed on the sawdust.

Results

Small blocks of blue-stained wood from beetle-infested lodgepole pines in several locations in the Gallatin National Forest, Montana were placed on the *Ceratocystis* selective medium. The organism growing out of these fitted the taxonomic description of *Ceratocystis montia* (Hunt 1956), and *C. ips* according to Upadhayay 1981 (see Materials and methods). Although it is likely that other blue-staining organisms belonging to the *Ceratocystis* complex could have been present in beetle-infested trees, we consistently isolated *C. montia*. This may be due to its ability to survive and grow on the selective medium. The other organisms in this complex have never been tested on this medium.

At the end of the first season no symptoms appeared in any of the lodgepole pines (controls, inoculated, or girdled). However, in the middle of the second summer season, symptoms (chlorosis, local necrosis) began to appear on the majority of trees inoculated by the AFT and the girdled trees but on none of the others. At the end of the second season all girdled trees were dead and 3 of 8 trees inoculated by the AFT were dead, while 4 other trees in this group showed decline (chlorosis and some local necrosis in the crown) (Table 1) and 1 was symptomless. Two of the 3 trees that died (AFT) and many of the girdled trees had natural beetle infestations in their crown during the second season. None of the other trees were attacked. Symptoms developed on only 1 of 6 trees inoculated by the QFT. *Ceratocystis montia* was recovered at or near the site of inoculation, using the *Ceratocystis* selective medium, on 7 of 8 of the AFT-inoulated trees (Table 1) and from the 1 (QFT) tree showing symptoms. The fungus had not become established in the other (QFT) inoculated trees, nor was it isolated from any of the control trees that were sampled.

Each of the trees infected by *C. montia* was cut cross sectionally into 5 and 10 cm long pieces upwards and downwards from the point of inoculation. In each case, as judged by the blue-stained and discolored wood, the fungus had moved only 15–20 cm upward from the points of inoculation but had moved downward towards or into the roots (30–50 cm). In all cases, the discoloration in the sapwood at or below the site of inoculation or at ground level of the tree was contiguous with the discoloration at the original site of inoculation. In several cases it was apparent that only 10–20% (wedge shaped) of the sapwood was infected (cross-sectional area), as evaluated by discoloration, yet the trees showed symptoms.

Cultures of a blue-stain fungus were obtained from blue-stained wood samples placed on the *Ceratocystis* selective medium. The wood samples were acquired from three trees that had originally been inoculated with *C. montia* by the AFT (Table 1). The fungus was placed under bark flaps made in small log pieces (4 × 5 cm) and then placed into a closed chamber for 7–10 days (22°C). In each case, the fungus had penetrated the sapwood of the small log, causing a blue-stained appearance and it was possible to isolate successfully *C. montia*, on the *Ceratocystis* selective medium, from the infected log. The fungus we judged to be *C. montia* based on its strict similarity in colony morphology on the *Ceratocystis* selective medium and conidial structure to authentic *C. montia*.

It appeared that the ability of *C. montia* to produce symptoms or death in lodgepole pine is related to its ability to establish itself in the inoculated tree. For instance only 1 of 6 trees inoculated by the QFT developed blue stain and crown symptoms and 1 symptomless tree of 8 trees inoculated by the AFT method did not have successful establishment of the fungus (Table 1). It is also interesting that *C. montia* does not penetrate and grow into the heartwood of lodgepole pine. Furthermore, it is commonly observed that after a blue-stained tree is harvested, dried, and rewetted, there does not appear to be renewed growth of the fungus. Thus, since *C. montia* appeared to be pathogenic in lodgepole pine, we felt compelled to carry out more comprehensive studies on the growth of *C. montia* as it is supported by tissues from various parts of the tree. Of the untreated (not extracted) samples, *C. montia* produced the best rate of radial growth on freshly prepared inner bark and sapwood (Table 2). Dried sapwood sawdust that was rewetted produced significantly less growth than fresh inner bark. The best radial growth of the fungus was observed after ethanol extraction of the dried sapwood sawdust (Table 2). Conversely, ethanol extraction of fresh inner bark resulted in a significant reduction of fungal growth on the extracted inner bark. Water extraction did not appreciably influence fungal growth over that observed in the original bark or sawdust samples (Table 2).

The reduction of fungal growth on predried sawdust may be due to the formation of an inhibitor or the destruction of a vital growth substance. Therefore, according to the standard fungal growth assay, we mixed fresh sapwood with dried sapwood

TABLE 2. Radial growth (centimetres) of *C. montia* on sawdust or inner bark fragments 5 days after inoculation of the samples with an agar plug (5 mm) containing *C. montia* mycelium. Each result is an average of the radial growth on three plates

Treatment	Radial growth
Fresh sapwood sawdust	1.95±0.08*bc*
Fresh inner bark	2.45±0.13*dc*
Dried sapwood sawdust (rewetted)	1.50±0.13*ab*
Fresh sapwood sawdust after H_2O extraction	1.78±0.16*bc*
Fresh inner bark after H_2O extraction	1.97±0.24*bc*
Dried sapwood sawdust after H_2O extraction	1.3 ±0.13*ab*
Fresh sapwood sawdust after ethanol extraction	2.5 ±0.40*dc*
Fresh inner bark after ethanol extraction	0.76±1.3*a*
Dried sapwood sawdust after ethanol extraction	3.1 ±0.43*d*

NOTE: Radial growth was also measured at 9 days and the growth pattern differences among treatments were essentially the same as those occurring at 5 days. Multiple comparison of the means (0.05 LSD) was preceded by a significant *F* test having a *P* value of <0.0001 for main treatment effects (H_2O−ethanol extraction). Numbers followed by the same letters are not significantly different at $P = 0$.

sawdust in the proportions 1:0, 1:1, and 0:1 and followed fungal growth. The relative growth rates were 100, 70, and 50%, respectively, of that normally occurring on fresh sapwood sawdust.

Discussion

The data presented in this report show that *C. montia* is associated with lodgepole pine trees having blue-stained sapwood in the Gallatin National Forest, Montana. Other fungi involved in this complex were not isolated. More importantly, in connection with this observation, is the fact that, under certain artificial conditions of inoculation, *C. montia* is capable of causing chlorosis, local necrosis, and ultimately death of lodgepole pine. In this study, Koch's postulates were completely fulfilled, whereas in other studies on blue-stain fungi in pines some questions about the conclusions made can be raised (Basham 1970) since the pathogen was never reisolated and tested.

An alternating flap inoculation technique was more successful in allowing *C. montia* to become established in lodgepole pine than a smaller patch (quadrant) technique (Table 1). However, neither technique simulated inoculation of trees by the mountain pine bark beetle and such experiments need to be done. Although many of the girdled trees and 2 of 8 trees inoculated by the AFT did experience some natural beetle infestation, this occurred during the second season. As such, this was during or after the pathological effects of girdling or fungal infection were being manifested.

On a relative scale, when compared with other members of the *Ceratocystis* group that attack trees such as *C. ulmi*, *C. montia* must be considered a relatively weak pathogen. Furthermore, the fungus did not induce symptoms any more rapidly in trees than did complete girdling of trees. If, for instance, *C. montia* were an aggressive pathogen, we might have expected symptoms during the first season. Nevertheless, the fungus appears to be affecting the entire complex relationship between the tree and the beetle; thus its ability to cause disease symptoms may have some influence on the life cycle of the beetle.

When *C. ips* (Upadhayay 1981) was massively inoculated on ponderosa pine trees (ca. same diameter as this study), it grew upwards in the trees and killed them within 30 days by precluding water movement (Mathre 1964). In contrast, the AFT is a much milder form of inoculation requiring at least two growing seasons for symptoms to appear (Table 1). Also, in contrast to Mathre's (1964) observations, *C. montia* grew downwards from the point of inoculation. Furthermore, in several cases since symptoms were noted on trees having less than 20% coverage of the sapwood (blue stain), a suggestion of a phytotoxin being involved seems warranted. Recently, F. Sugawara and G. A. Strobel (unpublished) have observed inhibition of lodgepole pine seed germination in the *n*-butanol phase (dried and redissolved in H_2O of 2-week-old still cultures of *C. montia*. The presence of such inhibitor(s) leads us to wonder if these or other fungal metabolites have some role in causing decline of fungus-infected trees.

Extraction of dried wood with H_2O did not dramatically alter the growth response of *C. montia* (Table 2). However, extraction of the dried wood with ethanol caused a restoration in the growth of *C. montia* on the sawdust (Table 2). Also, mixing of dried sapwood sawdust with fresh sawdust caused a reduction in fungal growth. Collectively, these data suggest that the drying process results in the production of an inhibitor(s) to fungal growth. Such an inhibitor(s) seems to be ethanol soluble and is probably not related to the inhibitors reported by Shrimpton and Whitney (1967) since they worked with volatile sapwood extractives. These results may explain why *C. montia* fails to develop on dead or dying trees after the sapwood has completely dried and is rewetted and why *C. montia* is not a threat to poles, lumber, or other lodgepole pine products once drying of the sapwood has taken place. Just the opposite phenomenon, however, may occur in the inner bark which offers an excellent growth support for *C. montia*. Once it is extracted with ethanol, growth is dramatically reduced or eliminated, which may be the result of the loss of one or more nutrients that stimulated fungal growth (Table 2).

Obviously, this report indicates the need for more comprehensive chemical, biochemical, and physiological studies on growth inhibitor or promoter substances in lodgepole pine for *C. montia*.

In the past, *C. montia* and other blue-stain fungi have been implicated in facilitating the development of bark-beetle brood through such mechanisms as more rapidly drying out the infested tree (Amman and Cole 1983). However, we must now consider *C. montia* as a factor contributing to the death of the tree.

Acknowledgements

We acknowledge the U.S. Department of Agriculture, Forest Service, grant 22-C-21WT-65 and the Montana Agricultural Experiment Station for financial support of this project. The assistance of Dr. D. Mathre and Dr. R. Lund in fungal identification and statistical analyses, respectively, is appreciated.

AMMAN, G. D., and W. E. COLE. 1983. Mountain pine beetle dynamics in lodgepole pine forests. Part II. Population dynamics. USDA For. Serv. Gen. Tech. Rep. INT-145.

AMMAN, G. D., M. D. MCGREGOR, D. B. CAHILL, and W. H. KLEIN. 1977. Guidelines for reducing losses of lodgepole pine to the mountain pine beetle in unmanaged stands in the Rocky Mountains. USDA For. Serv. Gen. Tech. Rep. INT-36.

BALLARD, R. G., M. A. WALSH, and W. E. COLE. 1984. The penetration and growth of blue-stain fungi in the sapwood of lodgepole pine attacked by the mountain pine beetle. Can. J. Bot. **62**:

1724–1729.

BASHAM, H. G. 1970. Wilt of lobolly pine inoculated with blue stain fungi of the genus *Ceratocystis*. Phytopathology, **60**: 750–753.

COLE, W. E., and G. D. AMMAN. 1980. Mountain pine beetle dynamics in lodgepole pine forests. Part I. Course of an infestation. USDA For. Serv. Gen. Tech. Rep. INT-89.

HUNT, J. 1956. Taxonomy of genus *Ceratocystis*. Lloydia, **19**: 1–58.

MATHRE, D. E. 1964. Pathogenicity of *Ceratocystis ips* and *Ceratocystis minor* to *Pinus ponderosa*. Contrib. Boyce Thompson Inst. **22**: 363–388.

MILLER, R. J., D. C. SANDS, and G. A. STROBEL. 1981. Selective medium for *Ceratocystis ulmi*. Plant Dis. **65**: 147–149.

PFISTER, R., B. KOVALCHIK, S. ARNO, and R. PRESBY. 1977. Forest habitat types of Montana. USDA For. Serv. Gen. Tech. Rep. INT-34.

ROBINSON, R. C. 1962. Blue stain fungi in lodgepole pine (*Pinus contorta* Doug. var. *latifolia* Engelm.) infested by the mountain pine beetle (*Dendroctonus monticolae* Hopk.). Can. J. Bot. **40**: 609–614.

RUMBOLD, C. T. 1941. A blue stain fungus, *Ceratostomella monticum* n. sp. and some yeasts associated with two species of *Dendroctonus*. J. Agric. Res. **62**: 589–601.

SHRIMPTON, D. M., and H. S. WHITNEY. 1967. Inhibition of growth of blue stain fungi by wood extractives. Can. J. Bot. **46**: 757–761.

UPADHAYAY, H. P. 1981. A monograph of *Ceratocystis* and *Ceratocystiopsis*. University of Georgia Press, Athens, GA.

WHITNEY, H. S. 1971. Association of *Dendroctonus ponderosae* (Coleoptera: Scolytidae) with blue stain fungi and yeasts during brood development in lodgepole pine. Can. Entomol. **103**: 1495–1503.

This article has been cited by:

1. Christopher J Fettig, A Steven Munson, Donald M Grosman, Parshall B Bush. 2013. Evaluations of emamectin benzoate and propiconazole for protecting individual Pinus contorta from mortality attributed to colonization by Dendroctonus ponderosae and associated fungi. *Pest Management Science* n/a-n/a. [CrossRef]
2. Hayato Masuya, Yuichi Yamaoka. 2012. The Relationships between the Beetle^|^rsquo;s Ecology and the Pathogenicity of their Associated Fungi. *Journal of the Japanese Forest Society* **94**:6, 316-325. [CrossRef]
3. Jae-Jin Kim, Alex Plattner, Young Woon Lim, Colette Breuil. 2008. Comparison of two methods to assess the virulence of the mountain pine beetle associate, Grosmannia clavigera , to Pinus contorta. *Scandinavian Journal of Forest Research* **23**:2, 98-104. [CrossRef]
4. Adrianne V.RiceA.V. Rice, Markus N.ThormannM.N. Thormann, David W.LangorD.W. Langor. 2007. Mountain pine beetle associated blue-stain fungi cause lesions on jack pine, lodgepole pine, and lodgepole × jack pine hybrids in Alberta. *Canadian Journal of Botany* **85**:3, 307-315. [Abstract] [Full Text] [PDF] [PDF Plus]
5. A.V.RiceA.V. Rice, M.N.ThormannM.N. Thormann, D.W.LangorD.W. Langor. 2007. Virulence of, and interactions among, mountain pine beetle associated blue-stain fungi on two pine species and their hybrids in Alberta. *Canadian Journal of Botany* **85**:3, 316-323. [Abstract] [Full Text] [PDF] [PDF Plus]
6. YASMIN J. CARDOZA, KIER D. KLEPZIG, KENNETH F. RAFFA. 2006. Bacteria in oral secretions of an endophytic insect inhibit antagonistic fungi. *Ecological Entomology* **31**:6, 636-645. [CrossRef]
7. Xudong Peng, Hisashi Kajimura, Ei'ichi Shibata. 1996. Response of Japanese red pine to inoculation with a blue stain fungus,Ceratocystis piceae. *Journal of Forest Research* **1**:1, 41-44. [CrossRef]
8. Y. Yamaoka, Y. Hiratsuka, P. J. Maruyama. 1995. The ability ofOphiostoma clavigerum to kill mature lodgepolepine trees. *Forest Pathology* **25**:6-7, 401-404. [CrossRef]
9. Jean H. Langenheim. 1994. Higher plant terpenoids: A phytocentric overview of their ecological roles. *Journal of Chemical Ecology* **20**:6, 1223-1280. [CrossRef]
10. JONATHAN GERSHENZON, RODNEY CROTEAUTerpenoids 165-219. [CrossRef]
11. D. B. REDFERN, J. T. STOAKLEY, H. STEELE, D. W. MINTER. 1987. Dieback and death of larch caused by Ceratocystis laricicola sp. nov. following attack by Ips cembrae. *Plant Pathology* **36**:4, 467-480. [CrossRef]