Taxonomy and chemistry of a new fungus from bark beetle infested *Pinus contorta* var. *latifolia*. Part 1. *Arthrographis pinicola* sp.nov.

LYNNE SIGLER[1]

University of Alberta Microfungus Collection, Devonian Botanic Garden, Edmonton, Alta., Canada T6G 2E1

AND

YUICHI YAMAOKA[2] AND YASUYUKI HIRATSUKA

Forestry Canada, Northern Forestry Centre, Edmonton, Alta., Canada T6H 3S5

Received June 29, 1989

Accepted October 31, 1989

SIGLER, L., YAMAOKA, Y., and HIRATSUKA, Y. 1990. Taxonomy and chemistry of a new fungus from bark beetle infested *Pinus contorta* var. *latifolia*. Part 1. ***Arthrographis pinicola*** sp.nov. Can. J. Microbiol. **36**: 77–82.

Arthrographis pinicola sp.nov. (Hyphomycetes) is described; it was isolated from galleries and adult beetles of *Ips latidens* and from galleries of *Dendroctonus ponderosae* in *Pinus contorta* var. *latifolia* in western Canada. In *I. latidens* infested lodgepole pine, this species extensively colonizes nuptial chambers and egg galleries, characteristically forming floccose conidiomata composed of repeatedly branched hyphae which divide to form arthroconidia having schizolytic dehiscence. The fungus is antagonistic to some blue stain fungi *in vitro*. *Arthrographis pinicola* is compared with other species of *Arthrographis*, and with *Arthropsis microsperma* and the discomycete *Pezizella chapmanii*.

Key words: *Arthrographis pinicola*, Hyphomycetes, bark beetle fungi, antifungal compound, arthrographol.

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Arthrographis pinicola sp.nov. (Hyphomycètes) est décrit; cette espèce fut isolée de galeries et d'insectes adultes d'*Ips latidens* et de galeries de *Dendroctonus ponderosae* chez *Pinus contorta* var. *latifolia* de l'ouest du Canada. Chez le pin lodgepole infesté par *I. latidens*, *A. pinicola* colonise les chambres nuptiales et les galeries de ponte de façon extensive, formant des conidiomata floconneux caractéristiques composés de répétitions d'hyphes ramifiés qui se divisent et forment des arthroconidies dont la déhiscence est schizolytique. *In vitro*, ce champignon est antagoniste envers les champignons qui sont teints par les colorants bleus. *Arthrographis pinicola* est comparé à d'autres espèces d'*Arthrographis*, ainsi qu'avec *Arthropsis microsperma* et le discomycète *Pezizella chapmanii*.

Mots clés : *Arthrographis pinicola*, Hyphomycètes, champignons des insectes d'écorces, substance antifongique, arthrographol.

Introduction

For the past few years, two of us (Y.Y. and Y.H.) have been involved in a study of blue stain fungi (*Ophiostoma* H. & P. Sydow spp., also called *Ceratocystis* Ell. & Halst.) associated with the mountain pine beetle, *Dendroctonus ponderosae* Hopk. (Scolytidae), in lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm.) in western Canada. Part of the investigation included a survey for naturally occurring antagonists or mycoparasites of blue stain fungi.

An arthroconidial fungus was isolated from the gallery walls of *D. ponderosae* and from galleries and adult beetles of *Ips latidens* (LeConte) (Scolytidae). The fungus produced small cylindrical arthroconidia by schizolytic dehiscence of dendritic fertile hyphae borne in floccose conidiomata. The formation of arthroconidia from dendritic conidiophores is characteristic of *Arthrographis* Cochet ex Sigler & Carmichael, and we describe here *Arthrographis pinicola* sp.nov.

We also investigated antagonism between the new fungus and some species of blue stain fungi, and its potential pathogenicity to lodgepole pine seedlings. The chemistry of the antagonism is described in a separate report (Ayer and Nozawa 1990).

Material and methods

Isolation

Logs cut from beetle-infested lodgepole pine trees (about 80 years old) were brought to the laboratory where a few discs were removed. Bark was torn by a chisel to expose cambium with beetle galleries. For *I. latidens* infested trees, cultures were established by transferring small portions of the gallery wall (UAMH 6373, 6374, and 6376), conidia taken from conidiomata in the galleries (UAMH 6402, 6431–6433, 6402A (NOF-1447), 6402B (NOF-1450), and 6402C (NOF-1452), or an adult bettle (UAMH 6375) to 1% malt agar. For *D. ponderosae* infested trees, small pieces of gallery wall (UAMH 5983) and sapwood (UAMH 6005) taken from the discs were used as inoculum. Established strains were grown on Weitzman and Silva-Hutner medium (WSH) (Malloch 1981), 5 and 10% Pablum mixed cereal agar (CER) (Pablum mixed cereal, 50 or 100 g, and agar, 20 g/L distilled water), 1 and 2% malt extract agar (MEA) (malt extract (Difco), 10 or 20 g, and agar, 15 g/L distilled water), and potato dextrose agar (PDA) (Difco). Color terms are according to Kornerup and Wanscher (1978). To test resistance to benomyl and cycloheximide, the strains were grown on modified Melin-Norkrans (MMN) (Marx 1969) and on MMN amended with 2 ppm benomyl, and on Mycosel agar (BBL). All cultures were incubated at 20 or 25°C. Material for microscopy was mounted in lactofuchsin, lactophenol, or glycerin jelly.

Dual culture for antagonism

The antagonistic ability of one strain of *A. pinicola* (UAMH 5983) against blue stain fungi associated with mountain pine beetles was examined using a dual culture method on agar plates. Four species of blue stain fungi were chosen: *Ophiostoma clavigerum* (Robins.-Jeff. & Davids.) Harrington (≡ *Ceratocystis clavigera* (Robins.-Jeff. & Davids.) Upadhyay) (UAMH 4819 = NOF-838), *O. montium* (Rumb.) von Arx (UAMH 4910 = NOF-450), *O. minus* (Hedgc.) H. & P. Sydow (UAMH 4820 = NOF-839), and *O. huntii* (Robins.-Jeff.) de Hoog & Scheffer (UAMH 4825 = NOF-840). An inoculum disc (diameter, 6 mm) of *A. pinicola* and a disc of one of the blue stain fungi

[1]Author to whom correspondence should be addressed.

[2]Current address: Institute of Agriculture and Forestry, University of Tsukuba, Tsukuba, Ibaraki 305, Japan.

were cut from the periphery of each colony growing on MEA and placed opposite each other about 4 cm apart on MEA in a petri dish (diameter, 9 cm). Cultures were incubated at 20°C in the dark and were observed after 2 weeks for evidence of inhibition.

Mating tests

Six isolates (UAMH 5983, 6005, 6373, 6374, 6375, and 6376) were mated in all possible combinations. Agar discs from the colonies on WSH were placed on WSH and MEA plates approximately 3 cm apart and pieces of autoclaved bark of lodgepole pine placed between the inocula. Cultures were incubated at 20 or 15°C in the dark.

Pathogenicity to lodgepole pine seedlings

UAMH 5983 was selected to test pathogenicity to lodgepole pine seedlings. A band of bark 1 cm wide was girdled on the stem (at 5 cm above soil) of 1-year-old lodgepole pine seedlings planted in Spenser Lamella in a greenhouse controlled at about 25°C for 16 L:8D photoperiod. An agar block (about 5 mm^2) cut from a 3-week-old colony of UAMH 5983 on MEA was placed on the exposed cambium of the seedling. The inoculum was squashed onto the cambium by the replaced bark and the inoculated area sealed with Parafilm. Seedlings were also inoculated with an MEA block of *O. clavigerum* (NOF-838) and an agar block without fungus as controls. Three seedlings were used for each inoculum. The inoculated seedlings were kept in the same greenhouse and observed for 2 months.

Taxonomy

Arthrographis pinicola Sigler & Yamaoka sp.nov.

Coloniae in agaro ad 25°C moderatim lente crescunt, planae, albae vel cremeae, vel alutaciae, glabrae vel velutinae vel caespitosae. Incrementum nullum ad 37°C. Hyphae hyalinae, septatae, (0.5–)0.8–2.5 μm latae. Conidiophora caespitosa. Hyphae conidiogenae ramosae, septa in basipetalo successive occurrentia. Arthroconidia non-septata, laevia, hyalina vel massaliter alutacia, cylindrica, truncata, schizolytica, 1.5–4.0 μm longa × 1.5–2.5 μm lata. Teleomorphosis ignota est.

HOLOTYPUS: UAMH 6402. Specimen typi de exemplo *Pinus contortae* var. *latifoliae*, Nojack, Alta. leg. a Y. Yamaoka et P. Maruyama, Mar. 1989, continenti fungum in galleriis *Ipos latidentis*. Ex-typus de specimine holotypi constitutus est.

Description on the host

Growth in galleries is confluent and powdery (Fig. 1) or with discrete dome-shaped or floccose conidiomata (Figs. 2–5), white, yellow, or tan, up to 300 μm high, composed of septate, repeatedly branched, fertile hyphae, measuring (1.9–)2.1–2.7 μm in width. Fertile branches divide by transverse septa into small cells, with septation occurring in basipetal order, then break apart schizolytically to form arthroconidia without disjunctors or separating cells (Figs. 6–7). Rarely, a conidium may contain a longitudinal septum (Fig. 8, arrow), but whether it undergoes further division is unknown. Mature arthroconidia are hyaline, tan in mass, smooth, truncate, cylindrical, sometimes broader than long, 1.5–4.0 μm long and 1.5–3.2 μm wide, often remaining connected in chains of 3–4 (Fig. 6).

Description in culture

Colonies grown on WSH, 5 and 10% CER, and 2% MEA were similar in growth rate and colony appearance when grown in the dark at 20°C. They were flat, slow growing, measuring 15–24 mm after 2 weeks and 24–36 mm at 3 weeks, at first white to cream, appressed with little aerial mycelium, gradually darkening to greyish-yellow (4B3) and developing tan or yellowish-brown conidiomata (5C5–5E7) on the colony surface (Fig. 9). On CER, young colonies were glabrous with a purplish-brown surface pigmentation, reverse yellow, orange or purplish brown with scant tan diffusing pigment. Sporulation occurred by 2–4 weeks on CER (Fig. 9) and WSH. In some isolates, conidiomata occurred abundantly near the periphery, whereas in other isolates, conidiomata were sparse. Conidiomata were slow to develop and most sparse on MEA.

Growth was restricted (4–7 mm in 2 weeks) on MMN amended with benomyl; there was no growth at 37°C on PDA or on Mycosel agar.

Growth in culture is as on the host, except that the fertile hyphae are slightly narrower (1.5–2.5 μm wide) and the conidiomata are less well developed. Vegetative hyphae are narrow, septate and hyaline, (0.5–)0.8–2.5 μm wide, bearing narrow conidiophores which branch repeatedly to form floccose conidiomata (Figs. 10–11). The fertile branches are initially sparsely septate and of uniformly narrow diameter (Fig. 10), but, as arthroconidial development begins, the apical region broadens and septation occurs in basipetal sequence to form many small cells (Fig. 11). Arthroconidia secede by schizolysis (Fig. 12), often remaining connected in chains of 3–4, which then undergo further schizolysis. In slide culture preparations using 10% CER, the conidiomata are slow to develop and arthroconidia occasionally develop by fragmentation of more or less undifferentiated hyphae; these arthroconidia are often irregular in shape. There are no disjunctors or separating cells, but occasionally, in stained preparations, a cell within a chain of arthroconidia remains unstained. No rhexolysis was observed. Mature arthroconidia are smooth, hyaline, tan in mass, cylindrical, but often broader than long, 1.5–4.0 μm long × 1.5–2.5 μm wide. A teleomorph was not observed on any medium or in any of the mated strains. In paired cultures and in strains grown alone on 10% CER, dark brown sclerotium-like bodies were observed (Fig. 13, arrows). These structures were firm, but not hard, and composed of septate hyphae and many crystals (Figs. 14 and 15). No internal spores were observed after incubation periods of up to 11 weeks. No yeast stage was observed.

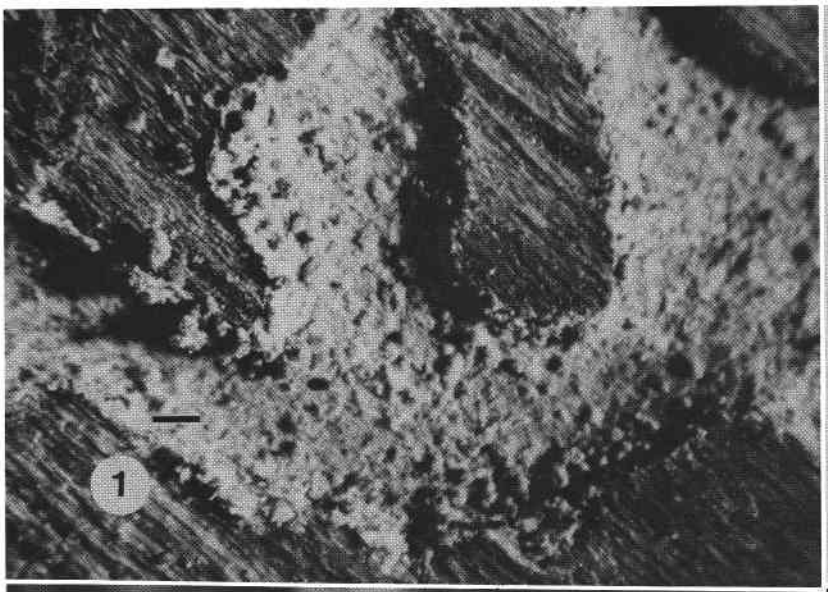
HABITAT: Wood of *P. contorta* var. *latifolia*, especially in galleries of *I. latidens*.

The holotype specimen, the ex-type culture, and other strains are maintained at the University of Alberta Microfungus Collection and Herbarium (UAMH). An ex-type culture, and several other specimens and cultures are deposited at the Northern Forestry Centre (CFB, NOF). A dried colony and ex-type culture have been deposited at the National Mycological Herbarium and National Fungus Culture Collection (DAOM 210573).

SPECIMENS EXAMINED: CBF-21920, *I. latidens* gallery in *P. contorta* var. *latifolia*, Whitney Creek, Alta., collected by Y. Hiratsuka, P. J. Maruyama and Y. Yamaoka, July, 1986; UAMH 6402 (TYPE), CFB-21922, 21923, 21924, 21925, and 21926, *I. latidens* galleries in *P. contorta* var. *latifolia*, Nojack, Alta., collected by Y. Yamaoka and P. Maruyama, March 1989.

LIVING STRAINS: UAMH 5983 (NOF-1222) and UAMH 6005

FIGS. 1–8. *Arthrographis pinicola* in galleries of *Ips latidens*. Fig. 1. Confluent powdery growth. Bar = 0.5 mm. Figs. 2–5. Floccose conidiomata on gallery wall. Figs. 2 and 5, bar = 50 μm; Figs. 3 and 4, bar = 100 μm. Figs. 6–8. Arthroconidia produced by schizolytic dehiscence. Note longitudinal septum in terminal arthroconidium in Fig. 8 (arrow). Bar = 10 μm. Figs. 1 and 8, UAMH 6402; Figs. 2, 3, 5, and 6, CFB 21920; Fig. 4, CFB 21925.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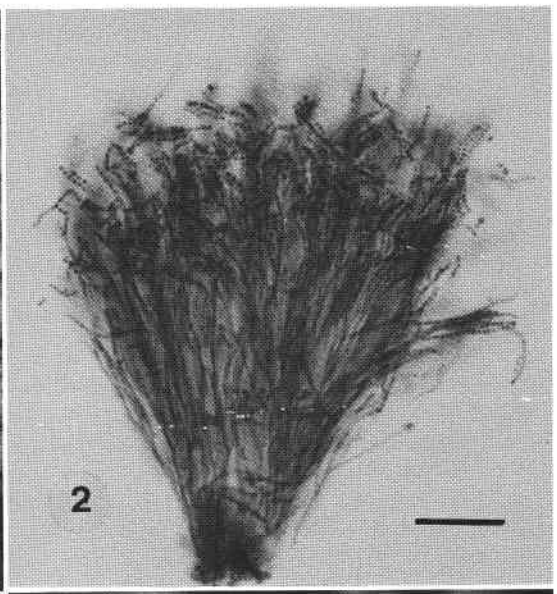
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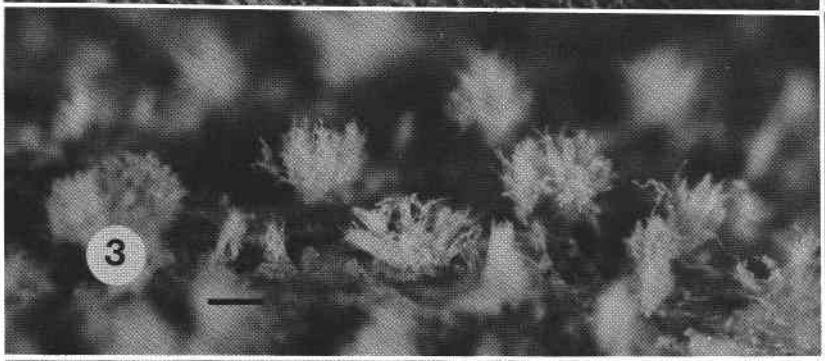
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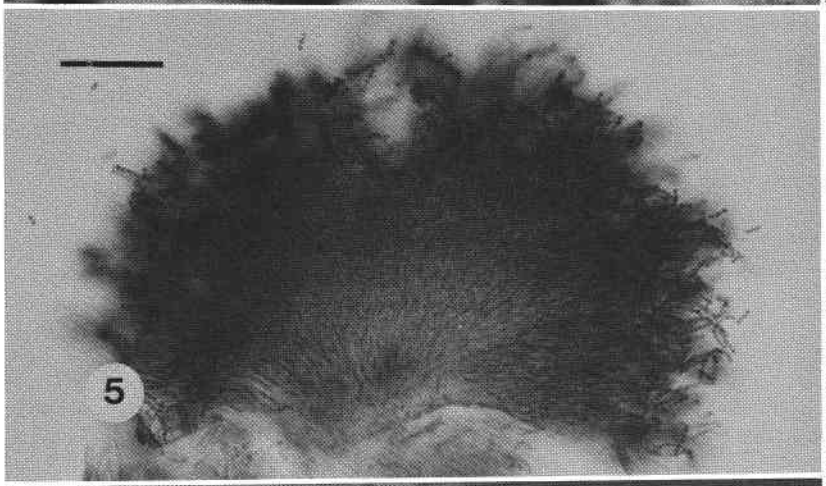
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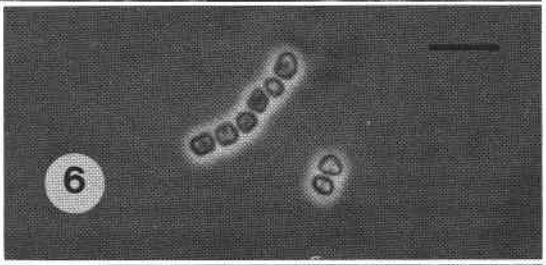
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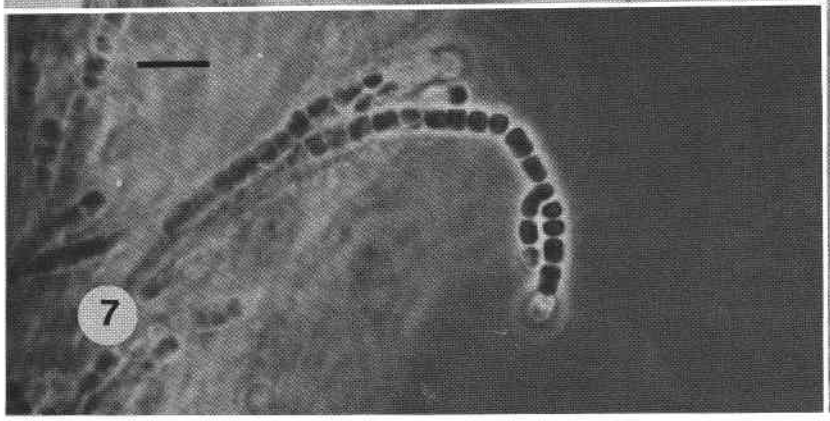
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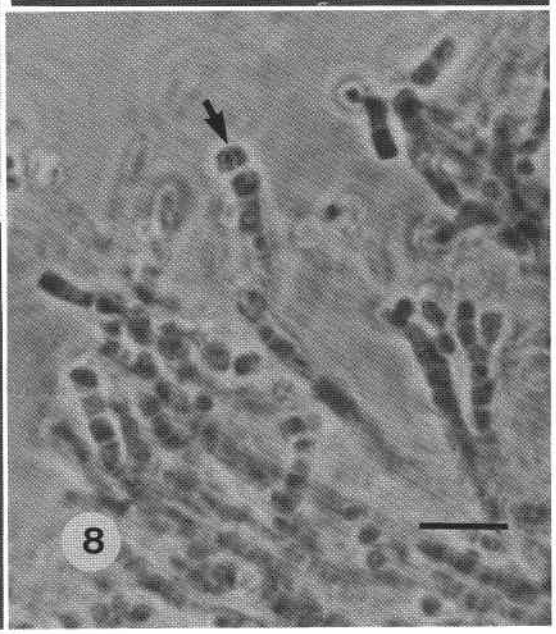
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