

## Conifer inhabiting species of *Phyllosticta*

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One species of *Macrophoma* and five *Phyllosticta* species isolated from symptomless and diseased conifer needles are described and illustrated. Information on growth, morphology, and enzyme production is included. *Macrophoma piceae* and *Phyllosticta pseudotsugae* are described as new. Endophytic isolates from *Abies balsamea* in Québec, Canada, have been identified as *P. multicorniculata* and strains derived from needles of *Abies grandis* and *Pseudotsuga menziesii* collected in Oregon, USA, have proved to belong to *P. abietis*. The *Phyllosticta* anamorph of *Discochora pini*, similar to the isolates from Asian *Pseudotsuga* spp. and to *P. cryptomeriae*, is included for comparison. A key to nine conifer-inhabiting *Phyllosticta* spp. is provided.

Keywords: Coelomycetes, taxonomy, pectinases, amylases, isozymes.

Species of *Phyllosticta* PERS. are leaf pathogens, mostly with a host spectrum limited to a number of congeneric host species; some *Phyllosticta* species, however, are known to occur as endophytes of various plants (PETRINI, 1986). The presence of *Phyllosticta* spp. in symptomless needles of *Abies amabilis* (DOUGL.) FORBES, *A. grandis* (DOUGL.) LINDL., *A. magnifica* A. MURR., *A. procera* REHDER, and *Pseudotsuga menziesii* (MIRB.) FRANCO was demonstrated by CARROLL & CARROLL (1978) in the Pacific Northwest. PETRINI & al. (1989) regularly isolated an unidentified species of *Phyllosticta* from healthy and galled needles of *A. balsamea* (L.) MILL. in Québec, Canada. In other studies *Phyllosticta* spp. appeared to be the dominant endophytes of fungal communities in needles of *Cryptomeria japonica* D. DON, *Pseudotsuga brevifolia* C.Y. CHENG, W.C. CHENG & L.K. FU, *P. gaussenii* FLOUS, *P. japonica* (SHIRAS.) BEISSNER, and *P. sinensis* DODE (G.C. CARROLL, unpublished). Although cultures of *Phyllosticta* derived from different hosts showed a very similar gross morphology, it soon became obvious that these isolates were distinct taxa.

*Phyllosticta* spp. occurring on angiospermous hosts were exhaustively monographed by VAN DER AA (1973); subsequent studies dealt

with nomenclatural problems of the teleomorphs ( BISSETT, 1986a,b), and with new species collected on mono- and dicotyledons (YIP, 1989; BISSETT, 1986b), but very little was published on conifer inhabiting *Phyllosticta* spp. (FUNK, 1985; KOBAYASHI & SASAKI, 1975; SIVANESAN, 1979). Recently, BISSETT & PALM (1989) described four new species of *Phyllosticta* from coniferous hosts in North America. BISSETT & PALM, however, based their work only on herbarium material and did not include any description of cultures. Some of the isolates encountered in this study could not be identified as any of the taxa included in the key provided by BISSETT & PALM (1989). Those have been determined to be two new taxa and are herein described. Additionally, information on growth, morphology, and extracellular enzyme production in culture is provided for five species treated in this paper.

### Material and methods

The material examined and the origin of the cultures studied is summarized in Tab. 1. Seventy-five endophytic strains from Canada, China, Japan, and Oregon, mostly isolated from apparently healthy needles and from needles showing light disease symptoms were obtained by the method of CARROLL & CARROLL (1978). All isolates were derived from hosts growing within their natural distribution range. Two cultures of *M. piceae* were obtained as single spore isolates from material collected in Switzerland.

To induce sporulation, cultures were either incubated under near UV-light (370 nm, 8 hours dark/16 hours light cycle) at 15 C or the mycelium was inoculated on sterilized needles of *Abies* sp. and incubated at 20 C in the dark.

Within each collection conidia or in some cases ascospores were mounted in water and the length and width of at least ten of them, chosen at random, were measured at 1000x magnification. Minimum, mean, standard deviation, and maximum values are given. Spore appendages, slimy caps and sheaths were studied by phase contrast microscopy and by mounting the spores in China ink diluted in water. Dried cultures and infected needles are deposited in ZT, selected living cultures are deposited at CBS.

To test growth on different substrates, the strains were grown in triplicate at 20 C during 30 days on 90 mm Petri-dishes containing either corn meal agar (CMA, Difco), Czapek - Dox agar (CZAPEK, Difco), malt extract agar (MA: 1 % malt extract, 2 % agar, Difco), or potato dextrose agar (PDA, Difco). Colony area, morphology, and colour were recorded. To determine temperature requirements, the strains were inoculated in triplicate on MA and incubated in the dark at 3 C, 10 C, 15 C, 20 C, and 27 C. The area of the colonies was

Tab. 1. — Origin of the *Phyllosticta* isolates studied. C: fructifications formed in culture; S: fructifications formed on sterilized needles. Unless otherwise stated, all were isolated as endophytes from the foliage of the respective hosts. Working numbers marked by \* have been used for substrate utilization tests and physiological studies.

no.	Species	Host	Locality	Date	Fructification substrate and other remarks
1	<i>P. cryptomeriae</i>	<i>Cryptomeria japonica</i>	JAPAN: Kumamoto Prefecture, Kumamoto City, Yakushima stram <i>Cryptomeria</i> , Tatsutayama	Dec. 1988	C
2	"	"	JAPAN: Kumamoto Prefecture, Kumamoto City, Aya Stram <i>Cryptomeria</i> , Tatsutayama	"	S
3	<i>P. pseudotsugae</i>	"	JAPAN: Kumamoto Prefecture, Kadoyama, Shimoshima, Amakusa	26 Oct. 1988	C, S
4	<i>P. cryptomeriae</i>	"	JAPAN. Kumamoto Prefecture, vicinity of Mt. Aso	4 Nov. 1988	C, S
5	"	"	JAPAN: Kyoto Prefecture, Kyoto City, Iwakura, Sakyoku Ward	12 Jan. 1989	C
6	"	"	JAPAN: Kyoto Prefecture, Kyoto City, near Sasari Pass, Sakyoku Ward	"	C
7	"	"	JAPAN: Kyoto Prefecture, Kyoto City, ca 2 km N of Kurama, Sakyoku Ward	Feb. 1989	C
8	"	"	"	"	C
9	"	"	JAPAN: Gifu Prefecture	Oct. 1988	C
10	"	"	JAPAN: Shiga Prefecture	"	C
11	"	"	JAPAN: Miyazaki Prefecture, close to Kagoshima Prefecture Boundary, Mt. Kirishima, Rokkan Ike	Nov. 1988	C, S
12	"	"	JAPAN: Kumamoto Prefecture, Mt. Yoshimuta, Yabe-cho	Sept. 1988	S
13	"	"	JAPAN: Yamaguchi Prefecture, Yamaguchi	Oct. 1988	C
14	"	"	JAPAN: Yamagata Prefecture, Higashine City	25 Nov. 1988	C

no.	Species	Host	Locality	Date	Fructification substrate and other remarks
15	"	"	JAPAN: Miyazaki Prefecture, Mt. Wanizuka	18 Nov. 1988	C, S
16	"	"	JAPAN: Ibaraki Prefecture, Tsukuba, Kukizaki-cho, Inashiki-gun	Oct. 1988	C
17	"	"	JAPAN: Akita Prefecture, Tsubakigawa, Yuwa-cho, Kawabe-gun, Akita Forestry Centre	"	C
18	"	"	JAPAN: Hokkaido Prefecture, Kikonai-cho, Kamiiso-gun	"	C
19a	<i>P. pseudotsugae</i>	"	JAPAN: Kumamoto Prefecture, Tatsutayama, Kumamoto City	Dec. 1988	C
19b	<i>P. cryptomeriae</i>	"	JAPAN: Kagoshima Prefecture, Mt. Kurino, Yoshimatsu-Cho	28. Sept. 1988	S
19c	"	"	JAPAN: Kumamoto Prefecture, Kumamoto	Dec. 1988	S
19d	"	"	"	Sept. 1988	S
20	<i>P. pseudotsugae</i>	<i>Pseudotsuga japonica</i>	JAPAN: Kino, Kamigamo Forestry Exp. Sta	Jan. 1989	C
21	"	"	"	"	C, S
22	"	"	"	"	C
23	"	<i>Pseudotsuga brevifolia</i>	P.R. CHINA: Guangxi Province, Ne-po	Fall 1988	S; Collections 23-27c are all derived from the same tree
24	"	"	"	"	C
25	"	"	"	"	C, S
26	"	"	"	"	C, TYPE; S, ISOTYPE
27	"	"	"	"	S

no.	Species	Host	Locality	Date	Fructification substrate and other remarks
28	"	<i>Pseudotsuga gaussenii</i>	P.R. CHINA: Zhejiang (Chekiang) Province, Lin-an Co.	"	C, S; Collections 28–31d are all derived from the same tree
29	"	"	"	"	C, S
30	"	"	"	"	C, S
31a	"	"	"	"	C, S
31b	"	"	"	"	S
31c	"	"	"	"	S
31d	"	"	"	"	S
32	"	<i>Pseudotsuga sinensis</i>	P.R. CHINA: Sichuan Province, Nan-chuan	"	C; Collections 32–35c are all derived from the same tree
33	"	"	"	"	C
34	"	"	"	"	C, S
35a	"	"	"	"	C, S
35b	"	"	"	"	S
35c	"	"	"	"	S
36 *	<i>P. abietis</i>	<i>Pseudotsuga menziesii</i>	USA: Oregon, Eugene	1987	C
37 *	<i>P. pseudotsugae</i>	<i>Pseudotsuga japonica</i>	JAPAN: Kyoto University, Botanical Garden	Feb. 1987	C

no.	Species	Host	Locality	Date	Fructification substrate and other remarks
38 *	<i>M. piceae</i>	<i>Picea abies</i>	SWITZERLAND: Kt. Aargau, Aarburg	10 Dec. 1987	C; single spore isolate; also material on host examined, TYPE
39 *	"	"	SWITZERLAND: Ramerenwald	21 Jan. 1988	"
40 *	<i>P. cryptomeriae</i>	<i>Cryptomeria japonica</i>	JAPAN: Kyoto City	Feb. 1987	C
41	<i>P. multicorniculata</i>	<i>Abies balsamea</i>	CANADA: Quebec, Parc des Laurentides, For. Montmorency		C
42	"	"	"	June 1987	C
43	"	"	"	"	C
44	"	"	"	"	C
45 *	"	"	"	July 1987	C
46	"	"	"	June 1987	C
47	"	"	"	Aug. 1987	C
48	"	"	"	June 1987	C
49	"	"	"	Aug. 1987	C
50	"	"	"	"	C
51 *	"	"	"	June 1987	C
52	"	"	"	"	C
53	"	"	"	Aug. 1987	C
54 *	"	"	"	July 1987	C
55 *	"	"	"	Aug. 1987	C
56 *	"	"	"	Aug. 1987	C
57 *	"	"	"	June 1987	C
58 *	"	"	"	June 1987	C
59	"	"	"	Aug. 1987	C

no.	Species	Host	Locality	Date	Fructification substrate and other remarks
60	"	"	"	Aug. 1987	C
61a	"	"	"	June 1987	C
61b	"	"	"	Aug. 1987	on galls of <i>Paradiplosis</i> <i>tumifex</i>
62	<i>P. abietis</i>	<i>Abies grandis</i>	USA: Oregon, Eugene	"	C
63	"	"	"	"	C
64	"	"	"	"	C
65	"	"	"	"	C
66	"	"	"	"	C
67	"	"	"	"	C
68	"	"	"	"	C

measured after 21 days with an IBAS-image analyzer (ZEISS) and the median value of the three replicates was computed. Observation of subjective parameters such as colour, appearance, and margin of the colonies was done by two observers independently and the results were then compared.

To study substrate utilization, the isolates were inoculated in triplicate on Bavendamm agar (NOBLES, 1958) to test for production of peroxidases, on starch agar (1 % corn starch, 1 % malt, 2 % agar) for detection of extracellular amylases and on a medium containing casein (0.5 % casein, 0.1 % yeast extract, 2 % agar) for extracellular protease production (PETRINI & al., 1984). Production of extracellular cellulases was tested on the medium described by SMITH (1977). Growth and reactions for all tests were recorded after 15 days incubation at  $15 \pm 2$  C in the dark.

For detection of pectic and amylase enzymes the cultures were grown as described by CRUICKSHANK & PITT (1987) and incubated for ten days. Electrophoreses were performed in vertical slab gels of 10% polyacrylamide containing either 0.1 % citrus pectin (SWEETINGHAM & al., 1986) or 0.1 % soluble starch solution. Staining of pectic enzymes and evaluation of the gels were done by the method of CRUICKSHANK & WADE (1980), and of  $\alpha$ -amylase according to VALLEJOS (1983). Zymograms were recorded graphically.

### Key to the identification of conifer inhabiting *Phyllosticta*

Species not treated here but described by BISSETT & PALM (1989) are also included.

Minimum, mean, and maximum values (except for *P. concentrica*) are given for the measurements.

1. Conidia with one or more appendages, or, if without, almost subglobose to globose and less than 12.5  $\mu\text{m}$  diam. Conidiomata on the host usually 75–425  $\mu\text{m}$ , in culture to 625  $\mu\text{m}$  diam. . . . . 2
1. Conidia without appendages, narrowly ellipsoidal, longer than 12, up to 41  $\mu\text{m}$ . Conidiomata on the host usually (80)350–850  $\mu\text{m}$ , in culture 600–2000  $\mu\text{m}$  diam. . . . . 9
  2. Conidia without appendages, or only rarely with a very short appendage, subglobose to globose to broadly ellipsoidal, 8.5–10.5–12.5  $\times$  7.2–9.2–11.5  $\mu\text{m}$  (on the host: 7–12.1  $\times$  6.6–8.8  $\mu\text{m}$ , *vide* BISSETT & PALM), on *Abies* spp. and *Pseudotsuga menziesii* . . . . . 1. *P. abietis*
  2. Conidia with one or more appendages . . . . . 3
3. Conidia with one to five appendages, 7–20  $\mu\text{m}$  wide; on *Abies* spp. . . . . 3. *P. multicorniculata*



3. Conidia with only one appendage, up to 10.5 (14)  $\mu\text{m}$  wide; on other hosts ..... 4
4. Conidia nearly globose to irregularly ellipsoidal, 9–11–13.5 x 6.3–8.4–10.5  $\mu\text{m}$ ; on *Cryptomeria* ..... 2. *P. cryptomeriae*
4. Conidia mainly obpyriform, narrowly ovoidal, irregularly ellipsoidal to almost cylindrical; usually not on *Cryptomeria* .... 5
5. Conidia (8)10–15(20) x (4)7–10(14)  $\mu\text{m}$ ; on *Araucaria*, *Taxus*, and non-coniferous hosts (if on *Araucaria* and conidia 5.8–11.8 x 4–6.6  $\mu\text{m}$ , see *P. acicola*) ..... *P. concentrica* SACC.  
(BISSETT & PALM, 1989; VAN DER AA, 1973)
5. Conidia 7–14.8 x 4–9  $\mu\text{m}$ ; on other hosts ..... 6
6. Conidial appendage in water less than 10  $\mu\text{m}$  long ..... 7
6. Conidial appendage in water 10–17  $\mu\text{m}$  long ..... 8
7. Conidia 6.5–9–11 x 4–6–7.5  $\mu\text{m}$ , the mucilaginous sheath always present and at the base of conidium usually 2–4  $\mu\text{m}$  thick; on *Pinus* ..... 6. *Phyllosticta* anamorph of *Discochora pini*
7. Conidia 6.7–9.5–13.5 x 5–6.7–9  $\mu\text{m}$ , the mucilaginous sheath, if present, at the base of conidium usually only 2  $\mu\text{m}$  thick; on *Pseudotsuga*, rarely on *Cryptomeria* ..... 5. *P. pseudotsugae*
8. Conidia 5.8–8.7–11.8 x 4–5–6.6  $\mu\text{m}$ , apical appendage in water up to 17  $\mu\text{m}$  long, filiform; on *Araucaria* ..... *P. acicola* BISSETT & PALM  
(BISSETT & PALM, 1989)
8. Conidia 7.1–10.4–14.8 x 4.8–5.6–6.8  $\mu\text{m}$ , apical appendage in water up to 15  $\mu\text{m}$  long, conoidal; on *Thuja* ..... *P. thujae* BISSETT & PALM  
(BISSETT & PALM, 1989)
9. Conidia 12–16–20 x 5–7–8.5  $\mu\text{m}$ ; on *Picea* ..... 4. *Macrophoma piceae*
9. Conidia 21–41  $\mu\text{m}$  long; on other hosts ..... *Macrophoma* spp.  
(BISSETT & PALM, 1989)

1. *Phyllosticta abietis* BISSETT & PALM, Can. J. Bot. 67: 3379. 1989. — Fig. 1.

Teleomorph. — Unknown.

Colony on MA grey to black in the center, dark brown to black towards the margin, sometimes with grey spots, mycelium felty to woolly, appressed, margin lobed, reverse black. — Conidiomata

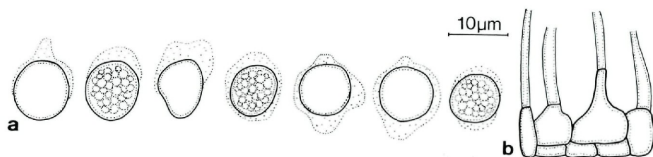


Fig. 1. — *Phyllosticta abietis*. — a. Conidia formed in culture. — b. Conidiophores formed in culture.

in culture 150–475 µm diam., globose, black, hairy when young, in dense clusters embedded in the mycelium and formed over the whole colony, conidial mass white to cream. — Conidiogenous cells 10–23 × 3 µm, holoblastic, cylindrical, hyaline, soon disappearing, sessile or rarely on short conidiophores. — Conidia  $(8.5)10.5 \pm 0.9$   $(12.5) \times (7.2)9.2 \pm 0.7$   $(11.5) \mu\text{m}$ , almost globose, subglobose to broadly ellipsoidal, sometimes with a slightly flattened basis, rarely with a very short apical appendage, hyaline, filled with numerous guttules, in young stage completely surrounded by a mucilaginous sheath which is gradually reduced or absent in old material.

Characters on the host. — See BISSETT & PALM (1989).

Habitat. — Needles of *Abies grandis*. Isolated as endophyte from needles of *A. grandis* and *Pseudotsuga menziesii*.

Distribution. — USA, Pacific Northwest.

Material examined. — USA, Idaho, Coeur d'Alène, In foliis *Abietis grandis* Lindl., 24.8.1983, leg. R.S. HUNT, DAOM 188797 (TYPUS). Nos 36, 62–68 (s. Tab. 1).

Substrate utilization (Tab. 2). — The strain investigated for enzyme production (no. 36) was able to decompose starch and showed a positive Bavendamm reaction. No proteolytic or cellulolytic activity was observed. No growth occurred at 3 C or 27 C, optimum growth was at 20 C. The largest colony diameters were observed on CMA, followed by MA, CZAPEK, and PDA. Production of pectin esterase was detected by electrophoresis (Fig. 7).

*P. abietis* differs from *P. pseudotsugae* by its cultural appearance and by the size and the morphology of the conidia, as well as by the pectic and amylase isoenzyme patterns (Fig. 7). The conidia of *P. abietis* are slightly larger in culture than on the host, an observation already made by SIVANESAN (1979) for the anamorph of *Discochora pini* (SIVANESAN) BISSETT & PALM. The small conoidal appendage described by BISSETT & PALM (1989) was not observed in the isolates studied here.

Tab. 2. – Results of physiological tests carried out on selected strains of *Phyllosticta* spp. and *Macrophoma piceae*. T<sub>MIN</sub>: minimum growth temperature; T<sub>OPT</sub>: optimum growth temperature; T<sub>MAX</sub>: maximum growth temperature; -: no reaction or enzyme production; +: reaction or enzyme production present; (+): weak reaction or enzyme production.

Taxon	Strains examined	T <sub>MIN</sub> [C]	T <sub>OPT</sub> [C]	T <sub>MAX</sub> [C]	Growth CMA [cm <sup>2</sup> ]	Growth PDA [cm <sup>2</sup> ]	Growth MA [cm <sup>2</sup> ]	Growth CZAPEK [cm <sup>2</sup> ]	Bavendamm reaction	Proteases	Amylases	Cellulases
<i>P. abietis</i>	36	10	15	20	8.8	1.2	3.5	2.6	+	-	+	-
<i>P. cryptomeriae</i>	40	10	20	27	6	7	7.5	1.2	+	+	+	-
<i>P. multicorniculata</i>	45, 51, 54, 55, 56, 57, 58	10	20	27	2.7	15.1	2.4	0	+	-	+	-
<i>M. piceae</i>	38, 39	10	20	27	23	52.7	23.7	2.3	+	+	(+)	(+)
<i>P. pseudotsugae</i>	37	10	27	>27	5.8	20	10.9	7.4	+	+	+	-

2. *Phyllosticta cryptomeriae* KAWAMURA, Bull. Gov. For. Exp. Stat. 10: 97. 1913. — Fig.2.

Teleomorph. — *Discochora sawadae* (KOBAYASHI) BISSETT & PALM, Can. J. Bot. 67: 3378. 1989. 1975.

Colony on MA cream, grey or light brown in the center, cream to dark brown towards the margin, mycelium felty, appressed, margin entire, in old cultures lobed, reverse black in the center, light brown towards the margin. — Conidiomata in culture 125–350  $\mu\text{m}$  diam., up to 800  $\mu\text{m}$  high, globose to cylindrical, black at maturity, grey and hairy when young, formed in the center in dense clusters or singly at the margin, covered by the mycelium, partly or completely immersed in the agar. — Conidiomata on sterilized needles 125–300  $\mu\text{m}$  diam., 150–250  $\mu\text{m}$  high, globose to cylindrical, black, in dense clusters, conidial mass white to cream. — Conidiogenous cells 8–14.5–27  $\times$  2.3–3–5.5  $\mu\text{m}$ , holoblastic, cylindrical, tapering towards the apex, sessile or rarely on very short conidiophores. — Conidia (9)11  $\pm$  1(13.5)  $\times$  (6.3)8.4  $\pm$  0.8(10.5)  $\mu\text{m}$ , nearly globose to irregularly ellipsoidal, slightly broadening towards the apex, hyaline, filled with numerous guttules, with a 5–10  $\mu\text{m}$  long and 1–2  $\mu\text{m}$  wide flexuous cellular appendage at the apex, up to 25  $\times$  1  $\mu\text{m}$  when mounted in KOH. In young material the whole apex and the appendage are embedded in a mucilaginous sheath.

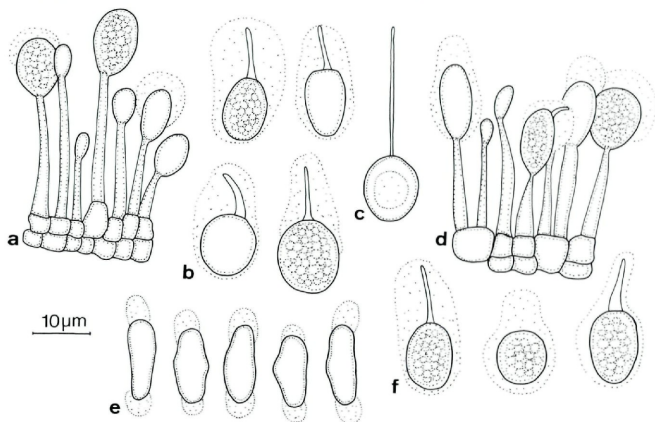


Fig. 2. — *Phyllosticta cryptomeriae*. — a. Conidiophores formed in culture. — b. Conidia formed in culture. — c. Conidium mounted in KOH showing the elongated appendage. — d. Conidiophores formed on inoculated needles. — e. Ascospores formed on inoculated needles (19d). — f. Conidia formed on inoculated needles.

Ascomata 150–175  $\mu\text{m}$  diam., 100–125  $\mu\text{m}$  high, subglobose, black. — Asci not seen. — Ascospores 11–14.5  $\times$  4.5–5.5  $\mu\text{m}$ , ellipsoidal, widest in the middle, constricted at each end, hyaline, with slimy caps at each end.

Habitat. — Endophytic in needles of *Cryptomeria japonica*.  
Distribution. — Japan.

Material examined. — Nos 1, 2, 4–18, 19b,c,d, 40 (s. Tab. 1)

Strain no. 5 produced conidia typical for *P. cryptomeriae*, but the olive grey colour of that culture is unusual for this species.

Substrate utilization (Tab. 2). — The strain investigated (no. 40) was able to decompose starch and casein but not cellulose and scored positively in the Bavendamm test. Minimum growth temperature was 10 C and optimum 20 C. The strain grew equally well on PDA, CMA, MA and only scant growth was observed on CZAPEK. Polygalacturonase and amylase were detected (Fig. 7).

*P. cryptomeriae* differs from *P. acicola* BISSETT & PALM and *P. thujae* BISSETT & PALM by its wider, more rounded conidia with a shorter conidial appendage, and from *P. concentrica* SACC. by the smaller and more rounded and regularly shaped conidia of *P. cryptomeriae*.

3. *Phyllosticta multicorniculata* BISSETT & PALM, Can. J. Bot. 67: 3382. 1989. — Fig. 3.

Teleomorph. — Unknown.

Culture on MA dark grey to black, margin white, sometimes with yellow mycelium, felty to woolly, appressed, margin lobed and fringed, reverse black. — Conidiomata in culture 125–350  $\mu\text{m}$  diam., subglobose to globose, black, hairy when young, in the center densely crowded and embedded in the mycelium, or scattered all over the mycelium in small clusters, conidial mass white to cream. — Conidiogenous cells 9–16–25.5  $\times$  2–3.5–4.8  $\mu\text{m}$ , holoblastic, cylindrical, tapering towards the apex, sometimes with indistinct proliferations. — Conidia (11.2)16  $\pm$  2.1(20.7)  $\times$  (7.2)12.2  $\pm$  2.5 (19.8)  $\mu\text{m}$ , irregular in shape, nearly globose to irregularly ellipsoidal, broadening towards the apex, hyaline, filled with numerous guttules, when young surrounded by a mucilaginous sheath, with up to five, 3–7  $\times$  1–2  $\mu\text{m}$  long cellular appendages.

Characters on the host. — See BISSETT & PALM (1989).

Habitat. — Needles of *Abies balsamea*, *A. concolor*. Isolated as endophyte from needles of *A. balsamea* and apparently associated

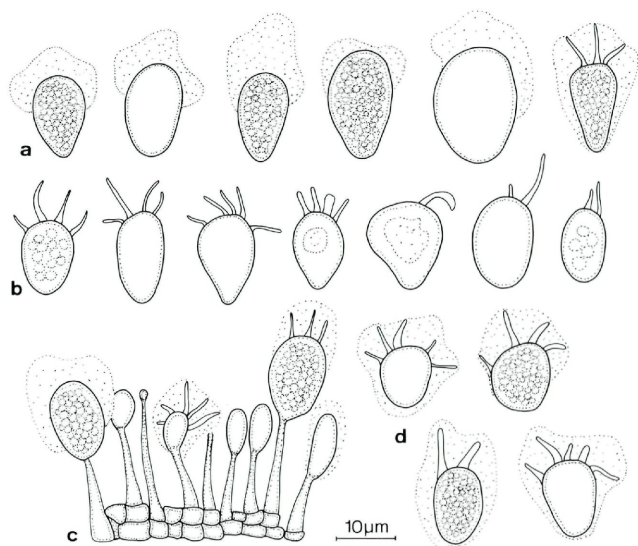


Fig. 3. — *Phyllosticta multicorniculata*. — a. Conidia formed in culture. — b. Conidia formed in culture mounted in KOH. — c. Conidiophores formed on galled needles infected by *Paradiplosis tumifex*. — d. Conidia formed on galled needles infected by *Paradiplosis tumifex*.

with galls of *Paradiplosis tumifex* GAGNÉ (PETRINI & al., 1989, sub *Phyllosticta* sp.).

Distribution. — North America.

Material examined. — Nos 41–61b (s. Tab. 1).

The appendages and the mucilaginous sheath are present only in very young material and very soon disappear. The conidia become larger with increasing age of the culture. Moreover, the conidia of *P. multicorniculata* are slightly larger in culture than on host tissue, as already described for *P. abietis*. BISSETT & PALM (1989) give 10–14.2 x 8.6–11.2 µm as the size of the conidia on the host. They also associated *P. multicorniculata* with needle blight of *Abies*. PETRINI & al. (1989) isolated it from symptomless needles of *A. balsamea* and from those infected with galls of *Paradiplosis tumifex*.

Substrate utilization (Tab. 2). — The strains investigated (nos 45, 51, 54–58) tested positive for amylase and Bavendamm reaction. No proteolytic and cellulolytic activity was found. Mini-

mum growth temperature was 10 C with a flat optimum between 20 C and 27 C. All strains grew well on PDA, with only very little growth on CMA and MA, and no growth at all on CZAPEK. The citrus pectin remained unaltered on the pectic isoenzyme gel (Fig. 7).

*Phyllosticta multicorniculata* is conspicuous in its conidial morphology. This is confirmed by the pectic and amyolytic isoenzyme patterns (Fig. 7).

4. *Macrophoma piceae* L. E. PETRINI, sp. nov. — Fig. 4.

Teleomorph. — Unknown.

Culturae in agaro maltoso albae, aetate provecta griseobrunneae ad brunneae. Conidiomata in cultura 600–1200 (2000)  $\mu\text{m}$  lata, ad 1000  $\mu\text{m}$  alta, semiglobosa vel cylindrica, nigra. Cellulae conidiogae 4–18  $\times$  3–6  $\mu\text{m}$ , cylindricae, apicem versus angustatae, holoblasticae. Conidia 12–16–20  $\times$  5–7–8.5  $\mu\text{m}$ , anguste ellipsoidea, basi truncata, hyalina, rare vagina mucosa tenuissima circumdata. Conidiomata in foliis hospitis 350–850  $\mu\text{m}$  lata, 125–625  $\mu\text{m}$  alta. Cellulae conidiogae 7–10  $\times$  3–4.5  $\mu\text{m}$ , ampulliformes vel cylindricae, apicem versus angustata. Conidia 12–16  $\times$  5–7  $\mu\text{m}$ .

Habitat in foliis ramisque *Piceae abietis*.

Culture on MA initially white, later turning grey brown to brown, mycelium woolly with powdery appearance, becoming felty with age, margin fringed, reverse olive to black. — Conidiomata in culture 0.6–1.2(2) mm wide, to 1 mm high, subglobose to cylindrical, black, sometimes fused together, in old cultures some conidiomata to 3 mm high and 2 mm wide, hairy and columnar in shape, conidial mass white. — Conidiogenous cells 4–18  $\times$  3–6  $\mu\text{m}$ ,

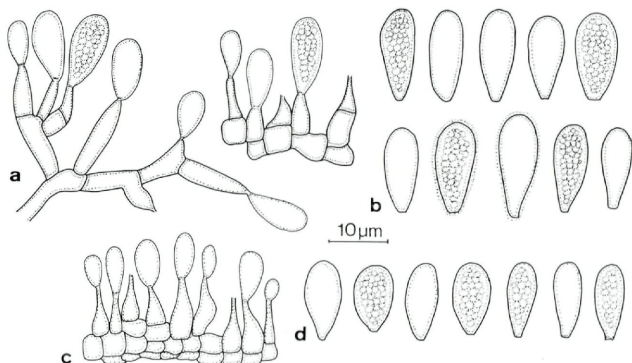


Fig. 4. — *Macrophoma piceae*. — a. Conidiophores formed in culture. — b. Conidia formed in culture. — c. Conidiophores formed on the host. — d. Conidia formed on the host.

holoblastic, cylindrical, tapering towards the apex. — Conidia  $(12)16 \pm 1.4$  (20)  $\times$   $(5)7 \pm 0.8$  (8.5)  $\mu\text{m}$ , narrowly ellipsoidal with a truncate base, widest near the apex, hyaline, filled with numerous guttules, rarely surrounded by a very thin, mucilaginous sheath.

Conidiomata on the host 350–850  $\mu\text{m}$  diam., 125–625  $\mu\text{m}$  high, semiglobose, often rostrate, black, breaking through the epidermis. — Conidiogenous cells 7–10  $\times$  3–4.5  $\mu\text{m}$ , ampulliform to cylindrical, tapering towards the apex. — Conidia 12–16  $\times$  5–7  $\mu\text{m}$ . Other characters as in culture.

Habitat. — Needles and small twigs of *Picea abies* (L.) KARST.  
Distribution. — Europe, Switzerland.

Material examined. — SWITZERLAND, Canton Aargau, Aarburg, on *Picea abies*, 10. Dec. 1987, leg. E. KANZLER, U. HEINIGER, no. 38 (TYPUS), ZT. No. 39 (s. Tab. 1).

A very thin mucilaginous sheath surrounding the conidia has been infrequently observed. The absence of a more conspicuous sheath and the large conidiomata suggest that this is a *Macrophoma* rather than a *Phyllosticta*.

Substrate utilization (Tab. 2). — The strains investigated (nos 38, 39) showed only weak amyolytic, proteolytic and cellulolytic activity, but a definitely positive Bavendamm reaction. Minimum growth temperature was at 3 C, optimum at 20 C and no growth was observed at 27 C. The colony growth was comparatively extensive on PDA, less on MA and CMA, and extremely reduced on CZAPEK. Polygalacturonase and amylase were detected on the pectic and amyolytic isoenzyme gels (Fig. 7).

##### 5. *Phyllosticta pseudotsugae* L. E. PETRINI, sp. nov. — Fig. 5.

Teleomorph. — *Discochora* sp.

Culturae in agar maltoso nigra vel olivaceonigra. Conidiomata 125–625  $\mu\text{m}$  lata, ad 650  $\mu\text{m}$  alta, primo globosa, dein cylindrica vel rostrata, coralliformia, nigra. Conidiomata in foliis *Pseudotsugae* 125–475  $\mu\text{m}$  lata, 125–400  $\mu\text{m}$  alta. Cellulae conidiogenae 6–10.5–25  $\times$  2–6.5  $\mu\text{m}$ , cylindricae, apicem versus angustatae, holoblasticae. Conidia 6.7–9.5–13.5  $\times$  5–6.7–9  $\mu\text{m}$ , irregulariter ellipsoidea, apicem versus lata facta, hyalina, appendice cellulari 3–7  $\times$  1–2  $\mu\text{m}$  praedita, apice appendiceque vagina mucosa circumdati.

Habitat endophytice in foliis *Pseudotsugae* spp.

Culture on MA black or olive grey, mycelium dense, felty, appressed, sometimes woolly, sometimes skin-like and shiny, margin deeply lobed and fringed, reverse black. — Conidiomata in culture 125–625  $\mu\text{m}$  diam., to 650  $\mu\text{m}$  high, first globose, later cylindri-



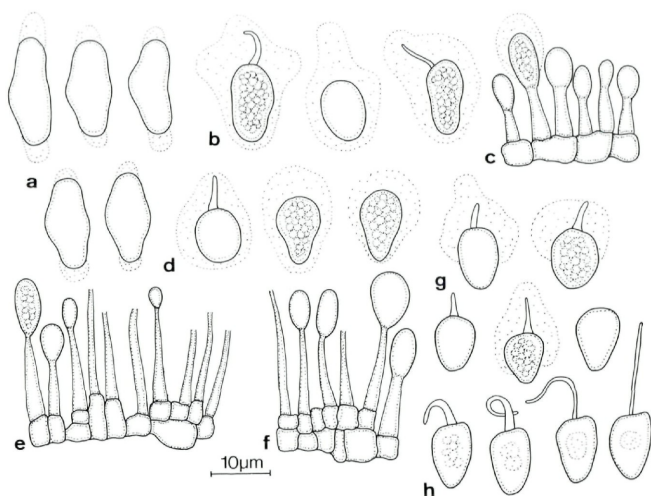


Fig. 5. — *Phyllosticta pseudotsugae*. — a. Ascospores formed in culture isolated from *Pseudotsuga brevifolia* (Type, 26). — b. Conidia formed in culture isolated from *Pseudotsuga brevifolia* (Type, 26). — c. Conidiophores formed in culture isolated from *Pseudotsuga brevifolia* (Type, 26). — d. Conidia formed on inoculated needles, strain isolated from *P. japonica*. — e. Conidiophores formed on inoculated needles, strain isolated from *P. japonica*. — f. Conidiophores formed in cultures isolated from *P. japonica*. — g. Conidia formed in culture isolated from *P. japonica*. — h. Conidium mounted in KOH, uncoiling its appendage.

cal and rostrate, forming coralloid structures, black, grey and hairy when young, often present in the center of the culture in dense clusters or forming a concentric ring around the margin. — Conidiomata on sterilized needles 125–475 µm diam., 125–400 µm high, subglobose to globose, black, in dense clusters, conidial mass white to cream. — Conidiogenous cells 6–10.5–25 x 2–3–6.5 µm, holoblastic, cylindrical, tapering towards the apex. — Conidia (6.7)9.5 ± 1.1(13.5) x (5)6.7 ± 0.7(9) µm, irregularly ellipsoidal to obpyriform, broadening towards the apex, hyaline, filled with numerous guttules, with a 3–7 µm long and 1–2 µm wide flexuous cellular apical appendage reaching 25 µm (45 µm) in KOH, entire apex and appendage embedded in a mucilaginous sheath when young.

Ascomata in culture sometimes scattered among the conidiomata and indistinguishable from them. — Asci not seen. — Ascospores 14.5–17 x 6.5–7.2 µm, ellipsoidal, widest in the middle and constricted at each end, hyaline, with slimy apical caps.

Habitat. — Endophytically in needles of *Cryptomeria japonica*, *Pseudotsuga brevifolia*, *P. gaussenii*, *P. japonica*, and *P. sinensis*.

Distribution. — Japan, Southwest China.

Material examined. — P. R. CHINA, Guangxi Province, Ne-po, isolated as endophyte from foliage of *Pseudotsuga brevifolia*, Fall 1988, leg. G. C. CARROLL, dried culture, no. 26, ZT (TYPUS), Nos 3, 19 – 35c, 37 (s. Tab. 1).

The microscopical characters of *P. pseudotsugae* in culture and on sterilized needles are almost identical. There is a tendency for *P. pseudotsugae* to produce larger conidia in culture but the differences are statistically not significant.

Ascospores have been found only in the original cultures of strain no. 26, isolated from *P. brevifolia*, as well as on sterilized needles and in cultures of isolate no. 3 from *Cryptomeria*. In isolate no. 3 the ascospores are smaller than those found in no. 26; they are similar in size to the ascospore from the collection 19d of *P. cryptomeriae* and are within the lower range of measurements given in the literature for *Discochora sawadae* (KOBAYASHI & SASAKI, 1975). *D. sawadae* may be a complex of more than one species that can be distinguished from each other on the basis of a combination of characters, the most important of which are conidial size and shape and cultural appearance. A similar situation has been demonstrated by PENNYCOOK & SAMUELS (1985) for *Botryosphaeria* and *Fusicoccum* species associated with Kiwi fruit rot in New Zealand.

All attempts to induce again teleomorph formation in strain no. 26 or to repeat the process in other strains failed even after inoculation onto sterilized needles. Because of our incomplete knowledge of the teleomorph we refrain from describing cultural types of *D. sawadae*.

Strains nos 3 and 19a, both isolated from *Cryptomeria*, show the typical characters of *P. pseudotsugae*. We have assigned these strains to *P. pseudotsugae* mainly because of their cultural characters and conidial morphology, even if this investigation has shown that *Phyllosticta* spp. on conifers are rather host-specific.

The isolates from the Asian *Pseudotsuga* spp. are culturally and morphologically diverse. The morphological and cultural differences found among the various isolates, however, are too small to justify the recognition of different taxa.

Substrate utilization (Tab. 2). — The strain investigated (no. 37) produced amylase, protease, but exhibited no cellulolytic activity. It also scored positively in the Bavendamm test. No growth was observed at 3 C, optimum at 27 C. Growth on PDA was very good, less on MA, CZAPEK, and CMA. The presence of polygalacturonase was detected on the pectinase gels (Fig. 7).

*Phyllosticta pseudotsugae* differs from *P. cryptomeriae* mainly by its smaller conidia and dark, almost black or olive-grey cultures. Also the isoenzyme patterns for pectinase and amylase (Fig. 7) justify the recognition of a new species. It is also different from *P. acicola* and from *P. thujae* by its slightly wider conidia and shorter conidial appendages. *P. pseudotsugae* has also smaller and more regularly shaped conidia than *P. concentrica*.

6. *Discochora pini* (SIVANESAN) BISSETT & PALM, Can. J. Bot. 67: 3378. 1989. — Fig. 6.

Basionym: *Guignardia pini* SIVANESAN, Trans. Br. Mycol. Soc. 73: 169. 1979.

Anamorph. — *Phyllosticta*.

Culture on PDA dark brown, grey black, with felty to woolly aerial mycelium, reverse black. — Conidiomata in culture 125–250  $\mu\text{m}$  diam., to 375 (700)  $\mu\text{m}$  high, globose to cylindrical, rostrate, forming coralloid structures. — Conidiogenous cells not seen. — Conidia (6.5)9  $\pm$  1 (11)  $\times$  (4)6  $\pm$  0.7 (7)  $\mu\text{m}$ , irregularly ellipsoidal, hyaline, filled with numerous guttules, surrounded by a 3–4  $\mu\text{m}$  thick mucilaginous sheath, cellular appendage to 5  $\times$  1–2  $\mu\text{m}$ .

Conidiomata on the host 125–250 (300)  $\mu\text{m}$  diam., subglobose, black, subepidermal. — Conidiogenous cells 6.3–8–11  $\times$  2.3–3.5–4.5  $\mu\text{m}$ , cylindrical, tapering towards the apex. — Conidia 7–9–10.5  $\times$  4.5–6–7.5  $\mu\text{m}$ , with a 4–10  $\times$  1  $\mu\text{m}$  long cellular appendage. Other characters as in culture.

Material examined. — CAMEROON: Mangombe Nursery, on *Pinus oocarpa*, seedling needles, 4. VI. 1983, leg. I. A. S. GIBSON, IMI 278452. — INDIA: Burnihat, State Forest Service, on *Pinus kesiya*, 20. VIII. 1981, leg. M. D. MEHROTRA, IMI 261600. — MALAYSIA: Negeri Sembilan, Behau, on *Pinus merkusii*, 21. VI. 1972, leg. M. H. IVORY, IMI 168712; Pahang, Lentang Nursery, on *Pinus caribaea* var. *hondurensis* (seedlings), 6. X. 1971, leg. M. H. IVORY, IMI 161902, IMI 161903; Sabah, isolated from *Pinus* sp., 14. XII. 1977, leg. WONG PUT HAM, dried culture, Typus, IMI 224009. — SOUTH AFRICA: Pretoria, isolated from *Pinus*, 23. IV. 1975, leg. J. M. DARVAS, dried culture, IMI 196422.

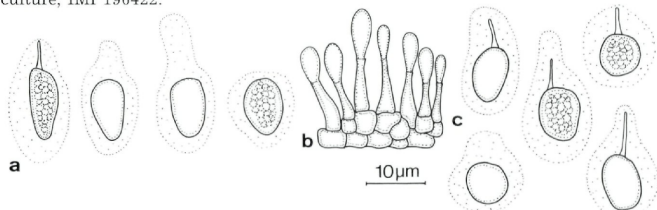


Fig. 6. — *Discochora pini*, *Phyllosticta* state. — a. Conidia formed in culture (IMI 196422). — b. Conidiophores formed on the host (IMI 278452). — c. Conidia formed on the host (IMI 278452).

The anamorph of *D. pini* is very similar to *P. pseudotsugae*. The conidia of the former, however, are narrower and possess an extremely thick (3–4  $\mu\text{m}$ ) gelatinous layer around the whole conidium. This layer is present on conidia produced on the host and in culture, but is visible only when the material is mounted in water.

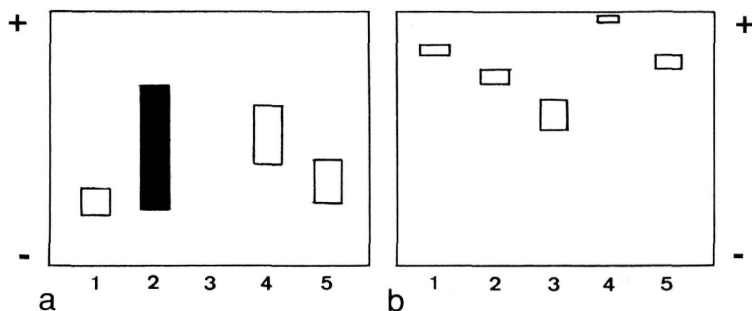


Fig. 7. — Diagrammatic representation of electromorphs for a) pectic enzymes, b) amyolytic enzymes. In the pectinase zymogram, the open squares represent polygalacturonase, the black squares pectin esterase. 1: *Phyllosticta cryptomeriae*. 2: *P. abietis*. 3: *P. multicorniculata*. 4: *Macrophoma piceae*. 5: *P. pseudotsugae*.

### Use of substrate utilization tests, growth experiments, and pectinase and amylase isoenzyme patterns to differentiate species of *Phyllosticta*

The results of the substrate utilization studies and of growth experiments are of no taxonomic relevance. Conversely, the study of the electrophoretic banding patterns of enzymes such as pectinases and amylases, both important for penetration and colonization of host tissues by mutualistic and antagonistic plant symbionts, has already proven to be useful in solving taxonomic problems in a number of fungal genera (CRUICKSHANK, 1983; CRUICKSHANK & PITT, 1987; NEATE & CRUICKSHANK, 1988; PATTERSON & al., 1989) and appear to provide some taxonomic clues on the taxonomic position of the species treated here.

Like other characters, the enzymes studied here represent only a small portion of the genome, and it would be inadequate to reach taxonomic conclusions on the basis of these characters alone. Moreover, for *P. abietis*, *P. cryptomeriae*, and *P. pseudotsugae* only one strain each has been investigated electrophoretically, thus allowing only limited conclusions to be drawn. Together with the results of the

morphological analysis, however, they contribute to a clear delimitation of the species investigated. The results of the enzyme analysis (Fig. 7) suggest that the five species can be distinguished on the basis of their pectic and amylolytic zymograms. *P. multicorniculata* did not produce any pectinase, polygalacturonase was detected in *P. cryptomeriae*, *M. piceae*, and *P. pseudotsugae*, with banding patterns specific for each species. *P. abietis* lacked polygalacturonase but produced pectin esterase, an enzyme otherwise absent in the other species. Pectin lyase was detected in no species.  $\alpha$ -amylases were produced by all strains studied, with banding patterns that allowed a clear differentiation among species (Fig. 7b).

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