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Pythium in the Swiss Alps

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Summary – A total of 107 *Pythium* isolates were obtained from soil samples collected from the Swiss Alps in the region around Davos at the altitude of 600–2300 m above sea level by baiting with cucumber seeds. The following species were identified: *P. intermedium* (46 isolates), *P. torulosum* (11), *P.* cf. *indigoferae* (5), *P. sylvaticum* (5), *P. vexans* (3), *P. rostratum* (2), and *P. ultimum* (2). Thirty-three isolates remained unidentified. Morphological descriptions and optimum temperatures for growth are given.

Key words: Alps, Davos, distribution, identification, morphology, optimum temperatures for growth, *Pythium*

Zusammenfassung – Aus Bodenproben, die in den Schweizer Alpen in der Region von Davos auf einer Höhe zwischen 600 und 2300 m ü.M. gesammelt wurden, konnten durch Ködern mit Gurkensamen insgesamt 107 *Pythium*-Isolate gewonnen werden. Die folgenden Arten wurden bestimmt: *P. intermedium* (46 Isolate), *P. torulosum* (11), *P.* cf. *indigoferae* (5), *P. sylvaticum* (5), *P. vexans* (3), *P. rostratum* (2) und *P. ultimum* (2). Dreiunddreissig Isolate konnten nicht bestimmt werden. Morphologische Beschreibungen und Temperaturoptima für das Wachstum werden präsentiert.

Schlüsselwörter: Alpen, Davos, Verbereitung, Bestimmung, Morphologie, Temperaturoptimum, Wachstum, *Pythium*

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The genus *Pythium* (Oomycota) plays a significant role in the soil environment. Several *Pythium* species are plant pathogens attacking preferentially seedlings of various plants (Hendrix & Campbell, 1973; Domsch et al., 1993). Because their isolation and identification require specific techniques, the occurrence of *Pythium* in natural habitats has been rarely reported. This is especially true for the alpine and subalpine zone. Therefore, during the post-congress foray held at Davos, Switzerland, during the 4th International Mycological Congress in September 1990, soil samples were collected and investigated for *Pythium* species.

Materials and methods

Samples were collected from several locations in the Alps in the region of Davos (46°48′ N, 9°52′ E), Switzerland, at altitudes between 600 and 2300 m above sea-level (Table 1). The samples were collected in various habitats (forests, meadows and river banks) and comprised five thoroughly mixed subsamples (each approx. 50 cm^3), which were taken randomly from the upper soil and litter layers (depth up to 15 cm) within a plot of approximately 30 m^2. The samples were stored for 6 days at 10 °C before processing.

Pythium was isolated by a baiting method using cucumber seeds (*Cucumis sativus* L., cv. Tokiwa-jibai) as a bait (Watanabe, 1984). Per sample two Petri dishes (diameter 9 cm) filled with 10 g of soil and 6 ml water were prepared. Ten seeds were then embedded in the soil in each Petri dish, one of them was incubated at 25 °C for 1 d and the other one at 10 °C for 7 d. Then, the seeds were removed, washed under running tap water for 1 h, air-dried, and placed on water agar (WA) (2 seeds per plate). After further incubation at 25 °C for 1–3 d, pure cultures of *Pythium* spp. grown out of the seeds on WA were obtained by transfer of hyphal tips. For mycological study, the cultures were grown on Difco cornmeal agar (CMA), potato dextrose agar (PDA), V-8 juice agar (Hawksworth et al., 1995), in potato dextrose broth (200 g peeled potatoes boiled in 1 l water for 60 min., filtered and filled with water to 1 l, 20 g glucose added, and autoclaved) and in sterile tap water.

Induction of asexual reproduction (zoospore production) was attempted with Petri's salt solution (Watanabe, 1984). The cultures were grown in potato dextrose broth, sterile tap water or on WA with inocula (1 cm^2) originating from cultures on PDA, CMA or V8-juice agar for up to 2–3 days. Then, the medium was replaced or covered by Petri's salt solution (4 ml/plate), incubated at 25 °C, and the cultures were observed daily under the microscope for up to 5 days.

All isolates were identified following the descriptions given by Middleton (1943), van der Plaats-Niterink (1981) and Watanabe (1994).

Tab. 1: Locations of collection in the Swiss Alps in the region of Davos. The samples were collected along the described routes. The map numbers refer to the Swiss topographical maps 1:25 000 (Bundesamt für Landestopographie, CH-3084 Wabern-Bern, Switzerland).

Name of location	Date	Map number	Route description	Elevation (m above sea level)	Dominant vegetation (P. = Picea, Pin. = Pinus, L. = Larix, F. = Fagus)	Soil and geology (Atlas der Schweiz, 1990)	Number of samples
Dischmatal	4.9.1990	1197	left side of the Dischma valley from Wildiwald to Stillberg	1600–1800	P. abies, L. decidua, pasture	podsol on silicate rocks	5
Malans	5.9.1990	1156	Malans – Winegg – Müli (Jenins)	650–700	F. sylvatica	brown soil on tertiary sediments	2
Zuoz	6.9.1990	1237	God Laviners (appr. 1.5 km SSE Zuoz) from Ova d'Arpiglia to Val Purschigl	1700–1850	L. decidua, P. abies, Pin. cembra, meadows	podsolic soil on silicate rock	4
Landwasser-schlucht	7.9.1990	1216	Monstein – Jenisberg – Wiesen – Cavja – Filisur	1050–1320	P. abies, Pin. mugo, L. decidua, Acer pseudo-platanus, Alnus incana	podsolic soil on silicate rocks and river sediments	4
Lavin – Susch	8.9.1990	1198 and 1218	Lavin – Craistas – Valplan – Fuora – Baldirun – Surpunt – Susch	1400–1420	L. decidua, P. abies, meadows	podsol on silicate rocks and river sediments	4
Albulapass	9.9.1990	1237	Ospiz – Val digl Diavel – Igls Plans	2040–2300	Pin. mugo, alpine meadows	clay rich brown soil and peat on silicate rocks	4
Ducantal	10.9.1990	1217	from Wasserfall along left side of Ducanbach	1900–2200	Pin. mugo, alpine meadows	rendzina on dolomite	3

Tab. 2: Results on Pythium isolations (for locations see Tab. 1).

Location	*P.* cf. *indigoferae*	*P. intermedium*	*P. rostratum*	*P. sylvaticum*	*P. torulosum*	*P. ultimum*	*P. vexans*	*Pythium* sp. A	Unidentified *Pythium* spp.
Dischmatal	3	11	0	0	9	0	0	0	3
Malans	2	17	0	0	0	1	0	0	2
Zuoz	0	0	1	0	2	0	3	0	5
Landwasser-schlucht	0	13	1	0	0	0	0	0	3
Lawin, Susch	0	2	0	0	0	0	0	0	1
Albulapass	0	0	0	5	0	1	0	1	9
Ducantal	0	3	0	0	0	0	0	0	9
Total	**5**	**46**	**2**	**5**	**11**	**2**	**3**	**1**	**32**

The growth rates of selected strains at six temperatures (19, 22, 25, 28, 31, and 34 °C) were determined by measuring the diameter on PDA 24 h after inoculation. The inocula were 4 mm diameter mycelial agar discs removed from the margin of one-day-old colonies grown on PDA at 25 °C, using an aseptic cork borer. Measurements of the colony diameter represent an average of at least six replicates in two experiments. Growth increments were compared by Wilcoxon's two-sample test (Sokal & Rohlf, 1981).

Representative isolates in this study were deposited in the American Type Culture Collection (ATCC), U.S.A.

Results

Morphology and distribution

Among a total of 107 *Pythium* isolates obtained in 19 out of 26 samples assayed, 16 isolates (15 % of the isolates) formed lobate or dendroid hyphal swellings, and the remaining 91 formed globose or subglobose hyphal swellings. Among the 16 isolates with lobate or dendroid hyphal swellings, five were identified as *P.* cf. *indigoferae* and 11 as *P. torulosum*. Zoospore discharge was observed only in *P. torulosum*. Among the 91 isolates with globose or subglobose hyphal swellings, nine formed sexual organs, and they were identified as one isolate each of the homothallic *P. sylvaticum* and an unidentified species designated as *Pythium* sp. A, two each of *P. rostratum* and *P. ultimum*, and three of *P. vexans*. Eighty-two isolates

never formed sexual organs on CMA and various other media tested under the laboratory conditions, and there were four isolates of heterothallic *P. sylvaticum*, 46 *P. intermedium*, and 32 of unidentified species. One to three species were isolated from each location, excluding unidentified isolates. *P. intermedium* was isolated from five out of seven locations (eight out of 26 samples), but others from one or two locations only (Table 2). They are described as follows.

Pythium cf. *indigoferae* Butler (Figs 1, 2)

Hyphae often showing dendroid branching, only sometimes slightly swollen, mostly 7.5 µm thick, often directly connected with sexual organs. No zoospore discharge was observed. Oogonia terminal, 17.5–20 µm, borne on thick oogoniophore, 5 µm wide, with single or occasionally double mostly monoclinous or rarely hypogynous antheridia. Oospores aplerotic, occasionally developed parthenogenetically, 12.5–15 µm in diameter, oospore wall 1.5–2 µm thick. Two isolates grew best at 25 °C with colony diameters of 22 and 28 mm after 24 h.

Isolates examined. SWITZERLAND, Dischmatal, 4 Sep. 1990, isolate 90–104 (ATCC 200701); Malans, 5 Sep. 1990, isolate 90–113 (ATCC 200702).

Pythium intermedium de Bary (Figs 3–5)

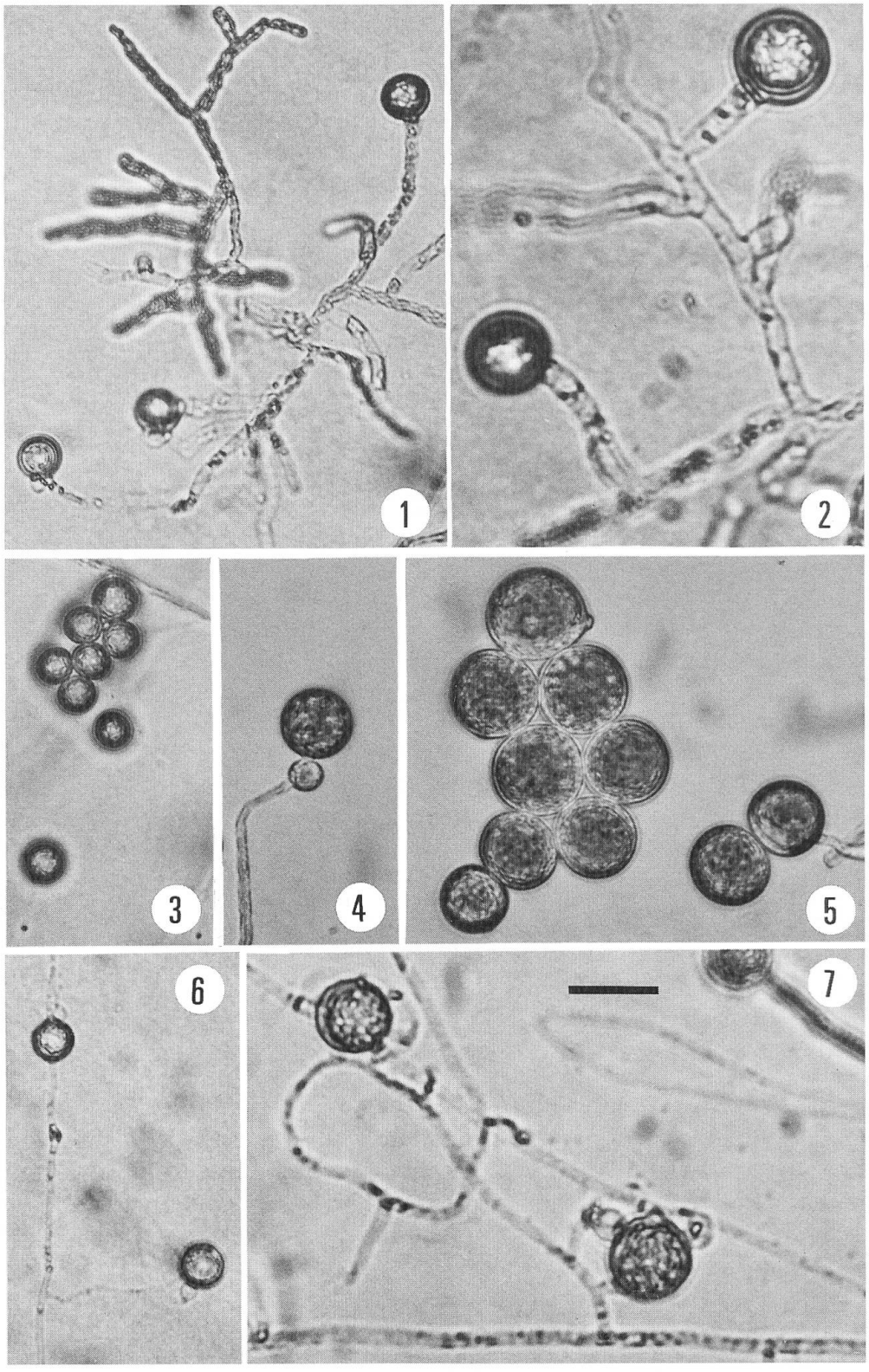
Hyphal swellings globose, (12.5-)15-20(-25) µm in diameter, characteristically catenulate, basipetally developed, often single, or in pairs, mostly terminal, occasionally intercalary, rarely deciduous. Most of the 17 tested isolates grew best at 25 °C. Ten isolates had a colony diameter between 47 and 45 mm, six between 16 and 31 mm. One isolate (90–109) grew best at 22 °C and had a colony diameter of 49 mm after 24 h.

Isolates examined. SWITZERLAND, Dischmatal, 4 Sep. 1990, isolate 90–107 (ATCC 200704); –109 (ATCC 200705); Malans, 5 Sep. 1990, isolate 90–115; Wiesen, 7 Sep. 1990, isolate 90–128 (ATCC 200706).

Pythium rostratum Butler (Fig. 6)

Hyphal swellings globose or subglobose, mostly intercalary, 15–25 µm in diameter. Oogonia terminal and intercalary, 15–17.5 µm in diameter bearing single or occasionally double monoclinous antheridia. Oospores plerotic, 15–16.3 µm in diameter. The temperature optimum of two isolates was at 28 °C, with a colony diameter of approximately 20 mm after 24h.

Isolates examined. SWITZERLAND, Zuoz, 6 Sep. 1990, isolate 90–124 (ATCC 200703); Wiesen, 7 Sep. 1990, isolate 90–131 (ATCC 200620).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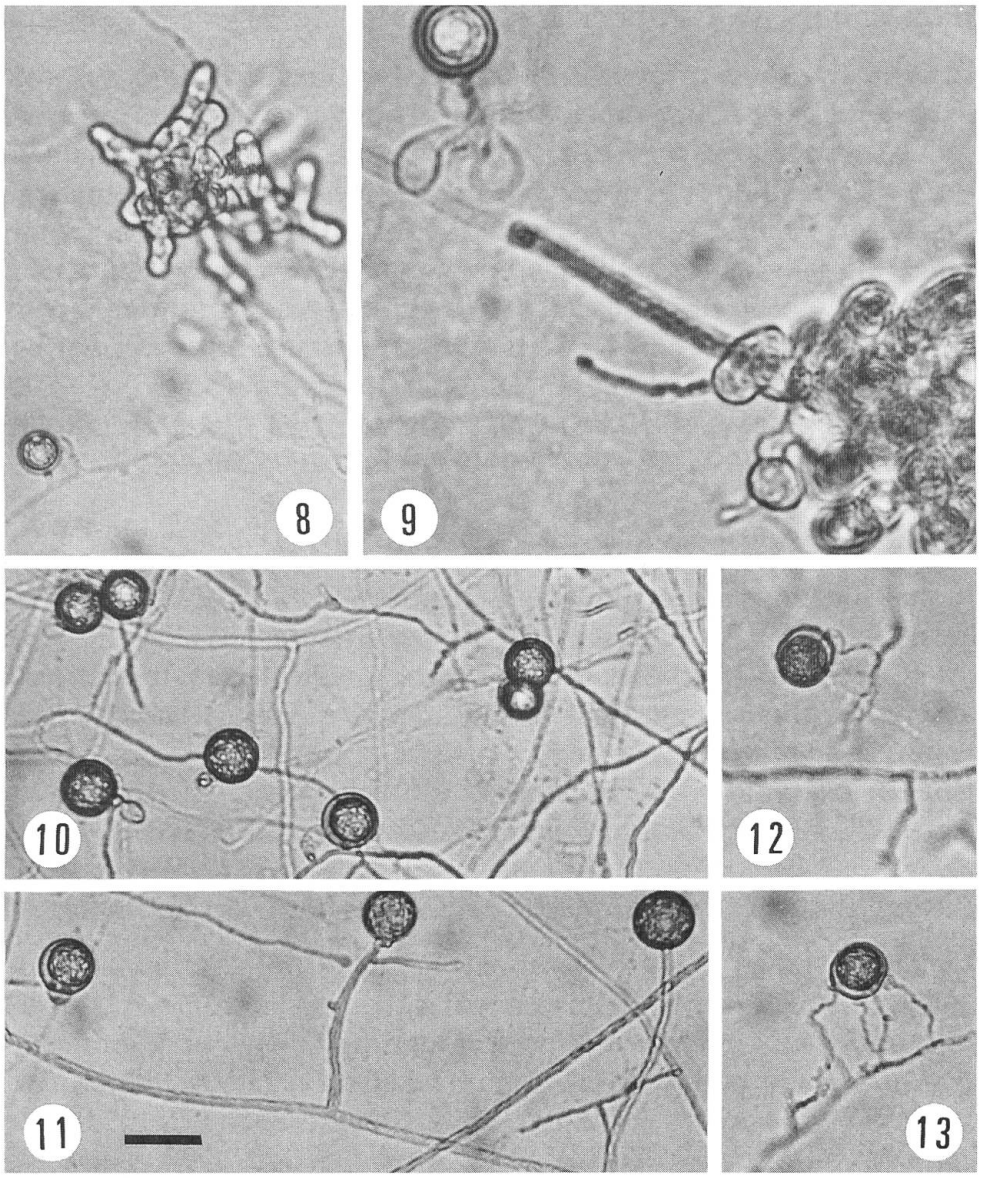

Figs 1–7. Pythium species *from the Swiss Alps. Figs 1, 2.* P. *cf.* indigoferae *(isolate 90–104). Lobate hyphal swellings (1) and sexual organs with aplerotic oospores (1, 2). Note parthenogenetically-developed oospores. Figs 3–5.* P. intermedium *(isolates 90–107; –109). Single (3), and two (4, 5) or several catenulate globose hyphal swellings (3, 5) basipetally developed (4). Fig. 6.* P. rostratum *(isolate 90–131). Sexual organs. Fig. 7.* P. sylvaticum *(isolate 90–142). Homothallic isolate, sexual organs with aplerotic oospore and globose hyphal swelling. (Scale bar in Fig. 7: for Figs 1, 3, 6, 40 µm; Figs 2, 4, 5, 7, 20 µm.)*

Figs 8–13. Pythium *species from the Swiss Alps. Figs 8, 9.* P. torulosum *(isolate 90–105). Lobate hyphal swellings and sexual organs with plerotic oospores. Figs 10, 11.* P. ultimum *(isolate 90–140), globose hyphal swellings and sexual organs with aplerotic oospores. Figs 12, 13.* P. vexans *(isolate 90–121), sexual organs with aplerotic oospores. (Scale bar in Fig. 11: for Figs 8, 10–13, 30 µm; Fig. 9: 15 µm.)*

Pythium sylvaticum Campbell & Hendrix (Fig. 7)

Hyphal swellings globose or subglobose, terminal or intercalary, (17.5–)20–27.5(–30) µm in diameter. Oogonia borne on short branches, bearing more than two diclinous and/or monoclinous antheridia, 18.8–22.5 µm in diameter. Oospores aplerotic, 15–17.5 µm in diameter. Among five isolates identified as *P. sylvaticum*, one isolate (isolate 90–142) (Fig 7) was homothallic, and the remaining four heterothallic. Five isolates tested for temperature response showed a growth optimum at 28 °C. The colony diameter after 24h of the strains 90–141 and 90–147 (41 and 16 mm) was significantly smaller compared with the others (90–142, 90–143, 90–145; 53–61 mm; $p < 0.05$).

Isolates examined. SWITZERLAND, Albula, 9 Sep. 1990, isolates 90–141 (ATCC 200621); –142 (ATCC 200622); –143 (ATCC 200623); –145; –147.

Pythium torulosum Coker & Patterson (Figs 8, 9)

Hyphal swellings lobate, branched, mostly 7–8 µm thick. Oogonia bearing single antheridia monoclinously, 12.5–15 µm in diameter. Oospores plerotic, mostly 10–12.5 µm in diameter. Two isolates were tested for temperature response. They showed a growth optimum at 31 °C, and the colony diameter after 24h was 30 mm.

Isolates examined. SWITZERLAND, Dischmatal, 4 Sep. 1990, isolate 90–105 (ATCC 200624); Zuoz, 6 Sep. 1990, isolate 90–122 (ATCC 200638).

Pythium ultimum Trow (Figs 10, 11)

Hyphal swellings globose, terminal, (15–)20–22.5(–25) µm in diameter. Oogonia terminal, 20 µm in diameter, with often sessile single or occasionally double monoclinous or rarely diclinous antheridia. Oospores aplerotic, 15–17.5 µm in diameter. Oospore wall 1.2–2 µm thick. One isolate tested for temperature response grew best at 25 °C. Its colony diameter was 39 mm after 24 h.

Isolates examined. SWITZERLAND, Albula, 9 Sep. 1990, isolate 90–140 (ATCC 200776).

Pythium vexans de Bary (Figs 12, 13)

Hyphal swellings mostly globose, 20–35(–37.5) µm in diameter. Oogonia subglobose, terminal, 20–22.5(–27.5) µm in diameter bearing mostly single bell shaped monoclinous antheridia. Oospores aplerotic, 15–17.5(–20) µm in diameter. One isolate tested for temperature response grew best at 25 °C and developed a colony of 6,7 mm diameter after 24 h.

Isolates examined. SWITZERLAND, Zuoz, 6 Sep. 1990, isolate 90–121 (ATCC 200775).

Pythium sp. A.

Hyphal swellings globose, predominantly intercalary, often ellipsoidal, often appearing empty, mostly 30 x 27.5 µm. Sexual organs developed on rather long hyphal branches characteristically. Oogonia mostly terminal, 20–22.5 µm in diameter, bearing mostly more than three antheridia, diclinously and monoclinously. Antheridia globose (7.5–10 µm in diameter), subglobose or elongated, broadly applied to oogonia. Oospores aplerotic, mostly 16–18 µm in diameter. This fungus may be identified as a homothallic strain of *P. sylvaticum*, but it grew significantly slower than *P. sylvaticum* (optimum at 28 °C, the colony diameter after 24 h was 16 mm).

Isolates examined. SWITZERLAND, Albula, 9 Sep. 1990, isolate 90–146.

Pythium spp.

No sexual organs were formed by these isolates. The hyphal swellings were mostly globose, or ellipsoidal. In eight isolates among 15 isolates studied, the swellings were up to 15 µm in diameter, in five up to 20 µm, and in two up to 30 µm. They were also not clearly separable on the basis of colony morphology and temperature-growth relationship. Among 15 isolates studied for temperature response, nine grew best at 25 °C and three each at 22 or 28 °C; the colony diameter after 24 h varied between 8 and 59 mm.

Isolates examined. SWITZERLAND, Dischmatal, Sep. 4, 1990, isolate 90–106; –108; Malans, Sep. 5, 1990, isolate 90–114; Zuoz, Sep. 6, 1990, isolate 90–119; isolate 90–123; Monstein, Sep. 7, 1990, isolate 90–130; Filisur, Sep. 7, 1990, isolates 90–134; –135; Susch, Sep. 8, 1990, isolate 90–137; Albula, Sep. 9, 1990, isolates 90–138; –139; –144; Ducantal, Sep. 10, 1990, isolates 90–150; –153.

Discussion

A total of 107 *Pythium* isolates, belonging to at least seven species, were isolated during this study. The rather high yield of *Pythium* isolates (19 out of 26 samples assayed) indicates high frequencies of *Pythium* in the examined habitats in the Swiss Alps.

To our knowledge, the *Pythium* flora in the Alps has not been studied before. Most species found in our study are known or supposed cosmopolites. The dominant species was *P. intermedium*, which is an ubiquitous soil inhabitant preferentially in the temperate zone (Domsch et al., 1993).The identification of *Pythium* spp. using morphological characters is complicated by their variability (Chen & Hoy, 1992; Shahzad et al., 1992; Barr et al., 1996), which is insufficiently known for most species reported in this study. For example, *P. inter-*

medium shows considerable variation in formation and deciduousness of sporangia (Watanabe, 1983). We also observed variation in growth rate within the species. This cannot be explained by the technique which we used, because all cultures started their growth simultaneously. In total, among 47 isolates studied for temperature response, 4, 30, 11 and 2 grew best at 22, 25, 28 and 31 °C, respectively. The occurrence of *P. torulosum* with rather high temperature optima in the Swiss Alps is noteworthy.

Only a few data are available from other mountainous regions. Among a total of 21 *Pythium* species isolated from water, soil and roots of various crops in the Kumaun region in the Himalayas, India, *P. rostratum*, *P. torulosum*, *P. ultimum*, and *P. vexans* were also reported, but *P. indigoferae*, *P. intermedium*, and *P. sylvaticum* were not found (Mer et al., 1984). Five (*P. intermedium*, *P. sylvaticum*, *P. torulosum*, *P. ultimum*, and *P. vexans*) out of six species recorded from a natural forest on Mt. Fuji, Japan, at an altitude of 1100 to 2350 m (Watanabe, 1985) were also found in the Swiss Alps.

Considering the frequently predicted climate change and the potential pathogenicity of several *Pythium* spp. on forest tree seedlings, we hope that our preliminary investigation might serve as a starting point for a more thorough examination of pythiaceous fungi in the Alps.

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