Effect of temperature on growth and survival of five aquatic hyphomycetes

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Three aquatic hyphomycetes from a Canadian stream and two species from an Indian stream showed maximum growth between 20–25 C. Only the Canadian species showed appreciable growth at 5 and 10 C, and they showed much better survival when exposed to freezing temperatures. Birch leaves pre–inoculated with a pure culture of a Canadian isolate before exposure in a stream were colonized by fewer fungi than sterile leaves, subsequent production of conidia was reduced, and most of the conidia belonged to the species used as inoculum. Generally, the same observations were made with leaves preinoculated with Indian species, though there were some exceptions when leaves were exposed in winter or spring.

Keywords: aquatic hyphomycetes, temperature preferences, transplant experiments, tropical and temperate species.

Aquatic hyphomycetes can be found in streams from the arctic circle to the equator (Bärlocher, 1992a). Some species are cosmopolitan, others are restricted to or more common in a narrow range of latitudes. Geographical occurrence of the fungi seems to be broadly correlated with optimal temperatures for in vitro growth and sporulation. In species common in temperate regions, vegetative growth is generally highest between 15–25 C, whereas sporulation often reaches a peak at somewhat lower temperatures (Webster & al., 1976; Suberkropp, 1984; Koske & Duncan, 1974). Agar cultures survived at –20 C for one month, and viable spores were found in frozen foam, and on snow above the tree limit in Sweden (Nilsson, 1964). Even though most fungi can be preserved frozen, however, this often requires special treatment, and it cannot be concluded that all fungi survive freezing under natural conditions. In five tropical species, maximum growth occurred around 25 C, and was much lower at 30 C (Singh & Musa, 1977).

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Seasonal temperature changes allow the periodic appearance of 'tropical' species (i.e., species more common in tropical regions) in temperate streams (Suberkropp, 1984; Webster & al., 1976). These generally disappear in winter, presumably due to their inability to grow at low temperatures. Nevertheless, their regular reappearance each summer indicates that they can survive low, possibly freezing, temperatures.

Temperature preferences and tolerance by themselves, however, are insufficient to fully explain the distribution or seasonal occurrence of aquatic hyphomycete species (Suberkropp, 1984; Webster & al., 1976). Interspecific interactions or substrate preferences have been suggested as complementary mechanisms. Of crucial importance seems to be the ability of a species to invade leaves already colonized by other fungi, and conversely, to resist invasion by competitors of leaves it occupies.

The present study investigated two species isolated from a tropical (Western Ghat area, India) and three species from a temperate stream (Nova Scotia, Canada). Our objectives were to determine in vitro growth and survival of the five fungi as a function of temperature. We also studied the effects of seasonal changes on their ability to persist in streams when introduced as pure cultures on oak leaf disks and to resist invasion by other fungi.

Materials and methods

The following five fungal species, all isolated from single conidia, were used in this study: *Articulospora tetracladia* Ingold, *Heliscus lugdunensis* Sacc. & Therry, *Varicosporium elodeae* Kegel (all isolated from *Picea glauca* (Moench) Voss roots submerged in the Boss Brook, N.S., Canada), *Phalangispora constricta* Nawawi & Webster (isolated from leaves decaying in the Sampaje stream, Kodagu, India) and *Tetracladium setigerum* (Grove) Ingold (from unidentified leaves in the Cauvery river, Kodagu, India). Cultures were maintained on 1% malt extract agar (MEA) at 18 C.

To study the effect of temperature on fungal growth, 5 mm disks were cut from the growing edge of colonies and inoculated on 1% MEA plates. For each species, four replicate plates were incubated at temperatures from 0 to 40 C (5 C intervals). After 35 days, the colony diameters (two measurements per plate at right angles to each other) were measured, and radial growth (mm per day) was calculated.

Field experiments were conducted in the Boss Brook, a softwater stream in Fenwick, Nova Scotia, Canada (Bärlocher, 1987). The stream is lined primarily by white birch (*Betula papyrifera* Marsh.), mountain maple (*Acer spicatum* Lam.) and white spruce (*Picea glauca*

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(Moench) Voss). The chemical characteristics of the stream water and its dominant fungi have been documented by Bärlocher (1987).

Oak [*Quercus petraea* (Mattuschka) Lieblein] leaf disks were used as substratum for the fungi. Autumn–shed leaves were collected from a single tree, soaked in distilled water for 30 min, cut into 1.5 cm disks and autoclaved. Sterile leaf disks were aseptically transferred to 500 ml flasks containing 250 ml of autoclaved mineral solution (KNO_3, 2.5 g; K_2HPO_4, 0.43 g; KH_2PO_4, 0.34 g; $MgSO_4 \bullet 7H_2O$, 0.49 g; NaCl, 0.37 g; distilled water 1000 ml; Suberkropp, 1984). Each flask was inoculated with a 5 mm MEA agar disk overgrown with a pure culture of one of the five aquatic hyphomycetes, and the flasks were incubated on a shaker at 18 C for two weeks. Leaf disks in mineral solution without fungal inoculum served as control.

Five leaf disks from each flask were rinsed and then aerated in distilled sterile water for 48 hr to induce sporulation. The suspension was filtered through a Millipore filter (8 μm) and stained with acid fuchsin in lactophenol. The conidia retained on the filter were identified and counted. Leaf disks were dried for two days at 105 C and weighed.

Additional colonized leaf disks were transferred to nylon bags (mesh size 1.5 mm; 10 disks per bag, all colonized by the same species). The bags were attached inside drain pipes which were tied to tree roots in the Boss Brook. Samples were recovered after one, two and four weeks of immersion. Leaf disks were rinsed and aerated. Released conidia were counted and identified as described above (5 replicates per sample). Colonized leaf disks were introduced on three occasions: 21 March (winter), 25 April (spring), and 1 August 1991 (summer). At weekly intervals, stream temperature was measured.

To determine the effect of temperature on fungal survival, oak leaf disks were inoculated as described above and transferred to 250 ml flasks containing 100 ml sterile distilled water (10 disks per flask). The flasks were kept at –15, 0, 5 and 15 C. After 1, 2 and 4 weeks five replicate disks per treatment were harvested, rinsed, and aerated. Released conidia were counted and identified as described above.

Results

Fig. 1 shows the effect of temperature on radial growth of the five fungi. Optimal temperatures were between 20 and 25 C in all cases. However, growth of the two Indian isolates was very limited or absent at or below 10 C, whereas all Canadian isolates grew well at 5 and 10 C. On the other hand, the only species to grow at a temperature as

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high as 30 C was one of the Indian isolates (*P. constricta*). No growth in any species was observed at higher temperatures.

Exposure to freezing diminished the reproductive potential of all five species, but the two Indian isolates were more severely affected (Fig. 2). Prolonged exposure to 0 and 5 C had a clearly negative effect on *P. constricta*.

The average stream temperature during the three field experiments was 0.75 (winter), 7.8 (spring) and 15.2 C (summer). Within any of the three periods, temperature varied by no more than 2.5 C.

Leaf disks preinoculated with a fungus were colonized by fewer species after they were placed in the stream (Fig. 3; exceptions: leaves inoculated with *P. constricta*). This difference was more pronounced in the winter and spring experiment.

The total numbers of conidia produced per unit weight of sterile or preinoculated leaves after stream exposure is shown in Fig. 4. Generally, preinoculation greatly reduced subsequent conidium production. The major exception was again *P. constricta* in the winter and spring experiment.

The percentages of conidia produced by species invading preinoculated leaves are summarized in Tab. 1. The highest values (up to 100%) were found in the winter and spring experiment on leaves ino-

Tab. 1.– Percentage of conidia produced by species invading leaf discs inoculated with pure cultures before stream exposure (n = 5; in parentheses: SE).

| Inoculum | | Days in streams | | |
		7	14	28
P. constricta	Winter	100 (0)	100 (0)	100 (0)
	Spring	3.2 (2.5)	12.1(6.2)	98 (1.2)
	Summer	21.5 (7.5)	45.0 (15.5)	6.7 (15.3)
T. setigerum	Winter	0.8 (2.5)	5.5 (3.4)	0.5 (2.1)
	Spring	3.2 (1.3)	0.3 (1.2)	1.2 (2.1)
	Summer	13.5 (6.4)	17.3 (5.3)	9.5 (5.2)
A. tetracladia	Winter	8.6 (3.4)	20.2 (5.5)	30.6 (12.3)
	Spring	1.2 (1.2)	4.1 (2.1)	3.9 (1.4)
	Summer	3.4 (2.5)	6.6 (3.1)	3.0 (2.1)
H. lugdunensis	Winter	0.4 (1.2)	1.8 (2.1)	2.9 (2.4)
	Spring	0 (0)	0.4 (0.4)	3.7 (0.9)
	Summer	6.0 (2.2)	5.9 (3.5)	2.6 (1.9)
V. elodeae	Winter	10.1 (3.2)	1.4 (1.7)	0.8 (1.3)
	Spring	12.7 (3.3)	4.4 (2.1)	12.4 (2.7)
	Summer	2.4 (1.1)	9.5 (3.4)	4.8 (3.0)
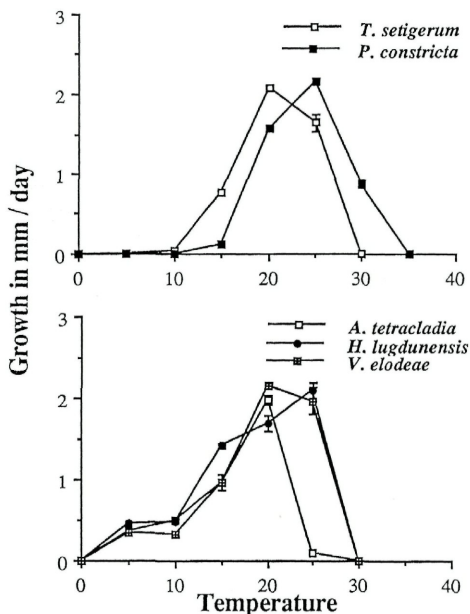
Fig. 1. – Effect of temperature on growth of five aquatic hyphomycetes. Top: Indian isolates; bottom; Canadian isolates (n=5, + SE).

culated with *P. constricta*. In most other instances, the contributions of invading species to total conidium production were considerably lower, typically between 0 and 10%.

Discussion

The effect of temperature on mycelial growth confirms earlier studies: with temperate species, the optimum generally occurs between 15 and 25 C and is close to 25 C for tropical species (Suberkropp, 1984; Singh & Musa, 1977; Koske & Duncan, 1974; Nilsson, 1964; Thornton, 1963). The differences between the two groups are more pronounced when their growth at the extremes are considered: low temperatures are tolerated better by temperate species, the opposite is true for tropical species. These preferences and tolerances clearly reflect the ecological conditions present in the streams from

which the isolates tested originated: in the Boss Brook the stream temperature varies between 0–17 C (Bärlocher, unpubl. obs.); stream temperatures in the Western Ghat (origin of the two tropical strains) vary between 17 and 30 C (Sridhar & al., 1992). It is obvious, however, that the optimal temperature *in vitro* is only vaguely related to the conditions under which most of the growth actually occurs in the stream. In Boss Brook, this would be after leaf-fall in autumn/early winter, when the stream temperature varies between 8 and 0 C (Bärlocher, unpubl. obs.); in Western Ghat streams, the stream temperature during periods of maximum substrate availability fluctuates between 17–22 (Sridhar & al., 1992). The ability to survive extreme conditions (heat in tropics, cold in temperate climates) may be more relevant than optimal growth temperature. This seems to be borne out by the second experiment: the three temperate species were clearly superior in their abiliity to survive freezing and exposure to 5 C (Fig. 2). Temperature fluctuations are generally more extreme in the terrestrial environment than in streams (Hynes, 1970); the ability to persist for limited periods of time outside the stream is believed to be important for the establishment and continued existence of aquatic hyphomycetes in a given region (Bärlocher, 1992b).

One of the tropical isolates, *T. setigerum*, showed limited survival at freezing temperature. This illustrates the fact that an unequivocal distinction between temperate and tropical species is not always possible. First, altitude often reproduces the effects of latitude, so that a stream originating on a tropical mountain offers much the same conditions as a temperate or even arctic stream (Hynes, 1970). These habitat islands may contribute to the fact that many aquatic hyphomycetes are cosmopolitan. *T. setigerum* is one of them: it is not uncommon in temperate regions (Nilsson 1964), and occurs naturally in the Boss Brook (Bärlocher, 1987). Its physiology, however, appears to be influenced by local conditions. For example, an isolate in British Columbia, Canada (Koske & Duncan, 1974), grew best at 20 C, like the Indian isolate of this study, but also grew well at 5 °C (no growth in the present study).

The response of the two tropical isolates to low temperatures seems sufficient to explain why they would have difficulty establishing themselves permanently in a temperate stream. The question remains whether they could compete successfully with native species at water temperatures more suitable for their growth. The experiments with preinoculated leaves were designed to shed some light on this. *P. constricta* again gave the clearest results (Fig. 3). It was unable to exclude competitors in winter and spring, but was able to lower the number of invaders from 7 to 4 in summer. The other tropical isolate, *T. setigerum*, consistently lowered the number of invaders; surprisingly, this effect was least pronounced in summer.
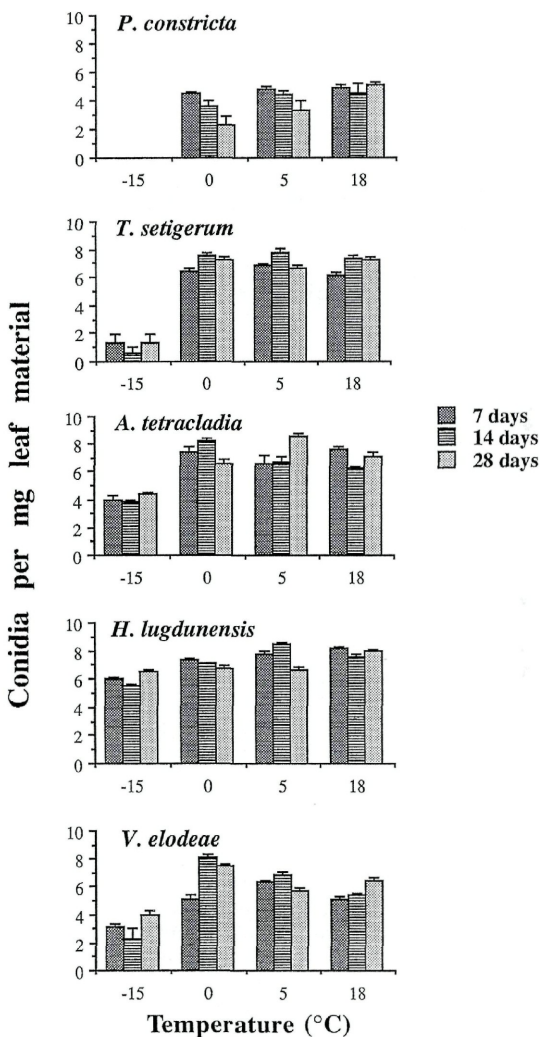
Fig. 2. – Conidium production by pure cultures on leaves after exposure to temperatures ranging from –15 to + 18 for 7, 14, or 28 days (n=5, + SE).
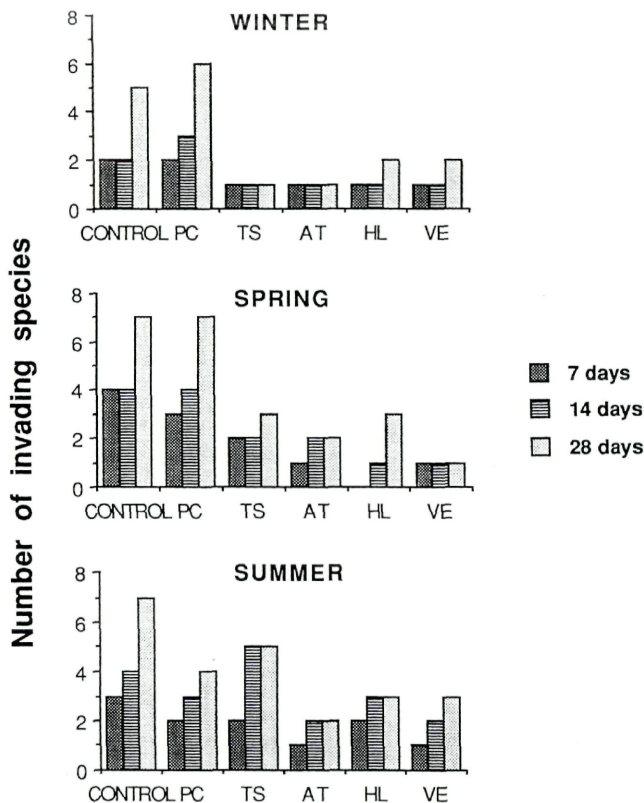
Fig. 3. – Cumulative number of fungal species invading sterile leaves (CONTROL) or leaves preinoculated with a pure culture after 7, 14 or 28 days in a stream. – PC = *P. constricta;* TS = *T. setigerum;* AT = *A. tetracladia;* HL = *H. lugdunensis;* VE = *V. elodeae.*

Pre-inoculation also lowered subsequent conidium production from leaves recovered from the stream (Fig. 4). The only exceptions were winter leaves with *P. constricta* or *T. setigerum* after 14 days of stream exposure. There was no obvious difference between tropical and temperate isolates in the summer experiment.

Temperature, of course, is only one of many factors distinguishing tropical from temperate streams. For example, riparian trees (and
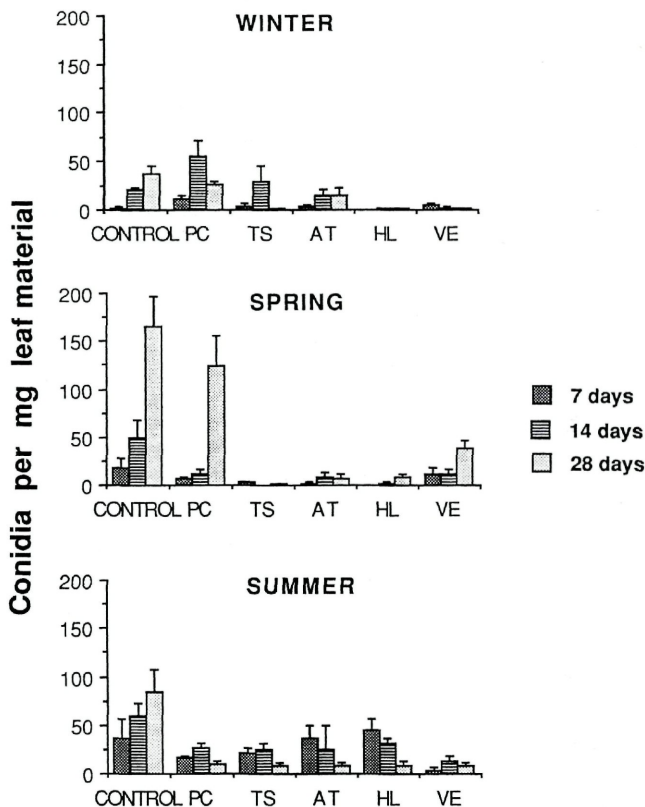
Fig. 4. – Total number of conidia produced from sterile (CONTROL) or preinoculated leaves after 7, 14, or 28 days in a stream (n = 5, + SE). – PC = *P. constricta;* TS = *T. setigerum;* AT = *A. tetracladia;* HL = *H. lugdunensis;* VE = *V. elodeae.*

therefore fungal substrates) as well as leaf–eating invertebrates (both predators and competitors of aquatic hyphomycetes) differ. From many studies, it appears that aquatic hyphomycetes generally have no strict substrate preferences. Changes in vegetation along a stream lead to shifts in fungal dominance patterns, but rarely to complete replacement of one community by another (Bärlocher, 1992b). In Boss

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Brook, shredding invertebrates are rare (Bärlocher, pers. obs.), and their influence on fungal occurrence is probably negligible.

Our results emphasize that the suitability of leaves for fungal growth deteriorates quickly, within two weeks of incubation with a pure culture. In most cases, the original colonizer produced the majority of the conidia. The major exception was *P. constricta*: In winter samples, it did not contribute a single conidium. In a study comparing species from soft and from hard water, Rosset & Bärlocher (1985) also found that pre–inoculation for four weeks at 10 C greatly reduced subsequent conidium production after stream exposure, and that the original fungus contributed between 20 and 100% of all conidia. Suberkropp (1984) exposed leaves at a relatively warm site in a stream for one or seven days, and then transferred them to a second, colder site in the same stream. The brief exposure was sufficient to establish a 'summer assemblage' that was able to maintain dominance until the temperature dropped. Therefore early resource capture is of paramount importance for an aquatic hyphomycete. Based on the almost immediate onset of conidium production, much of the energy is channeled into the release of more propagules (Suberkropp, 1991). This makes sense when the short residence times of fast decaying leaves is considered. It must be remembered, however, that aquatic hyphomycetes also occur on detritus that disappears much more slowly, such as wood (Shearer, 1992). Here, the resource quality changes more gradually, and even a small increase in the ability to invade already colonized areas (or to resist invasion) is likely to translate into a substantial gain over the residence time of the substrate. Some fungi colonize predominantly leaves or wood; others are less selective. It would be interesting to determine whether the competitive strategies of these non–specialists is influenced by the substrate on which they grow.

Acknowledgments

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