# Endophytic aquatic hyphomycetes of roots of plantation crops and ferns from India

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Roots of coffee and rubber plants and four ferns growing along two streams were examined for the presence of endophytic aquatic hyphomycetes by plating and aerating surface-sterilized root segments. No aquatic hyphomycete colonies could be isolated from plated root segments. Aeration yielded conidia belonging to 5–8 different aquatic hyphomycete species per plant. Average conidial production ranged between 3 and 410 per g root mass in five of the plants; in the fern *Christela dentata*, it reached 12,900. Between 8 (decorticated roots of *Coffea arabica*) and 96 % (entire roots of *C. dentata*) of root segments yielded aquatic hyphomycete conidia. The number of endophytic aquatic hyphomycetes species amounted to 14 and 38 % of the total species numbers previously found in the two streams. *Tetracladium furcatum*, *Triscelophorus acuminatus*, *T. konajensis* and *T. monosporus* are recorded for the first time as root endophytes.

Keywords: endophytes, aquatic hyphomycetes, ferns, plantation crops, India.

Aquatic hyphomycetes dominate decomposition of terrestrial leaves falling into streams (Bärlocher, 1992). In addition, they occur on a vast variety of other plant detritus, such as conifer needles, macrophytes, and wood. Recent studies demonstrated their presence as endophytes in aquatic roots of riparian vegetation (Fisher & al., 1991). To date, approx. 20 species have been reported as root endophytes of three angiosperms (*Acer spicatum* Lam., *Alnus glutinosa* (L.) Gaertner, *Betula papyrifera* Marsh.; Marvanová & Fisher, 1991; Marvanová & al., 1992; Sridhar & Bärlocher, 1992a; 1992b) and one gymnosperm (*Picea glauca* (Moench) Voss; Sridhar & Bärlocher, 1992a; 1992b). These are all trees occurring naturally along streams and rivers of temperate zones.

The objectives of the present study were to extend observations to two plantation crops (coffe and rubber trees) and four ferns in the vicinity of two streams of the Western Ghat forests of India.

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## Materials and methods

Five plants of coffee (*Coffea arabica* Linn.) and rubber (*Hevea brasiliensis* M.) were selected adjacent to Sampaje stream (tributary to River Payaswini, Kodagu District, Karnataka) of the Western Ghat forest (Sridhar & Kaveriappa, 1989). From each plant, sections of live roots (50–60 cm long , 5–6.5 cm diameter, 4–6 years old) immersed in the stream were procured during the post-monsoon season (November and December 1993). Five plants each of the ferns *Diplazium esculentum* (Retz) Sw. and *Macrothelypteris torresiana* (Gaudich.) Ching., along the margins of Sampaje stream, and *Angiopteris evecta* (Forst) Hoffin. and *Christela dentata* Brownsey & Jermy along the margins of Konaje stream were selected. Sections of live roots (10–15 cm long, 1.2–3.4 mm diameter) projecting from the rhizome into the stream water were procured during September and October 1993. Roots were brought to the laboratory in polythene bags and processed within 5–6 hours of collection.

Roots of coffee and rubber were rinsed in distilled water and cut into 1-cm segments. Bark was separated from the segments, and bark and decorticated root sections (xylem) were surface sterilized by exposure to 96 % ethanol (1 min), sodium hypochlorite (6 % available chlorine; 3 min) and again to 96% ethanol (0.5 min). They were immediately rinsed in sterile distilled water. Ten segments per plant of bark and xylem were aerated separately for 96 h in 500 ml flasks with 250 ml sterile distilled water. The water was then filtered through Millipore filters (8 µm), the ten filters were stained with cotton blue in lactophenol, and conidia on the filters were counted under a low power microscope. Dry masses of the root segments were determined after drying at 100 C for 24 h. Ten segments each of bark and xylem were plated individually on 1 % malt exract agar (MEA) supplemented with antibiotics (streptomycin sulphate, 0.4 mg $ml^{-1}$; penicillin G, 0.4 mg $ml^{-1}$). Plates were incubated at 24±2 C for 2–3 weeks. Each developing colony was periodically screened for sporulation. Non-sporulating colonies were subcultured on potato dextrose agar (PDA); agar plugs overgrown with mycelium were subsequently aerated for 48 hr in sterile distilled water in an attempt to induce conidium production.

Fern roots were rinsed in distilled water and cut into 10 segments (2.5 cm). They were surface sterilized, ten segments per plant were aerated and plated as described above.

The phenolic contents of dried root powder (steel mill, 250 µm screen) were determined by Folin-Ciocalteu's reagent, following the procedures by Rosset & al. (1982). For rubber and coffee, bark and xylem were analyzed separately.

Water temperature and pH were measured at the site of root collection. Water samples were collected from September through December to determine concentrations of oxygen (Winkler's method), $Ca^{2+}$ (EDTA titrimetric method), $Mg^{2+}$ (atomic absorption), $Na^{+}$ and $K^{+}$ (flame photometry). Standard techniques were used as outlined by Lind (1974).

## Results

Physicochemical characteristics of Sampaje and Konaje streams are given in Tab. 1. The data indicate that both are softwater streams with pH values close to neutrality and relatively high temperatures.

The highest phenolics content (in mg tannic acid equivalents per 100 mg dry root mass) was found in the bark of *Coffea arabica* (4.2±0.1, n = 5, ±SE). In the xylem, it dropped to 0.9±0.1. The corresponding values for *Hevea brasiliensis* were 0.69±0.03, and 0.52±0.02, respectively. In the four ferns (roots not subdivided), phenolics contents were 1.02±0.07 (*D. esculentum*), 1.06±0.05 (*M. torresiana*), 1.45±0.07 (*A. evecta*), and 1.19±0.11 (*C. dentata*).

The total number of aquatic hyphomycete species producing conidia from live roots of the six plants varied between 5 and 8 (Tab. 2). Three fungi could not be identified. They all produced sigmoid spores of the following dimensions: 35–65 x 2.5 µm (sp.1.), 70–120 x 2.5 µm (sp. 2),and 150–2200 x 2.5 µm (sp. 3). Average conidium production per unit mass was higher from bark than from xylem sections in both coffee and rubber trees. The values for five of the plants were close to within one order of magnitude (36–410); by contrast, a much higher value was found for the fern *C. dentata* (12,900).

Tab. 1.– Characteristics of Sampaje and Konaje streams between September and December 1993. All measurements except pH and Temperature in mg $l^{-1}$; n = 5 (±SE).

| | Sampaje | Konaje |
|---|---|---|
| Temperature | 23.4±0.6 (22.5–24.0) | 27.7±0.6 (26.5–28.5) |
| pH | 6.8±0.1 (6.7–7.0) | 6.9±0.2 (6.7–7.2) |
| Oxygen | 8.7±0.1 (8.6–8.8) | 10.3±0.1 (10.1–10.4) |
| $Ca^{2+}$ | 5.4±0.2 (5.2–5.6) | 3.0±0.2 (2.8–3.2) |
| $Mg^{2+}$ | 1.6±0.1 (1.3–1.7) | 0.13±0.04 (0.12–0.14) |
| $Na^{+}$ | 12.2±0.1 (12.0–12.4) | 16.0±0.1 (15.9–16.1) |
| $K^{+}$ | 7.2±0.1 (7.0–7.3) | 6.6±0.1 (6.5–6.7) |
| Alkalinity (as $CaCO_3$) | 13.4±0.5 (13.0–14.0) | 6.6+0.1 (6.0–7.0) |
| Total hardness | 19.9±1.0 (18.5–21.0) | 7.2±0.5 (6.5–7.6) |

Tab. 2.– Aquatic hyphomycete conidia produced per g dry mass of live aquatic roots of coffee and rubber trees (subdivided into B, bark and X, xylem) and four ferns (intact roots). Averages for 50 root segments, with range in parentheses, aerated for 96 hour.

| | *C. arabica* | | *H. brasiliensis* | | *D. esculentum* | *M. torresiana* | *A. evecta* | *C. dentata* |
|---|---|---|---|---|---|---|---|---|
| | B | X | B | X | | | | |
| *Lunulospora curvula* Ingold | 1 (0-3) | 1 (0-1) | 0 | 1 (0-3) | 0 | 0 | 13 (0-67) | 12 (0-59) |
| *Mycocentrospora* sp. | 8 (0-38) | 0 | 0 | 1 (0-2) | 5 (0-27) | 38 (0-188) | 0 | 0 |
| *Tetracladium furcatum* Descals | 0 | 0 | 0 | 0 | 0 | 0 | 1 (0-7) | 0 |
| *Tetracladium* sp. | 0 | 0 | 0 | 0 | 0 | 0 | 1 (0-7) | 0 |
| *Triscelophorus acuminatus* Nawawi | 3 (0-13) | 0 | 1 (0-2) | 1 (0-1) | 5 (0-17) | 127 (0-635) | 21 (0-87) | 11,000 (0-55,000) |
| *T. konajensis* Sridhar & Kaveriappa | 1 (0-3) | 0 | 0 | 0 | 0 | 79 (0-214) | 4 (0-20) | 470 (0-1380) |
| *T. monosporus* Ingold | 16 (0-36) | 1 (0-1) | 0 | 0 | 3 (0-13) | 110 (0-240) | 16 (0-54) | 800 (40-3300) |
| Unknown sp.1 | 5 (0-15) | 1 (0-2) | 53 (5-120) | 7 (0-31) | 39 (0-147) | 7 (0-20) | 0 | 110 (0-375) |
| Unknown sp. 2 | 2 (0-7) | 0 | 57 (0-240) | 23 (0-56) | 3 (0-17) | 47 (32-80) | 13 (0-56) | 340 (30-880) |
| Unknown sp. 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 (0-7) | 0 |
| Total number of species | 7 | 3 | 3 | 5 | 5 | 6 | 8 | 6 |
| Average conidial production | 36 | 3 | 111 | 33 | 55 | 410 | 72 | 12,900 |

Tab. 3. – Percentages of root segments yielding 0–5 aquatic hyphomycete species. B = bark, X = xylem.

| | | no. of fungi | | | | | |
|---|---|---|---|---|---|---|---|
| Host | | 0 | 1 | 2 | 3 | 4 | 5 |
| *C. arabica* | B | 44 | 36 | 12 | 4 | 4 | 0 |
| | X | 92 | 4 | 4 | 0 | 0 | 0 |
| *H. brasiliensis* | B | 48 | 20 | 28 | 4 | 0 | 0 |
| | X | 56 | 32 | 12 | 0 | 0 | 0 |
| *D. esculentum* | | 80 | 12 | 4 | 4 | 0 | 0 |
| *M. torresiana* | | 40 | 28 | 20 | 8 | 4 | 0 |
| *A. evecta* | | 60 | 8 | 16 | 8 | 8 | 0 |
| *C. dentata* | | 4 | 28 | 28 | 16 | 20 | 4 |

A higher percentage of bark than xylem segments yielded aquatic hyphomycete conidia (Tab. 3; *C. arabica*: 56 vs. 8 %; *H. brasiliensis*: 52 vs. 44 %). Among the four ferns, *C. dentata* had the highest percentage of colonized segments (96 %). Considerably lower values were found in the other ferns. Similarly, *C. dentata* was the only species where up to five aquatic hyphomycetes per segment were recovered.

*Triscelophorus monosporus* occurred on 28 % of *C. arabica* bark segments, and on 16 and 56 % of *A. evecta* and *C. dentata*, respectively. On *C. arabica* xylem, *T. konajensis* was the most widespread fungus, occurring on 8 % of all segments. Unknown sp. 1 was dominant on *H. brasiliensis* bark (48 % of all segments) and on *D. esculentum* (8 %), while unknown sp. 2 was the most common species on *H. brasiliensis* xylem (40 %) and on *M. torresiana* (40 %).

Tab. 4. – Percentages of plated root segments that yielded fungal colonies. In parentheses, percentages of segments that yielded non-sporulating colonies.

| Host | tissue | percentages (%) |
|---|---|---|
| *C. arabica* | bark | 48.0 (20.0) |
| | xylem | 28.0 (8.0) |
| *H. brasiliensis* | bark | 72.0 (4.0) |
| | xylem | 80.0 (8.0) |
| *D. esculentum* | | 96.0 (16.0) |
| *M. torresiana* | | 100 (28.0) |
| *A. evecta* | | 96.0 (0) |
| *C. dentata* | | 92.0 (8.0) |

Percentages of root segments that yielded colonies on MEA were generally higher than percentages of segments that released conidia of aquatic hyphomycetes (Tab. 4). Most of the colonies belonged to the genera *Aspergillus*, *Cladosporium*, *Mucor*, *Penicillium*, *Pestalotiopsis*, and *Rhizopus*. A variable number of isolates did not reproduce and could therefore not identified. None of these colonies produced

conidia when aerated under water, nor did they resemble the typical growth form of aquatic hyphomycetes (dense mycelia, lying flat on the medium, aerial hyphae almost completely absent).

## Discussion

Ingold (1942) discovered that deciduous leaves in streams are typically colonized by aquatic hyphomycetes, a fungal group characterized by producing multiradiate or sigmoid conidia when growing under water. Waid (1954) isolated one of these fungi, *Varicosporium elodeae* Kegel, from root surfaces of beech seedlings. Two additional species, *Anguillospora longissima* (Sacc. & Syd.) Ingold and *Tetracladium marchalianum* de Wild., were later isolated from the roots of strawberry plants (Nemec, 1969). Fisher & Petrini (1989) demonstrated for the first time the occurrence of two aquatic hyphomycetes as true root endophytes. Both, however, were isolated much less frequently through plating than the most common endophyte (in 1.6 and 0.7% of 300 root samples, vs. 19%). Subsequently, Fisher & al. (1991) compared aquatic and terrestrial roots of *Alnus glutinosa* (L.) Gaertn. Out of 66 isolates, 12 were known primarily from aquatic habitats. Only two of these were among the 21 most common endophytic fungi. In aquatic roots, an average of 31% of all colonies belonged to aquatic hyphomycetes.

The aquatic roots of three trees (spruce, birch, maple) were examined for aquatic hyphomycete endophytes by Sridhar & Bärlocher (1992a). For all three trees, more aquatic species were recovered by aerating rather than by plating root segments. Generally, bark segments yielded more species and/or conidia, suggesting that colonization proceeds from the root-water interface. Based on numbers of conidia and species, the density of aquatic hyphomycetes appeared to peak in 4–5 year old roots (Sridhar & Bärlocher, 1992b). Surprisingly, in view of their scarcity on conifer needles, aquatic hyphomycetes were most common in roots of *Picea glauca* (Moench) Voss. Thus, aquatic hyphomycetes make up a small but consistent component of the endophyte community of tree roots in temperate climates. Marvanová & Fisher (1991) and Marvanová & al. (1992) even described two new species from alder roots.

The current study extends these observations to two trees and four ferns in a tropical ecosystem. Five species *(Tetracladium furcatum*, *Tetracladium* sp., *Triscelophorus acuminatus*, *T. konajensis* and *T. monosporus)* were recorded for the first time as root endophytes. Total numbers of fungal species varied little among the six plants (5–8), but conidium production per unit mass was higher

from fern roots than from the two trees. Fungal invasion, however, as mentioned above, apparently proceeds from the outside. Fungal mycelium is therefore likely to be concentrated in a hollow cylinder, and root surface rather than total mass might be a more appropriate indicator of potentially available substrate. The surface/mass ratio of fern roots exceeded that of tree roots by a factor of between 4.8 to 10.3 (assuming cylindrical shapes, diameters 50–65 mm in trees, 1.2–3.4 in ferns). When conidium production values from the two trees are multiplied by this factor, they are no longer consistently below those of three of the ferns. Very high values, however, were found on *C. dentata*. They exceeded those of temperate tree roots (Sridhar & Bärlocher, 1992b) by a factor of approx. 6. Clearly, roots of certain species provide a more hospitable environment for aquatic hyphomycetes. The concentration of phenolics (which often inhibit aquatic hyphomycetes, e. g., Bärlocher & al., 1995) was not correlated with the spore production. Possibly, the kinds rather than absolute amounts, or, the cellular location, of the phenolics were more important.

No aquatic hyphomycetes were isolated with the plating technique. Undoubtedly, this was due to severe competition by fast-growing terrestrial fungi, which was exacerbated by a high incidence of air-borne contamination. Nevertheless, the results reinforce the earlier studies, suggesting that even in roots submerged in streams, aquatic hyphomycetes do not usually dominate the endophytic community. This suggests that their effects on the host plants may be small. By contrast, the availability of roots is likely to be of considerable importance to the fungi as a stationary refuge. It may allow them to persist in a given stream reach despite the unidirectional flow of water and the strongly seasonal supply of deciduous leaves. To date, 49 species of aquatic hyphomycetes have been recorded in Sampaje stream from litter, foam and water samples (Sridhar & al., 1992). On coffee and rubber leaves, 23 and 22 species, respectively, were identified. Thus, approx. 14 % of all species, or 30 % of those found on leaves also occurred in living roots of four riparian plants. In Konaje stream, only 18 species have been identified in total (Sridhar & al., 1992; Sridhar & Kaveriappa, 1984, 1989) and seven of these are now known to be present in the roots of two riparian ferns. Two other species (*Tetracladium furcatum* and *Tetracladium* sp., possibly an undescribed species, Roldan & al., 1989) were never previously observed in the stream. For comparison, approx. 25 % of all aquatic hyphomycete species ever found in Boss Brook (Nova Scotia, Canada) are known to occur in roots of three tree species (Sridhar & Bärlocher, 1992a, 1992b). Undoubtedly, these percentages will increase as the roots of more species are studied more thoroughly.

In addition to providing a refuge for metabolically largely inactive mycelia, roots might also be important as substrates in their own right. Their biomass in some streams equals or exceeds that of dead branches and twigs (unpubl. obs.). Taking into account the considerable turnover of root tissue (Waid, 1974), a substantial amount of dead root material will become available each year, and is potentially available for fungal colonization.

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