AQUATIC HYPHOMYCETES FROM MEDITERRANEAN STREAMS CONTRASTING IN CHEMISTRY AND RIPARIAN CANOPY

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Keywords: Hyphoinycetes, Mediterranean streams. water chemistry, riparian canopy.

SUMMARY

Hyphoinycete assemblages, conidia in foam aiid those produced on leal litter of three species (*Platanus orientalis*, *Populus nigra* aiid *Salix atrocinerea*), and reproductive activity of the fungi colonizing leaves, were studied at four sites located in two Mediterranean inotintaiii streams contrasting in water cheniistry aiid riparian canopy. The abundance and spccies richness of conidia in foam were consistently higher in the two cites with riparian canopy and highcr standing stock of beiithic CPOM thaii in the sites lacking riparian caiiopy. regardless of pH, alkalinity. nitrate or phosphate concentrations of the stream water. Consequently, species richness of the fungal assemblages in foam appenr to be deterrnined by substrate availability. However, the structure and composition of the fungal coniinunity colonizing leaves were essentially dependent on pH and/or alkalinity of the water. In the alkaline strearn species richness, diversity and evenness of the fungal assemblages on the three leaf species were lower compared to thosc in the circumneutral stream. Sporulation rates on the three leaf species acsayed were positively correlated witli dissolved iiutrient concentrations, particularly with phosphate.

INTRODUCTION

The detritus pathway is an essential component of the functioning of many lotic ecosystems, especially for those flowing throuph forested catchinents or within gallery forests. Large amounts of detritus (e.g., leaf litter) fall and are trapped in the bottom of thece ctreams; therefore the progress of decomposition of this material becomes a key determinant of the trophic structure and metabolism of such ecosystems (e.g., WALLACE *et al.*. 1997).

The role of the aquatic hyphomycetes has been recognized as critical for controlling the process of leaf litter breakdown and promoting the increase in leaf palatability to detritivores (e.g., GRAÇA *et al.*, 1993; GESSNER & CHAUVET, 1994). The activity of these fungi in the decomposition of leaf litter is affected by internal factors of the substrate, e.g., C:N ratio or lignin content, and by external factors such as water temperature, nutrient concentrations, pH and alkalinity (CHAMIER, 1992; SUBERKKOPP & CHAUVET, 1995). Among the external factors, water pH, alkalinity and nutrient concentration. havc been proposed as important determinants of the structure and composition of the hyphoinyccte assemblages in strearn water and of thosc colonizing leaves (review by CHAMIER, 1992). However. the pattern is not clear-cut, especially with regard to pH and alkalinity. While some studies concluded that species richness was higher in circumneutral than in alkaline ctreams (WOOD-EGGENSCHWILER & BARLOCHER, 1983; BARLOCHER, 1987; REGELSBERGER *et al.*, 1987), and that leaf hreakdown ratea tend to decline with increasing pH (THOMPSON & BARLOCHER, 1989), other studies found that hardwater streams had richer hyphomycete assemblages and higher activity on leaves than in softwater streams (SUBERKROPP & CHAUVET, 1995). Other external factors affecting abundance and species richness of hyphomycete assemblages are the structure and composition of the riparian canopy (BARLOCHER, 1992a).

Limnetica, 13 (2): 45-55 (1997)
J. Pozo & A Elosegi (eds),
Proceedings of the European Meeting on Litter Breakdown in Rivers and Streams

The aim of the present study was to investigate to what extent water chemistry, e.g., pH, alkalinity and nutrients, as well as riparian canopy affect the hyphomycete assemblages and their activity on leaves in two Mediterranean streams.

STUDY SITES

The field study wac conducted at four sites in two low-order non-polluted streams in eastern Andalucia, province of Granada, Spain (fig. 1). The headwaters of the River Genil flow over siliceous rocks belonging to the core of the Sierra Nevada mountains. Genil-I is Iocated upstream from the Canales reservoir, in the siliceouc region, and the banks are lined by a variety of deciduous tree species including *Salix atrocinerea* Brot., ***Salix*** *alba* L., *Populus nigra* L., *Castanea sativa* Miller, *Quercus pvrenaica* Willd. and *Juglans regia* L.. Genil-2 is located about 5 km downstream from Genil-l and 200 m downstream from the Canales reservoir in the area between the siliceous core and the limestone belt of Sierra Nevada. At this site the banks are devoid of riparian vegetation because this is a concrete-lined canal from the dam. The reservoir has a hypolimnetic outflow.

Vicario stream flows over the limestone coastal range of the Sierra de Almijara. The stream is mainly fed by ground water rich in calcium hydrogen carbonate and carbon dioxide which lead to the precipitation of travertine on the submerged substrates (CASAS *et al.,* 1994). Vicario-l is devoid of riparian canopy, although the alluvial surroundings have been planted recently with *Platanus orientalis* L., still scarcely developed. Towards the source of thc stream the riparian vegetation has **a** weak successional development, being mainly composed of scrubs of ***Salix*** *purpureu* L.. Vicario-2 ic located about 4.5 km downstream from Vicario-l and has a riparian vegetation which includes trees and scrubs of ***Salix*** *atrocinerea*, *S. purpurea*, *S. alba*, *Ruhus ulmifolius* Schott and *Populus nigra.* Dense patches composed of *Typha* sp. and *Phragmites* sp. are well represented in open lentic areas above this sampling cite.

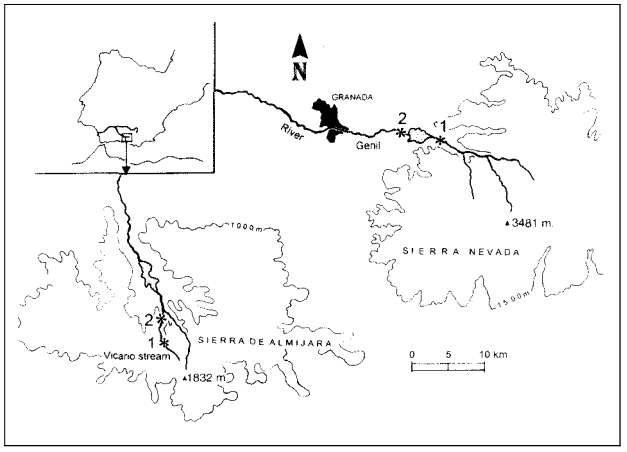

Figure 1. Location of ihe four sampling sites in southern Spain, Andalucia.

MATERIAL AND METHODS

On each sampling date water pH and conductivity were determined in the field by electrodes and a 1 l sample was transported on ice to the laboratory for chemical analysis. Alkalinity was determined by titrating with 0.02 N H_2SO_4 to an end point of pH 4.5. On 18 Nov. 1995 N-NO, and P-PO, were determined by the methods described in WETZEL & LIKENS (1991). The amount of benthic coarse particulate organic matter (CPOM, > 1 mm) was determined from three replicates collected with a Hecs sampler on 18 Nov. 1995 at each site. The material collected was sieved and the fraction over 1 mm was processed for ash-free dry mass (AFDM) determination *(550* °C; 5 h). The percentage canopy cover was determined at each site over 10 trancects perpendicular to the stream. of 5 m length on either side, and randomly distributed over a 200 m long reach. Water temperature ranges between sampling dates were determined with maximum-minimum thermometers at each cite. Discharge wac estimated on the first sampling date by measuring the speed of current with a propeller-type current meter along a known stream section.

Conidia in foam

Naturally occurring stream foam was sampled *(5* replicates of at least 100 ml foam per site and sampling date, on 18 Nov. and 9 Dec. 1995) from 200 m reaches. Foam samplec were introduced in sterilized plastic bags and preserved with 1 ml of 4 % formalin. In thc laboratory, after shaking the bags, 1 ml per sample was dispersed homogeneously on a microscope slide (9.7 cm') and dried at 40 °C. The spores on the slides were fixed and stained with trypan blue in lactophenol and heated for 30 min at 40 °C. Conidia were identified and counted in 50 microscope fields of 0.1 min' surface area (magnification 320 x) regularly distributed on three longitudinal transects per clide. An intensive cearch for infrequent or rare species wac carried out on one slide per site and sampling date. Additional foam from Vicario stream (12 Jan., 10 Feb. and 12 Mar. 1996) was sampled in the same wüy for unknown taxa, although this was iiot considered for comparison between streams. Unidentified spore forms and conidia of rare species have been drawn with camera lucida and reproduced as figs. 3-11.

Leaf hait colonization and sporulation

Leaves of *Platanus orientalis*, *Populus nigra* and *Salix atrocinerea* were collected before abscicsion in the vicinity of the 'Vicario stream in the autumn of 1995, air-dried and stored until needed. Leaves were assembled into packs of about 4 g dry mass, enclosed in nylon-mesh bags (0.5 cm mesh), attached to stones with rubber bands and submerged in the four cites on 5 Nov. 1995. Three leaf bags per date, leaf species and cite, were collected in separate sterilized bags after 13 and 34 days and transported to the laboratory in a cool box. The leaves were then rinsed with filtered distilled water and a fraction of ca. 1 g wet macs was uced to determine rates of conidium production by aerating in 100 ml filtered unchanged water from the corresponding site over 48 h at 15 "C in sterilized aeration chambers. The supernatant containing spores was filtered through 5 µm pore size polycarbonate filters, and these were fixed and ctained as above. Conidia were identified and counted in 50 microscope fields of 0.1 mm^2 area (magnification 320 x) distributed over two perpendicular diametrical transects. The remaining leaf debris was dried (60 °C; 48 h) and the ash-free dry mass determined (550 °C; 5 h). The Shannon diversity index (H') and evenness (E) were calculated following MAGURRAN (1991).

Statistical analysis

Data on density of conidia in foam and sporulation rates were log (x+1) transformed to account for normality and homoscedasticity, and analyzed by ANOVA; date and site were used as factors for conidial density in foam, and submergence time, site and leaf species as factors for sporulation rates. When differences were significant, post-hoc comparisons were carried out by meanc of Tukey's test (HSD, horiestly significant difference) (ZAR, 1984).

RESULTS

The two streams differed importantly in conductivity, pH and alkalinity (table 1). Genil is a circumneutral softwater stream and Vicario is a high pH hardwater stream. With regard to the concentrations of N-NO, and P-PO, (table 1), the sites can be ranked from oligotrophic (Genil-1) to eutrophic (Genil-2). The two sites at Vicario occupied a mesotrophic position in this ranking. The four sites also exhibited large differences in riparian canopy and standing stock of benthic coarse particulate organic matter (table 1): The two sites with riparian canopy, Genil-1 and Vicario-2, respectively averaged 120 and 70 times more CPOM than those without (Genil-2 and Vicario-1).

Conidia in foam

The concentration of conidia in foam (fig. 2) was significantly affected by the sampling site and date ($F = 83.20$, $p < 0.001$; $F = 17.36$, $p < 0.001$; respectively). The mean number of conidia per ml foam was significantly higher at the two sites with riparian canopy, Genil-1 and Vicario-2, than in Vicario-1, and the latter was higher than in Genil-2 ($p < 0.001$). The mean concentration of conidia on 9 Dec. was significantly higher than on 18 Nov. ($p < 0.001$). The same pattern was obtained for the most abundant species in foani, *Alatospora acuminata*. Conidia of *A. acuminata* dominated in foam from the four sites on the two sampling dates, except in Genil-1 on 18 Nov., when *Lemonniera cornuta* accounted for the majority of the conidia (table 2). Other relatively abundant species at all the sites and sampling dates were *Heliscus*

Table 1.- Physical and chemical characteristics of the streams. Values for pH, conductivity and alkalinity are ranges obtained over three sampling dates Discharge and nutrients were deterniined on 18 Nov. 1995. Data on CPOM are means ± 1SE. **AFDM:** ash free dry mass.

	Sampling sites			
Variables	Genil-1	Genil-2	Vicario-1	Vicario-2
Altitude (m a.s.l.)	920	800	940	860
Gradient (%)	5.0	4.5	3.9	2.0
Discharge (ls^{-1})	320	800	98	260
Temperature mean ("C)	10.5	10.0	12.7	12.0
Temperature range ("C)	8-13	9-11	7-19	9-17
pH	6.5-6.7	7.0-7.3	8.2-8.4	8.0-8.2
Conductivity (µS cm^{-1})	168-171	203-210	328-332	317-321
Alkalinity (meq l^{-1})	0.73-0.74	0.78-0.80	3.01-3.14	2.83-3.06
N-Nitrates (mg l^{-1})	148	536	305	291
P-Phosphates (mg l^{-1})	10	130	20	34
CPOM (g AFDM dm^{-2})	0.973 ± 0.154	0.008 ■0.003	0.011 10.007	0.772 ± 0.036
Canopy cover (%)	50.3	0.0	0.0	38.7

Table 2.- Fungal taxa seen as conidia in foam and relative abundance (%). Presence (+) or absence (-) of conidia recorded on one slide per sampling site and date. (ß) Spore form **A** in fig. 11. (*) Unidentified **spore** forms recorded on one slide per sampling site and date (see figs. 3-11).

TAXA	GENIL-1		GENIL-2		VICARIO-1		VICARIO-2	
	XI	XII	XI	XII	XI	XII	XI	XII
TAXA IDENTIFIED TO SPECIES								
Alatospora acuminata s.l.	8.37	50.38	33.51	62.07	87.50	49.63	59.97	44.11
Alatospora acuminata s.s.					+	+		+
Alatospora puichellu				+			0.81	+
Anguillospora crassa		+		+				
Anguillospora filiformis	3.54	2.31	5.94	+	+		1.35	4.21
Anguillospora furtiva sp. ined			+	+				
Anguillospora longissima	+	1.11	1.62	10.34				
Articulosporu proliferata		+						
Articulosporu tetracladia		+					+	
Campylospora chaetocladia		+						
Clavuriopsis aquatica	+							
Dendrospora polymorpha		+			+	2.22	0.40	0.10
Diplocladiella scalaroides			+					
Flagellospora curvula		5.93				+		0.88
Heliscella stellata								+
Heliscus lugdunensis	1.40	0.27	3.24		5.15	2.22	1.62	0.34
Lateriramulosa uniinflata		+				+		+
Lemonniera aquatica	+	+						
Lemonniera cornuta	82.21	11.62	3.24					+
Lemonniera terrestris	+	1.68						0.10
Lunulospora curvula		0.10					0.40	+
Mycofalcella calcarata				+				
Tetracladium apiense	+	6.78	27.03	+	4.80		24.26	4.88
Tetracladium marchalianum	+	7.78	20.00	15.70	2.49	4.44	9.84	6.48
Tetracludium maxilliforme		+						
Tetracladium setigerum		6.24	3.24			+	0.81	+
Tetrachaetum elegans	+							
Tricellula aquatica		+						+
Tricludium angulatum	0.73	1.88	1.62				0.40	0.55
Tricladium splendens	+	0.10	+	+				
Triscelosphorus monosporus	0.37	+						
Tumularia aquatica				+				
Varicosporium scoparium							+	+
Volucrispora graminea		0.27				+		25.46
Volucrispora ornithomorpha		+						+
TAXA UNIDENTIFIED TO SPECIES								
Curucispora cf. *ponapensis*								+
Cylindrocarpon cf. *ianthothele*		0.17						10.26
Dactylaria cf. *basitruncata*		+						+
?*Dactylaria obtriangularia*								+
Dicranidion sp.								+
Flabellospora cf. *verticillata*							+	
Fusarium spp				12.45		+	0.40	+
Goniopila / Margaritispora		0.44						+
Helicomyces sp.		+		+				+
Helicosporium sp.								2.32
Thrinacospora sp.		1.99						+
Tricellula sp.								+
Trinacrium sp.							+	
Triscelophorus sp.		+						
(ß) Unidentified (rod-shaped)						40.74		
TOTAL NUMBER OF TAXA	14	31	12	13	7	11	15	31
(*) NUMBER OF UNIDENTIFIED FORMS	0	12	2	2	0	7	13	22

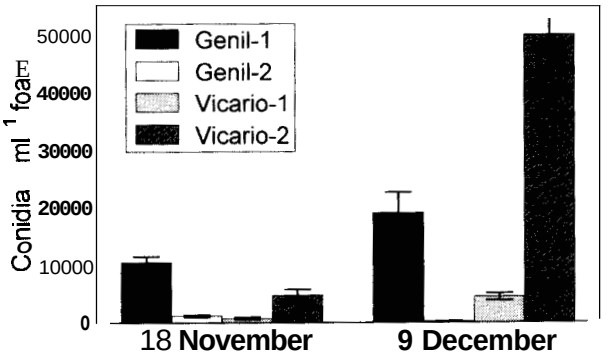

Figure 2. Denaity of conidia in foam for the four sites and two sampling dates.

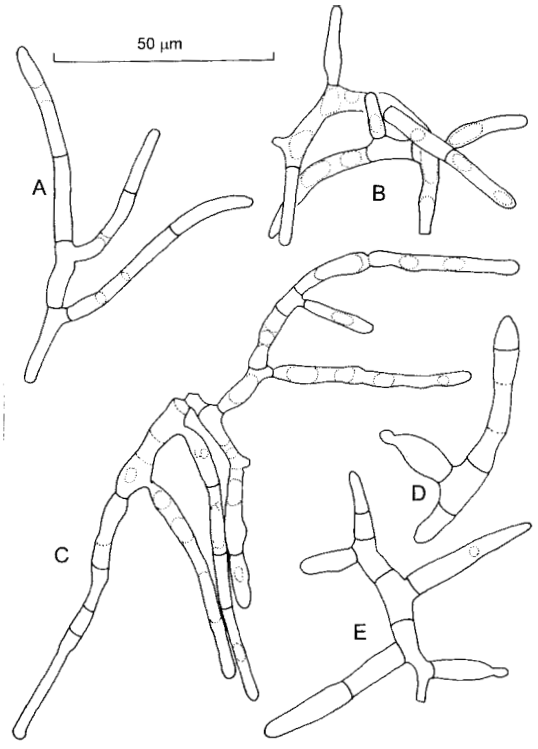

Figure *3*. **A:** unknown. B-E: *Dendrospora polymorpha*. B, C, E from Vicario-1 Feb. 96; **A** from Vicario-2 Feb. 96; D from Vicario-2 Nov. 95.

lugdunensis, *Tetrucladium apiense* and *Tetracladium marchalianum* (table *2)*. No clear pattern for the distribution of the most abundant species with regard to stream water chemistry (pH and/or nutrients) may be observed, except possibly in the case of *Anguillospora longissima*, *Tricladium splendens* and *Lemonnieru* species which seem to be more abundant and/or frequent in the circumneutral stream. Likewise, the pattern of variation of species richnesc seems to be independent from the water chemictry;

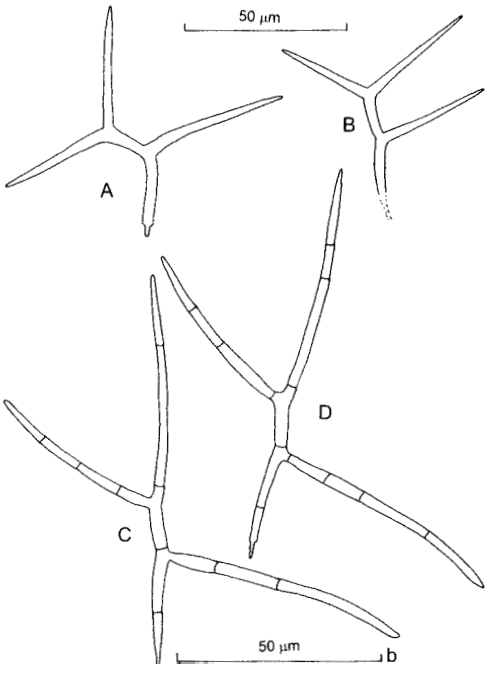

Figure 4. **A,** B: *Tricladium ?angulatum* (absence of septa may be due to poor staining). C, D: unknown (notice constricted branch insertions). **A,** B from Genil-2 Nov. 95; C, D from Genil-1 Dec. 95.

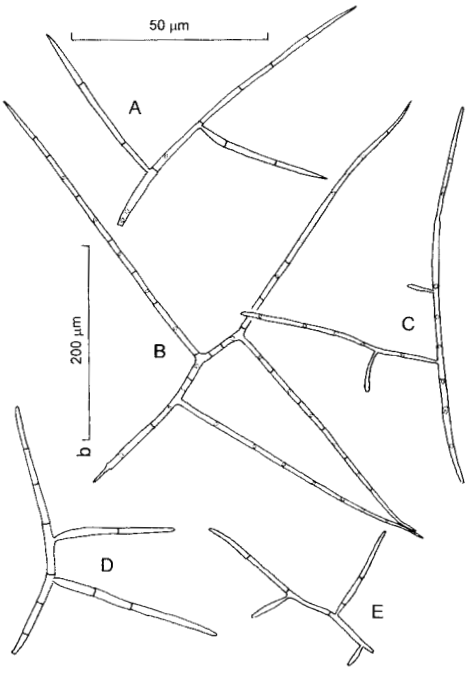

Figure *5*. **A,** C-E: unknown. B: *Varicosporium* cf. *tricladiiforme* (atypically large for the species). **A-C** from Vicario-2 Feb. 96; B also seen in Vicario-2 March 96; D **from** Genil-1 Nov. 95; E from Vicario-2 Nov. 95. **(A,** C-E to scale a: 50 µm; B to scale b: 200 µm).

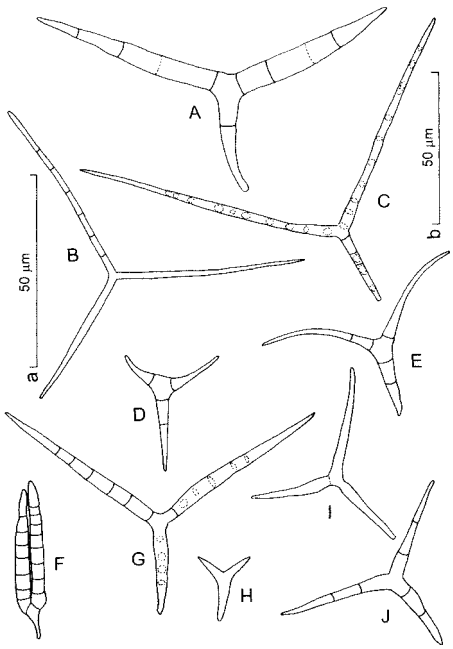

Figure 6. Spores with three elements, mostly forked. F: *Dicranidion* sp.. Remaining: unidentified, some probably *Trinacrium* spp. A-F, H, J from Vicario-:! Dec. 95; G from Vicario-1 Jan. 96, Vicario-2 Nov. 95; I from Vicario-2 Nov. 95. **(A,** B, D-J to scale a: 50 µm; C to scale b: 50 µm).

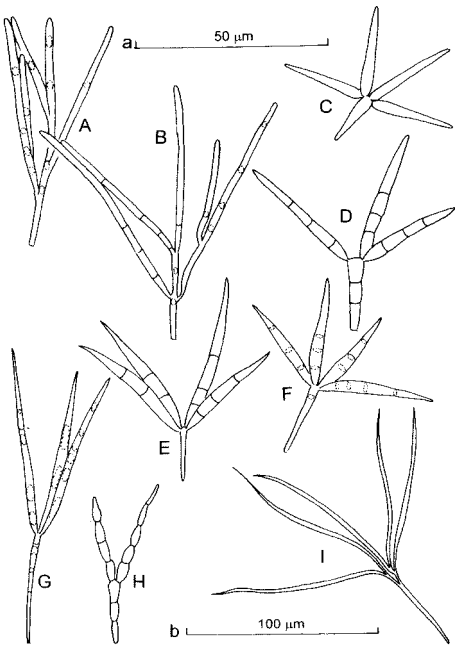

Figure 8. **A,** B: *Varicosporium scoparium.* C-I: unknown. **A,** B, E froin Vicario-2 Nov. 95; C from Vicario-] Jan. 96; D from Vicario-2 Jan. 96; F from Vicario-2 Nov. 96; G from Vicario-l Jan. 96, also seen in Vicario-2 Jan. 96, Vicario-? Feb. 96; H from Vicario-2 Nov. 95; I from Vicario-2 Dec. 95, also seen in Vicario-I Jan. 96. Vicario-I Feb. 96. **(A-H** to scale a: 50 µm; I to scale b: 100 µm).

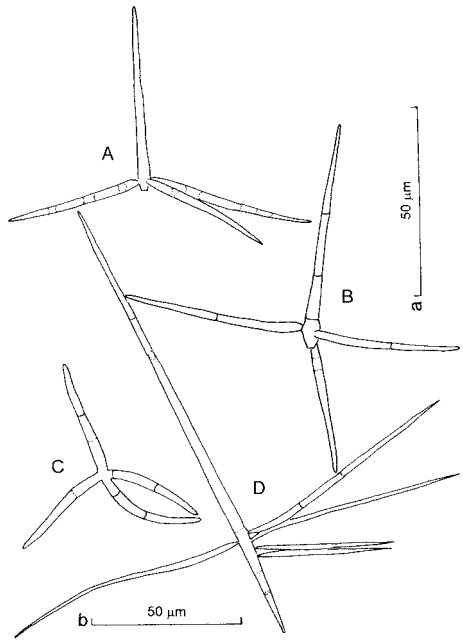

Figure 7. A: *Triscelophorus* sp. **B:** *Triscelophorus monosporus.* C. *Lemonnirru ?cornuta* (notice absence of central body). D: unknown. A from Genil-1 Dec. 95; B: source lost; C from Genil-2 Nov. 95; D from Vicario-2 Feb. 96. **A-C** to scale **a:** 50 pm; D to scale b: *50* µm).

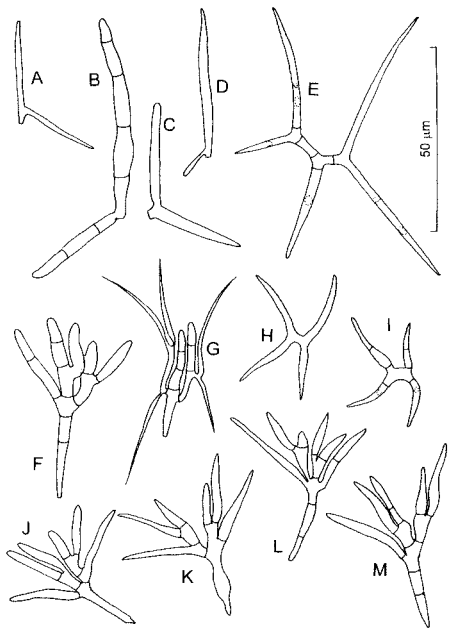

Figure 9. **A-D:** unidentified unbranched spores with excentric basal extension. B: possibly *Dendrospora polymorpha* or *Pachycladina* sp. **E:** unidentified spore with forked branching, possibly in *Dwayaangam.* F, G, J-M: unidentified *Tetracladium* spp. G: undeacribed sp. H, I: *Curucispora* cf. *ponapensis.* **A,** E, H, I from Vicario-2 Dec. 95; B froin Vicario-2 Nov. 95; C, G. K, M Vicario-2 March 96: C also seen in Genil-1 Dec. 95; D, F, J from Genil-1 Dec. 95: L from Vicario-2 Feb. 96. also seen in Vicario-2 March 96. (Scale 50 µm).

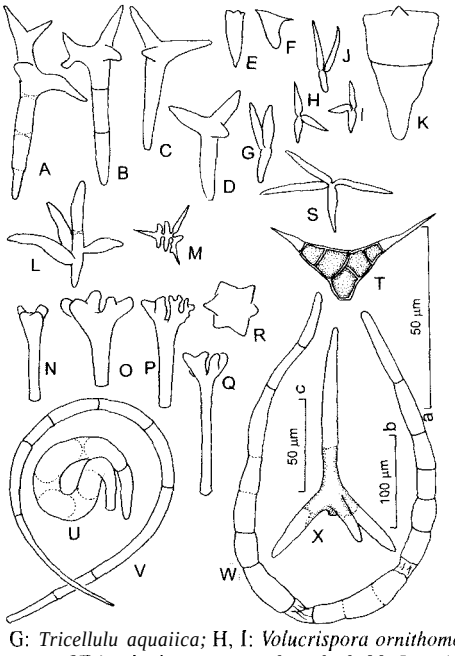

Figure 10. G: *Tricellulu aquaiica;* H, I: *Volucrispora ornithomorpha*; J: *Tricellula* sp.; L: ?*Triscelophorus* sp., undescribed; M: *Lateriramulosa uniinflata*; N-Q: ?*Thrinacospora* sp. (Coelomycetes); **R:** *Margaritispora aquatica* or *Goniopila monticola*; **S:** *Articulospora proliferata*; T: *Diplocladiella scalaroides*; U-W: unidentified scoiecospores. X: ?*Erynia* sp. (probably ***E. conica***) (Entomostorales). Remaining: unknown. **A, B** from Vicario-1 Feb. 96, **B** also seen in Vicario-2 March 96; C, G from Vicario-2 Nov. 95; D from Vicario-1 March 96; E, L, M, O, Q, S from Genil-1 Dec. 95; F, I, J, P, R from Vicano-2 Dec. 95; H, V, W from Vicario-2 Jan. 96: K, **U** from Vicario-1 Jan. 96; N from Vicario-2 Dec. 95, also seen in Vicario-1 March 96; T from Genil-2 Nov. 95; X from Vicario-2 Feb. 96. (A-U to scale a: 50 µm; V, W to scale b: 100 µm; X to scaie c: 50 pm).

however it was clearly consistent with presence of riparian canopy. Species richness was higher in the two sites endowed with riparian canopy (table 2).

Most of the unidentified forms were very scarce and recorded only by means of an intensive search carried out on one slide per site and sampling date. The richness of unidentified forms in Vicario-2 is remarkable (table 2; figs. 3, 5, 6, 7, 9, 10 and 11).

Leaf baits colonization and sporulation

In vitro sporulation rates (fig. 12) were significantly affected by sampling site, days of bait submergence and leaf species ($F = 9.37$, $p < 0.001$; $F = 78.15$, $p < 0.001$; $F = 4.68$, $p < 0.05$; respectively). Sporulation rates were significantly higher on leaf debris from the eutrophic site Genil-2 than on those from the oligotrophic sites Genil-1 and Vicario-2 ($p < 0.01$). No significant differences were obtained between Genil-2 and Vicario-2 ($p = 0.06$). The mean sporulation rate on *Salix atrocinerea* was

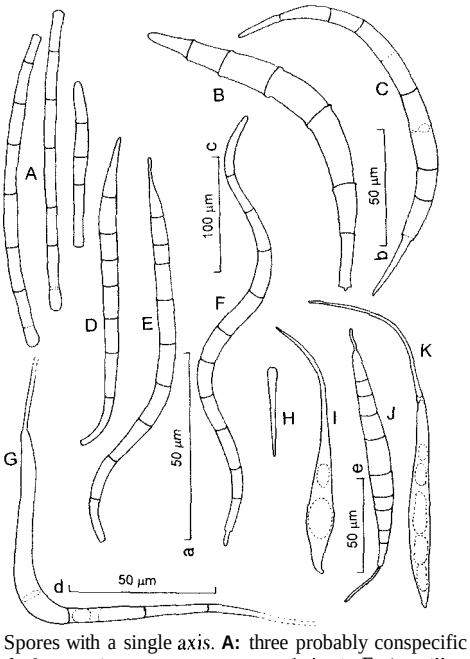

Figure 11. Spores with a single axis. **A:** three probably conspecific spores; B: unidentified spore (notice prominent septal rims); F: *Anguillospora* cf. *furtiva* sp. ined., atypically long; H: ?*Dactylaria obtriangularia.* Remaining: unidentified. **A:** very frequent in Vicario-i Jan. 96, Vicario-2 Feb. 96, Vicario-2 Nov. 95; B from Genil-1 Dec. 95; C-E, I, J from Vicario-2 Nov. 95; F from Genii-2 Nov. 95; G from Genil-2 Dec. 95; **H** from Vicario-2 Dec. 95; K from Vicario-2 Dec. 95, also seen in Vicario-2 Feb. 96. **(A,** D, E, **H,** 1, K to scale a: 50 µm; B, C to scale b: 50 µm; F to scale c: 100µm; G to scale d: 50 µm; J to scale e: 50 µm)

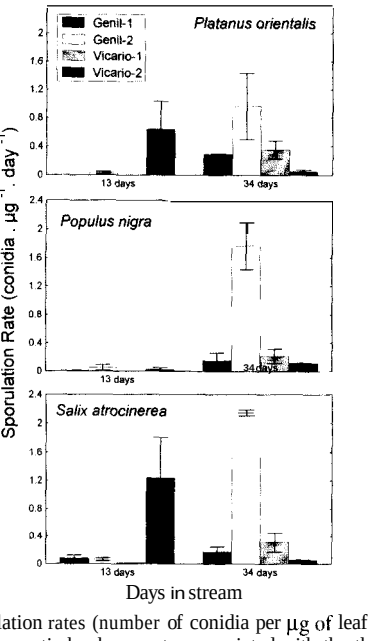

Figure 12. Sporulation rates (number of conidia per µg of leaf ash-free dry mass **per** day) of aquatic hyphomycetes asaociated with the three leaf species assayed.

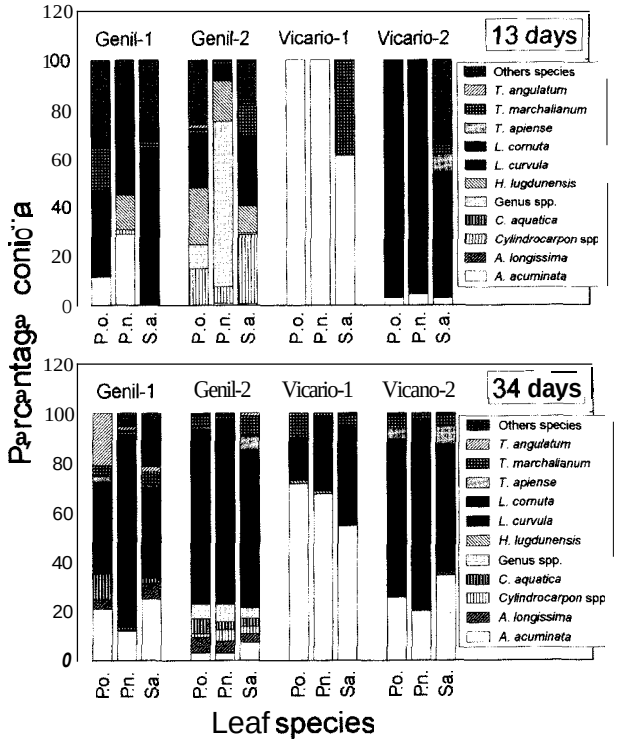

Figure 13.- Percentage of species of the fungal assemblages sporulating on leaves of sycamore (P.o.), poplar (P.n.) and willow (S.a.) at the four sites under study. Genus spp. similar to spore form B in fig. 11.

significantly higher than on *Populus nigra* ($p < 0.01$), but neither was significantly different from that on *Platanus orientalis* (for both comparisons $p > 0.05$). Sporulation rates mostly increased significantly from day 13 to day 39 of submergence of leaf substrates ($p < 0.01$), although the opposite trend could be observed in Vicario-2 for *Platanus orientalis* and *Salix atrocinerea* (fig. 12).

Conidia of *Lunulospora curvula* and *Alatospora acuminatu* accounted for 40 and 27 % respectively of the total, averaging all sampling sites, dates and leaf species (fig. 13). Conidia of *L. curvula* were best represented in leaf debris from the sites with higher phosphorus concentration (Genil-2, 44 %; Vicario-2, 79 %). On the other hand conidia of *A. acuminata* gave greater percentages from leaf debris incubated in the sites with lower phosphorus concentration (Genil-1, 16.7 %; Vicario-1, 75.6 %). Moreover, ***A. acuminata*** was a earlier colonizer in the two more oligotrophic sites (Genil-1 and Vicario-1) than *L. curvula.*

Species composition and relative abundance of conidia produced in bait colonization experiments seem to depend more on stream site than on leaf species (fig. **13).** Species richness **(S).** diversity (H') and evenness (E) (table 3) were higher at the two cites of the circumneutral stream than at those at the alkaline

Table 3.- Species richness (S), Shannon diversity index (H') and evenness (E) of the fungal assemblages sporulating on leaves from the four sites. Numbers represent means (two sampling dates and three leaf species per site) with ranges given in parentheses.

SITES	S	H'	E
Genil-1	9.0 (5-13)	1.53 (1.19-2.01)	0.73 (0.46-0.92)
Genil-2	9.0 (6-11)	1.34 (1.00-1.80)	0.61 (0.48-0.78)
Vicario-1	3.7 (1-6)	0.57 (0.00-0.99)	0.42 (0.00-0.96)
Vicario-2	5.5 (4-6)	0.56 (0.18-1.05)	0.32 (0.11-0.58)

stream. For instance, conidia of *Lemonniera cornuta, L. aquatica, L. terrestris, Tricludium angulatum* and *Tetrachaetum elegans* were exclusively obtained on leaf debris from the Genil, and those of *Anguillospora longissima* and *Clavariopsis aquatica*, well represented in leaf debris from the Genil, were scarcely produced on leaves from the Vicario.

DISCUSSION

The present results suggest that abundance and species richness of conidia of aquatic hyphomycetes are primarily correlated with substrate availability rather than with water chemistry (pH, alkalinity or nutrient concentration). This finding has been frequently reported by other authors in a variety of streams in temperate climates (IQBAL & WEBSTER, 1977; SHEARER & WEBSTER, 1985; BARLOCHER, 1987; CHAUVET, 1991; MOTHE-JEAN-LOUIS, 1997). Despite other potential factors, primarily rainfall events (WILLOUGHBY & ARCHER, 1973; MOTHE-JEAN-LOUIS, 1997), the peak nurnber of conidia and species in many streams flowing through deciduous forests generally seems well correlated with the prevailing litter fall pattern (review by BARLOCHER, 1992a). Furthermore, drastic anthropogenic reduction in the riparian vegetation results in a clear impoverishment of the fungal community (METVALLI & SHEARER, 1989), this being attributed to the dilution of the available food resources (BARLOCHER, 1992b). A comparable phenomenon may occur in our two sites lacking riparian canopy: at Genil-2, highly impacted by concrete lining, and at Vicario-1, due possibly to a long history of fires in the area. However, possibly the higher species richness at the two sites with riparian canopy may be not only related with in-stream substrate availability but also in the terrestrial surroundings. This hypothesis is suported by the finding of a substantially higher number of unidentified forms recorded in the two sites with riparian canopy. These forms may enter the stream during rain events.

Water chemistry seems to be much less important in determining conidial abundance and species richness in foam in the present study. Several studies have reported a decline in the number of fungal species in alkaline compared with circumneu-

tral streams (WOOD-EGGENSCHWILER & BARLOCHER 1983; MARVANOVÁ, 1984; BARLOCHER, 1987; REGELSBERGER *et al.*, 1987). However, in many of the above field studies pH covariates with other environmental factors of potential importance for aquatic fungi (e.g., riparian vegetation). In the review by CHAMIER (1992) this trend is far from clear-cut, and she concluded that site and substrate availability are as important determinants of aquatic hyphomycete communities as pH. Moreover, CHAUVET (1991), studying the environmental factors affecting community composition in foam from streams in south-western France, concluded that the importance of pH was lower than that of altitude, which in turn can determine important changes in quality, quantity and the timing of leaf litter imputs. This author suggested that pH may, nevertheless, affect the dictribution of some species. Our data agree with this suggestion, since conidia of several species were exclusively or more frequentiy recorded in the circumneutral stream (*A. longissima, T. splendens* and *L. cornuta*), while *D. polymorpha* was more frequent at the alkaline stream. A large number of unidentified spores were only present in the latter. But data available in the literature on pH tolerance of hyphomycetes are somewhat conflicting, especially when comparing field with iaboratory studies (CHAMIER, 1992). In fact, none of the species commonly cited in the literature as prefering high pH or alkalinity, e.g., *L. curvula, C. chaetocladia, L. aquatica, T. elegans, T. rnonosparus* and *T. rnarchalianurn* (IQBAL & WEBSTER, 1973a, 1977; SHEARER & WEBSTER, 1985; THOMPSON & BARLOCHER, 1989; CHAUVET 1991; SUBERKROPP & CHAUVET, 1995) have been recorded in the present study to be particulariy abundant in the alkaline stream. Therefore, this finding adds to the body of evidence supporting the general inconclusiveness on the role of pH/alkalinity, as a primary factor determining species composition in hyphomycete communities. A highly detailed study on this subject, that of SUBERKROPP & CHAUVET (1995), reported consistent lower species richness and a contrasting species composition in the circumneutrai streams with regard to those in the high pH streams studied. However, their circumneutral streams featured much lower dissolved nutrient concentration than their alkaiine streams and a lower alkalinity than in the circumneutrai stream studied in the present work (Genil).

Levels of dissoived nitrate and phosphorus, as well as pH or alkalinity, seem to be of minor importance determining higher abundance of conidia and/or species richness in foam in the present study. The site with the highest nutritional status, Genil-2, showed a relatively impoverished fungal community and the lowest conidial abundance in foam. The species composition in this site was not much different of that in the other three sites, although the high relative abundance of the supposedly saprotrophic *Fusariurn* spp is noteworthy.

Sporulation rates seem to be positively affected by dissolved nutrient concentrations, especially phosphate in the aikaline stream, where levels of nitrate were equally high at both sites. Similar observations concerning the role of nutrients on sporulation rates have been made in other streams from temperate ciimates, suggesting that higher nutrient concentrations, especially nitrates, stimuiates fungal sporulation (BÄRLOCHER, 1982; ROSSET *et al.*, 1982; SUBERKROPP, 1991; SUBERKROPP, 1995; SUBERKROPP & CHAUVET, 1995). However, alkalinity and pH were not major factors affecting fungal sporulation rates. ROSSET *et al.* (1982) concluded that when concentration of nutrients were similar, hyphomycete activity and leaf breakdown rates were greater in hardwater streams than in softwater streams. The present results are somewhat conflicting with the above conciusion. Sporulation rates were not significantly higher at Vicario-1 than at Genil-1, the former site featuring higher levels of alkalinity, pH and nitrate than the latter. Perhaps other factors are involved in the lack of significant differences in sporulation rates between the two cites, e.g., phosphorus limitation at the two sites and/or the impoverished fungal community at Vicario-1, which might delay leaf colonization. With regard to the second possibility, CHAUVET (1989) (in BARLOCHER, 1992a) attributed the poor fungal colonization of leaves in large rivers to the scarcity of conidia in water. However, a similar scarcity as well as low species richness of conidia in foam were recorded at Genil-2, where higher sporulation rates were obtained. Therefore. the hypothesis of phosphorus limitation seems to be more likely.

The fungal communities colonizing leaves at the two streams were subctantialy different from those obtained in foams. One of the major differencec between the two fungal assemblages was the dominance of *L. curvula* on leaves from the four sites, a species rare or absent in foams. This might be partially juctified by the bias that foam samples entail, since morphologically more complex conidia are more efficiently trapped in foams than sigmoid spores such as those of *L. curvula* (IQBAL & WEBSTER, 1973b). Furthermore, species richness of the fungal assemblages sporulating on leaves was iower than the assemblages in foams. Several authors have already observed the low correlation between types and numbers of conidia from both sources. This might be attributed partly to the presence of some spores that are swept in from the hanks, and partly due to the misleading comparison of propagules with propagule-producing structures (review by BARLOCHER, 1992a). In this respect, BARLOCHER (1992a) suggected that the above fact indicates a very selective admiscion policy. In the present study,

such a policy seems to be stronger for the alkaline stream, since at both sites species richness, diversity and evenness of the fungal community colonizing leaves of the three assayed species were lower compared with those from the circumneutral stream, independently of the species richness in foam. In fact, the structure of the fungal community on leaves from Vicario-2 was simple and similar to that from Vicario-1, despite the first site exhibiting the highest species richness in foam of all four sites studied. Therefore, contrary to the results obtained for conidia in foam, the low species richness encountered on leaves from the alkaline stream is in agreement with the conclusions by other authors that softwater streams typically contain higher numbers of species than hardwater streams (BARLOCHER & ROSSET, 1981; BARLOCHER, 1982). However, the reason as to why fungal assemblages on decomposing leaves from the alkaline streams are species poor is not known. This is somewhat surprising, since aquatic hyphomycetes isolated from softwater streams survived as well as hardwater species in either type of stream (ROSSET & BARLOCHER, 1985) and fungal metabolism is known to be enhanced in alkaline streams (CHAMIER & DIXON, 1983). Perhaps, the high alkalinity and/or pH allow certain species to behave more aggressively against other species, thereby determining a higher dominance of a few species in the assemblages colonizing leaves. Additionally, in our alkaline stream we observed travertine deposition seen as a crust of calcite on the surface of the leaves. This crust, although not thick during the initial stages of decomposition, might act as a selective physical barrier to the colonization and/or sporulation of certain fungal species, hence affecting the progress of leaf litter breakdown. In fact, in this alkaline stream with travertine deposition breakdown rates for four leaf species were among the lowest registered in the literature (CASAS & GESSNER, unpublished manuscript). However, the extent to which poor fungal assemblages on leaves may determine a reduction of breakdown rates is doubtful, possibly owing to the functional redundancy that hyphomycete species seem to have. In this respect, CHAMIER (1992) argued that species richness is not a reliable guide to physiological performance; for instance, at pH 6.8 two species of aquatic hyphomycetes in a treeless moorland stream degraded aider leaves four times faster than five species in an acid stream of pH 4.9 (CHAMIER, 1987). To ascertain the role of travertine precipitation on fungal activity and biomass and its implications on leaf breakdown a longer experiment encompassing the later stages of breakdown would be necessary. The crust of travertine would then probably be more effective in coating the surfaces of the leaf debris, therefore limiting the activity of aquatic hyphomycetes.

ACKNOWLEDGEMENTS

This recearch was partially supported by Grant AMB93-0551 from the Comision lnterministerial de Ciencia y Tecnología (CICYT), Spanish government. Enrique Descals contributed to this paper during tenure of Grant DGICYT PB01695.

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