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Evolution of microfungal community on *Chamaerops humilis* leaf litter in a Sardinian Mediterranean maquis

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Abstract – The microfungal community on *Chamaerops humilis* L. leaf litter was monitored from March 1999 to Jannuary 2001, using the litter bag method. The succession of microfungi was analised, and then primary and secondary colonisers were identified. The co-occurrence analysis between major colonisers was performed and their biotic interactions were investigated during leaf litter decomposition.

microfungi / *Chamaerops humilis* / leaf litter / Mediterranean maquis

INTRODUCTION

Litter decomposition is a critical process in the nutrient cycle strongly related to soil fertility, the phenomenon is controlled by a number of factors, of which the most importants are probably the environmental ones and the physico-chemical properties of the substratum (Gillon, 1994).

In Mediterranean ecosystems, growth of natural vegetation is often limited by availability of water and nutrients (Specht & Moll, 1983), and sclerophyllous vegetation is an adaptation to strong hydrological and edaphic constraints. The sclerophyllous leaves are considered to be a physiological adaptation against drought (Rundell, 1988), they contain a number of peculiar substances, as i.e. essential oils and waxes, with different degrees of antimicrobial and antifungal activity (Magiatis *et al.*, 1999).

Many papers have dealt with the decomposition of plant substrata in different ecosystems with the aim to define the microfungal succession (Vardavakis, 1988; Lunghini & Quadraccia, 1990, 1991; Mulas *et al.*, 1990, 1995). Same have been concerned sclerophyllous species in Mediterranean areas: Vardavakis (1988) on *Arbutus unedo* L., *Quercus coccifera* L. and *Cistus incanus* L.; Pasqualetti *et al.* (1999) on *Myrtus communis* L.; Fioretto *et al.* (2000) on *Myrtus communis* L. and *Cistus incanus* L.; Tempesta *et al.* (2003) on *Phillyrea angustifolia* L. In general all

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these studies have shown haw the climatic conditions, the type of the litter and the fungal flora already present on the fallen leaves, characterise the beginning of the substratum decomposition process.

The aims of this study were to observe the succession and seasonal variation of the microfungal communities on *C. humilis* litter as well as to investigate the ecological spatial relationships among major colonisers during substratum decomposition. Fungal saprotrophs colonising *C. humilis* leaf litter are of high interest because of the complete absence of mycological studies on this plant.

MATERIALS AND METHODS

The study was carried out on a coastal site of Mediterranean maquis at Torre del Sevo (110 hectares, central-western Sardinia, Italy). Soil was made up of eolic sands intercalated with numerous levels of clay quaternary paleosoils. Vegetal cover consisted of 358 species with *Compositae* (48 species), *Gramineae* (46 species), *Leguminose* (43 species); Mediterranean maquis was made up of *Pistacia lentiscus* L., *Phillyrea angustifolia* L., *Olea europaea* var *sylvestris* (Mill.) Brot., *Rosmarinus officinalis* L., *Cistus* spp. and *Chamaerops humilis* L. (Mulas, 1993). Precipitations were very low and concentrated in the winter months, and drought conditions occurred from May to September (Fig. 1), (Bocchieri & Mulas, 1992).

Microfungal succession, associated with *C. humilis* leaf litter decomposition, was studied from March 1999 to January 2001 using the litter bags method (Visser & Parkinson, 1975; Ariantosou, 1993; Pasqualetti *et al.*, 1999; Tempesta *et al.*, 2003). Fifty senescent fragments of leaves (1 × 2 cm), directly removed from the plants, were placed inside each bag (bag 10 × 10 cm; mesh 1 × 1 mm) and posi-

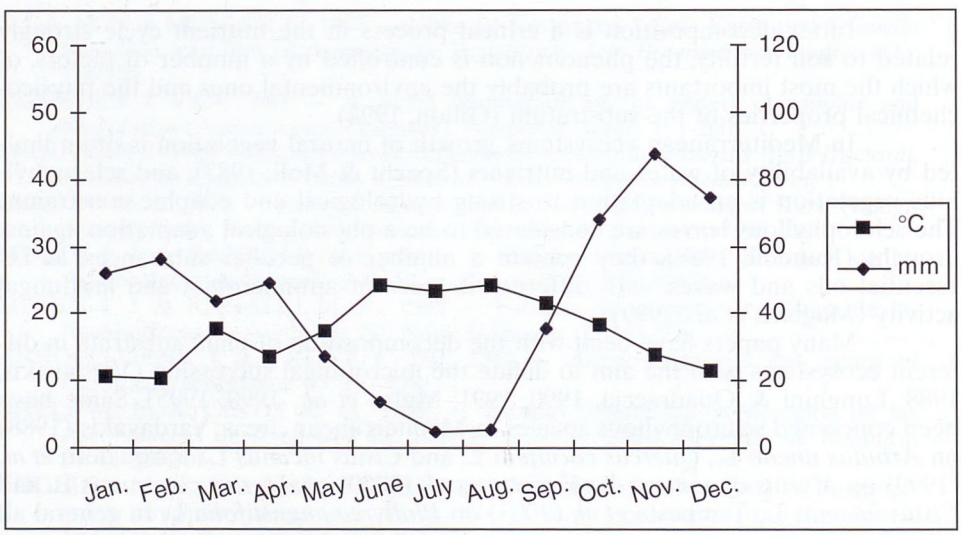


Fig. 1. Montly rainfall and mean temperature for Capo Frasca station (1962-1998), according to Bagnouls & Gaussen (1956).

tioned in four sites on the litter surface just below the respective plants. A further 12 bags containing 5 g of leaf fragments were placed in a fifth site to monitor dry weight loss which was measured with an infrared dryer at 60°C to complete dryness. Samples were collected at two-month intervals; for each sample, 40 steril damp chambers with ten leaf fragments each were set up and incubated at 25°C for 15 days. On each leaf fragments all the fungi were identified and the number of colonies of each species was calculated.

The relative frequency (Rf = n/Nx100, where n = number of fungal colonies of one species in a collection, N = total number of fungal colonies of all species in the same collection) of each species (Pasqualetti *et al.*, 1999), Pearson's index, multidimensional scaling (MDS) and cluster analysis (Wilkinson *et al.*, 1992) were performed.

The co-occurence analysis was carried out for each paired combination of the major colonisers (Rf > 5), in each sample and in the whole community (all samples). For each paired combination of the most frequent species, it was determined whether they occurred together on the same leaf fragment in a random manner, or whether they were associated each other more or less frequently than at random. Yule's coefficient of association Q and the asymptotic standard error were calculated and the χ^2 test was performed to determine whether the values of Q where significantly different from 0 at $P < 0.01$ (Wardle & Parkinson, 1991).

RESULTS

A total of 4800 *C. humilis* leaf fragments was examined; 41 fungal species belonging to 31 genera, and 5758 colonies were identified. Table 1 shows a list of colonisers identified, their relative frequency (Rf), and the total number of species and colonies for each collection. Figure 2 shows the dry weight of *C. humilis* leaf fragments over the 24 month period of the study. In the first two months of inclusion in litter there was a scantly reduction in dry weight (1.4 %), with the greatest

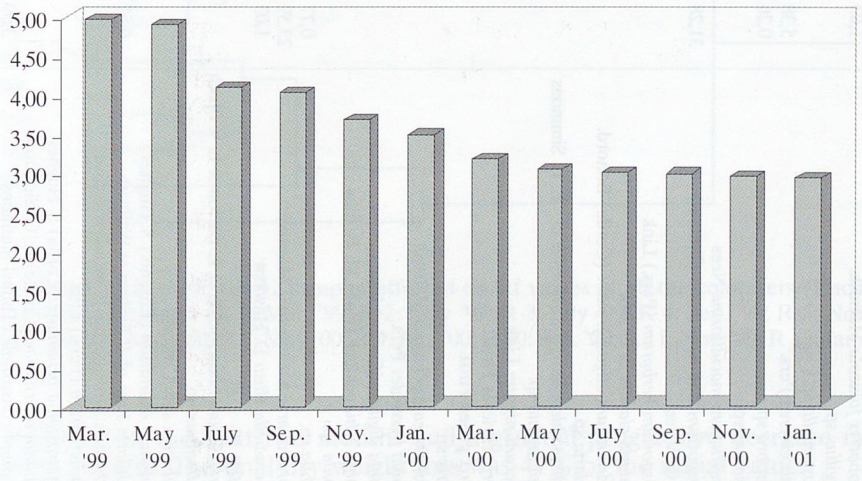


Fig. 2. Leaf litter dry weight (g) recorded over the 12 collections.

Table 1. Relative frequencies (Rf) of each species in each collection; for each collection the number of species and colonies is also shown.

	Mar. '99	*May. '99*	*July '99*	*Sep. '99*	*Nov. '99*	*Jan. '00*	*Mar. '00*	*May. '00*	*July '00*	*Sep. '00*	*Nov. '00*	*Jan. '01*
Acremonium sp.	3,85	2,58	0,4	0,96								
Alternaria alternata (Fr.) Keissl.	28,72	14,49	16,77	30,22	16,52	7,08		7,16	1,78	2,81	0,23	
Artrhobotrys foliicola Matsush.	1,03	4,45									0,71	
Aspergillus sp.		0,14										
Asterostomella sp.1					1,5	2,28	0,47	8,72	20,07	12,01	31,44	31,08
Botrytis cinerea Pers.	5,38										0,23	
Cercospora sp.	0,26											
Chloridium sp.									0,19			4,82
Circinotrichum maculiforme Nees					0,43		0,23			0,56		
Cladosporium cladosporioides (Fresen.) G. A. de Vries	31,28	16,36	11,72	13,91	13,95	10,73	17,91	6,94	2,38	6,75	4,02	0,77
Cladosporium herbarum (Pers.) Link				0,24								
Curvularia clavata B. L. Jain				0,48								
Cylindrotichum oligospermum (Corda) Bonord.									0,59			2,12
Dactylaria sp.									0,19			
Embelissa chlamydospora (Hoes *et al.*) E. G. Simmons		1,58										
Endophragmia sp.											0,23	
Epicoccum nigrum Link					0,64							
Gyrotrix citricola Piroz.												0,38
Gyrotrix grisea Piroz.						0,46	2,79	0,22		0,19		
Gyrotrix sp.										0,38		0,78
Matsushimaea fasciculata Matsush.												0,38
Periconia byssoides Pers.				0,24								
Periconia digitata (Cooke) Sacc.			0,2									
Periconia echinochloae (Bat.) M. B. Ellis			0,61		3,43	0,23	0,23	0,22	1,19	0,38		0,19
Periconia sp.		0,43										0,38
Pestalotia sp.	0,77	0,14										
Phaeoramularia hachijoensis Matsush.	23,59	32,71	40,2	30,7	24,25	20,32	43,95	38,48	29,42	37,15	20,8	17,37
Rhinocladiella ellisii D. Hawksw	1,03	26,26	28,69	21,34	36,27	56,16	33,72	37,58	42,3	39,77	42,32	41,5
Rhizopus sp.			0,2									
Scolecobasidium tshawytschae G. L. Barron & L. V. Busch												0,19
Sporidesmium sp.									0,19			
Stachybotrys atra Corda								0,22				
Stachybotrys chartarum (Ehrenb.) S. Hughes												
Stemphilium state of *P. herbarum* Wallroth	0,51		0,4		2,15							
Stemphylium sarciniforme (Cavara)Wiltshire						1,14						
Torula herbarum (Pers.) Link						0,46		0,22	0,59			
Trimmatrostoma betulinum (Corda) S. Hughes				0,24	0,86	0,91						
Ulocladium alternariae (Cooke) E. G. Simmons				0,72								
Ulocladium consortiale (Thum) Simmons	3,59	0,86	0,81	0,96			0,7	0,22	0,19		0,23	
Ulocladium oudemansii E. G. Simmons						0,23						
Zygosporium gibbum (Sacc., M. Rousseau & Bommer) S. Hughes									0,19			
No. of colonies	390	697	495	417	466	438	430	447	503	533	424	518
No. of species	11	11	10	11	10	11	8	10	14	9	9	12

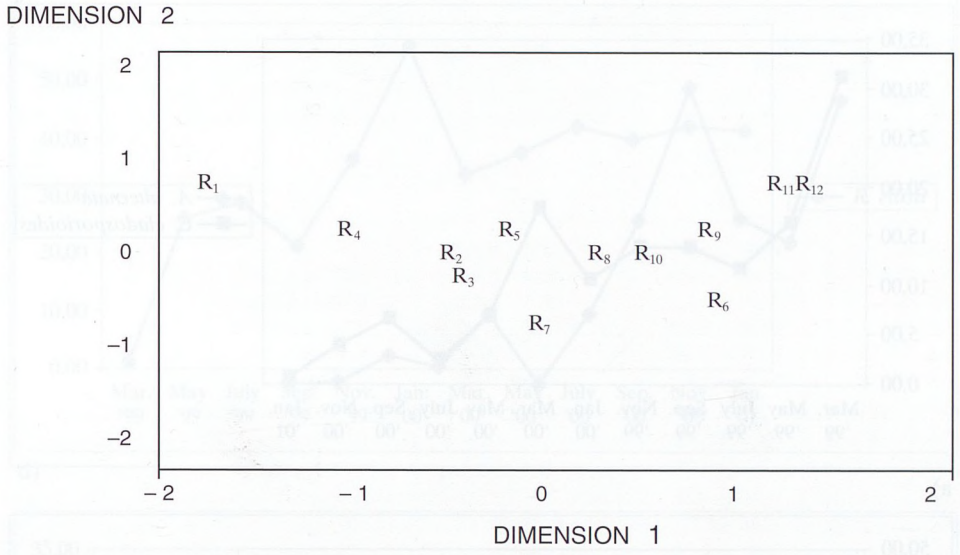


Fig. 3. Results of ordination by multidimentional scaling (MDS) of the Rf values of all the colonisers found in each collection (R 1: Mar. '99; R 2: May '99; R 3: July '99; R 4: Sep. '99; R 5: Nov. '99; R 6: Jan. '00; R 7: Mar. '00; R 8: May '00; R 9: July '00; R 10: Sep. '00; R 11: Nov. '00; R 12: Jan. '01); stress of final configuration is: 0.08891, proportion of variance (RSQ) is: 0.96440.

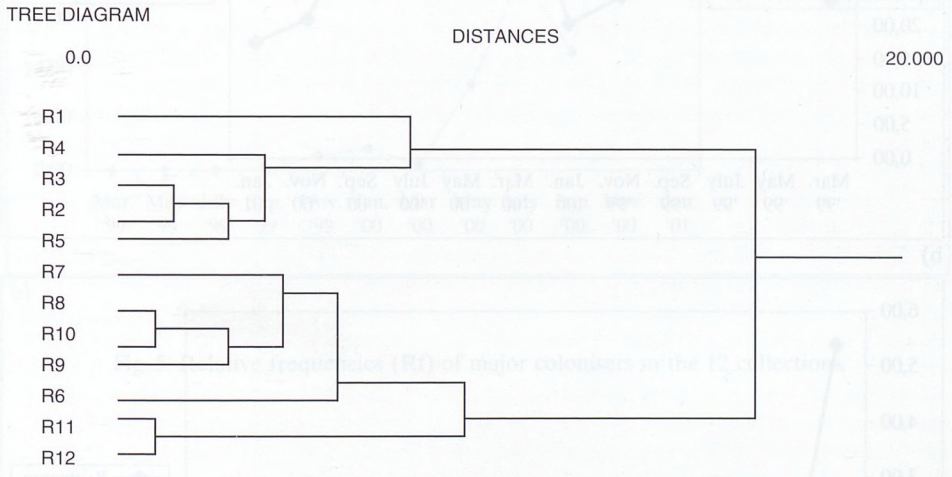
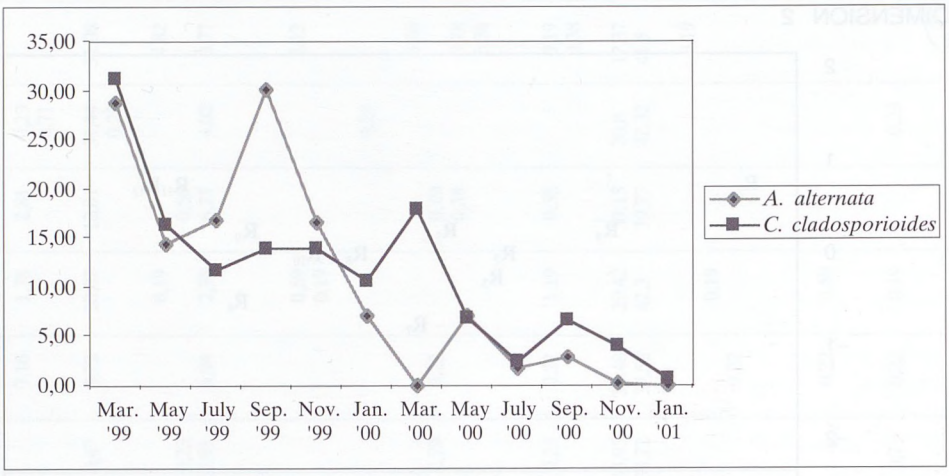


Fig. 4. Cluster Analysis of the 12 samples effected on Rf values of all the colonisers (Euclidean distance, Ward method). (R 1: Mar. '99; R 2: May '99; R 3: July '99; R 4: Sep. '99; R 5: Nov. '99; R 6: Jan. '00; R 7: Mar. '00; R 8: May '00; R 9: July '00; R 10: Sep. '00; R 11: Nov. '00; R 12: Jan. '01).

loss recorded in the next two months and a gradual progressive decrease in the following months. The final dry weight loss was 41% of the initial value.

Figure 3 and 4 show, in order, the multidimensional scaling ordination (MDS) and the cluster analysis of the twelve samples collected during the study


35,00
30,00
25,00
20,00
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Mar. '99
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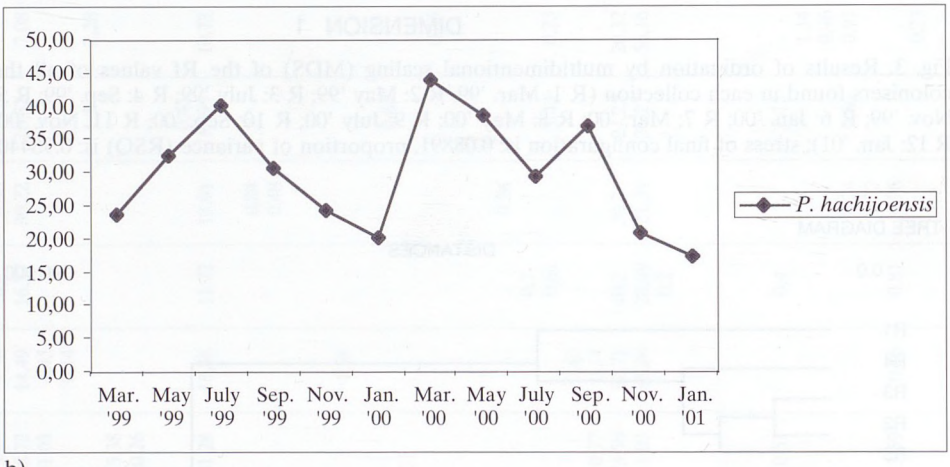
July '99
Sep. '99
Nov. '99
Jan. '00
Mar. '00
May '00
July '00
Sep. '00
Nov. '00
Jan. '01
A. alternata
C. cladosporioides

a)


50,00
45,00
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July '99
Sep. '99
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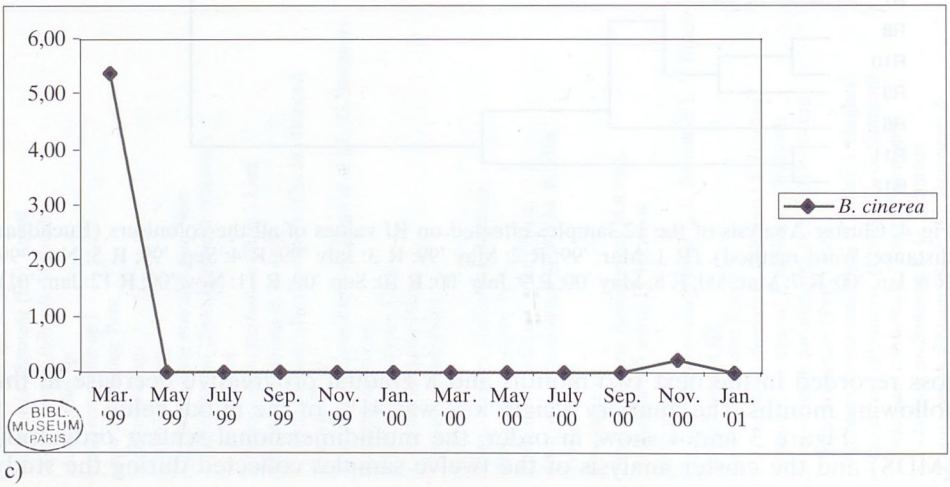
Mar. '00
May '00
July '00
Sep. '00
Nov. '00
Jan. '01
P. hachijoensis

b)


6,00
5,00
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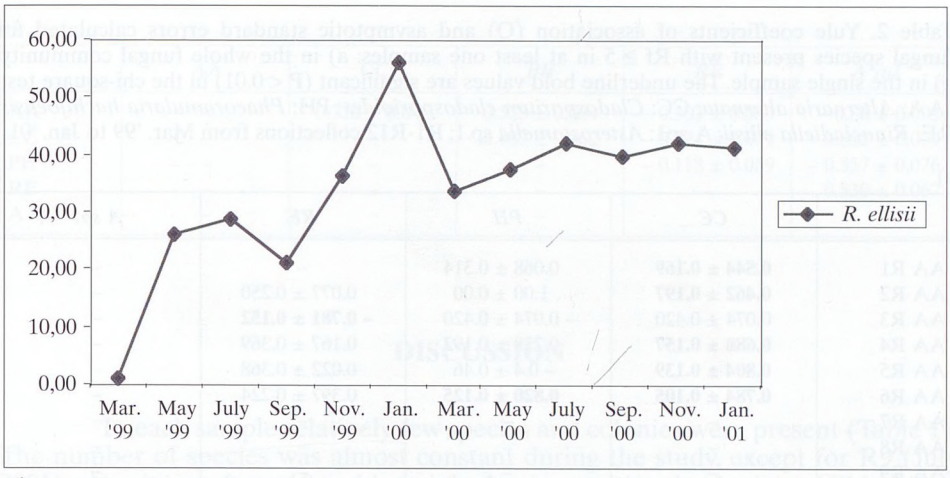
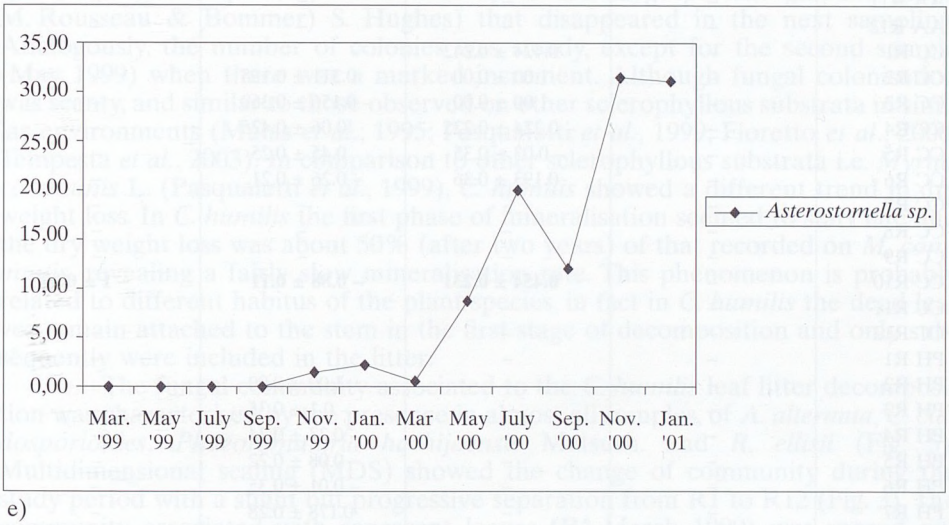
Jan. '00
Mar. '00
May '00
July '00
Sep. '00
Nov. '00
Jan. '01
B. cinerea
BIBL. MUSEUM PARIS

c)



d)



e)

Fig. 5. Relative frequencies (Rf) of major colonisers in the 12 collections.

(R1-R12); the cluster shows two main groups, the first ranging from R1 to R5, and the second from R7 to R12.

The fungal species present with Rf > 5 (major colonisers) in at lest one sample were: *Alternaria alternata* (Fr.) Keissl., *Cladosporium cladosporioides* (Fresen.) G. A. de Vries, *Botrytis cinerea* Pers., *Rhinocladiella ellisii* D. Hawksw. and *Asterostomella* sp. 1; their relative frequency (Rf) fluctuation was reported in figure 5. Finally, Table 2 shows the analysis of co-occurence carried out between major colonisers in the single sample and in the whole fungal community (all samples).

Table 2. Yule coefficients of association (Q) and asymptotic standard errors calculated for fungal species present with Rf ≥ 5 in at least one samples: a) in the whole fungal community; b) in the single sample. The underline bold values are significant (P < 0.01) in the chi-square test. (AA: *Alternaria alternata*; CC: *Cladosporium cladosporioides*; PH: *Phaeoramularia hachijoensis*; RE: *Rinocladiella ellisii*; A sp1: *Asterostomella* sp.1; R1-R12 collections from Mar. '99 to Jan. '01.)

a)

	CC	*PH*	*RE*	*A sp1*
AA R1	**0.544 ± 0.169**	0.068 ± 0.314	–	–
AA R2	**0.462 ± 0.197**	1.00 ± 0.00	0.077 ± 0.250	–
AA R3	0.074 ± 0.420	– 0.074 ± 0.420	**– 0.781 ± 0.152**	–
AA R4	**0.688 ± 0.157**	0.228 ± 0.192	0.167 ± 0.369	–
AA R5	**0.804 ± 0.139**	– 0.4 ± 0.46	0.022 ± 0.368	–
AA R6	**0.784 ± 0.105**	**0.820 ± 0.125**	0.397 ± 0.224	–
AA R7	–	–	–	–
AA R8	–	–	–	–
AA R9	–	–	–	–
AA R10	–	–	–	–
AA R11	–	–	–	–
AA R12	–	–	–	–
CC R1	–	0.424 ± 0.242		–
CC R2	–	1.00 ± 0.00	0.311 ± 0.195	–
CC R3	–	1.00 ± 0.00	– 0.156 ± 0.360	–
CC R4	–	– 0.224 ± 0.225	0.06 ± 0.425	–
CC R5	–	– 0.03 ± 0.35	0.45 ± 0.25	–
CC R6	–	0.193 ± 0.36	– 0.26 ± 0.21	–
CC R7	–	–	–	–
CC R8	–	–	–	–
CC R9	–	–	–	–
CC R10	–	**0.454 ± 0.231**	**– 0.88 ± 0.11**	**– 1 ± 0.00**
CC R11	–	–	–	–
CC R12	–	–	–	–
PH R1	–	–	–	–
PH R2	–	–	1.00 ± 0.00	–
PH R3	–	–	– 0.1 ± 0.35	–
PH R4	–	–	– 0.33 ± 0.34	–
PH R5	–	–	– 0.08 ± 0.22	–
PH R6	–	–	– 0.01 ± 0.35	–
PH R7	–	–	0.178 ± 0.28	–
PH R8	–	–	**– 0.51 ± 0.18**	**– 0.43 ± 0.23**
PH R9	–	–	0.33 ± 0.2	– 0.17 ± 0.28
PH R10	–	–	**– 0.36 ± 0.18**	**– 0.495 ± 0.171**
PH R11	–	–	**0.872 ± 0.14**	– 0.29 ± 0.51
PH R12	–	–	**0.53 ± 0.21**	– 0.25 ± 0.24
RE R1	–	–	–	–
RE R2	–	–	–	–
RE R3	–	–	–	–
RE R4	–	–	–	–
RE R5	–	–	–	–
RE R6	–	–	–	–
RE R7	–	–	–	–
RE R8	–	–	–	**0.917 ± 0.05**
RE R9	–	–	–	0.180 ± 0.24
RE R10	–	–	–	**0.643 ± 0.14**
RE R11	–	–	–	0.057 ± 0.25
RE R12	–	–	–	0.278 ± 0.208

b)

	AA	*CC*	*PH*	*RE*	*A sp1*
AA	–	0.710 ± 0.045	0.523 ± 0.064	– 0.303 ± 0.083	– 0.86 ± 0.093
CC	–	–	0.487 ± 0.062	– 0.221 ± 0.079	– 0.882 ± 0.079
PH	–	–	–	– 0.118 ± 0.059	– 0.557 ± 0.076
RE	–	–	–	–	0.530 ± 0.067
A sp1	–	–	–	–	

DISCUSSION

In each sample relatively few species and colonies were present (Table 1). The number of species was almost constant during the study, except for R9 (July 2001) when it rose from 10 to 14, mainly due to occasional colonisers (*Chloridium* sp. 1, *Dactylaria* sp. 1, *Sporidesmium* sp. 1 and *Zygosporium gibbum* (Sacc. M. Rousseau & Bommer) S. Hughes) that disappeared in the next sampling. Analogously, the number of colonies was steady, except for the second sample (May 1999) when there was a marked increment. Although fungal colonisation was scanty, and similar to those observed on other sclerophyllous substrata in similar environments (Mulas *et al.*, 1995; Pasqualetti *et al.*, 1999; Fioretto *et al.*, 2000; Tempesta *et al.*, 2003). In comparison to other sclerophyllous substrata i.e. *Myrtus communis* L. (Pasqualetti *et al.*, 1999), *C. humilis* showed a different trend in dry weight loss. In *C. humilis* the first phase of mineralisation seemed to start late and the dry weight loss was about 50% (after two years) of that recorded on *M. communis*, revealing a fairly slow mineralisation rate. This phenomenon is probably related to different habitus of the plant species, in fact in *C. humilis* the dead leaves remain attached to the stem in the first stage of decomposition and only subsequently were included in the litter.

The fungal community associated to the *C. humilis* leaf litter decomposition was characterised by the presence in almost all samples, of *A. alternata*, *C. cladosporioides*, *Phaeoramularia hachijoensis* Matsush. and *R. ellisii* (Fig. 5). Multidimensional scaling (MDS) showed the change of community during the study period with a slight but progressive separation from R1 to R12 (Fig. 3). The community associated with senescent leaves (R1-March 1999) was spaced out from the others and characterised by *A. alternata*, *C. cladosporioides* and *P. hachijoensis* that, together, made up an Rf> 80%. The May and July 1999 samples (R2-R3) were very similar (Pearson = 0.98) and characterized by *R. ellisii* and *P. hachijoensis* with high Rf values and by a strong reduction of *A. alternata* and *C. cladosporioides* (Fig. 5a). The R4 sample (September 1999) was plotted between R1 and R2-R3. It differs from R1 for the *R. ellisii* presence and from R2-R3 for an high presence of *A. alternata* (Fig. 5a). The sample R5 (November 1999) was plotted between R2-R3, with a Pearson index R2-R5 = 0.94 and R3-R5 = 0.93.

R1, R2, R3, R4 and R5 samples were characterised by the same fungal species (with exception of *R. ellisii* in R1) and differed exclusively for the Rf fluctuation of the single species. The R6 sample (January 2000) was separated in the right part of the plot. In this sample a peach of *R. ellisii* and a strong reduction of *A. alternata* were observed. The R7 sample, plotted in the central part, was characterised by the disappearence of *A. alternata* and the increasing of *P. hachijoensis*. R7 and R3 were found to be closed and this was probably due to analogous

Rf values of *P. hachijonensis*. The saprotrophs associated with R8, R9 and R10, were fairly similar. A new coloniser *Asterostomella* sp. 1 appeared and remained on the subdtratum until the end of the study. R11 and R12 were also similar and characterized by an increasing of *Asterostomella* sp. 1, high Rf values of *R. ellisii* and a strong reduction of *P. hachijoensis*. A similar sampling trend was observed by cluster analysis (Fig. 4), in particular two main groups were identified R1-R5 and R7-R12.

The trend of major colonisers in the samples were investigated to define the ecological role of each species. *A. alternata* and *C. cladosporioides* were detected from the first sample with high Rf values and progressively decreasing from R7 to R12. These species probably play no important role in the decomposition of complex structural components, as they are common ubiquitous species (Moore, 1998) probably able to utilise only simple organic substances present on the *C. humilis* (Fig. 5a). *B. cinerea* was only observed in R1 with rather low Rf values. Its presence on the senescent leaves could be considered an occasional contamination. This species is commonly present as hemiparasite on *C. humilis* fruits. *P. hachijoensis* showed high Rf values from the beginning up to the end of the study period, it was probably a primary saprotroph. This species was also recorded as primary saprotroph on *Phillyrea angustifolia* leaf litter in the same ecosystem (Tempesta *et al.*, 2003). *R. ellisii*, also primary saprotroph (Fig. 5d), appeared from May 1999 (R2) and gradually increased over the first 12 months, with a peack in January 2000 (R6), it remained with elevated Rf values until the end of the study. *Asterostomella* sp. 1 appeared in R8 with rather low values that increased progressively until R12 (January 2001), on a highly transformed substratum (Fig. 5e). This species showed a clear role as a secondary coloniser, probably utilising the decomposition products of the previous colonisations.

The major colonisers were in general not affected by seasonal variations, the only species that showed a seasonal behaviour was *P. hachijoensis*, with a strong reduction in the cold period (Fig. 5b).

Some information on the ecological spatial relationships between major colonisers were detected by co-occurrence analysis (Table 2); significant positive or negative association may be due either to resource heterogeneity or the indirect or direct effects of species on each other (Wardle & Parkinson, 1991). These analysis allow to identifie the relationships among colonisers at the fragment leaf to observe direct biotic interaction. The common ubiquitous species *A. alternata* and *C. cladosporioides* exhibit an high co-occurrence, both present positive and significant correlation whit *P. hachijoensis*. This could be due to their different ecologic role. The *P. hachijoensis* activity could be determined by release of simple substances, presumably removed by *A. alternata* and *C. cladosporioides*. The co-occurrences analysis calculated on the single samples confirms the previous observations. A low negative co-occurrence was observed between *P. hachijonensis* and *R. ellisii*. Detailed analysis carried out on each samples showed a different behaviour of the two species; i.e. they showed a negative and significant relationships in R8 and R10 while an high and significant positive interaction in R11 and R12. In the first relationship, the two species could present an antagonistic behaviour, probably related to common nutritional requirements. This antagonism disappears in the last period of research on advanced decomposed substratum. The last species *Asterostomella* sp.1 results correlated positively only with *R. ellisii* and negatively with the other species. The data of co-occurrence in each samples showed an high positive correlation of *Asterostomella* sp. 1 whit *R. ellisii* only in R8 and R10; the nutritional behaviour of *Asterostomella* sp. 1 probably depends by the presence and activity of *R. ellisii*. On the contrary there isn't any significant

correlation in R11 and R12 on a substratum in advanced decomposition stages; in this phase *Asterostomella* sp.1 resulted independent by the presence of other colonisers.

In conclusion the *C. humilis* mineralization litter process is characterized by the succession of primary and secondary colonisers; it is interesting to observe that the biotic interactions between major colonisers change during the process, presumably related to substratum composition.

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