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We investigated how habitat characteristics and in particular the level of disturbance was affecting colonisation processes and population dynamics of an ectomycorrhizal fungus. The basidiomycete *Hebeloma cylindrosporum* associated with *Pinus pinaster* trees occurs in two distinct habitats within the forest dune ecosystem along the Atlantic coast of France. The 'dune habitat' (D habitat) is characterised by scattered trees growing in almost pure sand at the extreme fringe of the forest marking the transition with the bare dune. The 'forest habitat' (F habitat) is within the mature forest in places of high human frequentation which prevents litter and humus accumulation. We studied over several years (up to ten) populations of *H. cylindrosporum* present in three geographic sites. Within each site we studied two areas corresponding each to one of the two possible habitats. In all sampled areas fruit bodies occurred within discrete patches of ground. Although an interaction between habitat and geographic site existed, habitat had significant effects on patch sizes and patch internal densities. Compared to the F habitat, patches in the D habitat were significantly larger and presented a lower density of fruit bodies. Patches very seldom occurred at the same place from one year to the next in the F habitat when in most cases they were perennial and increased in size in the D habitat. Fruit body size, which reflects reproductive output of the genets, differed between the two habitats (means of the cap diameters of 25.6 and 40.4 mm in respectively the F and D habitats). Almost all patches in the F habitat were made of several annual genets each producing on average 1.6 fruit bodies while most patches in the D habitat were dominated by one perennial genet which could simultaneously produce up to 60 fruit bodies. Under-representation of small sized genets in the D habitat suggests a low level of sexual recruitment in this habitat despite the large number of fruit bodies (and therefore of spores) produced by each of the existing genets. We therefore suggest that *H. cylindrosporum* could be considered as a fugitive species. Genets of this species survive in the D habitat in which few ectomycorrhizal fungal competitors occur. This habitat could represent the optimal habitat for this species. In the F habitat frequent recruitment could occur in 'empty patches' resulting from the local and transient elimination of competitors induced by human disturbance.

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The ectomycorrhizal symbiosis between fungi and the roots of woody plants is a conspicuous component of temperate and boreal forest ecosystems. Several features distinguish this type of symbiosis from other plant root symbioses such as the nitrogen fixing ones. In terms of 'host-plant benefit', the fungal partners contribute to functions as diverse as mobilisation of soil nutrients, protection against water stress, heavy metal toxicity or attacks by soil-borne pathogens (Smith and Read 1997). Furthermore, individual ectomycorrhizal fungal species (ECM species) have not evolved towards a narrow functional specialisation and different ECM species can each contribute to various extents to the different beneficial effects listed above. In terms of diversity and host specificity of the fungal partners, at the scales of a single monospecific forest stand, of the root system of a single tree or of soil cores, several (up to 50 and probably more per ha, Horton and Bruns 2001) species which belong to different clades of the Asco- and Basidiomycotina are present and cohabit. At the scale of a forest stand this functional community is spatially structured, the presence and/or abundance on the root system of an individual ECM species is not uniform, many of the species showing a clumped distribution especially those representing less than 1% of the mycorrhizal root tips (Gardes and Bruns 1996, Dahlberg et al. 1997).

The evolutionary dilemma of maintenance of complex communities of horizontally acquired symbionts competing for access to a common host (Douglas 1998, Genkai-Kato and Yamamura 1999, Wilkinson 2001) has led to numerous studies on the dynamics of ECM fungal communities. The structure of ECM fungal communities has long been interpreted in terms of fungal species succession (Last et al. 1987). According to this view, the composition, species richness and diversity of ECM communities largely reflect the age of the studied forest stands. This interpretation forwards the biological traits of individual fungal species as one of the main determinants of their abundance within a community. Among the major traits are the abilities to establish following spore dispersal and to survive and spread by vegetative growth of the mycelium in the soil. Based on surveys of fruit bodies in different forest ecosystems this has led to various 'ecological classifications' of ECM fungal species, which in some respects match the different strategies of Grime (1977) or Pugh (1980). ECM fungi have for instance been classified as early, late or multistage species (Last et al. 1987, Danielson 1984). An early stage fungus can be defined as a good coloniser but a poor competitor which quickly colonises from spores a newly established forest site depleted of an endogenous fungal inoculum. As a poor competitor it is replaced later in the forest succession by late stage species whose mycelia persist and spread in the soil by vegetative growth. In addition, a long-standing view was that ECM communities present in old forests become 'resistant' to invasion by incoming spores and therefore the major source of inoculum within these forests is pre-existing mycelium.

It was expected that the population structure of an ECM species should partly reflect the strategy it adopts and its position within the succession. Among the dozen of ECM fungal species for which studies of local populations have been carried out, significant differences have indeed been reported. These differences concern the age structure, clonality and genet recruitment within the populations. However, the unravelled structures did not necessarily match the initial expectations concerning the studied species. For example, in old forests, depending on the species, populations are characterised by either a low density of large genets (Dahlberg and Stenlid 1990, De La Bastide et al. 1994, Dahlberg 1997, Bonello et al. 1998, Sawyer et al. 1999, Gryta et al. 2000) or a high density of small genets (Gryta et al. 1997, Gherbi et al. 1999, Zhou et al. 1999, Redecker et al. 2001). The first structure fits well with the model which postulates that in late successional stages recruitment by spore germination is low and that long-established genets (which occupy large territories as a result of subterranean vegetative mycelial growth) predominate. However, the second structure which was recently shown to characterise populations of three 'typical' late stage species belonging to the genera *Amanita*, *Lactarius* and *Russula* contradicts this model (Redecker et al. 2001).

Almost all studies dealing with population structures of ECM species have each been carried out by sampling fruit bodies in only one or a very few forest sites over a period of time ranging from one to at best three years. This introduces several potential biases that should be evaluated before drawing any definite conclusions on the colonisation strategies adopted by an ECM fungus. First, by limiting studies to one site it appears difficult to conclude if the results obtained reflect intrinsic species traits or alternatively local species responses to various characteristics of the study site. Indeed, studies on plant populations have demonstrated in many cases a strong habitat effect on the balance between genet recruitment and survival of individuals (Eriksson 1993, Mandujano et al. 1998, Kudoh et al. 1999). Second, by sampling populations over too short a period of time it is difficult to distinguish, in the case of the exclusive occurrence of small genets, between (i) a recent colonisation event which could evolve in the long term towards a clonal population structure and (ii) recurrent recruitment associated with low survivorship values of individual genets.

In this paper we report of a study on different populations of the agaric *Hebeloma cylindrosporum* Romagnesi which aims at evaluating potential habitat effects on population structures of this species. The choice of *H. cylindrosporum* associated with *Pinus pinaster* (Ait.) Sol. along the southwest Atlantic coast

of France is justified by previous contrasted results reported for three of its local populations. Indeed, in one 200 m^2 site, two fruiting genets of this fungus, which persisted and extended in size by vegetative growth of their below-ground mycelia, over a five-year sampling interval, were observed (Gryta et al. 1997, 2000). At the opposite, in two other 500 and 100 m^2 sites sampled twice at a three year interval it was shown that populations of this fungus were composed of numerous small genets and that genets identified during the first sampling year were not re-identified three years later on the same sites (Gryta et al. 1997). This structure does not seem to be an artefact resulting from occasional random fruit body formation by perennial below-ground mycelia. Indeed, Guidot et al. (2001) established that the diversity of the genets of this species forming the fruit bodies matched the below-ground diversity of the genets forming the mycorrhizas and that disappearance of a genet above ground followed the disappearance of its corresponding mycorrhizas.

In this study, in order to unravel potential habitat effects on the dynamic and spatial structures of populations of *H. cylindrosporum*, we identified two distinct habitats in which this species occurs (see Material and methods) and followed during several years (up to 10) local populations present in both habitats (defined here as the biotic and abiotic characteristics of a local stand). To minimise potential geographic site effects, the analysis was performed in three sites located along the south-west coast of France and at each site two areas each representing one of the two habitats were studied. In addition to spatial and temporal surveys of the populations, we include in this study an analysis of fruit body size, which can be used to infer the reproductive investment of genets growing under contrasted environmental conditions. Beyond the case of *H. cylindrosporum*, the data presented could be used to infer and identify some of the mechanisms that lead to the recruitment and persistence of ECM species within a community. These data could also be used as guidelines to devise future field experiments necessary to test hypotheses related to the establishment, maintenance and functional impact of the local diversity of ECM fungal populations and communities.

Material and methods

Species studied

Hebeloma cylindrosporum Romagnesi is an agaric basidiomycete with a homokaryotic/dikaryotic life cycle controlled by a multiallelic bifactorial mating system (Debaud et al. 1997). In the field, the dikaryotic (genetically diploid) mycelia growing in the soil form the mycorrhizas and the fruit bodies. This species produces neither vegetative spores nor resting structures and long distance dispersal is assumed to be performed by the wind-dispersed haploid meiospores. *H. cylindrosporum* is a late fruiting species; in the study sites, fruit bodies form as a single synchronised flush, which starts end of October–early December depending on the weather conditions, and lasts about three weeks.

Habitat characteristics

H. cylindrosporum is commonly found in the European Atlantic sand dune forest ecosystem. In south-west France, where the sampling sites are located, most of the dune system has been planted by a monospecific forest of *Pinus pinaster* trees and *H. cylindrosporum* occurs in two distinct habitats. The first habitat (hereafter referred to as the dune habitat) is the extreme western fringe of the forest, which makes the transition with the bare dune. It is characterised by scattered planted trees with a low growth rate and often a bushy appearance due to sand erosion on the top branches. The soil is an almost pure non-calcareous sand (less than 0.5% of organic matter, pH around 6.1) with no distinguishable horizons. Ground vegetation, when present, is dominated by lichens and tussocks of graminae. In this habitat, the first ten or more cm of soil is almost devoid of *Pinus* roots which are deeply buried. Few other ECM fungal species fruit in this habitat, the most abundant being *Rhizopogon roseolus*, a species not to be found anywhere else in the forest. The transition between this marginal habitat and the regularly planted forest characterised by straight growing trees and litter and humus accumulation is usually sharp and well defined.

The second habitat where *H. cylindrosporum* occurs (hereafter referred to as the forest habitat) is more inland, within the forest, but exclusively in places where litter and humus accumulation on the floor is disturbed. This is characteristic of places of high human frequentation such as road and car park borders, recreational areas or camping sites located within the forest. In this habitat there is no ground vegetation, the percent organic matter in the top 10–20 cm of soil can reach 3% (pH around 5.5) and a dense network of roots is present just below the surface (unpublished observations). Visual inspection of fungal fruit bodies indicates a more diverse ECM community compared to that of the dune habitat.

The two habitats ('dune' and 'forest') can be contiguous or separated by several hundreds of metres of undisturbed forest characterised by a well developed humus layer and in which fruit bodies of *H. cylindrosporum* have never been observed.

Study sites and fruit body sampling

In this study, three geographic sites distributed along a 22 km South-North transect of the Atlantic coastline ca 50 km west of Bordeaux (France) were selected (Table 1). In each of these three sites, fruit body sampling was performed in two distinct areas, each one corresponding to one of the two habitats where *H. cylindrosporum* can be found. Each geographic site received a two letter code to which was added the letters D or F to respectively identify the dune and forest habitats (for example LPF and LPD for respectively the forest and dune habitats of the 'Le Porge' site, Table 1). Of the three dune habitats sampled, the LPD one was the most 'unstable' and corresponded to the border of an old-grown forest whose floor had been covered by several dm of sand during the last decade as a result of wind erosion of the opposite mobile dune.

All sites were visited during one week, once a year in autumn at the peak of the fruiting season. Three areas had been previously sampled in 1990 and 1993 (TVF and LPF) and during the period 1993 to 1997 (GCD) (Gryta et al. 1997, 2000). In this paper we include these data and report of additional data obtained in 1994, 1995, 1997, 1998 and 1999 (Table 1). Except for the GCF area, which was sampled on one occasion in 1998, all other areas were sampled at least during two consecutive years (Table 1).

In all sampled areas, fruit bodies were not evenly distributed, but, instead, were grouped within discrete patches which were usually several metres away from each other. Therefore, in each area, we adopted a two level sampling design of the fruit bodies. We firstly, haphazardly selected patches, and, secondly, mapped all fruit bodies in the patch, and, thirdly, sampled some of the fruit bodies within these pre-selected patches. Within a sampled area no attempt was made to map all patches, the patches we selected were the first we saw irrespective of the number of fruit bodies they encompassed. The only exception was the TVF area in which all patches were precisely mapped within a predefined 500 m^2 area (Fig. 2). Within a selected patch, all fruit bodies, irrespective of their degree of maturity, were precisely mapped and sampled regularly over the whole patch surface (Fig. 1). Depending on patch size and on

Table 1. Number of patches of *Hebeloma cylindrosporum* fruit bodies analysed each year in the dune and forest habitats of the three studied geographic sites.

Site	Habitat	Surface area (m^2)	Study year	No. of patches analysed	No. of patches genotyped[1]	Reference
Le Truc Vert (44°43′N, 1°15′W)	dune (TVD)	1000	1998–1999	2	2	this study
			1998	3	3	this study
	forest (TVF) (camping site)	3000	1990	4	4	Gryta et al. 1997
			1993	2	2	Gryta et al. 1997
			1994	2	2	this study
			1995	2	0	this study
			1997	3	3	this study
			1998	5	2	this study
			1999	11	0	this study
Le Grand Crohot (44°48′N, 1°14′W)	dune (GCD)	1000	1993–1994–1995–1997–1998–1999	2	2	Gryta et al. 2000 and this study
			1997–1998–1999	1	1	this study
			1997–1998	1	1	this study
			1998–1999	2	2	this study
			1998	2	2	this study
	forest (GCF) (recreational area)	2000	1998	4	4	this study
Le Porge (44°53′N, 1°13′W)	dune (LPD)	1000	1998–1999	2	2	this study
			1998	4	4	this study
	forest (LPF) (car park border)	500	1990	2	2	Gryta et al. 1997
			1993	4	4	Gryta et al. 1997
			1994	5	5	this study
			1997	1	1	this study
			1998	1	1	this study
			1999	5	3	this study

Surface area values are approximate. Not all patches of fruit bodies present in these areas were mapped except for a pre-defined 500 m^2 area within the TVF area.
Sampling of the same patches over several consecutive years (years written on the same lane) was possible only in the dune habitat. In the forest habitat the patches were not observed at the same position from one year to the next.
[1] Fruit body genotypes were identified either by analysis of polymorphism within the nuclear rDNA IGS sequence (in this study) or by combining four different molecular methods including IGS polymorphism (Gryta et al. 1997, 2000).

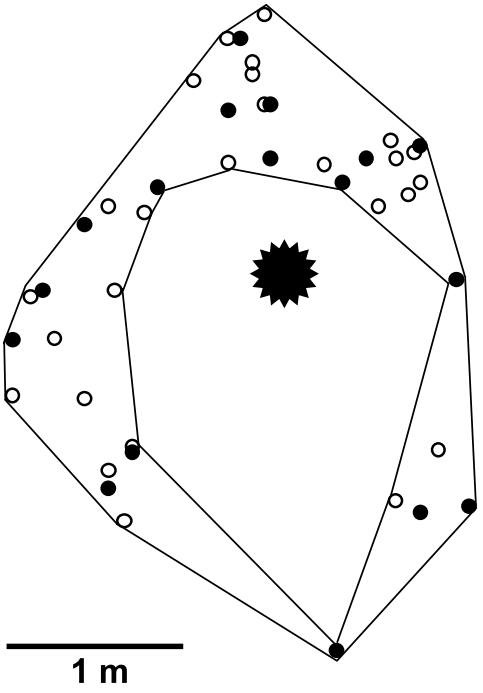


Fig. 1. An illustration of a patch of fruit bodies of *Hebeloma cylindrosporum* observed in the dune habitat and of the inner and outer convex polygons used to calculate patch area. Fruit bodies are represented by circles, filled circles represent fruit bodies whose genotypes were determined. The large star marks the position of a *Pinus pinaster* tree.

the number of fruit bodies within a patch, between 10 and 100% of the fruit bodies were sampled per patch. In total we analysed 56 patches in addition to the 14 studied in Gryta et al. (1997, 2000) (Table 1). Sampled fruit bodies were frozen and freeze-dried until further analyses.

Genet identification

The mycelium of a genet grows in the soil and can form fruit bodies several cm or metres apart from each other. We used a molecular method to identify fruit bodies formed by different genets within a patch of fruit bodies. The method used is a diagnostic method for genets and cannot be used to infer the genetic structure of the populations present in the different study sites. The method is based on the analysis of polymorphism within the hyperpolymorphic nuclear ribosomal DNA intergenic spacer (IGS) sequence as described in Guidot et al. (1999, 2001).

DNA was extracted from freeze-dried fruit bodies according to the method of Van Kan et al. (1991) and resuspended in water. PCR amplification of the entire ca 3.4 kb-IGS sequence and of the ca 1.5 kb-IGS2.1 sequence was performed as described in respectively Gryta et al. (1997) and Guidot et al. (1999). Appropriate amounts of the amplified sequences were digested with *Hae*III and the restriction fragments separated by agarose gel electrophoresis. Ethidium bromide stained gels were photographed. Differences in restriction profiles were interpreted as resulting from differences in DNA extracted from the different individuals.

Measurements and data analysis

Fruit bodies were grouped within discrete patches. The patch surface was defined as the surface within a convex hull delimited by the outermost fruit bodies. In cases where fruit bodies were not evenly distributed within the patch but formed a 'fairy ring' with no fruit bodies in the centre of the patch, the surface of the convex polygon delimited by the innermost fruit bodies was subtracted (Mohr 1947) (illustrated in Fig. 1).

Measurements of cap diameter and of fruit body dry weight were only performed on mature fruit bodies characterised by a fully expanded cap and dark brown sporulating gills. Measurement of cap diameter was performed on a random sample of 352 fruit bodies collected in the dune habitat and 143 ones collected in the forest one. These fruit bodies were collected in the six different sampled areas. Fruit body dry weight was measured for 88 fruit bodies collected in the two habitats. The allometric relation between cap diameter (D) and fruit body dry weight (W) was obtained from the linear regression between $\log_e$-transformed data: $\log_e(W) = a \log_e(D) + b$, i.e. $W = e^b D^a$.

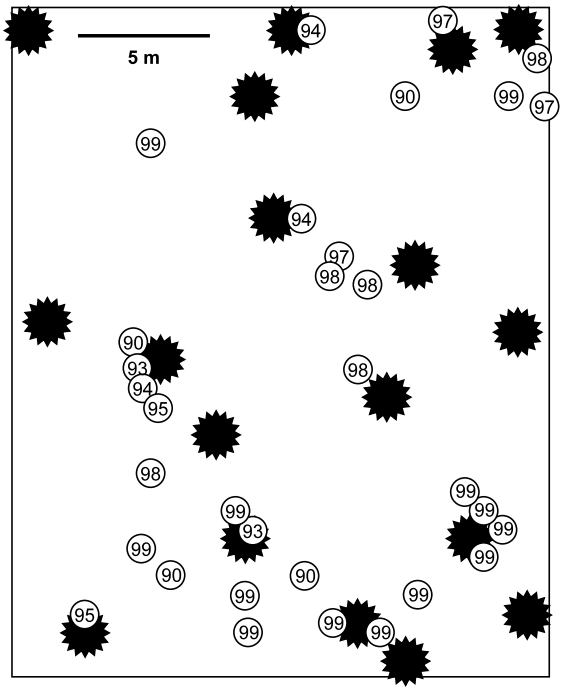
Within each site seven different parameters were measured (Table 2) and their values compared between the three geographic sites within a habitat (site effect) and between the two habitats (habitat effect). Site effect was analysed separately in the dune and in the forest habitat. Only the data for cap diameter followed a normal distribution and were subjected to analysis of variance (ANOVA). One-way ANOVAs were used to compare the three sites within a habitat. As some heterogeneity occurred between the three forest sites, a two-way non-repeated ANOVA (habitat, site) was conducted to evaluate the habitat effect; a weak habitat × site interaction was detected in this analysis ($P = 0.041$). For the other six parameters (Table 2) the Kruskal-Wallis and Mann-Whitney U non-parametric tests were implemented to evaluate the site and habitat effect respectively. In the case where a significant site effect existed ($P < 0.05$), differences between sites were evaluated using the Mann-Whitney U test. Data were analysed using the StatViewII (Abacus concepts, Inc.) and the R (version 1.3.1) softwares.

Table 2. Comparison of population spatial structures and of fruit body sizes of *Hebeloma cylindrosporum*: site and habitat effects.

Variable	Dune sites mean ± SE			Dune site effect	Forest sites mean ± SE			Forest site effect	Habitats mean ± SE		Habitat effect
	GCD	LPD	TVD		GCF	LPF	TVF		Dune	Forest	
Patch size (m^2)	$3.38^{a,b} \pm 1.59$ ($n = 7$)	$0.19^{a} \pm 0.07$ ($n = 5$)	$2.04^{b} \pm 0.52$ ($n = 5$)	n.s.	0.02 ± 0.01 ($n = 3$)	0.49 ± 0.22 ($n = 11$)	0.13 ± 0.04 ($n = 22$)	n.s.	2.05 ± 0.72 ($n = 17$)	0.23 ± 0.08 ($n = 36$)	$P = 0.0001$
Within patch fruit body density (fruit bodies/m^2)	$108^{a,b} \pm 75$ ($n = 7$)	$99^{a} \pm 18$ ($n = 5$)	$21^{b} \pm 3$ ($n = 5$)	n.s.	672 ± 55 ($n = 3$)	400 ± 127 ($n = 11$)	468 ± 78 ($n = 22$)	n.s.	80 ± 31 ($n = 17$)	464 ± 62 ($n = 36$)	$P = 0.0001$
Distance separating a fruit body from its nearest neighbour (cm)	$20.5^{a} \pm 1.8$ ($n = 153$)	$7.8^{b} \pm 0.8$ ($n = 32$)	$17.3^{a} \pm 1.4$ ($n = 51$)	$P = 0.0001$	5.4 ± 0.8 ($n = 22$)	4.0 ± 0.4 ($n = 22$)	4.9 ± 0.8 ($n = 52$)	n.s.	18.1 ± 1.2 ($n = 236$)	4.8 ± 0.5 ($n = 96$)	$P = 0.0001$
Fruit body cap diameter (mm)	40.75 ± 1.06 ($n = 216$)	38.52 ± 1.46 ($n = 54$)	40.57 ± 1.73 ($n = 82$)	n.s.	$33.13^{a} \pm 3.20$ ($n = 22$)	$20.31^{b} \pm 1.31$ ($n = 29$)	$25.47^{c} \pm 0.89$ ($n = 92$)	$p = 0.0001$	40.36 ± 0.80 ($n = 352$)	25.6 ± 0.85 ($n = 143$)	$P < 0.0001$
Average no of fruit bodies/genotype within a patch	$17.83^{a} \pm 5.10$ ($n = 8$)	$2.39^{b} \pm 0.74$ ($n = 6$)	$11.13^{a} \pm 2.64$ ($n = 5$)	$P = 0.0125$	2.04 ± 0.56 ($n = 4$)	1.46 ± 0.19 ($n = 16$)	1.56 ± 0.18 ($n = 13$)	n.s.	11.19 ± 2.67 ($n = 19$)	1.57 ± 0.13 ($n = 33$)	$P = 0.0001$
Within patch genet density (genets per m^2)	$24^{a,b} \pm 21$ ($n = 7$)	$24^{a} \pm 6$ ($n = 5$)	$1^{b} \pm 0.5$ ($n = 5$)	$P = 0.0384$	200 ± 58 ($n = 3$)	218 ± 74 ($n = 11$)	262 ± 80 ($n = 10$)	n.s.	17 ± 9 ($n = 17$)	234 ± 47 ($n = 24$)	$P = 0.0001$
Maximal distance between fruit bodies of the same genotype (cm)	$490^{a} \pm 54$ ($n = 20$)	$33^{b} \pm 7$ ($n = 13$)	$223^{c} \pm 42$ ($n = 8$)	$P = 0.0001$	16 ± 3 ($n = 4$)	17 ± 5 ($n = 19$)	13 ± 3 ($n = 24$)	n.s.	293 ± 42 ($n = 41$)	15 ± 3 ($n = 47$)	$P = 0.0001$

Only patches containing at least six fruit bodies were considered for comparisons of patch sizes and fruit body densities within patches. For the dune habitat, to avoid the repeated analysis of the same patches, only the 1998 data were used for the comparisons. In 1998, all the different patches in the dune habitat were analysed (Table 1). For one variable, mean values with the same superscript letters are not significantly different ($P > 0.05$).

All comparisons were performed using non-parametric tests (site effect was analysed using the Kruskal-Wallis test; habitat effect using the Mann-Whitney test) except for cap diameters. For this variable, one-way ANOVA was used for comparing the three sites of each habitat. As some heterogeneity occurred between the three forest sites, two-way non-repeated ANOVA was used to evaluate the habitat effect. n = sample size.



Year	1990	1993	1994	1995	1997	1998	1999
No of patches	4	2	3	2	4	6	13
Total no of fruit bodies	44	37	10	21	47	147	291
Sum of patch size (m^2)	0.27	0.08	0.03	0.02	0.07	0.68	2.07
(% of the sampled area)	(0.054)	(0.016)	(0.006)	(0.004)	(0.014)	(0.136)	(0.414)

Fig. 2. Positions of the patches of fruit bodies of *Hebeloma cylindrosporum* in the TVF forest area from 1990 to 1999. *Pinus pinaster* trees are represented by stars and each patch of fruit bodies by a circle with its corresponding year of observation. For each year, the number of patches and of fruit bodies as well as the total surface covered by these patches on the TVF area are given.

Results

Fruit body distribution

In all six sampled areas, whatever the study year, fruit bodies of *H. cylindrosporum* were not evenly distributed but showed a clumped distribution within discrete patches. In the 500 m^2 TVF sampled area which was the only area precisely mapped between 1990 and 1999, patches were on average 3.4 m away from each others and covered on average 0.1% of the area with remarkable between-year variations (Fig. 2).

If, for measurement accuracy, we only consider patches with more than five fruit bodies and only the 1998 ones for the dune habitat (see below), patches in the dune habitat were significantly larger and almost five time less crowded compared to those sampled in the forest habitat. Another figure which reflects the within patch fruit body density is the distance separating a fruit body from its nearest 'neighbour'. The mean value was 4.8 cm (SE = 0.5; estimated on a random sample of 96 pairs) in the forest habitat and 18.1 cm (SE = 1.2, $n = 236$) in the dune one. This habitat effect on patch size and internal density was supported by an absence of significant differences between the sampled areas located in the forest habitat (Table 2). This was less obvious for the dune habitat, specifically the LPD area had for several of the studied parameters mean values intermediate between those measured in the two other dune areas and those measured in the forest ones (Table 2). Other discrepancies could be explained by the specific contribution of one or a few patches to the mean value of the corresponding site. This was the case for the GCD area where one patch out of the 7 analysed had characteristics similar to patches of the forest habitat (0.02 m^2 and 550 fruit bodies per m^2). When it was omitted from the calculations, the mean patch size in the GCD area was 3.94 ± 1.76 m^2 with an internal density of 35 ± 17 fruit bodies per m^2; a value significantly different of the density estimated for the LPD area. Similarly, the high patch size of the forest LPF area resulted from 4 patches out of the 11 analysed; when their values were omitted the mean patch size for this area was 0.05 ± 0.03 m^2 with an internal density of 618 ± 145 fruit bodies per m^2.

Patches in the dune and forest habitats also differed with respect to their durability. In the dune habitat, 10 of the 19 patches analysed in the three sites were found at the same position the year after they were first identified (in most cases 1999 vs 1998 or 1998 vs 1997, Table 1). This was also the case in the two patches of the GCD area studied from 1993 till 1997 in Gryta et al. (2000) and which were re-sampled for the present study in 1998 and 1999. As patches in this habitat were perennial and, for most of them, were occupied by a single genet (see below genet distribution), we only used the 1998 data for comparison of patch size and density to avoid the repeated analysis of the same patches in the dune habitat (Table 2). On the contrary, for the LPF and TVF forest areas which were precisely mapped on seven occasions between 1990 and 1999 the positions of the patches seldom overlapped from one year to the next ones (illustrated for TVF in Fig. 2). For these two last areas, between year comparisons did not reveal significant differences in patch size and internal density and consequently all of them were included in the previous analysis.

Biometric analysis of fruit body size

Fruit body size reflects both the amount of biomass allocated to sexual reproduction and reproductive potential (number of meiospores produced). In the case of soil-borne fungal species, reproductive effort (proportion of biomass allocated to reproduction) cannot be inferred as biomass of below ground vegetative structures (mycelium and mycorrhizas) cannot be measured.

Analysis of 88 mature fruit bodies sampled in both habitats showed considerable variation in both cap diameter (D in mm, from 10 to 100 mm) and dry weights (W in mg, from 10 to 2400 mg) (Fig. 3). An allometric relationship was discovered to exist between these two parameters: $W = 0.058D^{2.31}$ (i.e. $\log_e(W) = 2.31 \times \log_e(D) - 2.84$ with $r = 0.96$, ($P = 0.0001$)). This relationship was supported by a high correlation coefficient between fitted (computed using the above formula) and observed values for the fruit body dry weight ($r = 0.92$, $P = 0.0001$). Therefore it can be considered that cap diameter alone reflects both the biomass allocated to reproduction and the reproductive potential of this species.

We observed a significant difference in cap diameter between fruit bodies sampled in the dune (mean = 40.4 mm, mini. = 15 mm, maxi. = 89 mm, SE = 0.8, $n = 352$) and those sampled in the forest (mean = 25.6 mm, mini. = 8 mm, maxi. = 60 mm, SE = 0.85, $n = 143$) habitat (Table 2). This difference between habitats (habitat effect in the two-way ANOVA: $P < 0.0001$) (Table 2) was not affected when the three sampling years or the three geographic sites were analysed separately. A significant difference was, however, observed between the three forest sites (one-way ANOVA, $P = 0.0001$) but not between the dune sites (Table 2). The coefficients of variation for the fruit body cap diameter values were similar for the two habitats ($CV_{dune} = 37.1$, $CV_{forest} = 39.9$). Although between patches comparisons were not performed due to the limited and variable numbers of fruit bodies per patch, it was found that within a single patch the same range of variation in fruit body size as the one recorded for the corresponding habitat could be observed. For example, within a patch in the GCD area fruit body cap diameters varied between 21 and 80 mm (range of variation in the GCD area: between 15 and 98 mm).

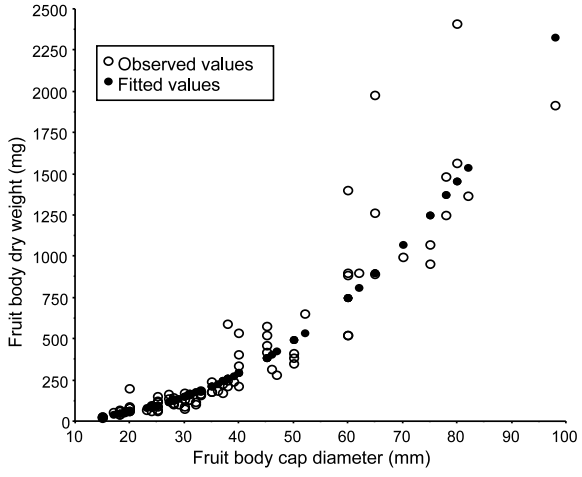


Fig. 3. The relationship between cap diameter and dry weight of the fruit bodies of *Hebeloma cylindrosporum*. Fitted values between fruit body dry weight (W) and cap diameter (D) were calculated using the allometric relationship $W = 0.058D^{2.31}$.

Genet distribution

The microscopic below-ground mycelium of an individual fungal genotype (genet) has the potential to cover surfaces ranging from a few cm^2 to several m^2 and consequently to form fruit bodies far apart from each other. We diagnosed fruit bodies formed by different genets by looking at polymorphism within a hypervariable segment of the rDNA intergenic DNA spacer sequence. This analysis allowed us to estimate the number of genets within patches but cannot be used to infer the genetic structures of the studied populations. It revealed that significant differences in within patch genet density and genet life-span existed between habitats.

The forest habitat

Different genotypes were identified within 32 of the 33 different patches analysed (276 fruit bodies analysed). The only exception was a patch in the LPF area in which the five fruit bodies were of the same genotype. Within a patch, the average number of fruit bodies per genotype was 1.6. This value was 1.8 if we only consider the 14 patches in which all fruit bodies were analysed (with a maximum of six fruit bodies per genotype) thus indicating that the within patch sampling strategy did not markedly underestimate genet density. On average, there were above 200 genets per m^2 in a patch and no significant difference was detected between the three studied sites (Table 2). For genets producing more than one fruit body, the mean distance between the two most distant ones was below 18 cm (Table 2), a value lower to the estimated 45–60 cm per year annual growth rate of a mycelium of *H. cylindrosporum* under natural conditions (Gryta et al. 2000).

The dune habitat

A completely different picture emerged of the analysis of the patches in the GCD and TVD dune areas. As opposed to the diversity of genets recorded within the patches in the forest areas, 10 of the 13 patches sampled in these two dune areas in 1997, 1998 or 1999 contained only one fruiting genotype. For five of these patches, all fruit bodies were analysed. Within a sampled area, none of the genets was found in two different patches, a situation expected for a fungal species which does not produce asexual propagules. For the five mono-genotype patches which were sampled twice at a one-year interval, only the genotype identified the first year was found the following year. The distance separating the two most distant fruit bodies of the same genotype on average exceeded 2 m (Table 2) and increased on average by 100 cm per year. This figure is consistent

with a previous estimate of the vegetative growth rate of *H. cylindrosporum* in the GCD site (45–60 cm per year, Gryta et al. (2000)). As shown in Gryta et al. (2000) and Guidot et al. (2001) the spatial distribution of the fruit bodies of *H. cylindrosporum* in the dune habitat reflects, to some extent, the spatial distribution of the corresponding below-ground mycelia. The remaining three patches in the GCD and TVD areas contained several genotypes. One was dominated by one genotype (represented by 26 of the 28 fruit bodies analysed) which was the only one persisting a year later. The two other patches were similar to patches described in the forest area (1.7 and 3.7 fruit bodies per genet). When these latter patches were omitted from the calculations, the mean within patch genet density at GCD was 3.4 genets per m^2 instead of 24.

In the LPD dune area, the situation appeared intermediate between the observations made in the forest sites and those reported above for the GCD and TVD dune areas. In only one of the six patches analysed in LPD was one genotype detected (six of nine fruit bodies analysed). The five others had multiple genets. The average number of fruit bodies sharing the same genotype within the LPD patches was 2.4 (Table 2). This value is significantly smaller than those observed in the GCD and TVD areas (Table 2) but not significantly different from those recorded in the GCF and TVF forest areas. As opposed to the forest areas, four of these five patches were observed in the same place in 1998 and 1999. Analysis of two of them during these two years showed that four of the seven genotypes identified in 1998 were present in 1999 and four new genotypes appeared the following year.

In conclusion, the populations of *H. cylindrosporum* present in dune habitat appeared dominated by perennial genets and in most cases one genet dominates within a patch.

Discussion

The results presented in this study show that habitat characteristics have a strong impact on the structure and dynamics of local populations of *H. cylindrosporum*. The comparative analysis of populations present in monospecific *P. pinaster* stands sampled in different sites allows us to conclude that the observed differences do not result from host plant or geographic site effects. Moreover, the recurrent observation of identical structures in a sampled area over several years (up to ten) indicates that these structures do not represent distinct stages in the colonisation of a forest site. Local populations present in the two habitats differ mainly with respect to genet life-span (as reflected by genet size), genet turn-over and recruitment intensity and finally with respect to the reproductive investment of individual genets (as reflected by the number and size of fruit bodies produced by the different individuals). Populations present in three areas of the dune habitat are characterised by long-lived genets which produce numerous large fruit bodies. In this habitat, sexual recruitment seems to be a rare event as suggested by the near absence of small size genets. Populations present in the forest habitat are characterised by short-lived (annual) genets each producing few, on average less than two, small fruit bodies. In this latter habitat, colonisation by new genets is frequent and occurs each year within discrete patches of ground of variable but small size.

One of the most likely explanations for such contrasted population structures lies in the characteristics and level of disturbance in the studied habitats. The dune habitat characterised by an extremely nutrient-poor sandy substratum, deeply buried roots and a species-poor ECM community appears favourable to the vegetative survival and growth of *H. cylindrosporum* genets. The dune habitat could represent the optimal habitat of this species. On contrast, in the forest habitat, human disturbance which prevents litter accumulation, could lead to random, localised elimination of resident symbionts on the root systems. These 'empty patches' could be favourable to the temporary colonisation by *H. cylindrosporum* whose mycelia are eliminated within a year (Guidot et al. 2001) as the disturbance, which led to the elimination of other competing ECM species, relaxes. Rapid changes in the ECM community following artificial removal of the humus layer in a forest stand had been reported by Baar and Kuyper (1998) thus suggesting that this type of disturbance may have general effects on ECM fungi. The forest habitat could therefore represent a marginal habitat for *H. cylindrosporum* in which its populations will exist as long as human disturbance persists. This interpretation implies that some symbiotic fungal species could be considered as fugitive species (Hutchinson 1951) defined as good colonisers but poor competitors (genets of *H. cylindrosporum* only survive for several years in the dune habitat characterised by an ECM species poor community), producing large amounts of sexual spores allowing them to transiently colonise empty niches. This interpretation suggests that local/regional ECM fungal species diversity could allow host plants growing in a disturbed environment to maintain their entire root systems associated with symbiotic ECM species. This is one of the many hypotheses put forward to interpret the diversity, lack of specificity and horizontal transmission of ectomycorrhizal symbionts; these characteristics would allow a host plant growing in a fluctuating environment 'to choose' locally for the best adapted symbionts (Genkai-Kato and Yamamura 1999) while 'single-partner' symbioses are restricted to environments favourable to both host and symbiont. This should, however, not be regarded as an exclusive interpretation as populations of other ECM species charac-

terised by short-lived genets have been reported in apparently undisturbed forest habitats (Gherbi et al. 1999, Redecker et al. 2001).

In each of the three studied geographic sites the dune and forest habitats are close to each other. It would be of interest to evaluate if a source/sink relationship exists between populations present in these two habitats. In other words, it can be asked if the recurrent colonisation of the forest habitat depends upon a continuous inflow of spores from populations present in the dune habitat. Dispersal and genetic differentiation of populations of ECM species at a local/regional scale has not been investigated so far. Our data cannot be used to infer the genetic structures of the studied *H. cylindrosporum* populations. This will necessitate the analysis of polymorphism at several loci which could be performed on fruiting genets but also on gametes (meiospores) trapped from the air as recently described for the wood rot fungus *Schizophyllum commune* (James and Vilgalys 2001). These aspects are particularly relevant in the case of *H. cylindrosporum* as we showed that habitat has a strong effect on sexual reproduction (as reflected by the size of the fruit bodies) and potentially on the relative contribution of individual genets to gamete (meiospore) production (as reflected by both the size of the fruit bodies and the number of fruit bodies produced per genet). We do not favour the hypothesis that fruit body size would be a genetically selected character in the two habitats. Although the genetic control of fruit body size has been demonstrated for basidiomycete species (Simchen 1966, Marmeisse 1989), it is also well established that for a single genotype fruit body size varies considerably depending upon environmental conditions (Takacs 1974, Horna and Royse 1983) or interspecific competition (Schmit 1999). While this has not been investigated for *H. cylindrosporum*, we observed that a single genet in the dune habitat can simultaneously form fruit bodies differing in size by a factor of 5 and we routinely obtained in microcosms in the laboratory mature miniature fruit bodies of a size far smaller than those observed in the field (unpublished data). As in the two habitats different parameters such as genet size and age, within patch genet density (suggesting potential intraspecific competition for resources), and soil composition are to some extent linked or correlated, it appears difficult to establish causal links between fruit body size and any of these parameters. Reciprocal transplantation in the dune and forest habitat of *Pinus* plants inoculated with the mycelium of genets isolated from the dune and forest habitats could be carried out to solve this problem.

Another feature of *H. cylindrosporum* populations, which deserves further studies, concerns genet establishment in the dune habitat. In this habitat we observed the predominance of large mono-genotype patches and apparently low level of recruitment both outside and within existing patches. Such a situation which indicates that the environmental conditions which favour colonisation by spores are different from those which favour the vegetative survival of the below-ground mycelia is a common feature in populations of several ECM (Dahlberg and Stenlid 1990, Dahlberg 1997, Bonello et al. 1998, Sawyer et al. 1999, Selosse et al. 1998, 1999, Gryta et al. 2000) as well as saprophytic or pathogenic (Smith et al. 1992, Legrand et al. 1996) soil-borne basidiomycete species despite the large number of fruit bodies (and consequently of spores) that the different genets form. Furthermore in the dune habitat, the sizes of the mono-genotype patches, often more than one metre across, indicate that the genets are more than one year old. In this habitat *H. cylindrosporum* could be compared to what was called 'initial seedling recruitment plant species' (Eriksson 1993) for which recruitment within a given area occurs during a limited favourable period of time which for *H. cylindrosporum* could correspond to the first years of forest establishment in the studied sites. Alternatively it could be hypothesised that in this habitat, as opposed to what is apparently observed in the forest one, genets display a reproductive waiting strategy, i.e. start forming fruit bodies after several years of vegetative growth thus masking the existence of young, newly established genets. The intermediate characteristics of the population present in the LPD area which differs to the ones sampled in GCD and TVD with respect to within patch diversity, patch size and minimal distance between two fruit bodies (Table 2) but not patch persistence, could be a clue to interpret population dynamics in the dune habitat. The initial stage of colonisation in the dune habitat could be in the form of scattered multi-genotype patches similar to the one observed in LPD (an old-grown forest whose floor had been covered up by sand during the last decade) and in the forest habitat. But ,in contrast to this latter habitat, the genotypes in the dune habitat are progressively eliminated, possibly through intraspecific competition, resulting in only one individual persisting as often observed in clonal plant populations. This possibility merits to be studied further as if this scenario could be confirmed it would offer an opportunity to evaluate if 'the fungal symbiotic effectiveness', and host plant responses to it affects competition between co-occurring symbionts. If it does, we would expect that samples of genets collected (i) within mono-genotype (after selection) and (ii) within multi-genotype patches (before selection) would differ with respect to symbiosis related traits thus suggesting that a host plant counter selects or sanctions ineffective ECM symbionts as suggested in the case of the *Rhizobium*-legume symbiosis (Denison 2000).

In conclusion our results suggest that disturbance in a forest ecosystem renders the local ECM fungal community prone to invasion by additional ECM fungal species naturally adapted to different forest habitats. The results obtained could be used to initiate new

studies in two poorly investigated fields. The first one concerns the genetic structures of ECM fungal populations and the spatial patterns of spore dispersal in heterothallic basidiomycetes. The second one concerns symbiotic effectiveness as a potentially selected character in ECM fungal populations.

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