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Seasonal Patterns of Spore Deposition of *Heterobasidion* Species in Four Forests of the Western Alps

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ABSTRACT

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Patterns of spore deposition by *Heterobasidion* species were studied between the spring of 1998 and December 2000 in four forests in the western Alps using woody traps. The maximum spore deposition rate (DR) ranged from 169 to 1,550 spores m^{-2} h^{-1}. Although spores were captured from February to October at most sites, inoculum concentration consistently peaked in the late summer or early fall. In one of the four study sites, similar patterns of DR were recorded in 2 years of sampling. A significant correlation ($r = 0.654$, $P = 0.001$) was found between DR and the average minimum air temperature in the 4 weeks before sam-

pling. Approximately 1,200 spores were isolated and identified at the species level by polymerase chain reaction-based methods. Single-spore isolates were consistently clampless, indicating the sampled airspora was almost exclusively composed of haploid basidiospores. No significant variations of basidiospore frequencies were detected for either *H. abietinum* or *H. annosum* among sampling periods. However, the frequency of *H. parviporum* spores was always significantly higher in the summer. These findings suggest different patterns of sporulation among *Heterobasidion* species.

Additional keywords: aerobiology, *Spiniger meineckellus*, spore dissemination.

Heterobasidion annosum (Fr.) Bref. *sensu lato* is a root and butt rot agent that severely affects coniferous forests worldwide. This pathogen is regarded as a species complex, comprising three intersterility groups in Europe (3,17), recently named *H. parviporum* Niemelä & Korhonen, *H. abietinum* Niemelä & Korhonen, and *H. annosum sensu stricto* (26). These species, although defined on the basis of partial reproductive isolation and morphology (4,5,17,21), are also characterized by differences in pathogenicity on a range of hosts (3,18,27,42). In Europe, *H. parviporum* primarily causes butt rots in Norway spruce (*Picea abies* (L.) Karst.), but it has also been reported to kill Scots pine (*Pinus sylvestris* L.) saplings. *H. abietinum* is commonly associated with root or butt rots in trees of the genus *Abies* (e.g., silver fir–*Abies alba* Miller), while *H. annosum sensu stricto* is typically associated with mortality of trees in the genus *Pinus*, but it can also attack *Picea*, *Juniperus*, and certain hardwood trees (18).

Heterobasidion sp. primarily infects its hosts through fresh-cut stumps or wounds by means of airborne meiospores and is capable of secondarily spreading from tree to tree through root grafts and contacts (30,41). Airborne infection through thinning stumps results in rapid and heavy infection of healthy stands in areas where *Heterobasidion* sp. is common (28,43), and aids its spread into new areas (1). The availability of inoculum is a necessary prerequisite for primary infection since inoculum potential is directly correlated with infestation levels (20). Thus, understanding the seasonal patterns of sporulation in *Heterobasidion* populations is pivotal when formulating control strategies for the disease this pathogen causes.

Although infection levels vary depending on site across the Alpine range, the pathogen is a major problem in the western Alps, especially where Norway spruce and silver fir grow together or independently. Up to 90% disease incidence was reported in a study of a subalpine forest in the western Alps (10), and incidence levels in the four study sites ranged from 30 to 50% as previously reported (11).

Aerobiology and temporal dynamics of spore deposition of *Heterobasidion* spp. have been studied in North America and northern and central Europe since the early 1950s (2,8,14,16,32,35,44). Seasonal trends in spore deposition have been found to vary greatly among different collecting periods within stands and among forests in different ecotypes. In Britain, for instance, infection occurs during most of the year (20,33), while in central and northern Europe, the main infection periods are autumn and spring (37,44) and summer (2,16), respectively. Regional differences in seasonal patterns of spore deposition have been reported in North America (6,8,22,35). However, no information on temporal dynamics of spore deposition is available for the Alps and southern Europe. The area studied has the characteristic of being affected by all three European species of *Heterobasidion*, often in the same forest stand, allowing for an unprecedented comparison in sporulation patterns among the three. Because each of the three species is ecologically quite unique, with distinct host and geographic ranges (18), we designed this study not only to describe the overall temporal variation in spore production of the entire complex, but also to highlight potential differences among the three species.

Environmental factors, and in particular the weather, are known to affect the amount and timing of both spore production and deposition for many plant-pathogenic fungi (13). Air temperature, air humidity, and rainfall (RF) have been reported to influence spore production and deposition for *Heterobasidion* spp. (30). A positive correlation between temperature and spore deposition has

been found in the northeastern United States and northern Europe (16,38). High summer temperatures have been reported to reduce sporulation in the southern United States (6,35). Hot dry weather in summer, combined with snow and low temperatures in winter, may locally restrict spore production to autumn (29,36). Weather factors affect sporulation in different ways in each respective region (30,31,44), complicating the task of developing local sporulation and spore dissemination models.

The goals of this study were (i) to determine temporal patterns of deposition of viable spores of *Heterobasidion* spp. in four forests of the western Alps; (ii) to compare temporal patterns of spore deposition among forests; (iii) to investigate the relationships between weather factors and the temporal patterns of inoculum availability; and (iv) to compare the patterns of deposition among the three European *Heterobasidion* species.

MATERIALS AND METHODS

Description of study sites. Spores deposition of *Heterobasidion* spp. was investigated in four naturally regenerated forests of the western Italian Alps: Chiusa Pesio, Jovençan, Charvensod, and Aymavilles (Table 1; Fig. 1). The stand at Chiusa Pesio (Piedmont) is characterized by a dominance of *Abies alba* including a significant component of various broad leaves such as *Corylus avellana* L., *Laburnum alpinum* (Miller) Berchtold et Presl, and *Sorbus aucuparia* L. The other three forests are located on the same mountain slope in the Aosta Valley. Jovençan is a forest dominated by *A. alba*, Charvensod is a pure *Picea abies* stand, and Aymavilles is a mixed *A. alba–P. abies* forest. The presence of other tree species is sporadic in these forests. Species list includes *Larix decidua* Miller and *Pinus cembra* L. at Charvensod, and *Betula pendula* Roth and *Populus tremula* L. in the remaining two forests, which are located at lower elevation. Soils are classified as Typic haplumbrept in Chiusa Pesio and Ochrepts/Umbrepts in the other three sites (45). All forests are characterized by several large, even-aged groups of trees, resulting in stands that, as a whole, can be considered uneven-aged. Silvicultural treatments are similar at all sites with precommercial thinnings occurring every 15 years in Chiusa Pesio and every 20 years in the other sites. On average, thinning is light at all sites and results in the cutting of 10% or less of the entire timber volume.

Chiusa Pesio is in close proximity to the Ligurian sea and the climate is characterized by mild temperatures and abundant RF that peaks in May and November. The other three sites are characterized by a colder, subcontinental climate with low average annual RF, with the highest precipitation occurring in October and November. Information about the airborne inoculum composition of *Heterobasidion* species was previously documented for the study sites (11).

Spore sampling. In each forest, two permanent plots were established at a distance of 100 m from one another as described previously (11). A plot comprised three transects, each including three sampling points placed along the transects at 7-m intervals, resulting in a plot size of approximately 0.1 ha. Spores of *Heterobasidion* spp. were trapped using a modified version of the wood-disk exposure method (14,34). Freshly cut wood disks of about 11 to 13 cm diameter, obtained from healthy spruce (Charvensod and Aymavilles) and fir (Chiusa Pesio and Jovençan), were sprayed, after bark removal, with 65% ethanol and placed onto a wet filter paper disk in a petri dish (11). One open petri dish per sampling point was placed on the ground or on snow from March 1998 to December 2000. Disks were exposed for approximately 24 h starting at about 8:00 a.m. Three closed petri dishes were included as controls at each collection time to check for possible contamination caused by either *Heterobasidion* isolates already present in the wood disks or by airborne spores that may have landed on the wood during trap preparation. In the first year, seasonal samples (one per season per site) were taken earlier in lower elevation sites. The first sampling (spring) was performed in March 1998 at Chiusa Pesio, in May at Jovençan and Aymavilles, and in June at Charvensod. A summer sampling was made in July at Chiusa Pesio, in August at Jovençan and Aymavilles, and in September at Charvensod. Fall samples were taken in October at Chiusa Pesio and Aymavilles, and in November at the other two sites. A winter sampling was conducted in all the forests in February 1999. Starting in March 1999, woody spore samplers were exposed at all sites at the beginning of each month. Spore sampling ended in August 2000, except for Charvensod and Aymavilles, where sampling was concluded in March and December 2000, respectively. Spore trappings were performed on calm days and were delayed when wind speed, measured at the beginning of each collection time by a portable anemometer (DO 9847K, Delta OHM s.r.l., Caselle di Selvazzano, Italy), exceeded 3.0 m s^{-1}.

After exposure, filter papers contained in the petri dishes were replaced and dampened with 3 ml of sterile water. Wood disks were sprayed with a benomyl solution (0.010 g of benomyl,
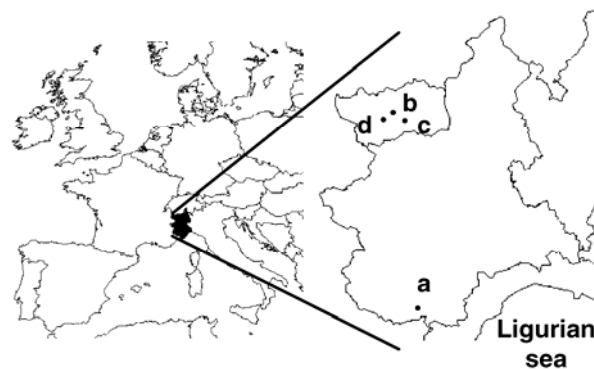
Fig. 1. Geographical location of the four study sites: Chiusa Pesio (a), Jovençan (b), Charvensod (c), and Aymavilles (d).

TABLE 1. Forest site locations and main stand characteristics

Location	Lat-long coordinates[a]	Aspect	Elevation (meters above sea level)	Mean annual rainfall (mm)	Tree host species and composition (%)	Approximate incidence of the disease[b]
Chiusa Pesio	44°12′48″ φ 7°39′34″ λ	N-W	1,042	1,445	*Abies alba* (100%)	50%
Jovençan	45°42′12″ φ 7°16′46″ λ	N	1,090	700	*A. alba* (95%) *Picea abies* (5%)	50%
Charvensod	45°41′36″ φ 7°19′43″ λ	N-N-W	1,780	700	*P. abies* (100%)	30%
Aymavilles	45°41′6″ φ 7°14′57″ λ	N-N-W	1,475	700	*A. alba* (50%) *P. abies* (40%) *Pinus sylvestris* (10%)	50%

[a] World Geodetic System 1984 (WGS84) datum.

[b] This estimate was based on the frequency of stumps displaying incipient and advanced decay on the stump top, as assessed by examining the stump surface immediately after last thinning.

500 μl of methanol, and 1 liter of sterile water) and incubated at 24°C for 5 to 7 days.

Disks were examined under a dissection microscope (×20) for colonies of *Spiniger meineckellus* (A.J. Olson) Stalpers, the co-nidial stage of the fungus. Colonies were counted and the number of spores was determined under the assumption that each colony resulted from deposition of one viable spore (14,34).

Weather monitoring. Weather measurements were taken in the forest of Aymavilles from March 1999 to the conclusion of the study. Air temperature and relative air humidity (RH) were re-corded continuously at the center of each plot using a hygrothermo-graph (MT 1500, SIAP, Bologna, Italy) installed in a weather shelter at 1.5 m above ground. RF was measured at the same loca-tion with a 7-day rain gauge (UM 8100, SIAP). In snowy winter conditions, snow height was used as a proxy for RF. Average values of the weather data were calculated at 1-h intervals.

Isolation and typing of spores of *Heterobasidion* spp. Iso-lations of putative single-spore colonies of *Heterobasidion* spp. were performed from disks exposed during the first year at all sites, and from trappings performed until July 2000 at Aymavilles. This site was selected for closer investigation because of the previously documented presence of all three European species of *Heterobasidion* (11).

Fungal colonies were isolated under a dissecting microscope (×20) by transferring infected wood pieces onto 5-cm petri dishes containing a selective medium for *Heterobasidion* (19). When three or more colonies per disk were visible, three colonies were ran-domly chosen for isolation. All isolates were subsequently grown at room temperature on 5-cm petri dishes with malt extract agar (20 g of malt extract, 20 g of glucose, 2 g of peptone, 20 g of agar, and 1 liter of distilled water). To determine the ploidy of colonies generated from spores, presence or absence of clamp connections was checked on a subset of 15% of all isolates by direct observa-tion of cultures in inverted petri dishes at ×300 magnification. Iso-lates displaying clamps at the septa were considered to be hetero-karyotic ($n + n$), while clampless isolates were regarded as homo-karyons (n). DNA extractions were made by a modified CTAB (cetyltrimethyl-ammonium bromide) extraction method (11).

Three methods were used to identify at the species level the isolates obtained during the first year of the study: (i) a taxon-specific competitive-priming-polymerase chain reaction (PCR) (9) combined with a PCR-mediated detection of species-specific introns in the ML5-ML6 DNA region of the mitochondrial large ribosomal RNA (mt LrRNA) gene; (ii) a PCR restriction fragment length polymorphism on the internal transcribed spacer; and (iii) sexual compatibility tests with homokaryon testers of the three European species of *Heterobasidion*. Because method one was deemed extremely accurate at the end of the first year and in a previous study (11), the isolates obtained from the monthly trappings at Aymavilles were typed exclusively by a modified ver-sion of this method (10).

Data interpretation and analysis. Based on the area of each trap of approximately 0.01 m^2, spore deposition values were stan-dardized and reported as (i) number of viable spores per square meter per hour (deposition rate [DR]) and (ii) percentage of in-fected disks per experiment (infection frequency [IF]). While DR provides information on the quantity of airborne spores, IF may be a more appropriate measurement when analyzing the spatial distribution of spore depositions within stands. For each year, sea-sonal and monthly DR values were compared using a one-way analysis of variance design and Tukey's honestly significant dif-ference test, considering values from transects as replicates.

Pearson product-moment correlation between DR and IF values was calculated for each stand, and simple linear regression analy-sis was used to determine the relationships between the two vari-ables. An $Ln(N + 1)$ transformation was used to normalize the distribution of the residuals for DR data (Shapiro Wilk's statistics, $P > 0.10$). An arcsine transformation was employed for IF data.

Pearson product-moment correlation analyses were used to relate DR values from the four forests studied monthly since March 1999, and DR values from the first and the second year of monthly samplings at Aymavilles. In addition, at Aymavilles, correlation analyses were used to determine the effects on DR of the following weather variables: the daily minimum, mean, and maximum temperature (°C); the daily minimum, mean, and maxi-mum RH (%); and the daily total RF (mm). DR values were cor-related with the above weather variables recorded over the 2-day, 7-day, and 4-week periods before the sampling period. Average values were used for temperature and RH, while cumulative values were employed for RF. Bonferroni's correction for multi-ple tests was used to adjust threshold levels of significance of correlation coefficients.

To define temporal trends of spore deposition for each species of *Heterobasidion*, both absolute frequencies and relative fre-

TABLE 2. Analysis of variance comparing spore deposition rate values of *Heterobasidion* spp. from different sampling periods in four forest stands of the western Italian Alps

Forest	Year[a]	Source	df	Sum of squares	Mean square	*F* value	*P*
Chiusa Pesio	A	Effect	3	10,685.83	3,561.94	1.89	0.1637
		Error	20	37,679.31	1,883.97	...	...
	B	Effect	9	1.24×10^7	1.38×10^6	214.88	<0.0001
		Error	50	3.21×10^5	6,420.28	...	...
	C	Effect	7	27,912.70	9,987.53	16.20	<0.0001
		Error	40	9,843.49	246.09	...	...
Jovençan	A	Effect	3	7.49×10^5	2.49×10^5	91.09	<0.0001
		Error	20	54,844.90	2,742.24	...	...
	B	Effect	9	1.21×10^6	1.35×10^5	12.01	<0.0001
		Error	50	5.61×10^5	11,214.02	...	...
	C	Effect	7	23,291.41	3,327.34	4.84	0.0005
		Error	40	27,485.62	687.14	...	...
Charvensod	A	Effect	3	1.25×10^5	41,772.27	8.89	0.0006
		Error	20	93,958.93	4,697.95	...	...
	B	Effect	9	33,036.27	3,670.70	18.69	<0.0001
		Error	50	9,823.19	196.46	...	...
	C	...	...	...	...	...	...
Aymavilles	A	Effect	3	1.71×10^6	5.69×10^5	60.99	<0.0001
		Error	20	1.87×10^5	9,327.10	...	...
	B	Effect	9	2.85×10^5	31,692.98	4.72	0.0001
		Error	50	3.35×10^5	6,709.94	...	...
	C	Effect	11	4.56×10^5	41,430.73	15.36	<0.0001
		Error	60	1.62×10^5	2,697.29	...	...

[a] A, B, and C refer to seasonal samplings of 1998 to 1999, monthly samplings of 1999, and monthly samplings of 2000, respectively.

quencies (percentage of spores of a given species relative to the total number of spores identified) were used. At Aymavilles, the data from two consecutive months were pooled. The Mann-Whitney U test was employed to compare absolute frequencies for each species from different collection periods. χ^2 tests and contingency tables were used to compare the relative abundance of spores of each *Heterobasidion* species obtained in different sampling periods. Spearman's rank-order correlation test was used to compare spore abundance of the three species in corresponding collection periods of 1998 to 1999 and 1999 to 2000. Statistical analyses were performed using the software Statistica (StatSoft Inc., Tulsa, OK).
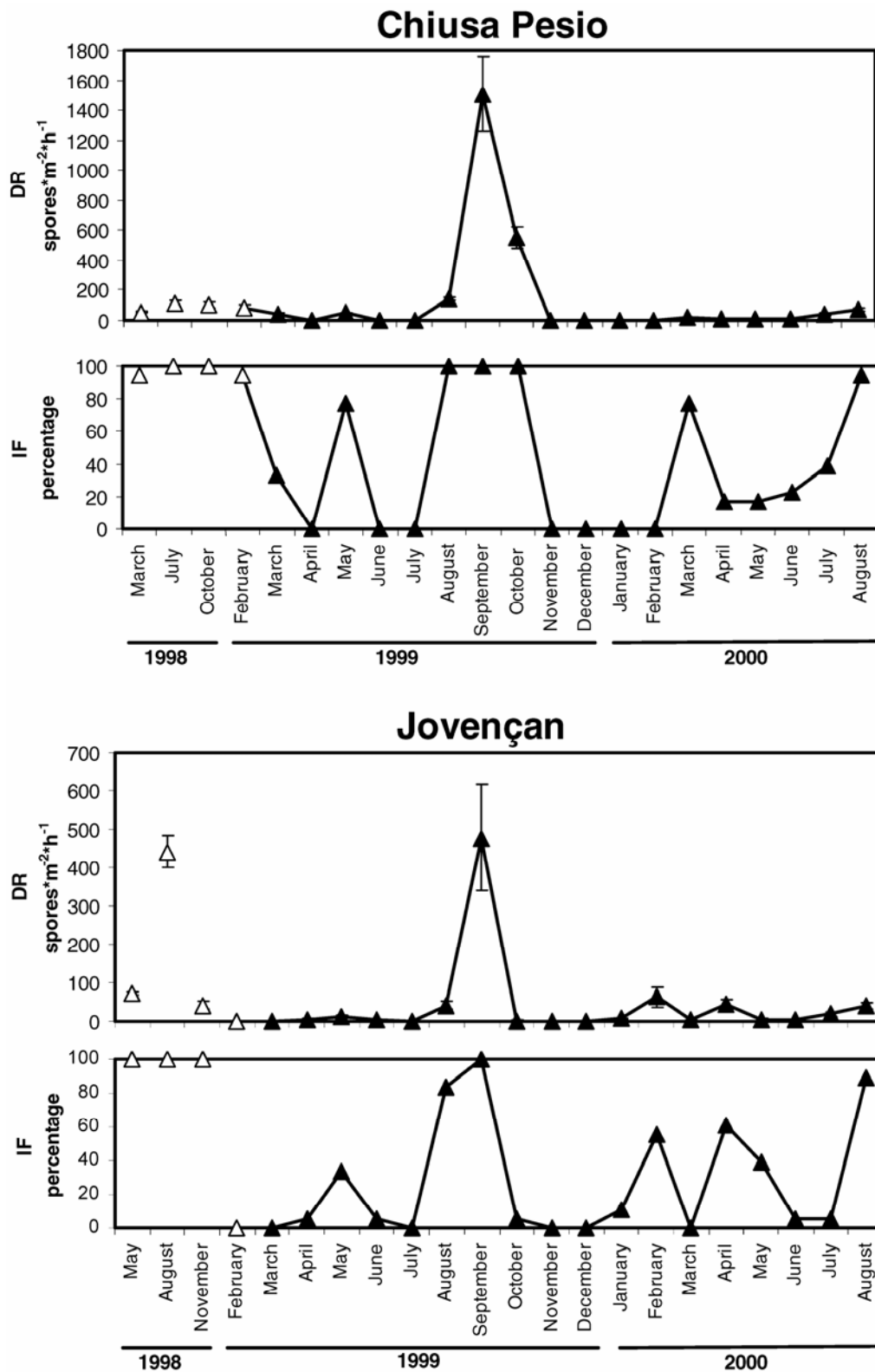
Fig. 2. Seasonal and monthly variation of spore deposition of *Heterobasidion* spp. over the years 1998 to 2000 in four forest stands in the western Alps. Deposition rates (DR) are expressed as the mean number of spores per square meters per hour, and infection frequencies (IF) represent the percentage of wood disks infected by spores of the pathogen. Bars show standard errors.

RESULTS

Spore deposition by all *Heterobasidion* spp. Spores of *Heterobasidion* spp. were collected on woody traps at all four study sites, while control disks were always uninfected.

Seasonal (1998 and 1999) data. Variation of DR values was highly significant in all stands, with the exception of Chiusa Pesio (Table 2). At this site, spores were trapped at each sampling time with a minimum IF of 94% (Fig. 2). At the other three sites, no spores were trapped during the winter
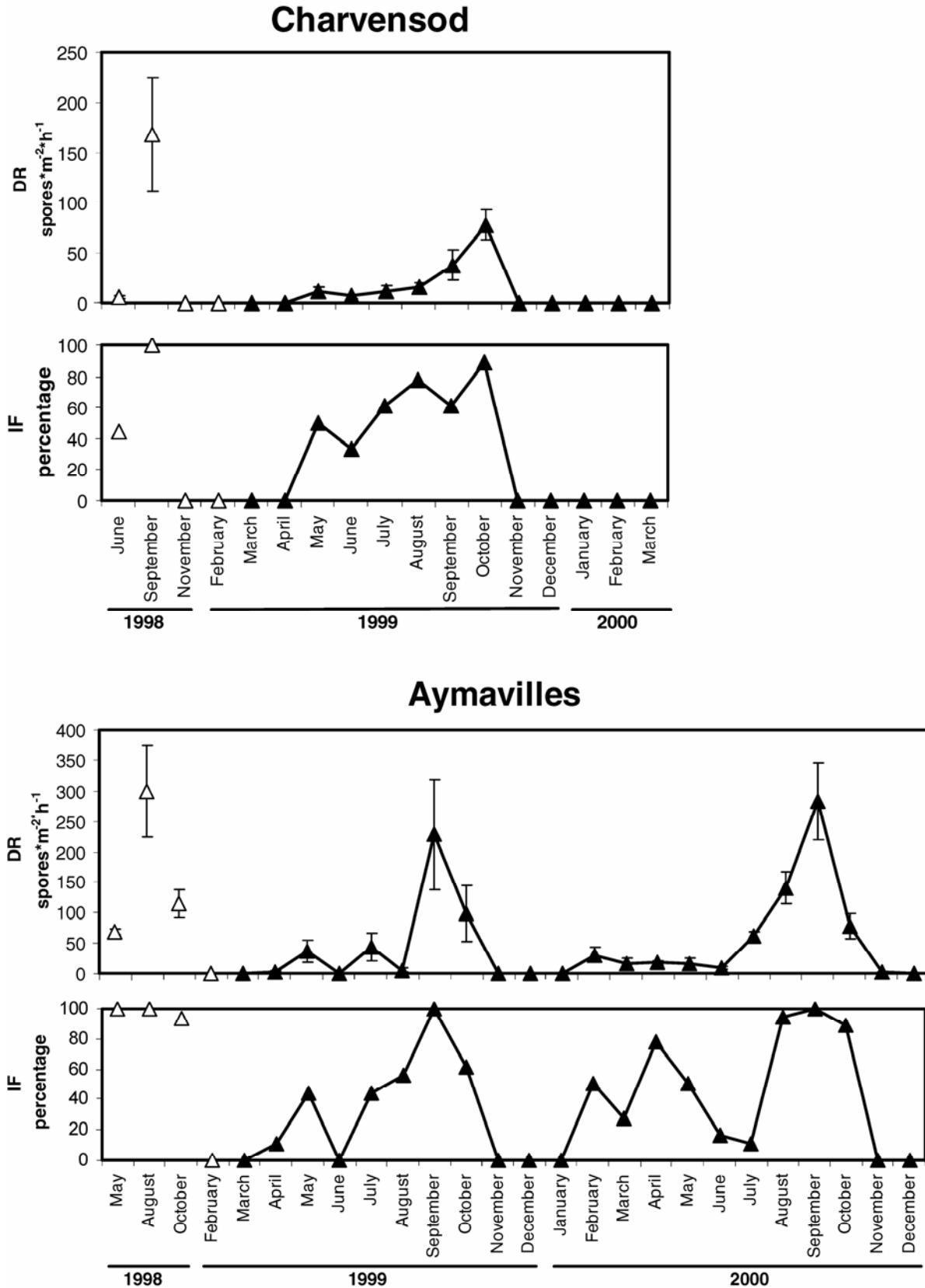

Fig. 2. (*Continued from preceding page*).

sampling. Maximum DR values were recorded in the summer in all forests.

Monthly (1999 and 2000) data. Monthly DR values peaked in September to October, and then declined (Fig. 2). DR values from trappings performed during winter, spring, and early summer were very low or zero. Although high IF values were always reached when high DR values were recorded, even slight increases in DR values resulted in relatively high peaks for IF measurements. During 1999, IF increased in June or July, reached a peak (IF > 90%) in August to October depending on the site, and then dropped to zero at all sites. The same pattern was evident in 2000. A smaller peak was attained around May at all sites and in both years.

Correlation between DR and IF was highly significant in all stands ($P < 0.001$), with r ranging from 0.917 at Jovençan to 0.985 at Charvensod. In the regression of DR versus IF, intercept values did not significantly differ from zero at any site, and regression slopes did not significantly differ from one another. Regression analysis performed on pooled DR versus IF data (Fig. 3) indicated that IF values of 50 and 90% were attained when DR values were >22 and 231 spores m^{-2} h^{-1}, respectively.

Temporal patterns of spore deposition were similar across all sites, both in terms of DR and IF. Significant correlations were found among all forests, with the exception of the Jovençan-Charvensod comparison (Table 3).

Monthly patterns of spore deposition in 1999 and 2000 at Aymavilles were well correlated with one another. Pearson's correlation coefficients for DR and IF were 0.718 ($P = 0.013$) and 0.759 ($P = 0.007$), respectively.

Abundance of spores produced by individual *Heterobasidion* spp. No clamp connections were found in 93% of analyzed cultures, suggesting that the majority of colonies were derived from basidiospores rather than from conidia. The remaining 7% was clamped, the likely result of mating of homokaryotic colonies generated by basidiospores landing near one another on the same trap. A total of 1,191 out of 1,232 isolates were successfully typed: 556 were *H. parviporum* (41%), 421 were *H. abietinum* (43%), and 214 (16%) were *H. annosum*.

H. abietinum was the most frequent species recovered at the silver fir-dominated stands of Chiusa Pesio and Jovençan. In Charvensod, the dominant fungal species was *H. parviporum*. At this site, which had the highest elevation among the four studied, spore population was most concentrated in September (Fig. 4).

In Aymavilles (Fig. 5), the abundance of *H. parviporum* spores was significantly higher in August 1998, in May/June to September/October 1999, and in July 2000 than in the other two species. Spore production by *H. parviporum* and *H. annosum* showed a significant peak in the September/October 1999 sampling.

The proportion of spores of *H. abietinum* and *H. annosum* did not show significant variations over time at any of the sites. The same was true for *H. parviporum* in Chiusa Pesio and Jovençan. At Aymavilles, a significantly higher proportion of *H. parviporum* spores resulted in summer samplings relative to the other sampling periods of respective years (χ^2 26.17, df = 1, $P < 0.005$ in August 1998; χ^2 26.59, df = 1, $P < 0.005$ in July to August 1999; and χ^2 10.10, df = 1, $P < 0.005$ in July 2000). Relative fungal species abundance recorded in 1998 was significantly correlated to species abundance recorded in analogous periods of 1999 (Spearman $\rho = 0.794$, $P = 0.011$).

Effects of weather. Both the mean and minimum air temperature in the 4 weeks before sampling were significantly correlated with DR, with Pearson's correlation coefficients of 0.632 and 0.654, respectively (Table 4). DR was not correlated with maximum temperature.

Correlations between DR and RH were not significant. Basidiospore capture was positively, but only weakly, correlated with the amount of monthly RF.

DISCUSSION

The results presented in this study indicate that the airborne inoculum of *Heterobasidion* spp., although present starting in February at most sites, is higher in August to October, reaching a peak in September. This observation is true in terms of both DR and IF. A relative peak, lower than the one in late summer, appears in late spring at all sites. This peak is more evident when evaluating results in terms of IF than in terms of DR.

Correlation analyses using DR or IF values indicated that temporal trends of spore deposition were homogenous among forests. This is noteworthy because our sites were characterized by diverse climatic conditions (Chiusa Pesio versus the other three sites), disease incidence, and inoculum concentration. Our sampling at Aymavilles also showed that spore deposition patterns were similar year after year. These findings indicate that *Heterobasidion* spore dissemination follows consistent seasonal patterns, not just at the local, but at the larger regional scale. A peak in
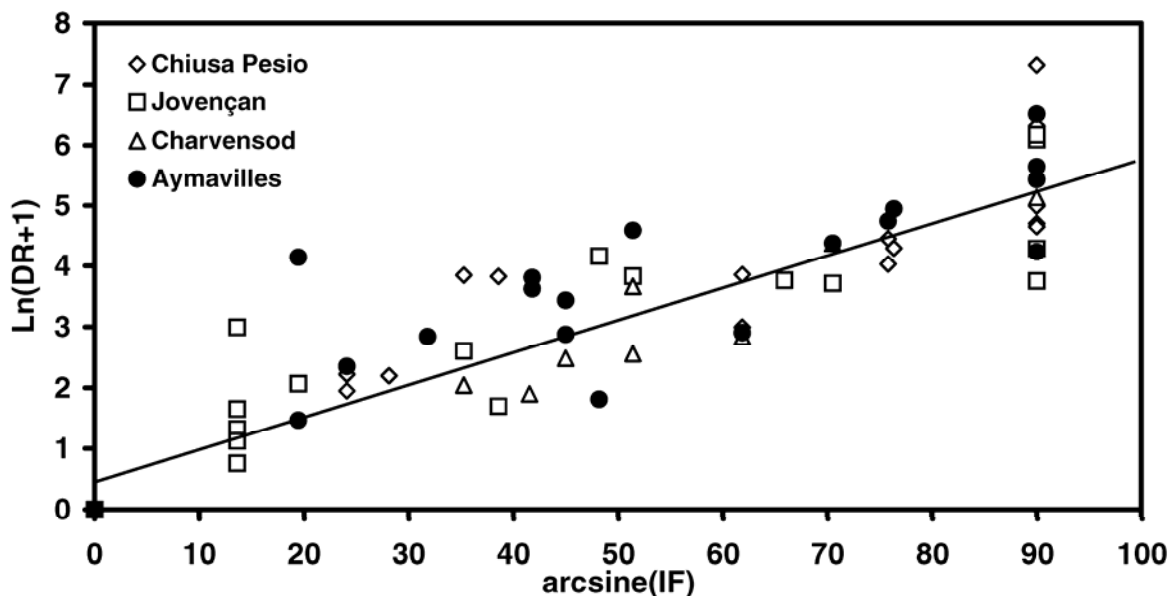
Fig. 3. Regression analysis between deposition rates (DR) and infection frequencies (IF) of spores of *Heterobasidion* spp. trapped on wood disks from 1998 to 2000 in four Alpine forests. The regression line is based on pooled data ($r^2 = 0.875$; $P < 0.001$).

spore deposition is not evident in the 1998 sampling at Chiusa Pesio. It is likely the peak was missed because of our limited sampling in that year.

A review of the literature suggests that differences in spore dissemination patterns are encountered when comparing different climatic zones (2,20,33,37,44). In the western Alps, inoculum production is largely concentrated in a period of 2 to 3 months. The spore inoculum in winter, spring, and early summer is generally low, with concentration levels comparable to those found in healthy stands of northern Europe (16,24). Our findings indicate (i) a marked seasonality of spore deposition and consequently of potential for infection of stumps and trees, and (ii) that the highest risk of infection may be concentrated in a relatively short period.

Data from Charvensod and Jovençan were not significantly correlated. However, the deposition patterns of the two sites were similar, with the exception of the main peak, which occurs a month later in Charvensod than in Jovençan. The two forests are on the same mountain slope, but the former site is approximately 700 m higher in elevation. Such lag in the presence of the peak can be explained by the more severe climatic conditions of the site at higher elevation.

The presence of airborne spores is a prerequisite for primary infection by most fungi (13), and the abundance of the airspora, often referred to as inoculum potential, is directly correlated with infection potential (20,38,39). Actual infection is bound to be lower than the estimated infection potential for a variety of reasons, including environmental factors, virulence of fungal isolates, competition with other isolates and/or other fungi, and the ability of the host to fence off the colonization process.

The maximum DR values in our study ranged between 169 to 1,550 spores m^{-2} h^{-1}, measured at Charvensod and Chiusa Pesio, respectively. These levels are comparable to the levels obtained from North America and Europe, using similar experimental approaches (7,14,44). Because the heterogeneity in the surface of wood disks or stumps leads to differential germination success of the pathogen on different sectors (12,23,25), it is likely that the actual concentration of airborne inoculum may be higher than that reported in this study.

DR and IF are correlated with each other, and both appear to be suitable indicators for describing the spore deposition process (Fig. 3). The relationship between the two variables is nonlinear. Large amounts of spores (i.e., over 230 spores m^{-2} h^{-1}) would be necessary for 90% of traps to be infected. On the other hand, small increases in DR may lead to large increases in IF. When biological and environmental conditions are favorable to the pathogen, IF values may predict infection better than DR because each spore has a high chance of successfully causing infection. As conditions become less favorable to the pathogen, DR values may predict infection better than IF. In adverse conditions, in fact,

successful infections by the pathogen may depend on the presence of multiple individuals.

DR values above 10 spores m^{-2} h^{-1} at the time of cutting significantly increase the risk of stump infection (16,23). In our study, that threshold was exceeded in all stands 17 to 150 times. A previous study performed in Charvensod determined that 100% of stumps had been colonized (25).

A geographically limited range of dispersal of *Heterobasidion* basidiospores has already been suggested (11,40); therefore, larger spore numbers would be expected where larger fruiting body production occurs. At Chiusa Pesio, the site where the highest levels of DR were recorded, basidiospore-producing fruiting bodies were several times more abundant than in the three other sites (data not shown).

The average minimum air temperature of the preceding 4-week period is the variable with the most significant correlation value with DR, and may thus be considered a suitable predictor for modeling *Heterobasidion* spore deposition. Positive correlations between temperature and basidiospore production have been previously reported (38). Our regression analysis (data not shown) indicated that at temperatures below –5.5°C, DR values are negligible. In Fennoscandia, the risk of stump infection at temperatures below +5°C is nil (15), and the air temperature must be >0°C in
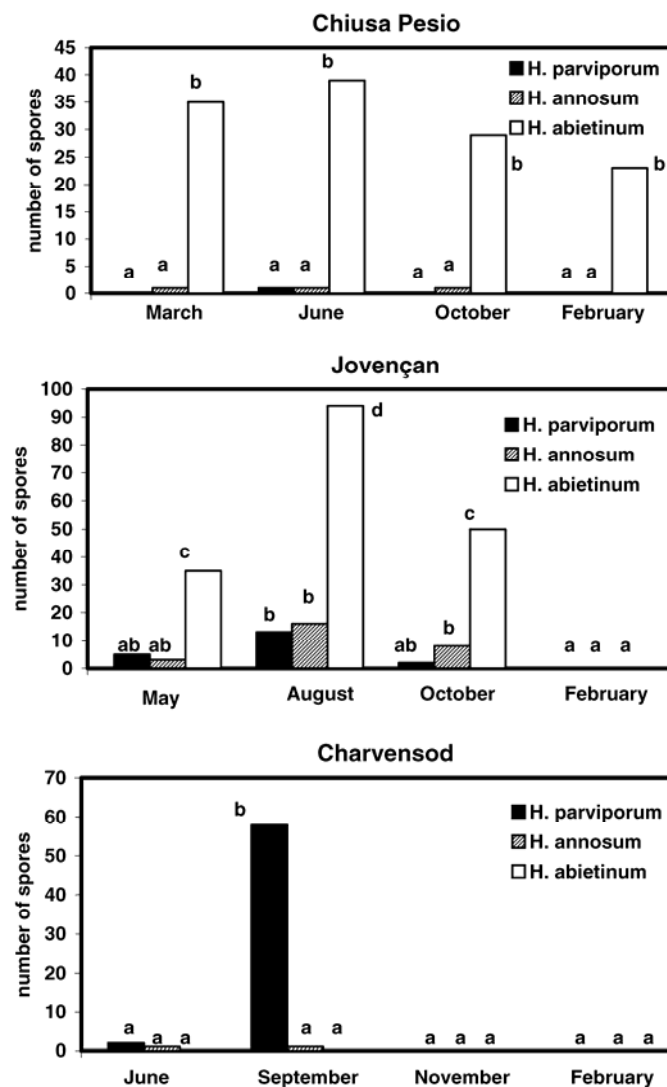

Fig. 4. Frequency of spores of the three species of *Heterobasidion* in three forests in the western Alps in different time periods determined with wood disk samples. Samplings were performed in 1998 and 1999. The abundance of spores was compared by the Mann-Whitney *U* test (*n* = 418); bars with the same letter are not significantly different (*P* ≤ 0.05).

TABLE 3. Correlation of monthly patterns of spore deposition of *Heterobasidion* spp. among the four forest stands in 1999 to 2000

Stands		Jovençan	N	Charvensod	N	Aymavilles	N
		Pearson's correlation coefficient[a]			
Chiusa Pesio	DR[b]	0.480*	18	0.691**	13	0.622**	18
	IF[c]	0.580*	...	0.656*	...	0.662**	...
Jovençan	DR	...	...	0.337 NS	13	0.579*	18
	IF	...	...	0.428 NS	...	0.832**	...
Charvensod	DR	...	...	...	...	0.675*	13
	IF	...	...	...	...	0.657*	...

[a] ** And * indicate significant at *P* = 0.01 and 0.05, respectively; NS = not significant.

[b] Deposition rate (DR) is the number of viable spores per square meter per hour; for analysis, values were transformed with the Ln(*N* + 1) transformation.

[c] Infection frequency (IF) is the percentage of infected wood disks; for analysis, values were transformed with the arcsine transformation.

order for spore production to occur, and above +5°C to be abundant (16,20,32). In our study, spore deposition stopped in autumn at a minimum monthly air temperature below +5°C and occurred again in winter/spring when mean temperatures exceeded +4°C.

Further studies are needed to assess whether precipitation events, including snowfall, may influence release and deposition of *Heterobasidion* airborne spores. However, we agree with authors of previous studies (16,38), that the low temperatures rather than the possible dispersal barrier posed by a snow cover may explain the lack of availability of inoculum during winter. Both in 1999 and 2000, spore deposition declined rapidly and stopped before the first snowfall. In California, spore production has been reported even in snowy conditions (14).

The fact that all three European *Heterobasidion* species are present at our study sites allows for a direct comparison among them. Our extended sampling at Aymavilles indicated that the numbers of spores of *H. annosum* and *H. abietinum* were always similar to one another and showed only small fluctuations throughout the year. On the other hand, sporulation by *H. parviporum* increased significantly during the summer. This was true in terms of both absolute number and relative abundance of spores. These patterns of spore deposition, which differentiated *H. parviporum* from the other two species, were analogous when comparing corresponding periods of different years. In areas where *H. parviporum* was not the dominant species, sharp changes in spore deposition were not recorded, potentially because such changes may have been partially masked by the abundance of other species (i.e., *H. abietinum*).

Our results show for the first time that different *Heterobasidion* species differ in their potential for spore dissemination even when found in the same forest. The ecological implications of these findings are numerous and deserve further studies. The presence of a rather short-lived but marked peak in spore production for *H. parviporum* appears as a useful adaptation for a pathogen species found at high altitudes and latitudes where favorable weather conditions may be limited to a short period in the summer months. The other two European *Heterobasidion* species are generally found in more mesic areas (18), where weather conditions may not be as harsh. Nevertheless, our results also provide

valuable information that could be used to design silvicultural guidelines aimed at minimizing the risk of infection of trees by this pathogen. Based on our study, thinning operations in spring rather than in summer should result in a drop in infection of stumps of Norway spruce by *H. parviporum*. In the spring, in fact, not only were the DR values significantly lower than in other seasons but the relative abundance of *H. parviporum* was also lower when compared with the two other species. The opportune timing of thinning operations may be most useful within an integrated disease management program including preventive stump treatments, but it should be regarded as a pivotal tool for disease control in areas where stump treatments are not allowed or economical.

Winter thinning and logging operations have been used in Fennoscandia to take advantage of the low inoculum pressure during the cold season. The same approach may also be useful in the western Alps, where inoculum pressure is also very low in the winter months. Although winter operations may not be feasible at all sites in the Alps, their advantages include limiting infection of spruce stumps not only by *H. parviporum* but also by *H. anno-*

TABLE 4. Correlation coefficients of monthly deposition rates of spores of *Heterobasidion* spp. with weather variables (temperature [T], relative humidity [RH], and rainfall [RF]) recorded daily for each of the 2-day, 7-day, and 4-week periods preceding the date of spore collection at Aymavilles from March 1999 to December 2000

Variable	Correlation coefficients[a]		
	2 days[b]	7 days[b]	4 weeks[b]
Mean T (°C)	0.425 NS	0.551 NS	0.632*
Minimum T (°C)	0.388 NS	0.465 NS	0.654*
Maximum T (°C)	0.412 NS	0.425 NS	0.450 NS
Mean RH (%)	0.321 NS	0.428 NS	0.245 NS
Minimum RH (%)	0.080 NS	0.196 NS	0.393 NS
Maximum RH (%)	0.513 NS	0.479 NS	0.009 NS
RF (mm)	0.042 NS	0.136 NS	0.302 NS

[a] * Indicates significant and NS = not significant, after Bonferroni's correction ($P = 0.002$).
[b] Average values were used for T and RH, and cumulative values were employed for RF.
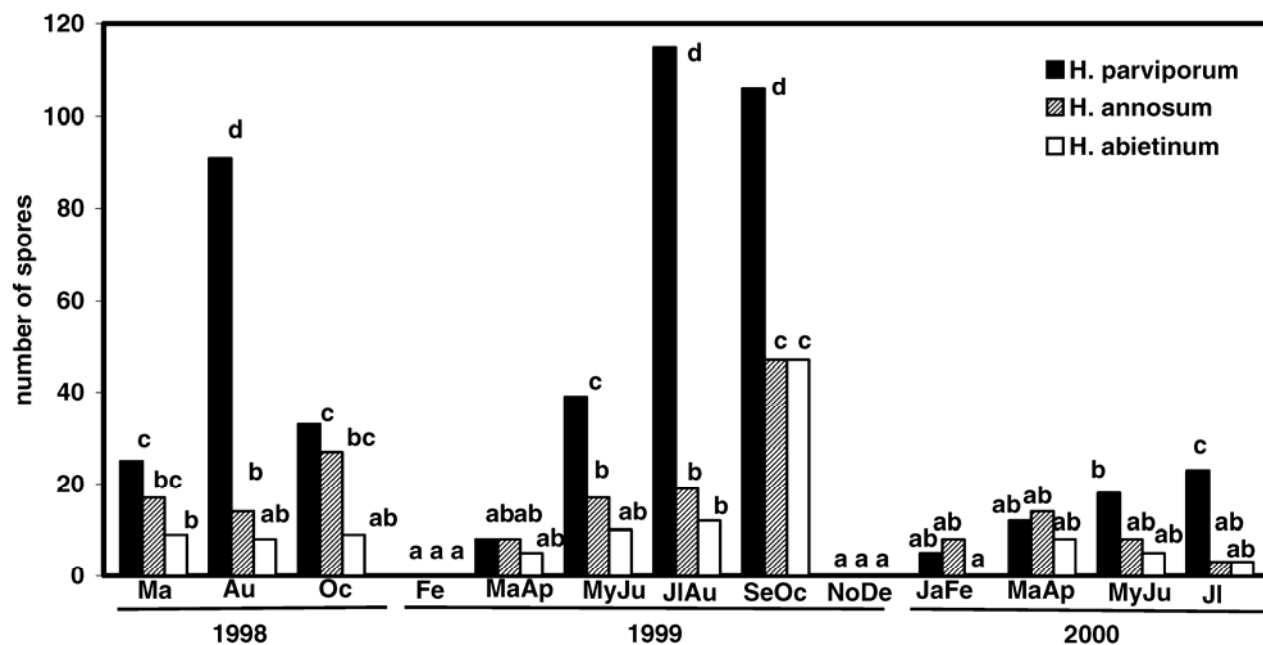

Fig. 5. Frequency of spores of the three species of *Heterobasidion* at different periods at the Aymavilles site determined with wood disk samples. Samplings were performed in 1998 to 2000. The abundance of spores was compared by the Mann-Whitney *U* test ($n = 773$); bars marked by the same letter are not significantly different ($P \leq 0.05$). Labels are as follows: Ja = January, Fe = February, Ma = March, Ap = April, My = May, Ju = June, Jl = July, Au = August, Se = September, Oc = October, No = November, and De = December.

sum. The increase of the latter species in spruce stands is well documented and may negatively affect the overall health of forest stands, given the aggressiveness in decaying wood by *H. annosum*, not only in pines but also in spruce stands.

ACKNOWLEDGMENTS

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