Seasonal patterns of spore deposition of Heterobasidion species in four forests of the western Alps

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Seasonal patterns of spore dispersal and deposition of *Heterobasidion* species in four forests of the Western Alps

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ABSTRACT

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Patterns of sporulation by *Heterobasidion* species were studied between the spring of 1998 and December 2000 in four forests in the Western Alps. The maximum inoculum concentration, defined as Deposition Rate (DR), ranged from 169 spores*m^{-2}*h^{-1} to 1,550 spores*m^{-2}*h^{-1}. Although spores were captured, using woody traps, 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 fluctuations of DR were recorded in two years of sampling. A significant correlation was found between DR and the average minimum air temperature of the four weeks before sampling. Approximately 1,200 spores were isolated and identified at the species level by PCR-based methods. 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: control, epidemiology, model

INTRODUCTION

Heterobasidion annosum (Fr.) Bref. *sensu lato* is a root and butt rot agent that severely affects coniferous forests worldwide. This pathogen has long been regarded as a species complex, comprising three intersterility groups (ISGs) in Europe (4,23), recently named *H. parviporum* Niemelä & Korhonen, *H. abietinum* Niemelä & Korhonen, and *H. annosum sensu stricto* (*s.s.*) (34). These species, although defined on the basis of partial

reproductive isolation and morphology (5,6,23,29), are also characterized by differences in pathogenicity on a range of hosts (4,24,35,51). 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 and attack exotics. *Heterobasidion 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 s.s.* is typically associated with mortality of trees in the genus *Pinus*, but it can also be found on *Picea*, *Juniperus* and even on deciduous trees (24).

Heterobasidion primarily infects its hosts by means of airborne meiospores, normally through fresh-cut stumps or wounds, and is capable of secondarily spreading from tree to tree through root grafts and contacts (39,50). Airborne infection through thinning stumps may not only result in a rapid and heavy infection of a healthy stand in areas where *Heterobasidion* is common (37,52), but may also aid the spread of the fungus into new areas (2). The availability of inoculum is a necessary prerequisite for primary infection. Inoculum potential is directly correlated with infestation levels (28). Understanding the seasonal patterns of sporulation in *Heterobasidion* populations is pivotal knowledge when formulating control strategies for the disease this pathogen causes.

Aerobiology and temporal dynamics of spore deposition have been studied in North America and Northern and central Europe since the early 1950's (3,9,19,21,41,44,53). Seasonal trends in spore deposition have been found to vary largely among different collecting periods in the same stands and, especially, among forests in different ecotypes. In Britain, for instance, spore infection occurs during most of the year (28,42), while in central and Northern Europe the main infection periods are autumn and spring (53), and summer (3,21), respectively. Regional differences in seasonal patterns of spore deposition have been reported in North America (7,9,30,44). However, no information on temporal dynamics of spore deposition is available for the Alps and Southern Europe.

Concentrations of airborne spores are the result of the entire sporulation process which includes spore formation, release, and dispersal. 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 (18). Air temperature, relative air humidity, and rainfall have been reported as factor influencing spore production and deposition for *Heterobasidion* spp. (39). A positive correlation between temperature and spore deposition has been found in the Northeastern United States and Northern Europe (21,47). High summer temperatures have been reported to reduce sporulation in the Southern United States (7,44). Hot dry weather in summer, combined with snow and low temperature in winter, may locally restrict spore production to the autumn (38,45). The fact that weather factors affect sporulation in different ways in each respective region (39,40,53) complicate the task of developing local sporulation models.

Disease-warning models predicting inoculum availability and weather conditions conducive to infection have been developed for several plant pathogens (1,10,13,16,22,26,27,36). Predictive models can assist forest managers in the development of integrated pest management strategies aimed at either intensifying silvicultural activities in periods of low sporulation, or at increasing preventative practices when operating in periods of high sporulation.

The goals of this study were (i) to determine rates of sporulation and special patterns of deposition of viable spores of *Heterobasidion* spp. on seasonal and monthly basis, 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, such as air temperature, relative humidity and rainfall, and the temporal patterns of inoculum availability, and (iv) to compare the patterns of deposition among the three European *Heterobasidion* species.

MATERIALS AND METHODS

Spore trapping

The deposition of spores of *Heterobasidion* spp. was investigated in four natural forests of the Western Italian Alps: Site 1 is a pure silver fir stand (Chiusa Pesio, Cuneo), Site 2 is a forest with about 95% of silver fir (Jovençan, Aosta), Site 3 is a pure Norway spruce stand (Charvensod, Aosta), and Site 4 is a mixed spruce-fir forest (Aymavilles, Aosta). Site 1, due to the close proximity to the Ligurian sea, is characterized by mild temperatures and abundant rainfall peaking in May and November. The other three sites are characterized by a colder sub-continental climate with low average annual rainfall, peaking in October and November. Information about the airborne inoculum composition of *Heterobasidion* species was already available for the study sites (15). Site locations and stand characteristics are shown in Table 1.

In each forest, two permanent plots were established, each including 3 transects with 3 collection points as described previously (15). Spores of *Heterobasidion* spp. were trapped using a modified version of the wood-disk exposure method (19,43). Freshly-cut wood disks, obtained from, healthy spruce (sites 3, and 4) and fir (sites 1, 2, and 4) were sprayed after bark removal with 65% ethanol, and placed onto a wet filter paper disk in a Petri dish (15). 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 used as traps or by airborne spores landed on the wood during trap preparation. Spore trappings were performed in calm days, and they were delayed when the 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}.

Sets of open Petri dishes, one dish per collection point, were placed on the ground or on snow from March 1998 to December 2000 at intervals specified below. In the first year, seasonal samplings (one per season per site) were performed earlier in lower elevation sites characterized by milder climate, to allow for a meaningful comparison across sites. A first sampling (spring) was performed in March 1998 at Chiusa Pesio, in May at Jovençan and Aymavilles, and in June at Charvensod. A second sampling (summer) was made in July at Chiusa Pesio, in August at Jovençan and Aymavilles, and in September at Charvensod. A third sampling (fall) was made in October at Chiusa Pesio and Aymavilles, and in November in the other two sites. A fourth sampling (winter) was performed in all the forests in February 1999. Starting on March 1999, woody spore traps were exposed at all sites at the beginning of each month, as described above. Spore trappings ended in August 2000, except for Charvensod and Aymavilles, where studies were concluded in March and December 2000, respectively.

After exposure, filter papers contained in the Petri dishes were replaced and dampened with 3 ml of sterile water. Disks were sprayed with a benomyl solution (0.010 g benomyl, 500 μl ethanol, 1 liter sterile water) and incubated at about 24°C for 5 to 7 days.

Disks were examined under a dissection microscope (x20) for colonies of *Spiniger meineckellus*, the conidial stage of the fungus. Colonies were counted and the number of spores was determined under the assumption that each colony had resulted from deposition of one viable spore (19,43).

Monitoring of weather variables

Weather measurements were taken in the forest of Aymavilles since March 1999. Air temperature (T) and relative air humidity (RH) were recorded continuously at the center of each plot using a hygrothermograph (MT 1500, SIAP, Bologna, Italy) installed in a weather shelter at 1.5 m above ground. Rainfall (RF) was measured in the same location with a 7-

day rain gauge (UM 8100, SIAP, Bologna, Italy). In snowy winter conditions, snow height was used as a proxy for rainfall. Average values of the weather data were calculated at 1-hour intervals, and recorded on electronic sheet files for analysis.

Isolations and typing of spores of *Heterobasidion* spp.

Isolations of putative single spore colonies of *Heterobasidion* spp. were performed from disks exposed during the first-year seasonal samplings at all sites, and from monthly trappings performed until July 2000 at Aymavilles. This site was selected for further investigations because of the presence of all the three European species of *Heterobasidion* (15).

Fungal colonies were isolated under a dissecting microscope (x20), by transferring infected wood pieces onto 5-cm Petri dishes filled with a selective medium for *Heterobasidion* (25). When three or more colonies per disk were visible, three randomly chosen colonies were isolated. All isolates were subsequently grown at room temperature on 5-cm Petri dishes filled with MEA (20 g malt extract, 20 g glucose, 2 g peptone, 20 g agar, 1 liter distilled water). A subset of 15% of isolates were checked for presence/absence of clamp connections by direct observation of colonies in inverted Petri dishes at x300 magnification.

DNA extractions were made by the CTAB (cetyltrimethyl-ammonium bromide) extraction method (12) previously modified (15).

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 (TSCP) - PCR (11) 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 RFLP on the Internal Transcribed Spacer (ITS), and, finally, (iii) sexual compatibility tests with homokaryon testers of the three European species of *Heterobasidion*. Because method (i)

was deemed extremely accurate at the end of the first year and in a previous study (15), the isolates obtained from the monthly trappings at Aymavilles were typed exclusively by a modified version of this method (14).

Data interpretation and analysis

Deposition of spores was evaluated in terms of: (i) number of viable spores per square meter per hour (**deposition rates or DR**), and (ii) percentage of infected disks (**infection frequency or 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. Seasonal and monthly DR values were compared by transect using ANOVA and the Tukey's honestly significant difference (HSD) test.

Pearson Product-Moment Correlation between deposition rates and infection frequency values was calculated for each stand, and simple linear regression analysis was used to explore the relationships between the two variables. A Ln(N+1) transformation was used for DR values to normalize the distribution of the residuals (Shapiro Wilk's statistics, P always > 0.10). An arcsine transformation was employed to normalize IF data.

Pearson Product-Moment Correlation analyses were used to compare 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. At Aymavilles, correlation analyses were used to determine the effects on DR of the following weather variables: the daily minimum, mean and maximum temperature (T, °C), the daily minimum, mean and maximum relative humidity (RH, %), and the daily total rainfall (RF, mm). DR values were correlated with the above weather variables recorded 2-, 7-days, and 4-weeks periods before sampling. Average values were used for T and RH, while cumulative values were employed for RF.

To define temporal trends of spore deposition for each species of *Heterobasidion*, both absolute frequencies and relative frequencies (percentage of spores of a given species on

the total number of spores identified) were used. At Aymavilles, the analyses were performed on data from two consecutive months pooled together. The Mann-Whitney *U* test, was employed to compare, for each species, absolute frequencies 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. Finally, the Spearman's rank-order correlation test was used to compare the abundance of spores of the three species in corresponding collection periods of 1998-1999 and 1999-2000.

Statistical analyses were performed using the software Statistica (StatSoft Inc., Tulsa, Oklahoma).

RESULTS

Analyses of 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. DR and IF values are shown in Fig. 1.

Seasonal (1998 and 1999) data. Seasonal variations in spore deposition were recorded at all sites but Chiusa Pesio. In Chiusa Pesio, spores were trapped at each sampling time with a recorded minimum IF of 94%. Large numbers of spores were collected in March 1998 (first sampling), July 1998 (second sampling), October 1998 (third sampling), and February 1999 (fourth sampling). At the other three sites, no spores were trapped during the winter sampling (fourth sampling). Maximum DR values were recorded in the summer in all forests. Recorded DR values were as follows: 109 spores*m^{-2}*h^{-1} at Chiusa Pesio, 442, 169, and 669 spores*m^{-2}*h^{-1} at Jovençan, Charvensod and Aymavilles, respectively.

Monthly (1999 and 2000) data. Monthly DR values peaked in the early fall (i.e., September), and then declined at most sites, including Chiusa Pesio. DR from trappings

performed during winter, spring, and early summer (i.e., June and July) were very low, or in many cases no spores were trapped at these periods. 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 from June or July, reached a main peak (IF> 90%) from August to October depending on the site, and then decreased sharply to 0% at all sites. The same pattern was evident in 2000. A relative peak around May was attained at all sites and in both years.

Correlation between DR and IF was highly significant in all stands ($P< 0.001$), with Pearson r ranging from 0.917 at Jovençan to 0.985 at Charvensod. In the regression analysis, intercept values did not significantly differ from zero at any site, and regression curves did not significantly differ from one another (not shown). Regression analysis performed on overall data (Fig. 2) indicated that 50% and 90% IF were attained when DR was over 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 2).

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

Temporal analysis of abundance of spores produced by each *Heterobasidion* spp.

Clamp connections were not found in 93% of analyzed cultures. 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*.

The frequency of spores produced by different *Heterobasidion* species at each collection periods is shown in Figs. 3 and 4. *Heterobasidion abietinum* was always the dominant fungal species both at Chiusa Pesio and Jovençan, where silver fir is the dominant tree species. In the pure spruce stand of Charvensod, the dominant fungal species was *H. parviporum.* At this site, the one with the highest elevation, among the four studied, spore trappings were mostly concentrated in the September trapping.

In the mixed forest of Aymavilles (Fig. 4) the abundance of *H. parviporum* spores was significantly higher than the other two species in the sampling of August 1998, in the period starting in May-June and ending in September-October 1999, and finally in the sampling of July 2000. These sampling times also corresponded to peaks in the spore deposition by this species. Spore production by *H. parviporum* and *H. annosum* showed a significant peak in the sampling of September-October 1999.

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 sites where this species is not the dominant one (i.e., Chiusa Pesio and Jovençan). At Aymavilles, a significant higher proportion of *H. parviporum* spores resulted in summer samplings with respect 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-August 1999, 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 ρ= 0.794; P= 0.011).

Effects of weather variables

Both the mean and minimum air temperature of the 4 weeks before sampling were correlated with DR (P< 0.01), with Pearson coefficients of 0.632 and 0.654, respectively

(Table 3). Mean temperature of the seven days preceding spore trappings was also significantly correlated with DR ($P< 0.05$). DR was never significantly correlated with maximum temperature, nor with minimum and mean temperature of the two days preceding samplings.

Correlations between DR and relative humidity in general were weak and not significant, with the exception of DR with maximum relative humidity recorded on the preceding 2 days.

Basidiospore capture was positively but only weakly correlated with the amount of monthly precipitations.

DISCUSSION

The presence of airborne spores is a prerequisite for primary infection by many fungi, and the abundance of the airspora, often referred to as inoculum potential, is directly correlated with infection potential (28,47,48). Actual infection is bound to be lower than the estimated infection potential for a variety of reasons including weather and environmental factors, fitness of fungal isolates, competition with other isolates and/or other fungi, and the ability of the host to fence off the colonization process. In a study of *H. annosum* infection of stumps of *Pinus jeffreyi* in two sites of the Sierra Nevada (California, USA), although 100% of stumps were initially infected at both sites, none of them were colonized in the drier of the two sites, while only 20% were colonized in the more mesic site (M.M. Garbelotto and W. Otrosina, *unpublished data*).

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 consistent with the records obtained from North America and Europe, using similar experimental approaches (8,19,53). Because the heterogeneity in the surface of disks or stumps leads to differential germination success of the pathogen on different sectors (17,31,33), it is

likely that the concentration of inoculum in the air may be higher than that reported in this study.

The results presented in this study indicate that the airborne inoculum of *Heterobasidion*, although present since February in most forests, is higher in August-October, with a peak in September. The above observation is true both in terms of deposition rates and infection frequency. A relative peak, lower than the late summer one, appears in late spring at all sites. Such peak is evident while evaluating results in terms of infection frequency rather than in terms of deposition rates.

Correlation analyses using either DR or IF values, indicated temporal trends of spore release and infection were homogenous among forests. This finding is noteworthy because our sites were characterized by quite diverse climatic conditions (Chiusa Pesio vs the other three sites), disease incidence, and absolute inoculum concentration. Our prolonged sampling at Aymavilles showed that a similar sporulation pattern was present year after year. All these findings indicate that *Heterobasidion* spore release and infection follow consistent seasonal patterns, not just at the local, but at the larger regional scale.

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. It should be noted that the two forests are on the same mountain slope, but the first site is approximately 700 m above the other one.

An overview of the pertinent literature suggests that significant differences in sporulation patterns may be encountered when comparing different climatic zones. For instance, while in Northern Europe infections seem to follow a bell-shaped curve with no deposition during winter (3), in some areas of central and Southern Europe, the risk of infections by spores is high in spring and fall, and low in summer (46,53). In Britain, however, spore infection occurs through most of the year (28,42).

In the Western Alps, we observed a very marked seasonality in spore deposition, due to the fact that inoculum production is largely concentrated in a period of 2-3 months. The spore inoculum in winter, spring, and early summer is, with the exception of a few periods, very low, with concentration levels comparable to those found in healthy stands of Northern Europe (21,32). Our findings have possible implications for control, since they indicate: (i) a marked seasonality of spore deposition and consequently of risk of stump/tree infection, (ii) that the highest risk of infection is concentrated in a relatively short period.

DR and IF are well correlated to each other, and both of them appear suitable as indicators for describe the spore deposition process. Regression patterns between these two variables were similar comparing one forest to another. An exploration of such relationship indicate that 50% of spore traps are infected when the amount of spores in the air is still very low, just over 20 spores*m^{-2}*h^{-1}. On the other hand, large amounts of spores (i.e., over 230 spores*m^{-2}*h^{-1}) would be necessary for 90% of traps to be infected. It should be noted that even small increases in DR may lead to large increases in IF. When conditions are favorable to the pathogen (environment, host, lack of microbial competition, etc.), IF values may predict infection better than DR. 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 significantly on the presence of multitudes of individuals, each characterized by different fitness levels.

Based on a previous report (21), Möykkynen and Kontiokari (31) have estimated that DR above 10 spores*m^{-2}*h^{-1} at the time of cutting, significantly increase the risk of stump infection. In our study, that threshold was exceeded in all stands by 17 to 150 times, thus it is not surprising that a study performed in Charvensod determined that 100% of stumps had been colonized (33).

Variations of absolute DR values in between years were evident both at Aymavilles and Charvensod, where the amount of spores in 1998 was at least twice as large than that recorded in 1999.

A geographical limited range of dispersal of *Heterobasidion* basidiospores has already been suggested (15,49), therefore, larger quantities of spores would be expected where larger fruit-body production occurs. At Chiusa Pesio, the site where the highest levels of DR were recorded basidiospore-producing fruit bodies were several times more abundant than in the three other sites (data not presented).

Our samplings were made in calm days, in the attempt to measure variations of inoculum availability in the air not affected by daily climatic changes (8), but rather determined by seasonal effects or general weather conditions affecting spore production and release. It should be noted that the contribution of seasonal effects vs. weather effects in determining inoculum availability cannot be estimated in the present experiment. We found the minimum air temperature to be the environmental variable with the most significant correlation value with DR (i.e., the variable with the highest predictive value with regards to DR). Positive correlations between temperature and basidiospore production have been previously reported (47), and the daily average temperature at thinning has been demonstrated to affect the incidence of stump infection in Northern Europe (20).

The average minimum air temperature of a four week period may thus be considered a suitable predictor variable for modeling *Heterobasidion* spore deposition. Our regression analysis (not shown) indicated that at temperatures below –5.49°C, DR values are negligible. In Fennoscandia, the risk of stump infection at temperatures below +5°C is nil (20), and the air temperature must be over 0°C in order for spore production to occur, and over +5°C to be abundant (21,28,41). In our study, spore deposition stopped in autumn at a minimum monthly air temperature below +5°C and occurred again in winter-spring when this parameter reached –3°C, but mean temperatures had exceeded +4°C.

With the exception of the maximum relative humidity of the two days preceding sampling, all other tested weather parameters were not significantly correlated with DR. This finding was not surprising as RH is known to affect basidiospores release and germination (18). Further studies are needed to assess whether precipitation events, including snowfall, may influence release and deposition of *Heterobasidion* airborne spores. However, we suggest, in agreement with other authors (21,47), 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 in fact, spore deposition declined rapidly and stopped before the first snowfall. Moreover, in California, spore production has been reported even in snowy conditions (19).

The rare fact that all three European species are present at our study sites (19) allow for a direct comparison amongst them. Our extended sampling at Aymavilles indicated that the number 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 both in terms of absolute number of spores and, in terms of 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 to sporulate even when found in the same forest. The ecological implications of these findings are numerous and deserve further studies. Nevertheless our results provide valuable information that could be used to design silvicultural guidelines aimed at minimizing the risk of infection of new trees by this pathogen. Based on our study, thinning

operations in spring rather than in summer should result in a significant drop in infection of stumps of Norway spruce by *H. parviporum*. In the spring, in fact, not only the DR values are significantly lower than in other seasons, but the relative abundance of *H. parviporum* is also lower when compared to 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 were stump treatments are not allowed or economical.

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TABLE 1. Site locations and main stand characteristics

Location	Lat-Long coordinates	Exposure	Elevation (m a.s.l.)	Mean annual rainfall (mm)	Tree host species and composition (%)	Incidence of the disease	Forest structure
1- Chiusa Pesio (Cuneo)	44°12'46.78''N -4°47'36.63''W (Roma 40)	N-W	1042	1445	*A. alba* (100%)	50%	uneven-aged by groups
2- Jovençan (Aosta)	45°42'19.48''N 7°17'1.77''W (Heyford ED50)	N	1090	700	*A. alba* (95%) *P. abies* (5%)	50%	Irregular to uneven-aged by groups
3- Charvensod (Aosta)	45°41'40.93''N 7°19'35.92''W (Heyford ED50)	N-N-W	1780	700	*P. abies* (100%)	30%	Irregular to uneven-aged by groups
4- Aymavilles (Aosta)	45°41'25.88''N 7°15'30.48''W (Heyford ED50)	N-N-W	1475	700	*A. alba* (50%) *P. abies* (40%) *P. sylvestris* (10%)	50%	Irregular

TABLE 2. Correlation of monthly patterns of spore deposition of *Heterobasidion* spp. among the four stands in 1999-2000

		Pearson correlation coefficients[a]					
Stands		**Jovençan**		**Charvensod**		**Aymavilles**	
			N		**N**		**N**
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 * = significant at *P*= 0.01 and 0.05, respectively; [NS] = not significant

[b] Deposition Rates; for analysis, values were transformed with the Ln(N+1) transformation

[c] Infection Frequency; for analysis, values were transformed with the arcsine transformation

TABLE 3. Correlation coefficients of monthly deposition rates (DR) of spores of *Heterobasidion* spp. with weather variables recorded daily for each of the 2-, 7-days, and 4-weeks periods preceding the date of spore collection at Aymavilles, from March 1999 to December 2000. Average values were used for T and RH, and cumulative values were employed for RF

	Correlation coefficients[a]		
	2 days preceding	**7 days preceding**	**4 weeks preceding**
mean temperature [°C]	0.425^{NS}	0.551*	0.632**
minimum temperature [°C]	0.388^{NS}	0.465^{NS}	0.654**
maximum temperature [°C]	0.412^{NS}	0.425^{NS}	0.450^{NS}
mean relative humidity [%]	0.321^{NS}	0.428^{NS}	0.245^{NS}
minimum relative humidity[%]	0.080^{NS}	0.196^{NS}	0.393^{NS}
maximum relative humidity [%]	0.513*	0.479^{NS}	0.009^{NS}
rainfall [mm]	0.042^{NS}	0.136^{NS}	0.302^{NS}

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

Figure captions

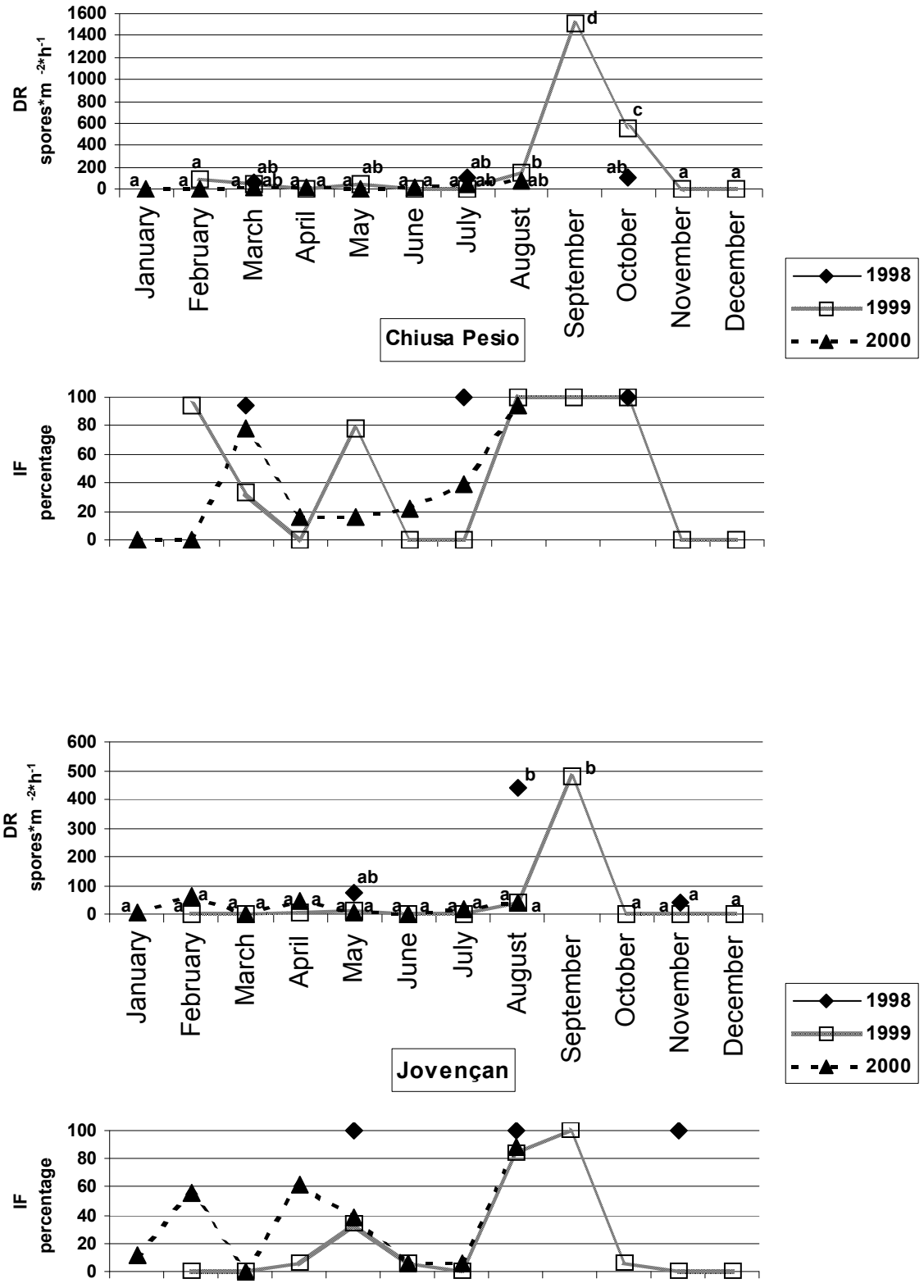
Fig. 1. Seasonal and monthly variation of spore deposition of *Heterobasidion* spp. over the years 1998 to 2000 in four stands in the Alps. Deposition rates (DR) are expressed as the mean number of spores per square meter per h, and infection frequencies (IF) represent the percentage of disks infected by spores of the pathogen. Values were calculated on the basis of spores trapped daily once a season until February 1999, and each month since March 1999 (details in the text). DR values were compared within each stand by the ANOVA Tukey HSD test ($P \leq 0.05$); the letters a, b, c, and d following means refer to the different groups that could be distinguished between means by this test.

Fig. 2. Regression analysis between Deposition Rates [Ln(N+1)] and Infection Frequencies (arcsine transformation of the percentage of infected disks) of spores of *Heterobasidion* spp. trapped seasonally and monthly from 1998 to 2000 in four Alpine forests. The regression curve was built on overall data ($R^2 = 0.875$; $P < 0.001$).

Fig. 3. Frequency of spores of the three species of *Heterobasidion* in three forests in different time periods. Samplings were performed in 1998 and 1999. The abundance of spores was compared by the Mann-Whitney *U* test; columns labeled with the same letter are not significantly different for $P \leq 0.05$.

Fig. 4. Frequency of spores of the three species of *Heterobasidion* at different periods at Aymavilles. Samplings were performed over the years 1998-2000. The abundance of spores was compared by the Mann-Whitney *U* test; columns marked by the same letter are not significantly different for $P \leq 0.05$. Labels are: Ja= January, Fe= February, Ma= March, Ap= April, My= May, Ju= June, Jl= July, Au= August, Se= September, Oc= October, No= November, De= December.

Fig. 1, Gonthier, *Phytopathology*



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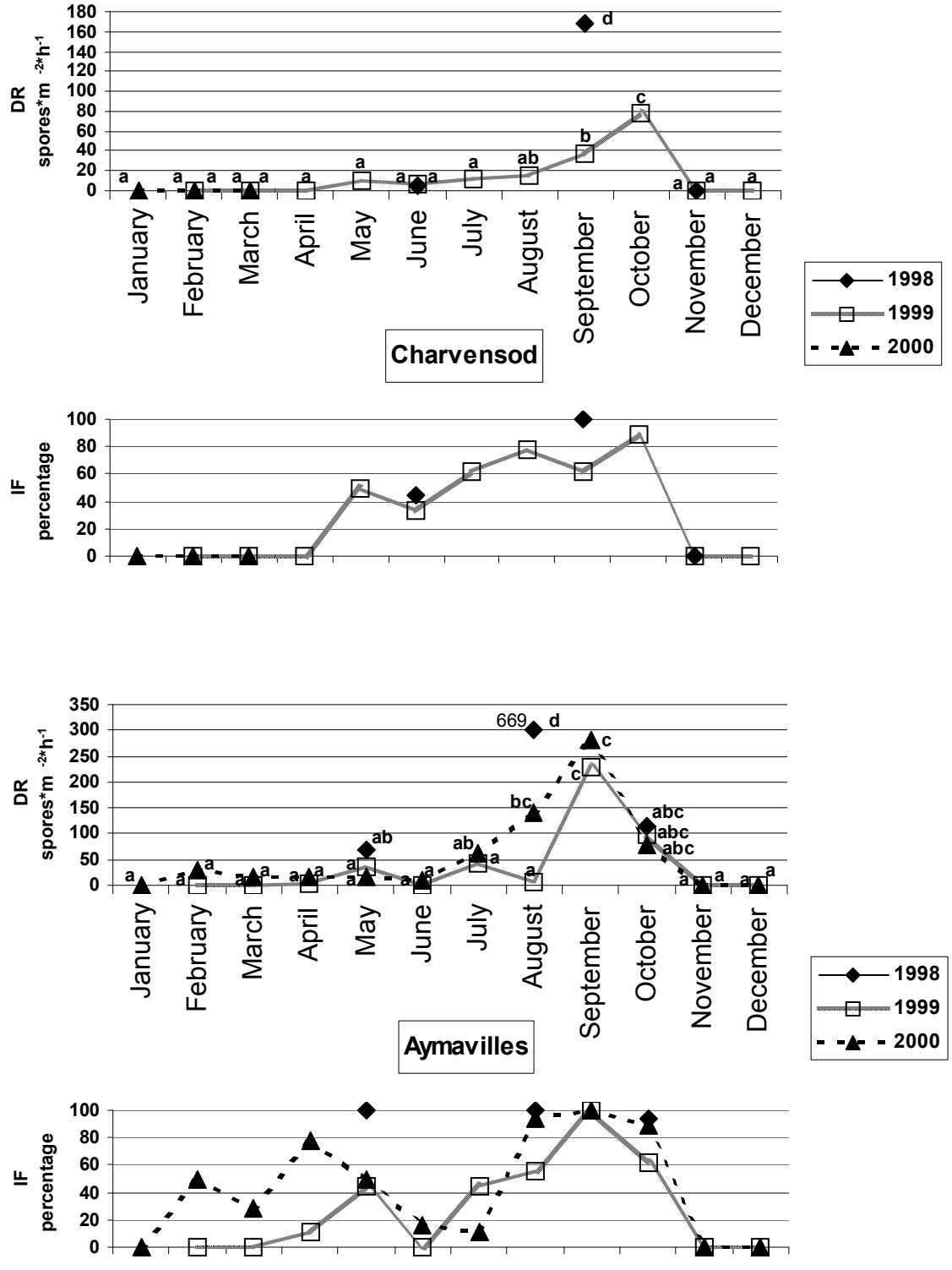

Fig. 2, Gonthier, *Phytopathology*

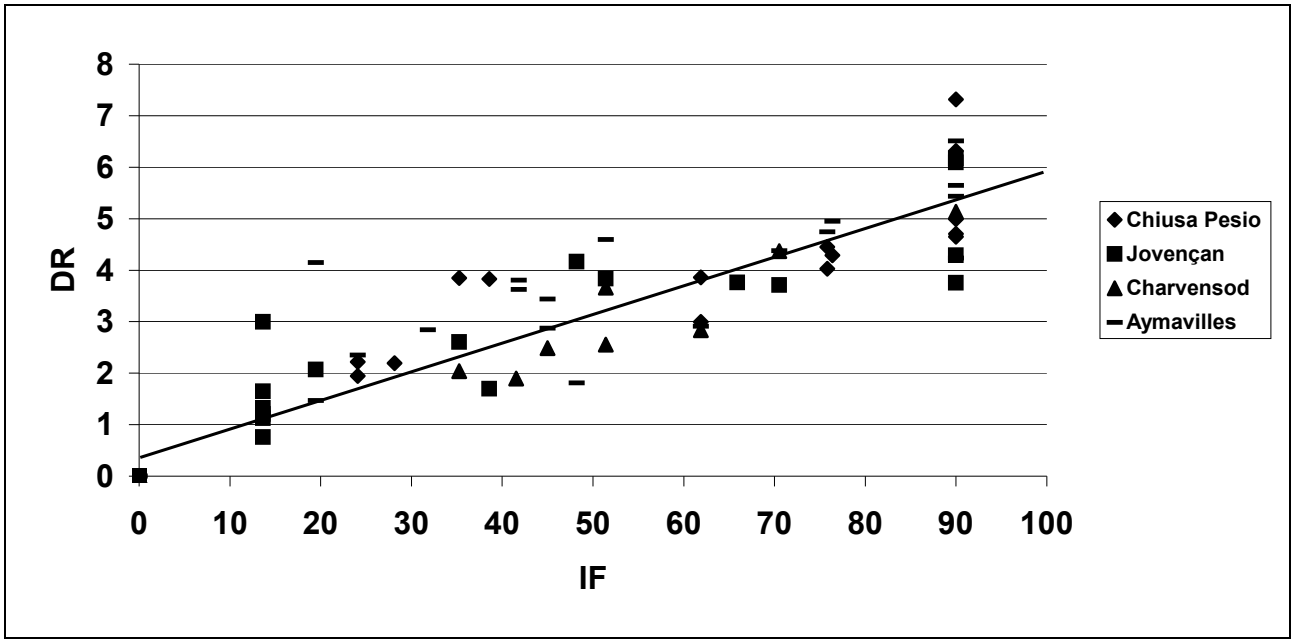

Fig. 3, Gonthier, *Phytopathology*

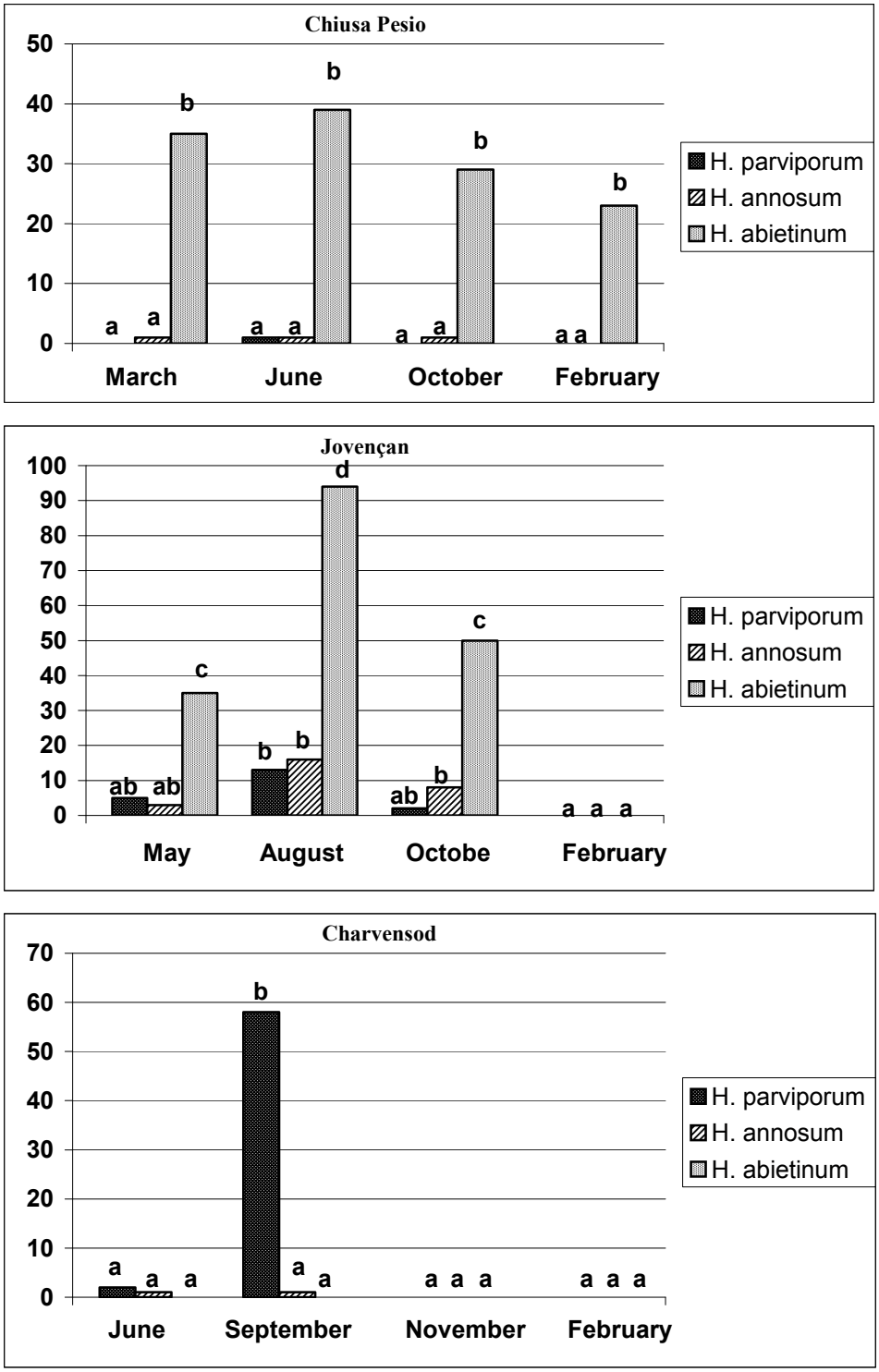

Fig. 4, Gonthier, *Phytopathology*