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THE AEROBIOLOGY OF THE ASCOSPORES

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Atmospheric ascospores have been monitored using volumetric spore trap. Spore concentration data were analysed using Spearman's correlation. Our results show that the meteorological factor with the greatest effect on spore concentration was the duration of rain. Temperature increase strongly reduced the ascospore concentration; but the length of windless periods resulted in an increase in spore count. The only measurable effect wind perse actually had on spore count, was registered when a strong wind blew after a long windless period. We observed that the count of ascospores during wet weather could surpass the total concentration of dry conidia measured on a typical, highly polluted summer day. Using selected air samples to study the effect of storms, certain aspects of long-distance spore transport were elucidated. We describe here three main strategies for long-range ascospore transport, "splash-off", "secondary emission" and "sporematrix projectiles".

Keywords: ascospore, airborne, biometeorology, long-range transport, wet weather spore

Introduction

Ascospore aerobiology is one of the least studied fields of aeromycology [1–5]. The life cycles and ecology of the fungi involved is more or less known; but there is little information on the spore transmission phase. For studying this phase taking place in the air, air capturing is the chosen adequate method. Of particular interest are the effects of meteorological events on the floating spores, including their interaction with other airborne particles, as well as on the types of primary and secondary emission and sedimentation.

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Materials and methods

Data on the daily concentration of airborne ascospores were obtained using a Burkard 7-day volumetric pollen trap and a Lanzoni VPPS 2000 Hirst-type air sampler with 10 l/min air respiration capacity. Samplers had been located at heights of 12 to 30 m above ground level (where the trap catches aeroparticles from an average 70 km radius) in the months May and June for five years (altogether 261 days had been monitored). Two longitudinal transverses along the length of the silicone-coated slides were scanned with a 100× objective of a DIALUX 20 microscope. We registered the number of spores/m^3 air of three ascomycete spore types (*Pleospora*, *Leptosphaeria*, *Lophiostoma*) [6, 7], and the relative concentrations of another two ascomycete spore types (*Ophiobolus* and fusiform spore group). In the morphological studies we used the Leica QWin Image Analyser Programme. We applied the Spearman's Correlation Analysis to examine the relations between changes of registered airborne fungal spore concentration and the following meteorological factors: wind speed – "W" [m/s], atmospheric pressure [MPa], "Tmin", "Tmax", "Tavg" minimum, maximum and average temperatures – [C^o], precipitation "P" [mm], rainfall intensity [mm/s], duration of precipitation [min], cloud cover [%], solar radiation – [Cal], relative humidity – "RH" [%], presence of fog and mist, daily temperature change – "Td" [C^o], number of sunny hours "S" [min], evaporation Piche/Wild. [mm] and windstorms [m/s]. The meteorological data were collected automatically, near the air samplers. Factors "RH", "W", "S", and "P" were generated from the meteorological data using 15-day time series (e.g. precipitation in the previous 9 days = "$P_{d\text{-}9}$"). To analyse the aeromycobiota diversity at the start of the precipitation, we compared the spore counts with the number of mycoparticle types, using Margalet's Biodiversity Index (Fig. 1) [8].

$$\frac{S-1}{\log N}$$

S = number of mycoparticle types
N = total concentration of mycoparticle

Fig 1. Margalet's Biodiversity Index

Results

Ascospore biometeorology

The appearance of normal wet weather aeromycobiota (WWS) correlated with high relative humidity meteorological events and included the presence in the air of ascomycete spores along with other fungal taxa (*Sphaeropsidales, Melanconiales, Endomycetales, Basidiomycetes*). The duration of rain displayed the strongest effect (Fig. 2) on the increase of spore count (Spearman's correlation coefficient = 341, strongest effect in the case of *Lophiostoma*). This effect was even stronger than that of rainfall intensity (0.303, strongest effect in the case of *Lophiostoma*), and high relative humidity (0.205, strongest effect in the case of *Lophiostoma*). Following a third consecutive day of rainy weather, spore emission decreased. Temperature increase strongly reduced the ascospore concentration (0.336, strongest effect in the case of *Leptosphaeria*). The only measurable effect wind actually had on spore count was registered when a strong wind blew after a long windless period (0.173, only in the case of *Pleospora*). Spore count increased with the length of the windless period (0.173, only in the case of *Pleospora*). Length of drought and number of sunny hours had an unfavourable effect on sporulation (0.227 and 0.171, only in the case of *Lophiostoma*). A high number of sunny hours, one to two weeks before spore emission, had a positive effect on the daily ascospore count (0.213, only in the case of *Leptosphaeria*). Statistical results are shown in Table I.

Table I

Descriptive statistics on the concentration of three airborne ascospore taxa collected above a vineyard [Spore/m^3 air]

Taxa/Spore cc.	*Pleospora*	*Leptosphaeria*	*Lophiostoma*
average	8.97	1.45	0.5
standard deviation	59.6	7.33	3.36
maximum	697	77	39

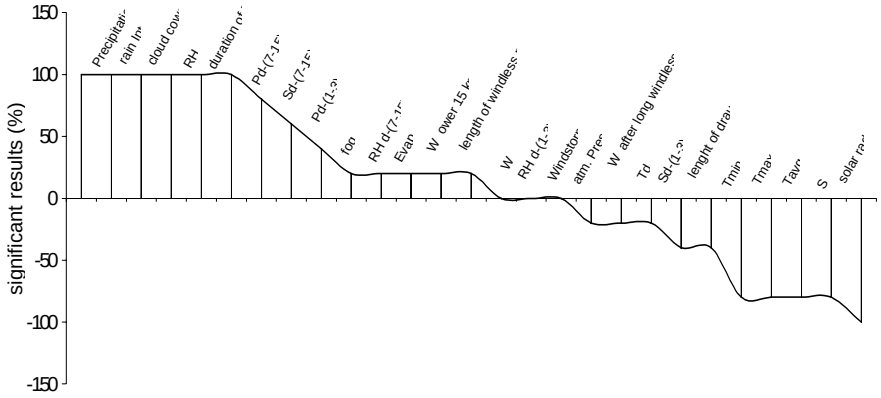

Fig. 2. Effect of various meteorological factors on the concentration of the airborne ascospores (100% means positive effect on all the 5 monitored taxa)

Changes in the aeromycobiota

Applying Margalet's Biodiversity Index (Fig. 1) to the aeromycobiota, the diversity of WWS proved to be seven times higher than that of Dry Weather Spores (DWS).

Sporematrices in the atmosphere

Ascospores tend to stick together. Sometimes sporeoctads and -hexads (sporematrix) were observed in air captured in upper air layers at sampling heights from10 to 30 m above ground level.

Splash droplets in the atmosphere

Intensive precipitation produces large raindrops (250–3500 μm diam.) that contain numerous mycoparticles (mycop.) in summer and early autumn, mainly wet weather conidia, basidio- and ascospores (500–1000 mycop./droplet, cca. 250,000–500,000 mycop./m^3 air). Droplets produced solid spore halos after drying on the silicone-coated slides of air samplers (Table II). During wet meteorological events, mainly storms, the splash-borne number of WWS would be extremely high (250,000–500,000 mycop./m^3), surpassing even the concentration of conidia on a highly polluted dry summer day (100,000–300,000mycop./m^3); however this extreme concentration lasts only one to two hours in a few days only within a season.

Table II

Concentration of ascospores in 74 splash droplets captured in the upper air layers

Spore cc/Taxa	max	St. dev.	Avg.	Spring avg.	Summer avg.
Leptosphaeria	24	4.11	1.43	0.24	2.43
Xilariaceae	25	3.75	1.30	0.08	2.32
Dyatripaceae	22	3.14	0.80	0.00	1.48
Pleospora	7	1.60	0.68	0.38	0.93
Lophiostoma	6	1.24	0.41	0.00	0.75
Chaetomium	7	0.88	0.17	0.32	0.05
Ophiobolus	4	0.52	0.11	0.00	0.20
Paraphaeosphaeria	2	0.35	0.07	0.05	0.09
Aglaospora	1	0.11	0.01	0.00	0.02

Discussion

Ascospore biometeorology

At the start of the rain, DWS disappears and the mycobiota changes into a mass of ascospores, that float in the atmosphere, until they are blown away or sedimented [9,10]. Following a long windless period, an incoming windstorm can blow away the concentrated airspore floating still in the air, decreasing the number of fungal colonies (diluent effect).

Mycobiota of splash droplets in the upper air layer

In our opinion, the source of the large droplets observed in traps is probably not the direct rainfall; but rather the thin water film on the surface of the soil. Falling rainwater splashing in the water film produces secondary droplets that splash to an average height of 0.5 meter (with a maximum height of 1 meter) [11]. Another possible source of the droplets may be the phylloplane mycobiota and its “wet shake-off” mechanism in which secondary emission is facilitated by twig interception. Because of the mucilaginous and sticky surface of the ascospores, they glue to barrier surfaces such as the foliage of trees, one of the best natural air purification systems [12]. One tree may reduce the aeroparticle amount by 66 to 80% [13]. Humidity increases the leaves’ filtration capacity. This is particularly true of sticky, hairy or unclean leaf surfaces [14, 15]. The living ascospore remains captured on the foliage and can only be

released during rainy events ("secondary emission"). This washing off mechanism itself is not part of the process of forming these large droplets caused by the rapid sweep-out of the DWS at the start of the precipitation [9].

Our observations show that ascospore transmission is very poor in mild wind conditions and after or during moderate rainfall; however by strong wind and large droplets these same ascospores may be transported for long distances.

Long-distance transmission by sporematrices

Sporematrices (or large spores) may move longer distances, as Ingold mentions [11], in accordance with the laws of aerodynamics. Ascospores are normally discharged to a distance of 1 cm or more, to 6 to 8 cm in hexads or octads; but *Podospora fimicola* projects its spores in octads to a distance of 50 cm. If the ascomycetes would not discharge their spores higher than the laminar boundary layer, the spores would not be blown away by the turbulent air layer. By different techniques (bipolar asymmetry of the spores, forming "giant" spores, sporaoctads, or long-necked ostiola), ascomycetes increase their shoot to overpass the 2 mm thick still air layer [11, 16]. Our samples captured in the upper air layer contained sporematrices which indicate that – contrary to the traditional theory [11] – the discharged sporeoctads or hexads are able to remain glued together also during aerial transport. We found this phenomenon to be of extreme significance in long-range aerial transport.

Our results indicate that allergy symptoms, reported after/during storms, may be caused by the high concentration of WWS, containing a significant number of ascomycetes. *Pleosporales*, the most frequent airborne ascospore taxon, was reported to be a respiratory allergen [17].

Heavy rain captures mycoparticles that play a major role in the local dispersal of WWS [18–20]; and our results indicate that it probably is one of the major reasons of greater epidemics. Splash-off droplets with ascospores may have been blown far away during windstorms in 1999, a particularly rainy year during which we registered 27 days with extra high WWS concentration ([21] over 13,000 mycop./m^3). That number was 15 times higher (and the yearly WWS concentration was 18 times higher) than the average of the three previous years. The main factor in the first appearance of *Pyllachora* and the later epidemic caused by it in 1999 was probably the high number of heavy rains [22].

The splash-off mechanism may cause aerial epidemics through long-range transport by heavy rain, splash-off, and wet-shake off as secondary emission from the phylloplane (Fig. 3).

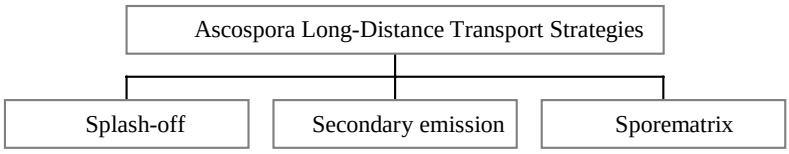

Fig. 3. Long-distance transport strategies of the ascospores

The current dogma holds that conidia are the main factors of long-distance plant disease epidemics [23]. We suggest that in wet meteorological events, mainly during storms, when WWS number should be very high, the effect of ascospore on long-range epidemics is as important as that of the conidia in dry weather.

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