

## Nocturnal Fungi: Airborne Spores in the Canopy and Understory of a Tropical Rain Forest<sup>1</sup>

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### ABSTRACT

Pathogens and other symbiotic fungi that infect above-ground plant parts commonly disperse as airborne spores. Here we present diel patterns of the density of airborne fungal spores in the canopy and understory of a tropical rain forest. Spores were 52-fold more abundant in the understory than in the canopy. Additionally, spores were 5- to 35-fold more abundant at night than during the day, associated with environmental conditions conducive to germination and plant infection.

### RESUMEN

Los hongos patógenos y otros hongos simbióticos que colonizan las hojas y tallos de plantas se dispersan comunmente como esporas en el aire. Aquí presentamos patrones diarios de la densidad de esporas de hongos en el aire en el dosel y en el sotobosque de un bosque tropical lluvioso. Las esporas son 52 veces más abundantes en el sotobosque que en el dosel. Además, las esporas fueron 5- a 35-veces más abundantes en la noche que durante el día, con la mayor abundancia de esporas asociada con las condiciones ambientales adecuadas para la germinación e infección de plantas.

*Key words:* Airborne fungi; Australia; canopy; fungal spores; Queensland; tropical rain forest.

TROPICAL ECOLOGISTS ARE ACCUSTOMED TO ORGANISMS ACTIVE during different times of the day—the raucous chatter of birds in the early morning, frog choruses in the evening, diurnal CO<sub>2</sub> acquisition for C<sub>3</sub> plants, and nocturnal acquisition for those with CAM photosynthesis, crepuscular mosquitoes, and prowling nocturnal predators. Like big cats, tropical fungi may lead a largely nocturnal life. Here we present quantitative diel patterns for airborne fungal spores in the canopy and understory of a lowland tropical rain forest.

We measured the concentration of airborne fungal spores during the early rainy season in the canopy (24 m above ground near the top of an *Acmena graveolens* (Myrtaceae) tree) and below it in the understory (1.5 m) of the tropical rain forest at the Australian Canopy Crane Research Facility, Cape Tribulation, Queensland, Australia (16°06'S, 145°27' E; alt. 31–55 m). The site is in the Wet Tropics World Heritage Area, and includes complex mesophyll vine forest with an irregular canopy from 15 to 33 m tall. The site averages 3600 mm rain annually, with 70 percent usually falling between December and April. See Stork and Cermak (2003) for a detailed description of the site and facilities.

We captured airborne fungal spores onto petroleum jelly-coated microscope slides using two Alergenco MK-3 volumetric air samplers (Alergenco, San Antonio, TX). The samplers automat-

ically captured spores for 10-min periods at either half-hour or hour intervals. The captured spores were quantified by staining slides with augmented Calberla's solution, then counting all fungal spores in four fields of view at 400 or 1000× (Olympus CH-2 microscope, Olympus Inc., Melville, NY), depending on spore density. Fields of view were chosen haphazardly within a spore trace by selecting the spot to sample while looking directly at the slide on the microscope stage, rather than through the lenses. Each field of view at 400× covered 0.1616 mm<sup>2</sup>, and 0.0259 mm<sup>2</sup> at 1000×. The spore sampler has a flow rate of 0.015 m<sup>3</sup>/min, and a total trace area of 15.95 mm<sup>2</sup>. Therefore, the total number of spores counted in the four fields of view at 400×, divided by 0.00608 provides the number of spores/m<sup>3</sup> air. We did not differentiate between the different species of fungi, but there was a great diversity of spore morphologies.

To measure corresponding daily patterns for microclimate in the canopy and the understory, we deployed three Hobo relative humidity and temperature sensors (Onset Computer, Inc., Bourne, MA) in the top of the canopy (at 25, 27, and 30 m; canopy openness at those sites was 71, 74, and 74%, determined with CID-110 Canopy Analyzer, CID Inc., Camas, WA), and three sensors at 1.5 m above the ground (canopy openness 19, 25, and 27%) immediately below each canopy sensor. Sensors were deployed from 25 to 31 January 2002 in those positions, data were collected every 10 min, and we present the mean values of the three sensors. However, after

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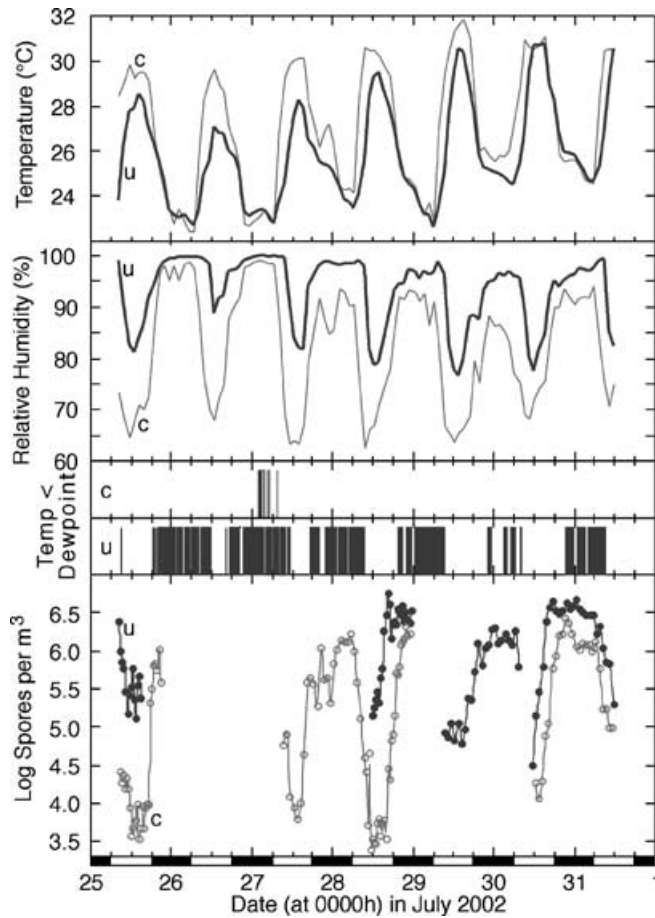


FIGURE 1. Diel patterns for fungal spores in the air in the canopy (c, fine line) and the understory (u, dark line) of the forest at Cape Tribulation, Queensland, Australia, and for temperature, relative humidity, and periods when the temperature fell below dew point temperature. Open and dark bars at bottom indicate times of daylight and darkness.

1030 h on 30 January, only one sensor (the middle in each list above) was used in each stratum.

Fungal spore densities showed striking spatial and temporal patterns. First, spore density was consistently much greater in the understory than in the canopy (Fig. 1). For all canopy–understory sample pairs taken within 10 min ( $N = 60$  pairs), there was a mean of  $6.06 \pm \text{SD } 0.51 \log_{10}(\text{spores})/\text{m}^3$  in the understory and  $4.95 \pm 1.02 \log_{10}(\text{spores})/\text{m}^3$  in the canopy (paired  $t = 11.9$ ,  $P \leq 0.0001$ ). On average, there were 52-fold more spores in the understory than in the canopy (range 1.3–125 $\times$ ). Greater spore abundance near the ground may reflect a greater abundance of fungi in the understory (G. S. Gilbert and D. R. Reynolds, unpublished data), the contribution of fungi active in litter decomposition, as well as better environmental conditions for spore production.

Second, within each forest stratum, spore density showed strong daily patterns, increasing sharply around sunset, remaining high throughout the night, and then declining sharply shortly

after sunrise (Fig. 1). In the understory, there was an average of  $6.36 \pm 0.23 \log_{10}(\text{spores})/\text{m}^3$  at night (1800–0600 h) compared to only  $5.6 \pm 0.8 \log_{10}(\text{spores})/\text{m}^3$  (unequal variance  $t$ -test,  $t = 8.21$ ,  $P \leq 0.0001$ ). In the canopy, there was an average of  $5.91 \pm 0.34 \log_{10}(\text{spores})/\text{m}^3$  during at night compared to  $4.37 \pm 0.73 \log_{10}(\text{spores})/\text{m}^3$  during the day ( $t = 14.3$ ,  $P \leq 0.0001$ ). These differences correspond to a 5.7-fold greater abundance of spores at night in the understory and a 34.7-fold greater abundance in the canopy. In agricultural systems, many pathogenic fungi have been shown to have periodic spore release. For a number of species, few spores are found in the air at night, with a sharp increase in spore release in the early morning after a moist night during which spores are produced (Langenberg *et al.* 1977, Couture & Sutton 1978). Other plant pathogenic fungal species release most spores later in the day (Leach *et al.* 1977, Raynal 1990, Carisse & Pillion 2002), in the late afternoon (Hock *et al.* 1995), or at night (Warner & Braun 1992, Stensvand *et al.* 1998, Fernando *et al.* 2000). In studies over sugar cane fields, Bhagawan and Pande (1988) found that fungal species differed in whether they produced spores primarily at night or in the early morning. Variation in periodicity likely reflects how different fungal species respond to environmental conditions for spore production, different mechanisms of spore release, and different environmental regimes among habitat types.

The spatial and temporal patterns observed in this tropical forest are consistent with fungal spores being produced and dispersed under those conditions generally most adequate for spore germination and plant infection—in particular, high relative humidity and cooler temperatures (Fig. 1). In particular, extended periods of free water on leaf surfaces from dew formation or remaining following rains is often essential for successful germination and leaf penetration (Bradley *et al.* 2003, and references within). Although we did not directly measure dew formation, air temperatures in the understory were cool enough for dew formation during most evening hours, but only for a few hours on one night in the canopy. Because nighttime leaf temperatures are often lower than ambient air temperature (Cooper 1966), conditions appropriate for dew formation, particularly in the understory, may be even more common than indicated by our data.

We know of only three previous estimates of patterns of abundance of aerial fungal spores in tropical forests. In seasonally moist tropical forest at Ft. Sherman, Panama, Hutton and Rasmussen (1970) measured air spore abundance in the forest understory or above the canopy, at different times of the day (1200, 1600, and 1900 h), and in wet or dry seasons. They placed sterile Petri plates inside horizontal 13-cm diameter tubes and exposed them for 1 h, overlaid the exposed plates with growth media, and counted developing fungal colonies. They used the same methods for a single mid-day sample in the canopy and understory in the forest at El Verde, Puerto Rico. Their findings are broadly similar to ours (recalculating from their Tables 1 and 3). In Panama, in both wet and dry seasons they found 1.03- to 23.7-fold more fungal spores in understory air than above the canopy. In Puerto Rico, they captured 2-fold more spores in the understory than in the canopy. During the wet season they also found about 1.7-fold more spores at night (1900 h) than during the day (1200 h), but that temporal pattern

was reversed in the dry season, with 1.3- to 5-fold more spores during the day.

More recently, on Barro Colorado Island in Panama, Gilbert (2002) and Arnold and Herre (2003) exposed sterile Petri dishes with fungal growth media to the understory air for short periods and counted the number of colonies that grew. Gilbert estimated  $9.3 \pm 3.3$  colony-forming units (CFU)  $\text{cm}^2/\text{h}$  (Gilbert 2002). Arnold and Herre (2003) found similar spore densities, and noted that sporefall was 5-fold greater in the forest understory (about 15 CFU/ $\text{cm}^2/\text{h}$ ) than in a forest clearing (3 CFU  $\text{cm}^2/\text{h}$ ). The difference between understory and forest clearing, as well as the present finding of strong differences between the canopy and understory separated by less than 30 m, indicate strong gradients in spore abundance and suggest that fungi are likely dispersal limited in tropical forests. Studies of individual fungal species are needed to evaluate the scale of dispersal limitation for fungi with different spore dispersal mechanisms.

Assuming that sporefall was constant throughout the day and night, a 100  $\text{cm}^2$  leaf in the understory would then encounter >36,000 spores per day (Arnold & Herre 2003). Our findings of a strong 5- to 35-fold greater abundance of spores at night than during the day suggest that these previous attempts might be significant underestimates of the true rate of encounters, and that tropical mycologists, like mammalogists, may need to adopt nocturnal habits.

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