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SPORE PRODUCTIVITY IN CLADOSPORIUM

by

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ABSTRACT

The high incidence of *Cladosporia* in the airspora indicates a prolific production of spores. Six species of *Cladosporium* were sampled over a period of 9 weeks, using dry and wet (mist-laden) air, and over a period of 4 weeks using humid air. Many more spores were released in wet air than in dry air: numbers released in humid air were generally intermediate between those of wet and dry samples. None of the cultures was exhausted of spores at the end of the sampling periods although samples generally decreased in size from the fifth or sixth week onwards. Removal of spores would seem to be conducive to further sporulation provided the substrate is not exhausted. Maximum productivities recorded for the six species (all in mist-laden air) ranged from 730 to 26 100 spores per mg dry weight of mycelium. Differences in the levels of spore production in culture by the six species do not correlate with their individual frequencies in the airspora, indicating that the latter are more dependent on the distribution and substrate relationships of each species.

There are numerous estimates of the level of spore output by fungi. These refer mainly to the more spectacular feats of spore production by the larger basidiomycetes (BULLER, 1909; WHITE, 1919). Others relate to plant pathogens such as *Tilletia caries* and wheat rust (STAKMAN & CHRISTENSEN, 1946) and *Sclerotinia sclerotiorum* (STEVENS, 1911). Estimates of spore productivity by fungi in culture appear to be much less numerous. INGOLD (1960) estimated the production of some 400 million conidia by a colony of *Penicillium*, 2.5 cm in diameter.

Such estimates clearly indicate a direct relationship between size of fruit and level of spore output, i.e. the larger basidiomycete fruits generally produce the greatest numbers of spores. Smaller ascomycete fruits produce fewer spores so that in certain cases it is possible, with fair accuracy, to estimate the total spore product of a single perithecium (WALKEY & HARVEY, 1967).

Analysis shows that although basidiospores and ascospores are prominent components of the airspora, the spores of certain saprophytic moulds are even more numerous. Surveys have established the predominance of the genus *Cladosporium* in the air, and culture plate isolations have shown that four species, *C. herbarum*, *C. cladosporioides*, *C. sphaerospermum* and *C. macrocarpum*, occur much

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more frequently than two other species, *C. elatum* and *C. resinae* (HARVEY, 1967). In particular, the very high incidence of *C. herbarum* in the air would seem to indicate an extremely prolific production and efficient release of spores.

In assessing the specific characteristics which contribute to the differing frequencies of these species in the airspora an attempt has been made to determine their individual levels of spore production in culture in relation to unit dry weight of mycelium.

The six species were grown in standardized cultures and their spores were harvested at regular intervals during the development of the cultures. It has been shown (DAVIS, 1959; HARVEY, 1967) that the release of *Cladosporium* spores into the air is much influenced by the presence or absence of mist or rain droplets in the air passing over the culture. As a further investigation of this feature three series of experiments were carried out in which cultures were sampled using dry, wet (mist-laden) or humid air for spore collection. The sampling extended over a period of nine weeks in the former two series (dry and wet air sampling) and for four weeks using humid air.

MATERIALS AND METHODS

a) Cultures

The six species of *Cladosporium* were grown on corn meal agar in tube cultures, using 20 cm lengths of 1.6 cm diameter tubing, open at both ends. Dry and wet air harvesting began on the fifth day after inoculation and was repeated four or five times on successive days in each of the following nine weeks. Humid air harvesting was delayed until the sixth week after inoculation so that the cultures used in this series of experiments experienced a protracted period of undisturbed growth.

b) Sampling or harvesting

This was carried out by sucking air through the culture tube, over the colony, into a slit sampler where the spores impinged on a plate of corn meal agar rotating once in 30 seconds immediately beneath the slit of the sampler. Sampling was at the rate of 28 l/min so that 14 litres of air passed through the tube on each occasion. The rate of air flow (approx. 4.7 m/s) must have decreased fractionally during later periods of sampling as the agar in the culture tubes dried and contracted. This was most noticeable in the tubes sampled with dry air.

In dry sampling the air was drawn through the culture tube directly from the surrounding air of the laboratory. In wet sampling the air was charged with mist droplets, using an atomiser, and in humid sampling the air was drawn through a series of wetting columns. During periods of incubation the culture tubes were closed at both ends by means of cotton wool plugs.

c) Counting

Culture plates used in the slit sampler were stored at room temperature and resultant colonies counted after 4 or 5 days. Where dispersion was light and widespread all the colonies were counted but in heavier, more crowded samples representative sectors were counted. The counts thus obtained were of dispersion units rather than single spores. Previous investigations have shown that in the airspora generally the average number of spores per dispersion unit of *Cladosporium* varies between 1.16 and 1.34, but that when samples are taken near the area of spore release (as in these experiments) then the dispersion units will tend to be significantly larger.

Average dispersion unit sizes were estimated for each species in the dry and wet series of experiments. At the end of the period of sampling the dry weight of individual cultures was determined and the spore products are recorded in terms of unit dry weight of the parent mycelium.

Results

The results of the three series of experiments (Table I) show that the number of spores released in wet air was very much greater than in dry air. This confirms the influence of mist droplets in effectively releasing larger numbers of spores, almost invariably in the form of larger dispersion units. Other, probably more important, effects of using wet air are considered in greater detail below.

Table I Spore release by Species of *Cladosporium* in dry, wet and humid air.

	C. herbarum	*C. cladosporioides*	*C. sphaerospermum*	*C. macrocarpum*	*C. resinae*	*C. elatum*
			DRY AIR			
Dispersion units	13030	7500	8120	2970	1960	720
Average size of dispersion units	1.77	1.6	1.4	1.0	1.68	1.18
Culture dry wt. (mg)	20.2	21.2	26.5	8.8	2.9	22.3
Estimated spores/mg	1140	570	430	340	1130	38
			WET AIR			
Dispersion units	106600	127300	84600	31100	11900	33700
Average size of dispersion units	2.47	2.24	6.05	1.8	2.68	4.48
Culture dry wt. (mg)	20.5	10.9	21.8	14.5	10.4	20.6
Estimated spores/mg	12900	26100	23500	3900	3070	730
			HUMID AIR			
Dispersion units	10630	11270	34500	1530	2970	1040
Culture dry wt. (mg)	56.1	45.4	30.3	16.9	14.0	12.9

Productivity in the humid air experiments cannot be compared directly, as in the dry and wet series, because of the differences in initial periods of incubation and the number of subsequent samples.

Estimates of total spore production in the 9 successive weeks (4 for humid series) of sampling show (Fig. 1) that, although there was a general tendency towards reduced productivity after 5 weeks, in none of the experiments were the cultures completely exhausted of spores. Thus the recorded spore products do not represent the full potential of the individual species and no direct comparison can be made with INGOLD's (1960) estimate for a colony of *Penicillium*.

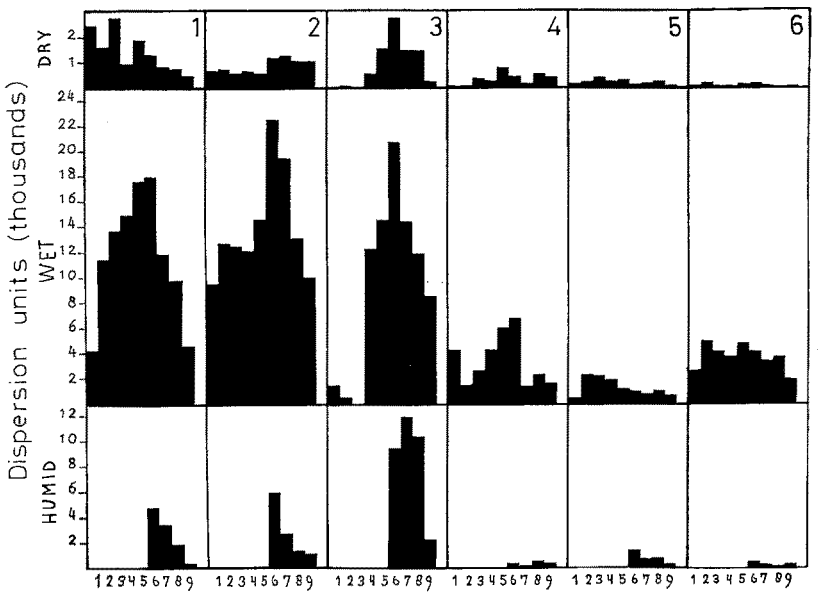

Fig. 1. Spore release by species of *Cladosporium* over 9-week sampling period in dry and wet (mist-laden) air and over 4-week period in humid air. Species 1: *C. herbarum*; 2: *C. cladosporioides*; 3: *C. sphaerospermum*; 4: *C. macrocarpum*; 5: *C. resinae*; 6: *C. elatum*.

DISCUSSION

Percentage frequencies for the incidence of the principal species of *Cladosporium* in the airspora at Cardiff (Table II) would seem

TABLE II Percentage frequencies of the principal species of *Cladosporium* in the airspora at Cardiff

	C. herbarum	*C. cladosporioides*	*C. sphaerospermum*	*C. macrocarpum*	*C. resinae*	*C. elatum*
1960—64	63.8	20.8	6.4	8.0	0.4	0.4
1966	72.0	8.9	14.8	3.0	0.7	0.5

to indicate the possibility that some species are able to maintain much higher levels of spore production than others. This applies particularly to *C. herbarum* which predominates in all analyses of the incidence of *Cladosporium* species in the air.

The results of the dry air experiments lend support to this suggestion, with *C. herbarum* producing at least twice as many spores per unit dry weight as any of the other species, except *C. resinae*. The disparity in frequency between *C. herbarum* and other species reaches its peak in the summer months when it dominates the spore content of the air as a typical member of the dry airspora. However the numbers of spores produced by individual species both in dry and in wet air do not compare in any way with the percentage frequencies of those species in the airspora.

The wet air experiments resulted in much higher levels of spore release than in dry air but culture weights were not generally increased as a result of the repeated wetting received by such cultures. Thus, in each species, the estimated spore product per unit dry weight is much greater in wet than in dry air. One obvious explanation of this is the visible delay in the drying out of the agar in the culture tubes as compared with that in the dry air tubes. The fact that more spores are released in wet air, indicating much higher levels of spore production than in dry air, can be interpreted as the result of more prolonged metabolic activity, the products of which are used in sporulation rather than mycelial development. However, at an early stage in all experiments the colonies covered practically the whole of the surface area of the agar, so that under less restricted cultural conditions further mycelial growth would have diverted resources from the process of sporulation, with corresponding effects upon the level of spore production. To a very limited extent the lower levels of spore release in dry air could be attributed to reduced rate of air flow over the culture as the agar dried up.

One of the most noticeable features of the humid air series is that the dry weights of the cultures (with the exception of *C. elatum*) were greater than in the other experiments. Apart from the use of humid air the treatment of these cultures also differed in that there was a time lag of 5 weeks before sampling started. Consequently estimates of spore productivity were based upon a significantly smaller number of samples. In spite of this more spores were recovered than in the dry air experiments, except in the case of *C. herbarum*. The period of undisturbed growth under humid conditions, in which the rate of drying of the agar was minimal, would presumably be conducive to both mycelial development and sporulation. Of the samples obtained after the 5 week period of incubation the first was the largest recorded in four of the six species, and in some cases amounted to approximately 50 % of all spores subsequently recovered. This suggests a 'saturation level' of sporulation in undisturbed cultures; the balance between mycelial growth and sporulation is disturbed by the process of sampling so that development of

further 'crops' of spores is stimulated by the removal of the first 'crop'. Thus, within certain limits, dispersal of spores may be conducive to higher levels of spore productivity.

One such limiting factor is the humidity of the air above the culture. Initially in all three series of experiments this must have been at, or very near, saturation level. In the wet and humid air experiments this saturated condition was maintained, but in dry air experiments the drying out of the agar must have been accompanied by a lowering of humidity in the culture tube. Observation of cultures grown in relatively dry atmospheres (r.h. 66 %) has shown that conidiophores produced at lower humidity are morphologically abnormal with restricted development and spore production.

The estimates recorded have an obvious relevance to the results of airspora surveys in indicating that the different frequencies of individual species of *Cladosporium* in the air cannot be explained simply in terms of different potentials for spore production. Airspora frequencies of *Cladosporia* must reflect more fundamental differences in the distribution and ecology of individual species. Thus *C. herbarum* has been recognized as a primary, and usually dominant, colonizer of plant tissues as they become moribund at or above the surface of the soil. On the other hand, PARBERY (1969) records that although *C. resinae* is widely distributed in the soil on a variety of substrates, it would seem to be more limited in its competitive saprophytic ability. In addition the spores of *C. herbarum*, produced at the surface of exposed decaying vegetation, will be more readily released into the air than those of *C. resinae* which are produced in the soil beneath the leaf-litter layer.

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