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WOODLANDS AS A SOURCE OF ALLERGENS

with Special Reference to Basidiospores

By

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BASIDIOSPORES IN THE GENERAL ATMOSPHERE AND AS ALLERGENS

This paper sets out botanical and clinical reasons for regarding woodlands as a source of certain fungal spore allergens *viz.* basidiospores of the toadstool and bracket and related fungi (Hymenomycetes).

It is generally recognized that the only kinds of plant spore which may be likely to produce epidemics of allergic disease are those which are antigenic and which occur widely in high concentrations regularly each year. In 1952 *Gregory & Hirst* working at Harpenden, England, announced their discovery that basidiospores were present in steady high concentrations in the air there during the period 1 June to 30 September 1951 (*Gregory & Hirst* 1952). Their detailed observations (*Hirst* 1953, *Gregory & Hirst* 1957) which had been rendered possible by the employment of the automatic volumetric spore trap (*Hirst* 1952) were amply confirmed at several other centres in England, Wales and Scotland *viz.* London (*Hamilton* 1959, *Hamilton & Denny* 1960, *Davies* 1962), Liverpool (*Davies, Denny & Newton* 1963), Cardiff (*Hyde & Adams* 1960), Glasgow (*Davies* 1965), Aberystwyth (*Adams & Hyde* unpublished data). In particular *Adams* (1964) showed that

these spores are present every year at Cardiff in greater or lesser abundance according to the higher or lower summer rainfall. During the five years 1962–66 basidiospores made up on average 29 per cent of the fungal aerospora, as against *Cladosporium*—an important mould allergen—25 per cent and ascospores 25.5 per cent (*Adams*, unpublished data). All the workers agreed that in England and Wales basidiospores were specially abundant in late summer and autumn, the peak coming usually in August, September or October according to weather; in 1962 and 1963 peak concentrations came earlier at Glasgow than London and Liverpool (*Davies* 1965).

Frankland & Hay (1951) showed that an indoor species of Basidiomycete *viz.* *Merulius* (*Serpula*) *lacrymans* (dry rot) could be an allergen. Apart from this, basidiospores were virtually disregarded by allergists until some years after *Gregory & Hirst* (1952) had stated bluntly that the high concentrations of basidiospores reported by them “obviously need investigation in connection with seasonal asthma”. Then *Bruce* (1963) found by intradermal skin testing and by bronchial testing that two different mixtures of basidiospores gave positive reactions (in 15 out of 20 patients skin tested with extracts 1:10 w/v and 12 out of 20 tested bronchially). Mixture 1 contained the spores of *Polyporus giganteus*, *Ganoderma appplanatum* and *Stereum hirsutum*: mixture 2 contained the spores of *Polyporus betulinus*, *Polystictus versicolor*, *Armillaria mellea* and *Fomes annosus*.

At Cardiff, from time to time over a number of years, individual patients have complained that being near trees adversely affected their asthma, but investigation revealed no cause. Many patients have had recurrent attacks of summer or autumn asthma or exacerbations of asthma; some were skin sensitive to one or another kind of mould spore prevalent in summer but this did not account satisfactorily for their symptoms; others were not skin sensitive to any airborne allergen then recognized as such and known to be in the air at the relevant time(s). These observations prompted systematic investigation of other possible allergens. As the result it has recently been

shown (*Herxheimer, Hyde & Williams* 1966) by skin testing (intradermally with extracts 1:1,000 w/v) and by inhalation tests that the spores of certain individual basidiomycetes, *viz.*: *Agaricus bisporus*, *Armillaria mellea*, *Coprinus micaceus*, *Ganoderma applanatum*, *Hypholoma fasciculare*, *Polyporus squamosus* and *Merulius (Serpula) lacrymans* were antigenic. (We have some evidence, on skin test results only, that the basidiospores of *Boletus elegans* also are potential allergens).

The patients tested were those whose asthma was confined to or exacerbated in the summer and autumn and recurred year after year; (those whose symptoms could be accounted for by sensitivity to grass pollen were excluded). The frequency in this group, of positive skin tests to some kinds of basidiospores *e.g.* *Coprinus* 13 per cent, *Armillaria* 9 per cent, *Hypholoma* 7 per cent are of much the same order as those obtained with similarly prepared extracts of moulds (*fungi imperfecti*) of accepted clinical importance, *e.g.* *Pullularia (Aureobasidium)* 11 per cent, *Cladosporium* 7 per cent, *Alternaria* 5 per cent. The investigation is being continued with a select group of some 20 patients; all of these have been observed over several years: their symptoms have either not been explained or have been explained only in part by sensitivity to airborne spore allergens other than basidiospores. These patients are only distinguishable by (summer or autumn) exacerbations of their asthma, without hayfever, at a time when other airborne spores could not properly account for their symptoms. Although this investigation is still incomplete it is already apparent, from correlation of clinical histories with atmospheric spore counts and with skin tests and inhalation tests, not merely that basidiospores are antigenic but also that they may well cause severe and incapacitating summer asthma.

All the species used by *Bruce* (1963) and all but two of the species used for bronchial testing at Cardiff *viz.*: *Agaricus bisporus* (cultivated mushroom) and *Merulius (Serpula) lacrymans* (dry rot) are found in woodlands.

The fruiting bodies of all the allergens tested at Cardiff are large enough to see and identify if an appropriate park or a

TABLE 1

Common Basidiomycetes Collected from Parks and Woods in and near Cardiff.

Name	Period of collection	Typical habitat (broadleaved trees unless otherwise stated)
<i>Armillaria mellea</i> (Honey fungus)	September–November	Parasitic on tree stumps and hidden roots of trees
<i>Boletus elegans</i>	June–August	Floor of coniferous plantations, (larch, <i>Larix</i>)
<i>Coprinus micaceus</i>	April–November	On and around old tree stumps
<i>Ganoderma applanatum</i>	All the year, but brackets particularly productive in late summer and autumn	Trees and tree stumps, especially beech (<i>Fagus</i>)
<i>Hypholoma fasciculare</i> and <i>H. hydrophilum</i>	May–November March–November	Old tree stumps
<i>Polyporus squamosus</i>	May–September	Tree stumps and logs
<i>P. giganteus</i>	July–October	Tufted on tree stumps
<i>Stereum hirsutum</i>	May–November	Logs, tree stumps and fallen branches

woodland district is searched. The fruiting bodies do not contain any store of pre-formed spores: spores must be collected as they are formed during the period immediately after gathering. This is done by placing the caps, with gills or pores downward, on sheets of glass. Many such collections are required before sufficient spores can be obtained from the caps or brackets to make the spore extracts.

All our gatherings were made in and around Cardiff. As will appear later it is difficult and often impossible to name specifically or even generically the great majority of the obvious basidiospores caught on the slides exposed in our Hirst traps. We think therefore it will be of interest to clinicians to know the times of year when we collected the fruiting bodies

and spores of the particular fungi under discussion. These facts are set out in Table 1.

INVESTIGATION OF THE WOODLAND AEROSPORA

The various fungal-spore censuses published between 1957 and 1964 and listed above have been carried out at more or less open sites and may perhaps be presumed to apply to the general atmosphere near the ground. *Gregory & Hirst* (1957) pointed out that their pioneer census of this kind might have given quite different results, had it been made in woodlands. Doubtless they had in mind the abundance of fungi and especially Basidiomycetes which flourish in woodland localities. Over 2,000 species of gilled and pored and related fungi (Hymenomycetes) occur in Britain. Of those species fully described and figured by *Wakefield & Dennis* (1950) more than 75 per cent are essentially woodland or tree-loving, including about 8 per cent which are more or less exclusively associated with coniferous trees. Some, notably the polypores (bracket fungi), are parasitic on tree trunks and branches (often on isolated trees) but probably the great majority vegetate in the rich soil of the forest floor, sending up their sporophores above the ground at the appropriate season.

No woodland spore census has been reported from Britain hitherto though *Gregory* (1954) observed high "spot" concentrations of basidiospores in an oakwood in Hampshire. *Lacey* (1962) made a study of two contrasting rural sites 450 m apart, one (S) a sheltered spot in a valley with trees near by, the other (M) a flat area 12 m higher on a small hill, open and wind-exposed, over the period of 14 May–24 September, 1958. She trapped nearly 3 times as many basidiospores at S as at M. Probably this result was influenced by other factors than the proximity of trees but at least it is suggestive. In view therefore of (a) the abundance of basidiospores in the general atmosphere, (b) the special frequency of their source plants in woodlands, and (c) *Gregory's* and

TABLE 2
Spores at Tintern (Woodland) and Central Cardiff Compared 1 May-16 September, 1964.

Classes of fungi	Cardiff (115 days)		Tintern (113 days)		Tintern as % of Cardiff
	Average mean 24 hr. concentration spores/m ³	% of total spores	Average mean 24 hr. concentration spores/m ³	% of total spores	
1. Basidiomycetes					
Basidiospores Coloured (sensu stricto)	293.8	11.8	1992.2	15.5	678
Hyaline	732.4	4.7	3475.6	27.4	474
Other basidiomycetes	118.5	1.9	164.5	1.3	139
Total basidiomycetes	1144.7	18.4	5632.3	44.2	490
2. Ascomycetes					
Including ascospores, Hyaline rods and Erysiphe conidia	1543.0	24.9	2676.4	21.0	173
3. Fungi imperfecti					
Cladosporium	2730.6	44.0	2921.2	23.0	107
Others	625.1	10.0	1307.9	10.2	208
Total	3355.7	54.0	4229.1	33.2	126
4. Phycomycetes	21.6	0.3	19.8	0.2	92
5. Unknown and damaged	149.7	2.4	182.8	1.4	122
Grand total	6214.7	100.0	12,740.4	100.0	205

Lacey's observations just referred to, it was apparent that a woodland spore census should be undertaken and its results compared with those obtained at an open site not too far away. Such a census was begun in 1964.

METHOD

Hirst traps have been operated at two woodland stations, viz. Tintern, Mon. (SO N.G. 527:018), 43 Km ENE of Cardiff, and Resolven, Glam. (SN 827:022) the same WNW of Cardiff, during part of the summer and autumn months of the years 1964 and 1965 respectively, as well as continuously at our principal station in Cardiff (ST 773:182).

At Tintern the apparatus was situated at 134 m altitude and 1 m above ground level, in a small valley, about 1.5 m from a stream and about 55 m from the edge of a wood (chiefly oak) to the east of the site. The area within 0.8 Km was occupied as to 80 per cent by woodlands, chiefly broadleaved but in part also coniferous, while broadleaved trees predominated in the hedgerows and around the boundaries of the (relatively few and small) fields and orchards. Further south, woodlands (predominantly broadleaved) stretched uninterruptedly for 1.6 Km and to the southwest, with some gaps, for over 3.2 Km.

At Resolven the trap was operated at 82 m altitude on a hillside which, except for a house and garden and a field of rough grass to the southwest, was entirely surrounded by plantations of conifers, viz. Sitka spruce, Douglas fir and Japanese larch. The trap stood at 2 m above ground level, 33.5 m away from the house at the end of a grassy paddock and immediately outside a spruce plantation.

The Cardiff apparatus is situated about 3.5 Km from the Bristol Channel at 13.7 m altitude and 13 m above ground level on a high roof in a public park containing numerous trees and is otherwise surrounded for an average distance of between 4 and 6 km by a built-up area.

The daily 24 hr. slides exposed at these three stations have been scanned for fungal spores, special attention being paid to various categories of basidiospores as described below. The results are summarised in Tables 2 and 3 and Fig. 1 and 2.

Categories of Basidiospores Recognized in the Present Survey

Basidiospores, in the narrow sense understood here, are derived from the Hymenomycetes (Agaricales and Tremellales, sensu Ainsworth 1966). They are scanned by us under two main groups (a) coloured, (b) hyaline.

(a) *Coloured*. A few unmistakable species and also certain genera viz. *Ganoderma*, *Boletus*, *Rhodophyllus* and *Thelephora*, can be recognized separately on the slides, but these only account for about 15 per cent: the remaining 85 per cent of coloured basidiospores are placed under various colour subcategories,

TABLE 3
Spores at Resolven (Woodland) and Central Cardiff Compared July–October 1965.

Classes of fungi	Cardiff		Resolven		Resolven as % of Cardiff
	Average mean 24 hr. concentration spores/m ³	% of total spores	Average mean 24 hr. concentration spores/m ³	% of total spores	
1. Basidiomycetes					
Basidiospores Coloured (sensu stricto)	530.2	8.3	1527.3	11.4	288
Hyaline	1945.8	30.8	5298.6	39.6	272
Other basidiomycetes	34.8	0.5	34.3	0.2	98
Total basidiomycetes	2510.8	39.6	6860.2	51.2	273
2. Ascomycetes					
Including ascospores, Hyaline rods and Erysiphe conidia	1448.1	22.8	4817.0	35.9	333
3. Fungi imperfecti					
Cladosporium	1259.2	19.8	811.5	6.1	64
Others	913.1	14.4	650.8	4.9	72
Total	2172.3	34.2	1462.3	11.0	67
4. Phycomycetes	23.2	0.4	18.7	0.1	80
5. Unknown and damaged	188.5	3.0	235.3	1.8	124
Grand total	6342.9	100.0	13,393.5	100.0	211

BASIDIOSPORES 1964
TOWN AND WOODLAND COMPARED
MONTHLY AVERAGE OF DAILY MEAN COUNTS
IN SPORES PER CUBIC METRE

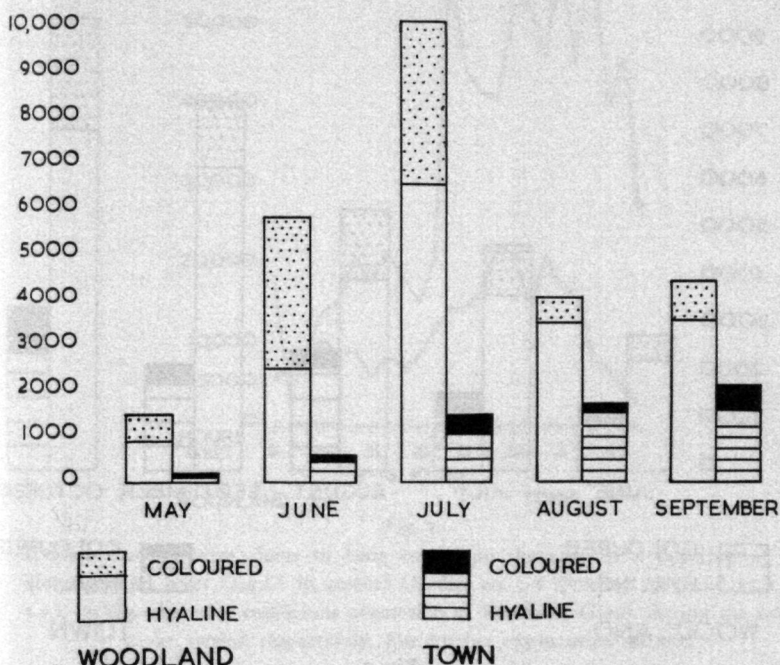


Fig. 1.

Basidiospores, coloured and hyaline, month by month at 13 m above ground level (a.g.l.) in central Cardiff and at 1 m a.g.l. in woodland country at Tintern, Mon. For further explanation see text.

"brown", "olive", "yellow" and "tan". Coloured basidiospores considered to be allergenic have been either identified specifically on our slides or grouped with other spores of similar appearance as follows:

i. *Ganoderma applanatum*. Spores rust coloured, distinctly marked and easily identified.

ii. *Boletus*. Spores (of the genus) distinctly spindle-shaped; those of the species vary in size and colour (cream, yellow or fawn).

iii. *Coprinus micaceus*. Spores dark brown, indistinguishable from other *Coprinus* species. During routine counting by us they are placed in the "brown"

BASIDIOSPORES 1965
TOWN AND WOODLAND COMPARED
MONTHLY AVERAGE OF DAILY MEAN COUNTS
IN SPORES PER CUBIC METRE

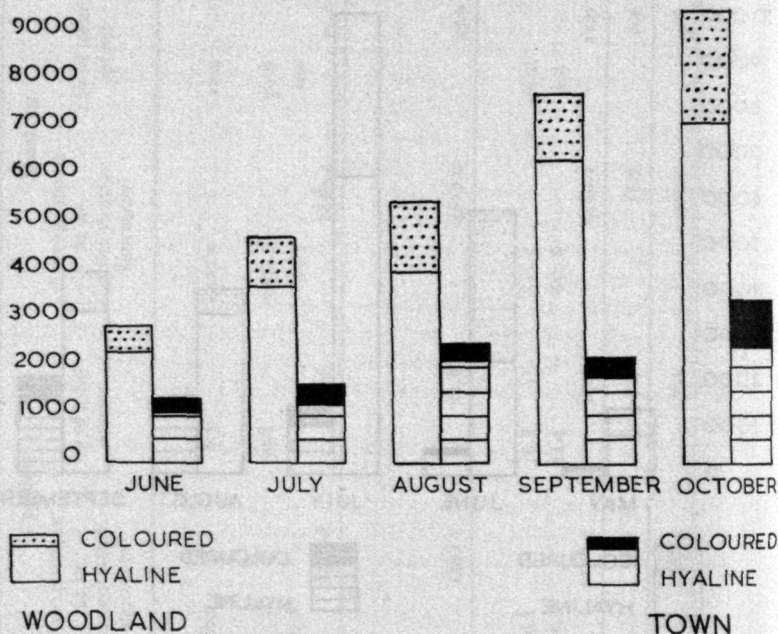


Fig. 2.

Basidiospores, coloured and hyaline, month by month at 13 m above ground level (a.g.l.) in central Cardiff and at 2 m a.g.l. on the edge of a spruce plantation at Resolven in Rheola Forest, Glam. For further explanation see text.

group. When a special investigation on Cardiff slides (1960) was carried out, the spores of *Coprinus* spp. formed about 45 per cent of all brown basidiospores (Adams, unpublished data).

iv. *Hypholoma fasciculare*. Spores olive-coloured, placed in the "olive" group; in the special investigation on Cardiff slides (1960) the spores of *Hypholoma fasciculare* formed about 40 per cent of the olive basidiospores.

v. *Agaricus bisporus* (cultivated mushroom). Spores are very unlikely to be caught on outdoor slides, but other species of *Agaricus* are observed during routine counting and their spores are placed in the "olive" subcategory. In the special investigation mentioned above they formed about 10 per cent of this group.

HYALINE BASIDIOSPORES
TOWN AND WOODLAND COMPARED
HOURLY COUNTS IN SPORES PER CUBIC METRE

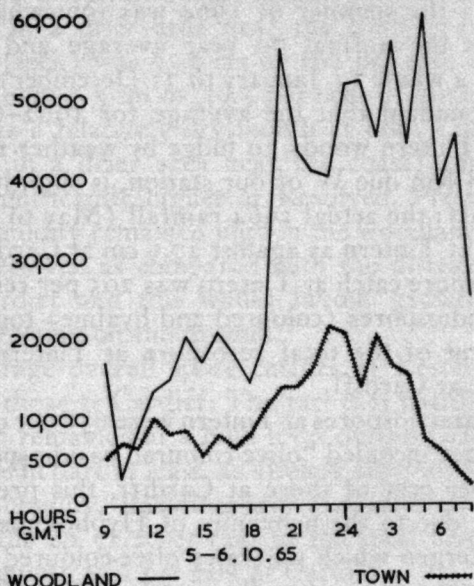


Fig. 3.

Hyaline basidiospores: hour to hour counts on the roof of a building 13 m above ground level (a.g.l.) in central Cardiff on 5-6 October 1965 and at 2 m a.g.l. on the edge of a coniferous plantation at Resolven, Glam. during the same 24 hr. period respectively. For further explanation see text.

(b) Hyaline (colourless) basidiospores: derived mainly either from polypores or from fungi belonging to the Tremellales, all of which grow on trees or logs. The spores of *Armillaria mellea* and *Polyporus squamosus* which were used for clinical testing at Cardiff and those of *Polyporus giganteus*, *Polyporus betulinus*, *Stereum hirsutum* and *Fomes annosus* which were used by Bruce (1963) cannot be analysed individually with any certainty on the exposed slides since they are similar to certain other hyaline basidiospores. A small proportion of hyaline spores (perhaps 1 per cent on average), ornamented with spines and ridges, are easily recognized as spores of either *Russula* or *Lactarius* spp.

The spores of certain other groups of Basidiomycetes are identified and counted under the heads of *Ustilago*: mainly cereal smuts (easily identified and counted), and others including spores of rusts (Uredinales), puff-balls (*Lycoperdals*) and smuts other than *Ustilago*.

RESULTS

1. *Cardiff/Tintern 1964*. (Table 2, Fig. 1)

At Cardiff the summer of 1964 was somewhat drier and warmer than the normal 70 year average and the fungal aerospora as a whole (1 January to 31 December) was 20 per cent more abundant than the average for 1962-66. The climate of the Tintern woods, to judge by weather records kept at a station 8 Km due W of our station, is slightly drier than that at Cardiff; the actual 1964 rainfall (May to September) was 22.2 cm at Tintern as against 27.9 cm at Cardiff.

The total spore catch at Tintern was 205 per cent of that at Cardiff. Basidiospores (coloured and hyaline) together made up 43 per cent of the total aerospora at Tintern as against 16.5 per cent at Cardiff.

Coloured basidiospores at Tintern were 678 per cent of those at Cardiff: they included "olive-coloured basidiospores" which were 1390 per cent of those at Cardiff, this preponderance being in part due to the proximity of *Hypholoma fasciculare* (a known allergen which possesses olive-coloured spores) on a stump near the apparatus. By contrast, *Ganoderma* spores were only 30 per cent those at Cardiff. This fungus in South Wales mainly affects old and unhealthy broadleaved trees which are unlikely to be frequent in well-tended woods like those at Tintern. Hyaline basidiospores were 474 per cent as numerous at Tintern as at Cardiff (Fig. 1).

2. *Cardiff/Resolven, 1965*. (Table 3, Fig. 2)

The normal annual rainfall at Cardiff is 105 cm, that at Resolven about 152 cm (*Rider* 1936). The weather at Cardiff during the period July to September 1965 on the whole was cooler and wetter than average and atmospheric humidity was above normal, average mean temperatures and hours of sunshine were below normal. October was dry and sunny. The actual rainfall received at Cardiff during the period July to September (incl.) was 35.0 cm or 131 per cent of normal

(in July it was 212 per cent of normal) but in October only 2.67 cm or 22.8 per cent of normal. No rainfall records are available for Resolven but at Neath (9.5 km WSW of Resolven and in a drier area near the coast) rainfall July to September (incl.) was 33.9 cm or 102 per cent of normal but in October only 5.3 cm or 39.5 per cent of normal. Although October was a relatively dry month at both Cardiff and Resolven and in a drier area near the coast) rainfall July to probably significantly higher at Resolven. Presumably therefore soil humidity remained high in the woodlands, more especially at Resolven as compared with the district and country around Cardiff and this would favour growth of Hymenomyces growing on the ground.

The average overall spore catches at Resolven were 211 per cent of those at Cardiff. The fact that basidiospore counts at Resolven remained at a high level during October was presumably due in part at least, as already suggested, to persistent high soil humidity. Basidiospores (coloured and hyaline together) made up 51.2 per cent of the total aerospora at Resolven as against 39.1 per cent at Cardiff.

Coloured basidiospores at Resolven were 288 per cent of Cardiff. Spores of *Boletus* spp. formed 655 per cent of those at Cardiff; several species of *Boletus* are confined to coniferous woods. Olive-coloured spores (40 per cent of them attributable at Cardiff to *Hypholoma fasciculare*, a very common woodland species) were 528 per cent of those at Cardiff. *Ganoderma* spores were only 18 per cent of Cardiff. Suitable habitats for this fungus were notably infrequent in the Resolven area.

Hyaline basidiospores at Resolven were 272 per cent of Cardiff: they included spores of the *Russula*—*Lactarius* type which were 1078 per cent of those at Cardiff; a species of *Russula* was growing under the trees near the site. Fig. 3 sets out the hourly counts of hyaline basidiospores at both sites for the 24 hrs. 5–6 October 1965 and shows that while the peak counts at both stations occurred during the night, as has been recorded previously for both hyaline and coloured basidio-

spores [cf. *Hirst* (1953), *Hamilton* (1959), *Adams* (1964)], the counts rose higher and were sustained for a longer period at the woodland site than in Cardiff.

DISCUSSION

i. *The Aerospora at the Three Stations Compared*

Basidiospores were conspicuously higher at both the woodland sites than at Cardiff, a finding which applies with even greater force to Tintern than to Resolven: Coloured basidiospores at *Tintern* (1964) were 673 per cent those at Cardiff, hyaline 474 per cent; the corresponding figures for Resolven (1965) were much lower, *viz.* 288 per cent and 272 per cent respectively. This was perhaps to be expected since the woods at Tintern are preponderantly broadleaved, those at Resolven purely coniferous, and pored and gilled and related toadstools are probably more prevalent as to species and more abundant as individuals in broadleaved woods than in coniferous plantations.

ii. *Applicability of Results to British Woodlands in General*

What few data are available (other than our own) by which to test the hypothesis that basidiospores are more abundant in woodlands generally than elsewhere, support it; Gregory's and Lacey's observations have already been quoted (*Gregory* 1954, *Lacey* 1962). Basidiospores were consistently much more abundant in central Cardiff than in central London in both the wet summer of 1958 and the dry summer of 1959 (*Adams, Denny, Hamilton & Hyde*, unpublished); a finding which was to be expected, since the London station was much farther removed from the country, and therefore from the woodlands, than the one in central Cardiff.

Basidiospores are more abundant in central Cardiff in wet summers than in dry ones (*Adams*, 1964). Probably this applies to Great Britain generally; however, the west of Britain (including Wales) has a wetter climate than the

east and it seems likely that the incidence of basidiospores will vary correspondingly. Thus in 1961 basidiospores were much more abundant at Liverpool than in central London (*Davies, Denny & Newton* 1963). *Adams* (1964) also estimated the abundance of basidiospores in 1961 at Cardiff and, though differences in presentation preclude direct comparisons between her figures and theirs, there were more spores in Cardiff than in London. Similar considerations may hold also for N.W. Europe generally. It may be added too that since recent climatic trends suggest that cool wet summers are likely to occur increasingly often in Britain in the years ahead (*Lamb* 1965), high average concentrations of basidiospores must also be expected.

SUMMARY AND CONCLUSIONS

1. The summer aerospora at two woodland sites in South Wales, one in an area of preponderantly broadleaved woodlands, the other among coniferous plantations, has been studied and compared with that of central Cardiff (taken as representative of the general aerospora of the region near ground level). Average numbers of fungal spores in the air at the woodland sites were respectively 205 per cent and 211 per cent of those obtained at Cardiff; the corresponding figures for basidiospores were 533 per cent and 376 per cent respectively.

2. It would appear from the special abundance in woods of the fungi concerned, from the aerobiological observations described here and from those previously reported by others, that hymenomycetous basidiospores are more abundant in the air of woodlands generally, especially in wet weather, than in any other type of vegetation in Britain, and that this probably applies also to N. W. Europe.

The clinical findings summarized in the Introduction and aerobiological findings summarized above make it probable that many cases of late summer asthma may well be due to inhaling fungal spores of kinds which are specially abun-

dant in the neighbourhood of woods and will explain exacerbations of summer asthma in or near woodland districts. The type of woodland concerned is of importance.

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