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IDENTITY OF AIRBORNE HYALINE, ONE-SEPTATE ASCOSPORES AND THEIR RELATION TO INHALANT ALLERGY

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Hyaline, one-septate ascospores (HIS) are known in high concentrations in the air, and have been reported as *Didymella exitialis*. HIS ascospores were isolated from the air, photographed and cultured. The cultures grew into six taxa in *Ascochyta*, the *Ascochyta* state of *D. exitialis*, an undescribed taxon close to *D. exitialis*, the *Ascochyta* states of *D. phleina*, *A. hordei*, *A. hordei* var. *europea* and *A. leptospora*. It is concluded that the majority of airborne HIS ascospores belong to various species of *Didymella*, some as yet undescribed. The late colouration and roughening of ascospores and conidia is discussed, as is the role of *Didymella* ascospores in inhalant allergy.

Airborne hyaline ascospores with one septum have been reported in the air spora by several authors (Meier, 1935; Hamilton, 1959; Kramer & Pady, 1960; Adams, 1964). The presence of such ascospores above fields of wheat and barley was reported by Last (1955), while Corbaz (1969) reported them in the air above a field of wheat, and identified them as the ascospores of *Didymella exitialis* (Morini) E. Müller. Frankland & Gregory (1973) reported that pseudothecia of *D. exitialis* were common on barley leaves, and that large numbers of ascospores resembling those of *D. exitialis* were present in the air close to barley fields at several sites.

Such hyaline-1-septate (HIS) ascospores were also found in the air spora of Cambridge during a survey of the air spora from 1969 to 1971. The highest concentrations were usually found in July and August, and this late summer peak concentration was particularly striking in 1971, when the daily average from 27 July to 16 Aug. was 9500 m^{-3} air, and the highest was approximately 90000 m^{-3} air for the 6 h period from 18.00 to 24.00 G.M.T. on 5 Aug.

It was thought possible that an airborne ascospore occurring in such high concentrations might be a cause of inhalant allergy, and there was evidence that some patients who had kept diaries of their symptoms of asthma and rhinitis in 1970 and 1971 experienced their worst symptoms when HIS ascospore concentrations were very high (Allitt, in prep.). Frankland & Gregory (1973) reported that the worst symptoms of a patient with 'barley asthma' coincided with a period of high airborne concentrations of HIS ascospores in the first 2 weeks of August 1972. They tested 100 patients with extracts from two isolates of the ascospores from barley leaves and observed an allergic reaction in twelve. Harries *et al.* (1985) showed that four patients had immediate skin-prick test reactions and specific IgE antibody to a mycelial extract of *D. exitialis*. These four patients displayed an immediate asthmatic response to an inhalation test of *D. exitialis* extract, whereas a control did not. Falls in the peak expiratory flow rate of one of these sensitive patients occurred when airborne counts of HIS ascospores, identified by Harries *et al.* (1985) as *D. exitialis*, increased. An increase in cases of acute asthma in Birmingham in 1983 was thought to be caused by high concentrations of *Didymella* ascospores (Packe & Ayres, 1985).

For the routine counting of HIS ascospores trapped in Cambridge an arbitrary classification into three visual types had been made, but the significance of the morphological differences used was unknown. It seemed desirable to discover two things: the identity of the airborne ascospores, and also to what extent the morphology of the airborne ascospores corresponded to the morphology of the ascospores of fungal species. Ascospores were therefore isolated from the air, transferred to agar, photographed, and then grown in culture. There were however problems in comparing the ascospores on spore trap slides and those isolated from the air, for the morphology of the ascospores was not easy to observe or photograph on the agar surface: hydration, especially laterally, affected the size and shape of the ascospores, roughening was not easily observed, and it was never possible to observe the colour. However, smooth ascospores

may be presumed to be hyaline, and roughened ascospores to be coloured, as this is almost always the case in ascospores on traces from spore traps observed under normal conditions of microscopic examination, using a mountant and coverslips.

METHODS

Visual examination of HIS ascospores

HIS ascospores were noted during routine counting of spores on traces obtained from a Burkard Automatic Volumetric Spore Trap (BAVST) exposed on the roof of Addenbrooke's Hospital, old site, near Fitzwilliam Street, from spring to autumn for 1969–71. Traces were mounted in a plastic mountant, 'Gelvatol', and examined microscopically.

Isolation of HIS ascospores from air

In the summer of 1972 HIS ascospores were isolated from the air, photographed, and cultured singly after photography, as follows. A BAVST, with its inlet about 6·5 m above ground level, and sited at the Cambridge University Botanic Garden, was used for sampling. Trapping was performed only on evenings when it had rained earlier in the day and relative humidity had remained sufficiently high, as previous trapping experience had shown that under such conditions concentrations of HIS ascospores were likely to be high, and the common components of the 'dry air' spora such as *Cladosporium*, *Alternaria* and *Botrytis* would be low. These evenings were those of 8, 22 June, 2, 9, 10, 11, 14, 16, 17, 22, 23, 24, 25, 30 and 31 Aug., 1, 6, 7, 15, 19 and 27 Sep.

In order to obtain a trace consisting of widely spaced spores, a number of precautions were taken. The 7-day clock was replaced by a 24 h clock, which moved the trace behind the orifice at a rate of 14 mm h^{-1}, rather than the usual 2 mm h^{-1}. Sampling with a BAVST is usually efficient and isokinetic because of the free rotation of the upper part of the trap so that the intake orifice points into the wind, and because of an air intake of 10 l min^{-1}. Sampling was made less efficient by fixing the upper part of the trap in one position and the reduction of the flow rate to 3 l min^{-1}.

Uncoated 'Melinex' tape was used as the trapping surface, and after removal from the drum was cut into sections 28 mm long, representing trapping over 2 h, the trace between 22.00 and 02.00 G.M.T. being examined for preference. For examination of the trace a section of tape was lowered face down on to the surface of minimal agar (MA) of agar and distilled water in a Petri dish. The back of the tape was rubbed gently, the position of the tape marked in the agar with a sterile needle, and the tape removed. The spores thus transferred to the agar surface were examined under a dissecting microscope. When a suitable HIS ascospore, separated from other spores, was found, its position on the agar surface was marked by a sterile needle. The Petri dish was transferred to the stage of a microscope, and the HIS ascospore was recorded photographically. A small portion of agar on which the ascospore rested was transferred to a tube of Potato Dextrose agar (PDA) and kept under laboratory conditions on a windowsill. Most ascospores grew into anamorphic states in *Ascochyta* and were sent to the Commonwealth Mycological Institute for identification.

As it was not possible to use the microscope and camera under sterile conditions the isolations were made in an open (non-mycological) laboratory. The first set of isolations, of spores trapped during the night of 8–9 June, were made during the afternoon of 9 June, and many were contaminated by *Cladosporium*. After this, isolations were made later than 18.00 G.M.T., almost eliminating the problem of contamination.

Subsequently attempts were made to induce the formation of ascocarps: in 1974 the cultures were revived and then subcultured on to Oat Agar (OA) and Minimal Agar with filter paper and kept under

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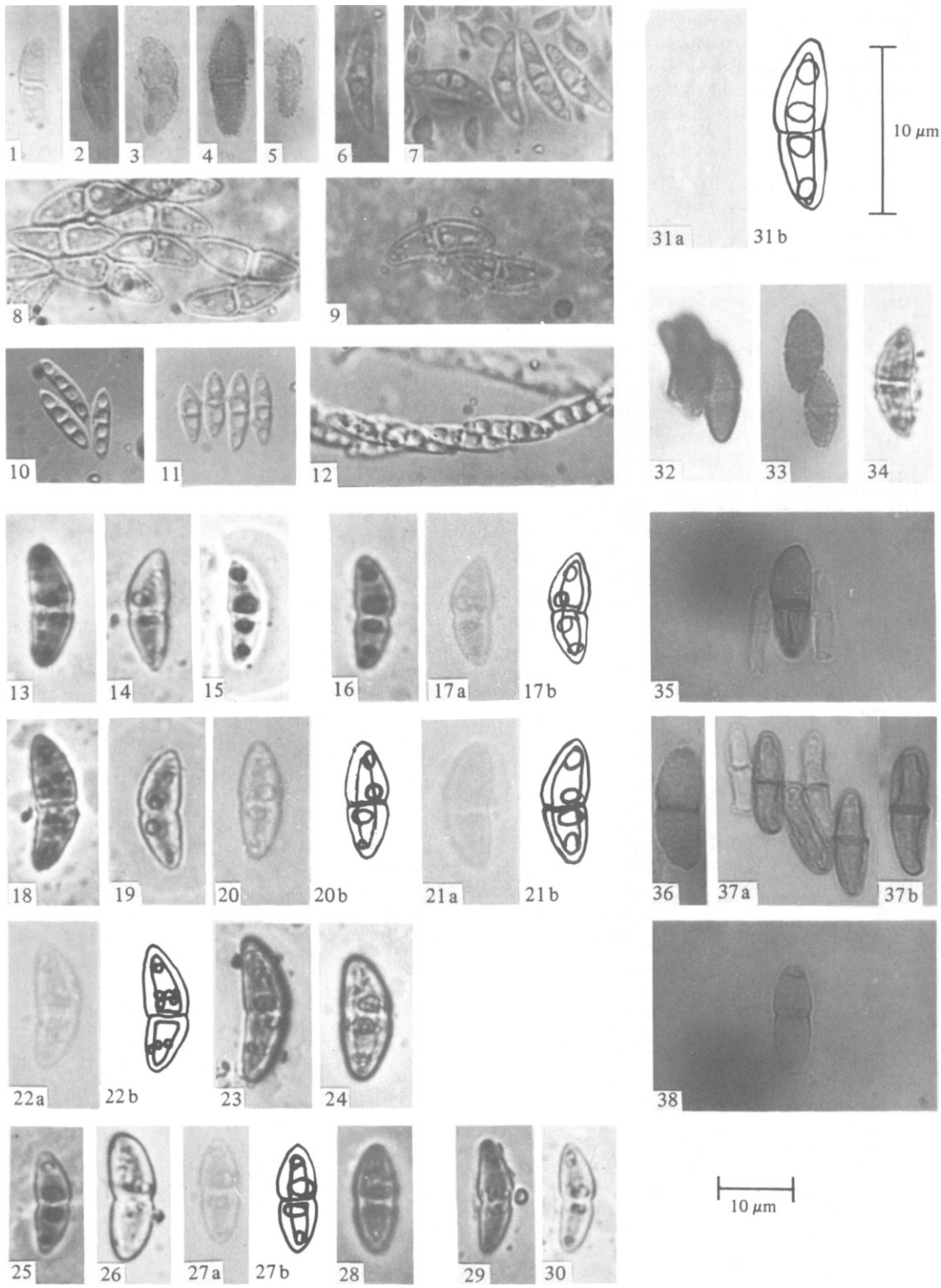
Figs 1–5. Airborne CRIS ascospores from spore trap traces. Figs 6, 7. Airborne HIS ascospores from spore trap traces. Figs 8, 9. Airborne CRIS ascospores from spore trap traces. Fig. 10. Ascospores of a *Diaporthe* species. Fig. 11. Ascospores of *D. arctii*. Fig. 12. Ascospores of *D. eres*. (Figs 6–12 were photographed under Nomarski interference contrast.) Figs 13–15. Airborne ascospores which grew into the *Ascochyta* state of *Didymella exitialis*. Figs 16–17*b*. Airborne ascospores which grew into an *Ascochyta* resembling the *Ascochyta* state of *D. exitialis*. Figs 18–21*b*. Airborne ascospores which grew into the *Ascochyta* state of *D. phleina*. Figs 22*a*–24. Airborne ascospores which grew into *A. hordei*. Figs 25–28. Airborne ascospores which grew into *A. hordei* var. *europea*. Fig. 29. Airborne ascospore which grew into *A. leptospora*. Fig. 30. Airborne ascospore which grew into cf. *A. leptospora*. Figs 31*a*, *b*. Airborne ascospore 148. Figs 32, 33. Ascospores from culture 148, grown from ascospore 148. Fig. 34. Rough, and apparently striate, airborne ascospore 136. Fig. 35. Conidia of the *Ascochyta* state of *D. exitialis*, cultured from Fig. 13. Figs 36–37*b*. Conidia of the *Ascochyta* state of *D. phleina*, Figs 37*a*, *b*; cultured from Fig. 19. Fig. 38. Conidium of *A. hordei*, cultured from Fig. 23.



Figs 1–38. For caption see opposite.

uv light (Philips uv tubes, TL. 40w/08) in an 18 h light/6h dark cycle. In 1975 the cultures were again revived and subcultured on sterile wheat straw and Tap Water Agar and kept under the same conditions as in 1974. In 1984 culture 148 was revived and grown on a variety of media.

RESULTS

Visual examination of HIS ascospores

Most HIS ascospores were smooth, hyaline, guttulate, fusiform, inequilateral, and with the upper cell a little longer and wider than the lower one. On some occasions ascospores were seen which were essentially similar, but which were coloured and roughened, not hyaline and smooth. This type of ascospore tended to appear in and disappear from the air spora rather abruptly. It occurred during 1969 and in very high concentrations in 1971, but was almost absent in 1970.

The airborne ascospores seemed of heterogeneous origin, displaying an array of variation in spore shape, size, number and distribution of guttules, and also in the degree of colouration and ornamentation (Figs 1–5). For routine counting an arbitrary division into three visual types was adopted, namely smooth slender hyaline ascospores, mostly 3·0–4·0 μm wide (Figs 6, 7), hyaline smooth wide ascospores, mostly greater than 4 μm wide, and wide, coloured and roughened one-septate (CRIS) ascospores (Figs 8, 9).

A number of ascospores commonly found in the air, which resembled HIS ascospores in also being hyaline and having one septum, were logged as separate visual types, and later identified by comparison with reference material of named fungi. Only two categories of ascospore were sufficiently similar to HIS ascospores to be confused with them: the immature ascospores of *Diaporthe* species, an unidentified *Diaporthe* (Fig. 10), *D. arctii* (Lasch) Nitschke (Fig. 11) and *D. eres* Nitschke (Fig. 12), and ascospores of *Mycosphaerella* species. It is possible that some of these were included in the 'smooth slender HIS' visual type. However, they were thought not to contribute large numbers of ascospores to the air spora, and not to occur in high proportions in the large peaks of HIS and CRIS ascospores.

Examination of cultures

A total of 81 isolations of HIS ascospores were made and 51 successful cultures resulted. Six of these were obvious contaminants, seventeen cultures produced no fructification, and twenty-eight cultures were those of the coelomycete genus *Ascochyta*. In 1972 these cultures could not be identified further, but because of the work of Punithalingam (1979), this became possible later, and twenty-four of the cultures were assigned to six taxa. The dates of isolation of ascospores, and the taxon into which they grew, are listed.

The cultures were identified as follows.

Ascochyta state of *D. exitialis*. Five ascospores grew into this fungus, nos. 54 and 114, and nos. 41, 50 and 59 (Figs 13–15). Ascospores isolated on 9, 10, 11 and 24 Aug.

An undescribed *Ascochyta* resembling *D. exitialis*. Ascospores nos. 63 and 83 (Figs 16, 17*a*, *b*), were isolated on 14 and 17 Aug.

Ascochyta state of *D. phleina* Punith. & Kåre Årsvoll. Seven ascospores grew into this fungus, nos. 29, 38 and 149, and also nos. 77, 78, 88 and 98 (Figs 18–21*b*). They were isolated on 2, 9, 16 and 22 Aug. and 15 Sep.

A. hordei Hara. Three ascospores grew into this fungus, nos. 85, 160 and 161 (Figs 22*a*–24). They were isolated on 17 Aug. and 27 Sep.

A. hordei Hara var. *europea* Punith. Four ascospores grew into this variety of *A. hordei*, nos. 49, 56, 82 and 155 (Figs 25–28). They were isolated on 10 and 17 Aug. and 19 Sep.

A. leptospora (Trail) Hara. Two ascospores grew into this fungus, no. 140 and also no. 68 (Fig. 29), while the culture resulting from ascospore no. 53 (Fig. 30), is probably also this species. They were isolated on 7 Sep., 16 and 10 Aug. respectively.

Other *Ascochyta* cultures. No further identification was made on another three *Ascochyta* cultures, or on the culture resulting from ascospore 148 (Fig. 31*a*, *b*), isolated on 15 Sep. Only a few additional ascospores classified as hyaline and slender germinated, and these were sterile.

Teleomorphs were found in very few cases; in culture 41, *D. exitialis*, where immature asci and ascospores were found on OA, and in culture 148. This culture was originally classed as sterile, but in a later re-examination of a microscope slide prepared from this culture on PDA several ascospores were seen. These were rough, brown, M 4, A 4–5 when young, and M 5, B 8 when older, and measured 12·5–15·5 × 4·0–5·0 μm, width measured at septum (Figs 32, 33). There was also one hyaline *Ascochyta* conidium, measuring 19·5 × 3·0 μm. Although the culture was revived and grown on various media in 1984 no fructification was formed and it could not be identified.

DISCUSSION

Identity of HIS and CRIS ascospores

It is already known that *Didymella* teleomorphs may be associated with *Ascochyta* anamorphs in a

Table 1. Didymella *species already known to have* Ascochyta *anamorphs*

Teleomorph	Anamorph	Reference
D. exitialis	*Ascochyta*	Müller (1952)
D. phleina	*Ascochyta*	Punithalingam (1979)
D. graminicola	*Ascochyta*	Punithalingam (1974)
Didymella	*A. avenae*	Obst (1984)
Didymella	*Ascochyta* identified as *A. brachypodii*	Zeiders (1979)
Didymosphaeria loliina (closely related to *Didymella*)	*Ascochyta*	Punithalingam (1979)

number of cases (Table 1). The existence of teleomorphs in four additional taxa, namely *A. hordei*, *A. hordei* var. *europea*, *A. leptospora* and an *Ascochyta* resembling the *Ascochyta* state of *D. exitialis*, is implied by the fact that these cultures resulted from ascospores. The unidentified culture 148 included the anamorph and the teleomorph. All the original airborne ascospores isolated resembled those of *Didymella*, and are thus presumed to belong to this genus, as are many of the airborne HIS and CRIS ascospores observed on spore trap traces.

It is unfortunate that so few of the *Ascochyta* cultures produced teleomorphs. Although the culture of ascomycetes on low nutrient substrates is often a successful method of inducing ascocarp formation it was not so in this case, presumably because pseudothecia of the *Didymella* species in this study are normally formed in living grass leaves, where the supply of nutrients must be high. Certainly Obst (1983) found that cultures of *A. avenae* (Petrak) Sprague & Johnson produced mainly pycnidia when grown on a low nutrient substrate, whereas on carrot juice agar more pseudothecia were produced. Zeiders (1982) found that on PDA and V-8 agar a proportion of the *Didymella* state occurred among the pycnidia of an *Ascochyta* identified as *A. brachypodii* (Sydow) Sprague & Johnson. The teleomorph was found in cultures of *D. graminicola* Punith. on PDA (Punithalingam, 1974) and in *D. exitialis* on PDA, *D. phleina* on OA and *Didymosphaeria loliina* Punith. on OA (Punithalingam, 1979).

This study also shows that airborne HIS ascospores are indeed heterogeneous, including at least the six taxa identified. Relatively few ascospores were isolated, and it is possible that only the most common taxa were detected. As well as the taxa isolated from the air there are another sixteen graminicolous species or varieties of *Ascochyta* recorded for the British Isles, *A. avenae* being the most commonly recorded of these taxa (Punithalingam, 1979). The teleomorphs of these taxa may well contribute ascospores to the air spora.

Teleomorphs have been described for two of the species which were isolated, *D. exitialis* and *D. phleina*, and in these species a direct comparison can be made between the airborne ascospores and the published description of the ascospores of the species into which they grew. The airborne ascospores which proved to be ascospores of *D. exitialis*, nos. 41, 50, 59 (Figs 13–15), and a taxon closely related to *D. exitialis*, nos. 63, 83 (Figs 16, 17*a*, *b*) match the description of Müller (1952) in being broadly fusiform, although the ends are not 'rather strongly tapered and often slightly bent', presumably because the ascospores (Figs 13–17*b*) are hydrated. They are larger (16·5–20 × 4·0–5·5 μm) than the size range given (13–16 × 2·5–4·0 μm), again presumably because of hydration. Similarly ascospores nos. 77, 78, 88 and 98 (Figs 18–21*b*), which proved to be *D. phleina*, resemble the description (Punithalingam, 1979) in shape, although their ends are not acute, and they are larger, 19·0–22·0 × 5·0–6·5 μm, than the size range given, 16–17(–18) × 4·5 μm. Two, possibly three of the ascospores which grew into *D. phleina* were apparently roughened, ascospores no. 149 and no. 77 (Fig. 18) and presumably also no. 78 (Fig. 19). This feature is absent from the description of the ascospores of *D. phleina* (Punithalingam, 1979).

It is possible to compare the morphology of the airborne ascospores which were cultured within and between sets of airborne ascospores which gave rise to the same species or variety of *Ascochyta*. It can be seen from the photomicrographs that within sets the shape and size of the ascospore is quite similar. However, within two sets there are both roughened and smooth ascospores; in the *D. phleina* set ascospore 77 (Fig. 18), and possibly ascospore 78 (Fig. 19) are roughened, and in the *A. hordei* set, one ascospore, no. 160 (Fig. 23) appears to be roughened over part of its surface. This distribution of roughening is shown by some airborne spores (Figs 1, 2).

The number, size and distribution of guttules varies markedly within the sets of airborne ascospores. Such differences in guttulation within

Table 2. *Measurements (μm) of numbered airborne ascospores (length × width at septum and width at broadest part), size range of each set of ascospores, and size range of conidia of corresponding taxon in* Ascochyta

(Conidial measurements from Punithalingam (1979).)

Ascospores	Conidial size range
Didymella exitialis	
41 20·0 × 5·5 (7·0)	
50 19·0 × 5·5 (7·0)	
59 16·5 × 4·0 (5·0)	
close to *D. exitialis*	
63 18·0 × 4·5 (6·0)	
83 17·5 × 5·0 (6·0)	
Range: 16·5–20·0 × 4·0–5·5 (5·0–7·0)	15·0–18·0 × 3·5–4·0 (on wheat straw)
D. phleina	
77 22·0 × 6·5 (7·0)	
78 19·0 × 5·0 (5·5)	
88 19·0 × 5·5 (6·5)	
98 20·0 × 6·5 (7·5)	
Range: 19·0–22·0 × 5·0–6·5 (5·5–7·5)	18·0–20·0 (–24·0) × 5·5–6·5 (–8·0)
Ascospores of *A. hordei*	
85 18·0 × 6·0 (6·5)	
160 20·0 × 7·0 (8·0)	
161 20·0 × 6·0 (7·0)	
Range: 18·0–20·0 × 6·0–7·0 (6·5–8·0)	(15·0–) 17·0–20·0 (–22·0) × 3·5–4·0 (–4·5)
Ascospores of *A. hordei* var. *europea*	
49 16·5 × 4·5 (5·5)	
56 20·0 × 5·0 (6·0)	
82 17·0 × 5·0 (6·0)	
155 17·5 × 5·0 (6·0)	
Range: 16·5–17·5 (–20·0) × 4·5–5·0 (5·5–6.0)	14·0–16·0 × 3·0–4·5 (–5·0)
Ascospores of *A. leptospora*	
53 17·0 × 4·0 (4·5)	
68 18·0 × 4·0 (4·5)	
Range: 17·0–18·0 × 4·0 (4·5)	(13·0–) 14·0–16·0 × 3·0–4·5 (–5·0)

a species are illustrated in different isolates of *D. exitialis* by Punithalingam (1979), and presumably the state of many small guttules and the state of four large guttules represent different stages of ascospore maturation.

It is possible to see some differences between the sets of ascospores: the size ranges are a little different (Table 2), while it can be seen that ascospore no. 68 (Fig. 29) *A. leptospora*, and ascospore no. 53 (Fig. 30) cf. *A. leptospora*, are much thinner than any of the other ascospores isolated. The outline of the ascospore is more or less straight on the shorter side in the *D. exitialis* set, whereas in the *D. phleina* set, and the closely related *A. hordei* set, the ascospores are somewhat bent.

Only the *D. phleina* and *A. hordei* sets include roughened or apparently roughened ascospores, but roughening of ascospores occurred in two additional instances, in the unidentified culture 148, which not only had roughened ascospores in culture (Figs 32, 33), but also grew from an ascospore noted as possibly rough and striate when observed on the agar surface (Figs 31 *a*, *b*), and in ascospore no. 136 (Fig. 34), which shows roughening, and also what may be striations. (This culture was discarded because of contamination by *Cladosporium*.) These features are reminiscent of a *Didymosphaeria* described by Hudson (1962) on the grass *Saccharum officinarum*.

Differences in size and shape may be useful in characterizing ascospores when the teleomorphs are eventually cultured or collected, but will not serve to identify single airborne ascospores visually. The difficulties are demonstrated by the indistinguishability of the ascospores of *D. exitialis* (Figs 12–14) and the ascospores of a closely related undescribed taxon (Figs 15, 16).

It is obvious that the ascospores of *Didymella* and

conidia of *Ascochyta* resemble one another in a general way, but if the morphology of sets of airborne ascospores is compared with the morphology of the conidia of the corresponding species or variety, as given by Punithalingam (1979), then it can be seen that there is a resemblance between the ascospores and conidia within a species (Table 2).

The occurrence of colouration and ornamentation in the conidia of some *Ascochyta* species may have some bearing on the identity of the CRIS ascospores known in the air spora. Late colouration and slight roughening of the conidia was observed in one of the five isolates of *D. exitialis* (Fig. 34), although this was not observed for the species by Punithalingam (1979). Of the other taxa isolated in this study it is known that the conidia of *D. phleina* (Figs 36, 37*a*, *b*), *A. hordei* (Fig. 38), *A. hordei* var. *europea* and *A. leptospora* are either coloured or may become so late in their development, while the conidia of *D. phleina* and *A. hordei* may also become ornamented late in their development (Punithalingam, 1979). Since ascospores and conidia resemble one another in *Didymella* this suggests that the airborne CRIS ascospores may be the ascospores of the species which have coloured rough conidia, namely *D. phleina* and the teleomorph of *A. hordei*. Some evidence from this study is consistent with this hypothesis, as cultures of *D. phleina* and *A. hordei* resulted from both smooth airborne ascospores and apparently rough airborne ascospores. However, one isolate of *D. exitialis* had a few coloured rough conidia, and it would be premature to suggest that all CRIS ascospores are those of *D. phleina* and *A. hordei*, as late colouration and roughening may occur in the conidia and ascospores of other species without this having been observed. There is also the possibility that *A. avenae*, not isolated in this study, which may have coloured rough conidia (Punithalingam, 1979) is also contributing ascospores to the air spora.

This late colouration and ornamentation in *Didymella* ascospores may explain why both HIS and CRIS ascospores occur in the air spora: the smooth hyaline guttulate ascospores being found frequently, golden brown rough ascospores without guttules such as those seen on 31 Aug. 1969 (Fig. 4) and in culture 148 (Figs 32, 33), being found only rarely, while from time to time ascospores with various degrees of guttulation, pale colouring and ornamentation (Figs 8, 9) are found, sometimes in high concentrations.

It is clear that the three artificial categories of ascospores which were adopted do not correspond precisely with any species or combination of species of *Didymella*. The smooth slender category may have included ascospores of *Mycosphaerella* species and immature ascospores of some *Diaporthe* species, as well as slender HIS ascospores such as those which developed into *A. leptospora* (Figs 29, 30), and possibly the narrower ascospores of other *Didymella* species. The smooth hyaline and broad ascospores would be a mixture of the ascospores of *D. exitialis*, *D. phleina* and the teleomorphs of the *Ascochyta* resembling *D. exitialis*, *A. hordei* and *A. hordei* var. *europea*. The broad coloured and rough ascospores would consist of at least the more mature ascospores of *D. phleina* and the teleomorph of *A. hordei*, and possibly other species. The composition of these artificial categories may well be different at different times of the season or in different years.

Allergy to HIS and CRIS ascospores

Although it was shown that the artificial visual categories of one-septate ascospores used were not taxonomically precise there was some evidence that there was a difference of allergenic significance between them, as the symptoms of different patients, while corresponding to airborne concentrations of *Didymella*-like ascospores as a whole, also showed a tendency to correspond to one artificial group rather than another (Allitt, in prep.). The late changes in the ascospore wall suggest there may be accompanying changes in which particular allergens are present. If this were so it could provide part of the explanation for patients responding differently to the different groups.

Allergy to the ascospores of *D. exitialis* has been referred to as 'barley asthma' by Frankland & Gregory (1973), but other species could be associated with barley crops, as seven taxa in *Didymella* or *Ascochyta* have been recorded on barley (Punithalingam, 1979), and indeed *A. hordei* var. *europea* has been isolated from the air over a barley field by Gregory (fide Punithalingam, 1979). Also, most *Didymella* and *Ascochyta* species have been recorded on several cereals or wild grasses, for example *D. exitialis*, *A. hordei* and *A. hordei* var. *europea* have been recorded on wheat as well as barley, while *A. avenae* occurs on barley, wheat and oats (Punithalingam, 1979). 'Barley asthma' would then seem to be a particular instance of allergy to the ascospores of graminicolous species of *Didymella*. This allergy need not be associated with close proximity to barley or other cereal crops, since the ascospores may be carried for some distances, as they presumably were in Cambridge and in Birmingham (Packe & Ayres, 1985).

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