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HUMICOLA STELLATUS SP.NOV., A THERMO- PHILIC MOULD FROM HAY

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(With Plate 26 and 2 Text-figures)

Humicola stellatus sp.nov., a thermophilic mould found in mouldy hay from England and Wales, is described. *Monotospora lanuginosa* (Griff. & Maubl.) Mason is transferred to *Humicola*.

Humicola stellatus sp.nov. (Pl. , text-fig. 1)

Hyphae 1-2.8 μ diam., thermophilae, 40° C. lente crescentes. Conidiophora solitaria, lateralialia, 3.0 μ (0.5-1.1 μ) longa, ab hyphis rectis aeriis oriunda. Conidia recentia—subglobosa et hyalina; matura-clara brunnea et saepe cadentia, levia, 4-6(-8) angustitrunculosus rotundatis protuberationibus (incluso hilo) stellata; minima diametros 5.3 μ (3.5-6.8 μ), maxima (trans protuberationes) 7.6 μ (4.5-10.5 μ).

Habitat faenum gramineum in Anglia et Cambria. Typus Herb. I.M.I. 77024 est.

The type specimen, isolated from hay from Rothamsted, Harpenden, Herts., is deposited in Herb. I.M.I. as no. 77024. Type material has also been deposited in Herb. Kew; Herb. DAOM, Ottawa. Cultures have been placed in the C.M.I. culture collection and at C.B.S., Baarn.

The specific epithet refers to the shape of the spores.

In 1959, during the early stages of a study of dust from mouldy hay, small irregularly shaped brown spores were seen in several samples. It was not until some 'hay' with a moisture content of 42% was incubated in a sealed tin at 40° C. that the fungus was seen in quantity. From this incubated hay the fungus was isolated on hay infusion agar (20 g. hay soaked in 1 l. water overnight, strained, and 2% agar added) at 40° as a dematiaceous mould apparently hitherto undescribed. It has since been found in numerous samples of mouldy hay that have obviously heated spontaneously during storage; the samples came from places as far apart as Cornwall, North Wales, Cumberland and Hertfordshire. After seeing a culture of this organism, Mr P. K. C. Austwick, Central Veterinary Laboratory, Weybridge, found sketches (drawn by him in July 1952) of some spores of an identical fungus from a sample of very mouldy hay sent to him from near Wolverhampton.

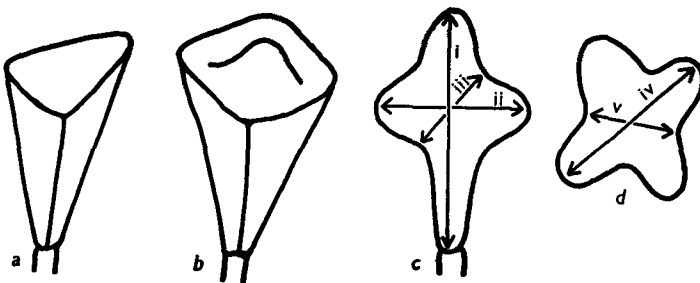
One spore occurred on a Hirst spore trap slide exposed on the afternoon of 25 July, 1958, at Imperial College Field Station, Silwood Park, Ascot, Berks.

Material was examined by Dr M. B. Ellis and Mr J. J. Elphick at the Commonwealth Mycological Institute, Kew, and by Dr S. J. Hughes, Plant Research Institute, Canada Department of Agriculture, Ottawa, all of whom agreed in referring it to the genus *Humicola* Traaen (1914).

On hay, its natural substrate, *H. stellatus* forms a superficial network of hyaline, septate hyphae, 1–2.8 μ diam. Aleuriophores arise on long aerial hyphae, are solitary, lateral, 1.2–2.8 μ diam. and 3 μ (0.5–11 μ) long, occasionally separated from the basal cell by a septum. Aleuriospores (Text-fig. 1) are borne singly and terminally on the aleuriophores; they



Text-fig. 1. *Humicola stellatus* sp.nov. Developing and mature spores grown at 40°. *a*, From hay, Rothamsted 1959; *b*, from hay, Rothamsted 1960; *c*, from 6-month-old culture on 'Oxoid' nutrient agar; *d*, from 5-day-old culture on 'Oxoid' nutrient agar; *e*, from Czapek + peptone agar; *f*, from Czapek + sucrose agar.



Text-fig. 2. *Humicola stellatus* sp.nov. Diagram showing basic shape of spores. *a*, With four projections; *b*, with six projections; *c*, side view of spore with six projections; *d*, top view of spore with six projections. (i–v, positions of measurements.)

are subglobose and hyaline when young, clear dark brown and stellate when mature (Text-fig. 2), and they often become detached. The spore wall is smooth and is thinner towards the tips of the 4-6(-8) projections (including the hilum). Table 1 shows the mean, standard deviation and range of sizes of spores from five different samples (each of twenty-five spores) measured in the five positions (i-v) of Text-fig. 2.

Table 1. *Measurements of spores of Humicola stellatus*

Specimen	View (Text-fig. 2c)		
	Length, μ (i)	Width, μ (ii)	Minimum, μ (iii)
Hay, Cornwall	7.2 \pm 0.69 (5.5-8.5)	7.2 \pm 0.92 (5.5-9.0)	5.4 \pm 0.94 (4.0-6.5)
Hay, Rothamsted 1959	6.6 \pm 0.90 (5.0-10.0)	7.2 \pm 0.87 (6.0-10.25)	4.9 \pm 0.46 (4.0-5.75)
C67 (a)	8.0 \pm 0.22 (6.5-9.0)	8.2 \pm 0.77 (7.0-9.5)	5.8 \pm 0.44 (5.0-6.75)
C67 (b)	8.0 \pm 0.99 (5.25-9.5)	8.0 \pm 0.74 (6.5-10.0)	5.6 \pm 0.71 (4.0-6.75)
Hay, Rothamsted 1960	6.4 \pm 0.90 (5.0-9.0)	6.9 \pm 0.80 (5.0-8.5)	4.8 \pm 0.42 (4.0-5.5)
Mean	7.2	7.5	4.9

Specimen	View (Text-fig. 2d)	
	Maximum, μ (iv)	Minimum, μ (v)
Hay, Cornwall	8.2 \pm 0.78 (7.0-10.0)	5.5 \pm 0.55 (4.75-6.75)
Hay, Rothamsted 1959	7.7 \pm 0.81 (6.5-9.0)	5.0 \pm 0.10 (4.5-5.75)
C67 (a)	8.8 \pm 0.65 (8.0-10.5)	5.8 \pm 0.70 (4.5-7.5)
C67 (b)	9.0 \pm 0.26 (7.5-10.5)	5.6 \pm 0.41 (5.0-6.5)
Hay, Rothamsted 1960	7.4 \pm 0.86 (5.5-9.0)	4.9 \pm 0.55 (3.5-5.75)
Mean	8.2	5.3

C67—6-month-old culture grown on 'Oxoid' nutrient agar for 14 days at 40°: (a) mounted in glycerine jelly; (b) mounted in lactophenol.

Table 2. *Growth rates (increase in diameter per day in mm.) of colonies of Humicola stellatus*

pH ...	40°			24°		
	4.5	6.5	8.5	4.5	6.5	8.5
'Oxoid' nutrient agar (mm.)	3.5	3.3	2.5	0.6	0.5	0.4
Czapek + sucrose agar (mm.)	1.0	0.7	0.2	0.2	0.2	0.3
Czapek + peptone agar (no sugar) (mm.)	3.1	2.0	1.4	0.5	0.3	0.2

H. stellatus grows and sporulates well, forming a dark colony, on hay infusion agar and other media containing peptone or beef extract. On media containing only salts and sugars it grows more slowly, forms a pale colony, sporulates sparsely and produces hyaline chlamydospores in the hyphae. Its optimum temperature for growth is 40°; it grows very slowly at 24° and it survives without obviously growing at 50°. On 'Oxoid' nutrient agar at 40° the colony has a black reverse with a slightly paler edge; the surface is dark brown to black with a grey velvety sheen (Pl. 26, figs. 1-9). Growth is increased by lowering the pH (Table 2). Tests made by growing the fungus on strips of blotting paper in different liquid media showed it was not cellulolytic. The spores do not germinate readily;

those that have been seen germinating were immature and produced a germ tube from the scar of attachment to the aleuriophore.

As this fungus was found in hay associated with some diseases in animals, Mr P. K. C. Austwick kindly did pathogenicity tests. Three weeks after inoculating it intravenously into mice he could not recover it from any of the organs, but 3 weeks after inoculating intraperitoneally into guinea-pigs he found some of the original inoculum unchanged and viable in the peritoneal cavity.

Monotospora lanuginosa (Griff. & Maubl.) Mason is very similar to *H. stellatus*; it also occurs in hay, is thermophilic and non-cellulolytic, the aleuriospores are produced in the same manner and are fairly easily detached; no phialospores have been found. It differs in having globose spores with thick, rough walls, and in growing and sporulating well on the usual mycological media. The two genera, *Monotospora* and *Humicola*, have been confused for many years (Mason, 1933, 1941). *Monotospora* was erected by Corda (1837) for the single species, *M. toruloides*, but authentic material of the species in Herb. Corda shows no fungus which can definitely be related to Corda's description. Saccardo (1880) excluded *M. toruloides* from his concept of *Monotospora*, regarding *M. sphaerocephala* Berk. & Br. as the type species. Traaen (1914) established *Humicola* for *H. fuscoatra* and *H. grisea* isolated from Norwegian soil. These species produced aleuriospores in the same manner as *H. stellatus* and *M. lanuginosa*, but they occur in soil, are mesophilic, cellulolytic, and have phialospores; their aleuriospores do not readily become detached and are often described as chlamydospores. As *Monotospora* Corda is not definable, it seems that *Humicola* Traaen is the most suitable genus for *M. lanuginosa* and this species is therefore transferred as

Humicola lanuginosus (Griff. & Maubl.) comb.nov. (Basionym: *Sepedonium lanuginosum* Griffon & Maublanc 1911, in *Bull. Soc. mycol. Fr.* 27, 70, figs. 1-3.)

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EXPLANATION OF PLATE 26

Humicola stellatus sp.nov.Figs. 1-6. 10-day-old cultures at pH 7.5 ($\times \frac{1}{2}$)

- Fig. 1. On malt extract agar at 40°.
- Fig. 2. On the same agar at 24°.
- Fig. 3. On potato-dextrose agar at 40°.
- Fig. 4. On the same agar at 24°.
- Fig. 5. On 'Oxoid' nutrient agar at 40°.
- Fig. 6. On the same agar at 24°.

Figs. 7-9. 11-day-old cultures at pH 6.5 and 40° ($\times \frac{1}{2}$)

- Fig. 7. On 'Oxoid' nutrient + sucrose agar.
- Fig. 8. On Czapek agar (without sugar).
- Fig. 9. On Czapek + peptone agar (without sugar).
- Fig. 10. Surface view of culture on nutrient agar ($\times 107$).
- Fig. 11. Mature spores ($\times 400$).
- Fig. 12. Spores in different stages of development ($\times 400$).

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