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AEROSPORA OF AN *ERAGROSTIS CURVULA* PASTURE IN SOUTH AFRICA*

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ABSTRACT

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A qualitative and quantitative survey was made of the aerospora above an *Eragrostis curvula* (Schrad.) Nees pasture. The pasture, which lay adjacent to a field of *Panicum coloratum* L. where an outbreak of ovine photosensitivity had occurred, was not grazed during the survey. A Burkard volumetric spore trap was used to sample the aerospora continuously for 10 months. Twenty components of the aerospora were recognized: spores of 9 genera of fungi (*Cladosporium, Alternaria, Epicoccum, Drechslera, Pithomyces chartarum* (Berk. & Curt.) M. B. Ellis, *Nigrospora, Sporormia, Curvularia, Spegazzinia*); 8 recognizable but unidentified spore types; rust spores; hyphal fragments and pollen grains. *Cladosporium* conidia comprised 83,37% of the total aerospora. Conidia of *Pithomyces chartarum* formed a small (0,29%) but significant component of the aerospora, since this fungus is known to cause hepatogenous photosensitivity.

Résumé

AÉROSPORE D'UN PÂTURAGE À ERAGROSTIS CURVULA *EN AFRIQUE DU SUD*

On a effectué un recensement qualitatif et quantitatif de l'aérospore au-dessus d'un pâturage à Eragrostis curvula (*Schrad.*) *Nees. Le pâturage, adjacent à un champ de* Panicum coloratum *L. où s'était produite une éruption de photosensibilité ovine, n'a pas été brouté pendant le recensement. On a utilisé un piège volumétrique à spores, type Burkard, pour procéder à un échantillonnage continu de l'aérospore pendant* 10 *mois. On a reconnu vingt composants de l'aérospore: des spores de* 9 *genres de champignons* (Cladosporium, Alternaria, Epicoccum, Drechslera, Pithomyces chartarum (*Berk. & Curt.*) *M. B. Ellis,* Nigrospora, Sporormia, Curvularia, Spegazzinia); 8 *types de spores reconnaissables mais non identifiées; des spores de rouilles; des fragments d'hyphes et des grains de pollen. Les conidies de* Cladosporium *représentaient* 83,37% *de l'aérospore totale. Les conidies de* Pithomyces chartarum *constituaient un pourcentage petit* (0,29%) *mais significatif de l'aérospore, car comme on le sait, ce champignon cause la photosensibilité hépatogène.*

INTRODUCTION

The investigation was carried out with the objective of obtaining information that might shed some light on the aetiology of *Panicum* photosensitivity. *Panicum* photosensitivity (Afr. "dikoor") is a hepatogenous photosensitivity syndrome (Clare, 1952, 1955) of sheep grazing *Panicum* grasses on cultivated lands in South Africa (Steyn, 1928; Rimington & Quin, 1937). It closely resembles geeldikkop (Quin, 1928, 1930, 1933; Van Tonder, Basson & Van Rensburg, 1972; Kellerman, Basson, Naudé, Van Rensburg & Welman, 1973) and has much in common with *Panicum* photosensitivity syndromes of sheep described abroad (Simpson, 1946; Clare, 1952, 1955).

Since outbreaks of dikoor occur under fairly specific meteorological conditions (Steyn, 1928; Rimington & Quin, 1937), and for other reasons connected with the epizootiology of the disease, it has been postulated that dikoor may be a mycotoxicosis comparable to facial eczema (Kellerman & Marasas, 1971). Facial eczema has been reported in South Africa in sheep grazing rye grass (*Lolium perenne* L.) and white clover (*Trifolium* sp.) pastures (Marasas, Adelaar, Kellerman, Minne, Van Rensburg & Burroughs, 1972). The causal fungus, *Pithomyces chartarum* (Berk. & Curt.) M. B. Ellis, has also been isolated from plant debris collected elsewhere in the country (Marasas & Schumann, 1972).

Although information is available on the quantitative composition of the mycoflora of pastures where outbreaks of ovine photosensitivity have occurred in South Africa (Marasas *et al.*, 1972; Marasas & Schumann, 1972; Marasas & Bredell, 1974; Papendorf & Jooste, 1974; Jooste, 1975; Sutton & Marasas, 1976; Eicker, 1976), detailed information on the quantitative and qualitative composition of the aerospora above such South African pastures is lacking. Since detailed information on the saprophytic mycoflora of pastures (Thornton & Ross, 1959; Di Menna, 1959, 1971; Smith, Lees & Crawley, 1965; Di Menna & Parle, 1970, 1972; Di Menna & Bailey, 1973) and the aerospora above such pastures (Brook, 1959, 1963, 1969; Smith, Crawley & Lees, 1961, 1962; Brook & Mutch, 1964; Smith & Crawley, 1964; Sinclair & Howe, 1968; McKenzie, 1971) in New Zealand has led to a better understanding of the aetiology of facial eczema, it was decided to conduct an investigation on the aerospora above such a pasture when the opportunity arose. This paper reports on the qualitative and quantitative composition of the aerospora above an *Eragrostis curvula* (Schrad.) Nees pasture adjacent to a field of *Panicum coloratum* in a stretch of old maize land on which an outbreak of ovine photosensitivity had occurred. Detailed quantitative results of the survey and information on the diurnal periodicity and seasonal variation of the aerospora will be reported elsewhere.

MATERIALS AND METHODS

Geographical location of the pasture surveyed

During January 1973, an outbreak of photosensitivity occurred in sheep grazing mainly *P. coloratum* plants in an old maize land on the farm Knoppiesfontein, Delmas district, (26 28 BA) Transvaal Province (Kellerman & Marasas, 1973, unpublished

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data). Since it was not possible to do a survey of the aerospora of this *Panicum* field over a period of 12 months because the farmer wanted to plough the land, it was decided to set up the spore trap in a permanent *Eragrostis curvula* pasture adjoining the maize land. The fact that the sheep which had become photosensitive had also grazed in the *Eragrostis* pasture provided additional justification for this decision.

Spore trapping

A Burkard* seven-day recording volumetric spore trap (Hirst, 1967) was used to survey the aerospora of the *Eragrostis* pasture continuously from 1974-02-01 to 1974-11-17. The Burkard is a suction-type spore trap equipped with a drum that rotates past the intake orifice, making 1 revolution in 7 days. Air was sucked in through the orifice (14 × 2 mm) at a rate of 10 ℓ/min and spores impinged on transparent cellulose tape mounted on the drum moving at a rate of 2 mm/h. The spore trap was converted to battery operation and mounted level on a wooden platform in the pasture with the intake orifice *c*. 50 cm above ground level.

Spore counting

The cellulose tape was removed from the drum after continuous exposure for 7 days and placed with the sticky side facing upwards on a perspex ruler* divided into 7 sections of 48 mm each. The tape was then cut into 7 sections corresponding to 24 hours each, and the sections mounted with the sticky side upwards on microscope slides (75 × 25 mm) in AH mounting medium (Cunningham, 1972). The mounted sections were covered with cover slips (50 × 22 mm) and left in a horizontal position to dry for 14 days. A rubber stamp and ink were used to mark a grid composed of 6 vertical and 3 horizontal lines on the cover slip of each 24-hour section. The 6 vertical lines corresponded to the times of the day when spore counts were made, i.e., 03h00, 06h00, 08h30, 14h00, 16h00 and 22h00, respectively. The 3 horizontal lines, 4 mm apart, were parallel to the direction in which the tape moved and represented 3 replicates. At each of the 6 times of the day, the spores present in 3 microscope fields (×400) located at the intersections of the vertical line with each of the 3 horizontal lines were counted under a Zeiss phase-contrast microscope. Consequently, the spores present in 18 microscope fields, representing 3 replicates of each of 6 different times of the day, were counted on each 24-hour section of tape. A total of 2 034 microscope fields were examined during the survey.

RESULTS

Spore types and other structures encountered

The following 20 components of the aerospora above the *E. curvula* pasture could be distinguished on the spore trap tapes:

Identifiable genera of fungi

Cladosporium (Fig. 1)
Alternaria (Fig. 2)
Epicoccum (Fig. 3)
Drechslera (Fig. 4)
Pithomyces chartarum (Fig. 5, 6)
Nigrospora (Fig. 7)
Sporormia (Fig. 8)
Curvularia (Fig. 9)
Spegazzinia (Fig. 10)

Recognizable but unidentified spore types

Hyaline amerospores (Fig. 11)
Dark amerospores (Fig. 12)
Didymospores (Fig. 13, 14)
Three-celled phragmospores (Fig. 17)
Four-celled phragmospores (Fig. 15, 16)
Six-celled phragmospores (Fig. 18)
Seven-celled phragmospores (Fig. 19)
Dictyospores (Fig. 20, 21)

Other structures

Rust spores (Fig. 22)
Hyphal fragments (Fig. 23)
Pollen grains (Fig. 24)

The only spores that could be identified to the species level were those of *P. chartarum* (Fig. 5, 6). The conidia of the latter were identified from the descriptions of Ellis (1960, 1971), Dingley (1962) and Marasas & Schumann (1972). This identification was confirmed by a comparison of the *P. chartarum* conidia on the Delmas tapes (Fig. 5) with those on a spore tape from New Zealand (Fig. 6) which was kindly supplied by Dr Margaret E. di Menna of Ruakura Research Station, Hamilton, New Zealand. Although Rees (1964) clearly saw the characteristic hyaline remains of the conidiophores attached to the base of *P. chartarum* conidia trapped in Australia, these basal frills were not visible on either the South African or New Zealand tapes (Fig. 5, 6).

Relative incidence of spore types

The number of entities of each of the 20 components of the aerospora described above were counted in each of the microscope fields examined and the total calculated as a percentage of the total number of spores counted during the entire period of the survey. The relative incidence and order of dominance of the different components of the aerospora are given in Table 1.

TABLE 1 Relative incidence and order of dominance of the different components of the aerospora of an *Eragrostis curvula* pasture.

Component	Number of entities	Percentage of the total number of entities
Cladosporium	9 277	83,37
Hyaline amerospores	506	4,54
Four-celled phragmospores	235	2,11
Hyphal fragments	196	1,76
Didyamospores	192	1,72
Alternaria	169	1,51
Dark amerospores	134	1,20
Pollen grains	107	0,96
Epicoccum	83	0,74
Drechslera	44	0,39
Rust spores	39	0,35
Pithomyces chartarum	33	0,29
Nigrospora	32	0,28
Sporormia	24	0,21
Curvularia	12	0,10
Three-celled phragmospores	10	0,08
Six-celled phragmospores	10	0,08
Seven-celled phragmospores	8	0,07
Dictyospores	8	0,07
Spegazzinia	5	0,04

* Burkard Manufacturing Co., Rickmansworth, England

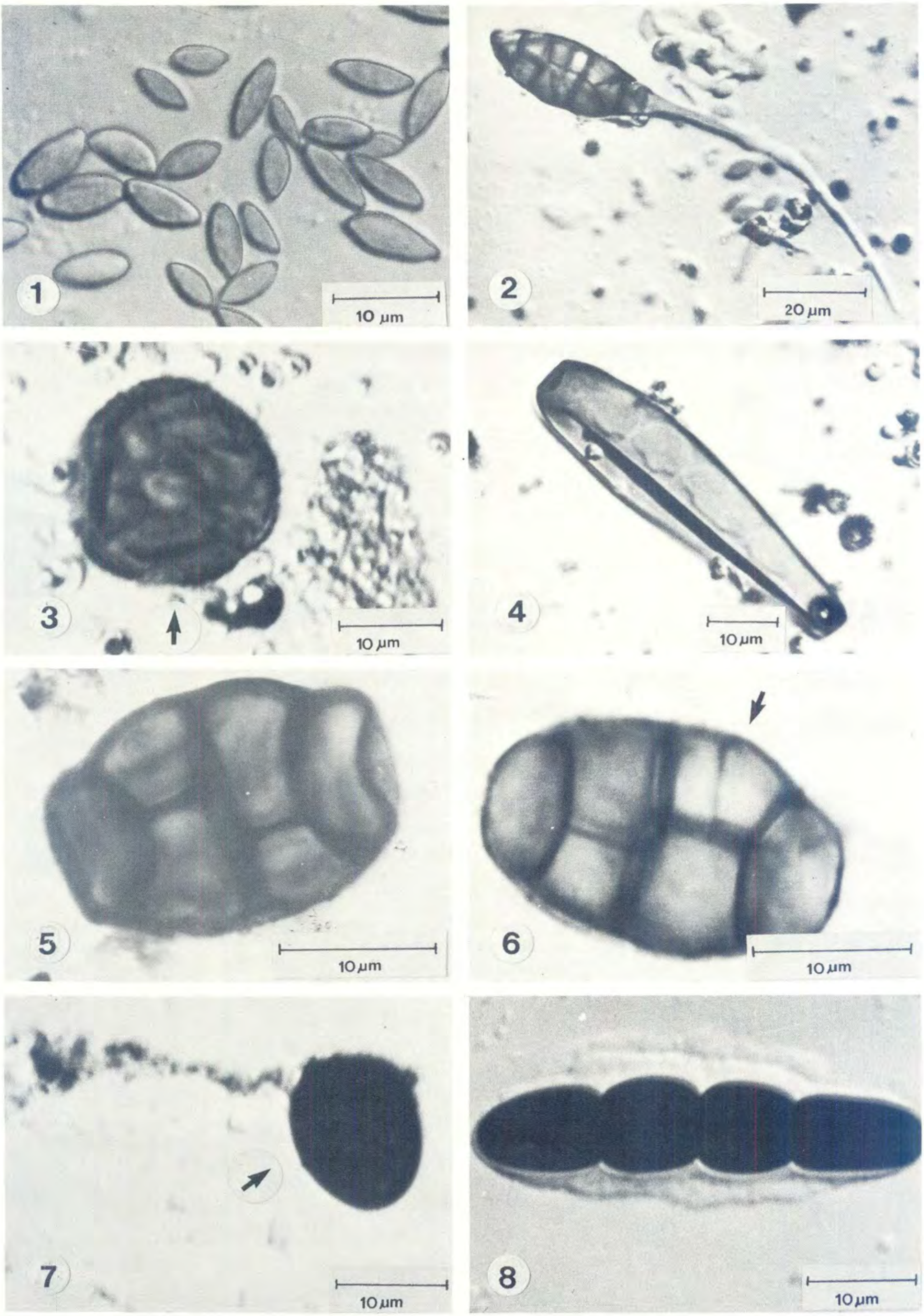

FIG. 1 *Cladosporium;* FIG. 2 *Alternaria;* FIG. 3 *Epicoccum;* FIG. 4 *Drechslera;* FIG. 5, 6 *Pithomyces chartarum;* FIG. 7 *Nigrospora;* FIG. 8 *Sporormia*

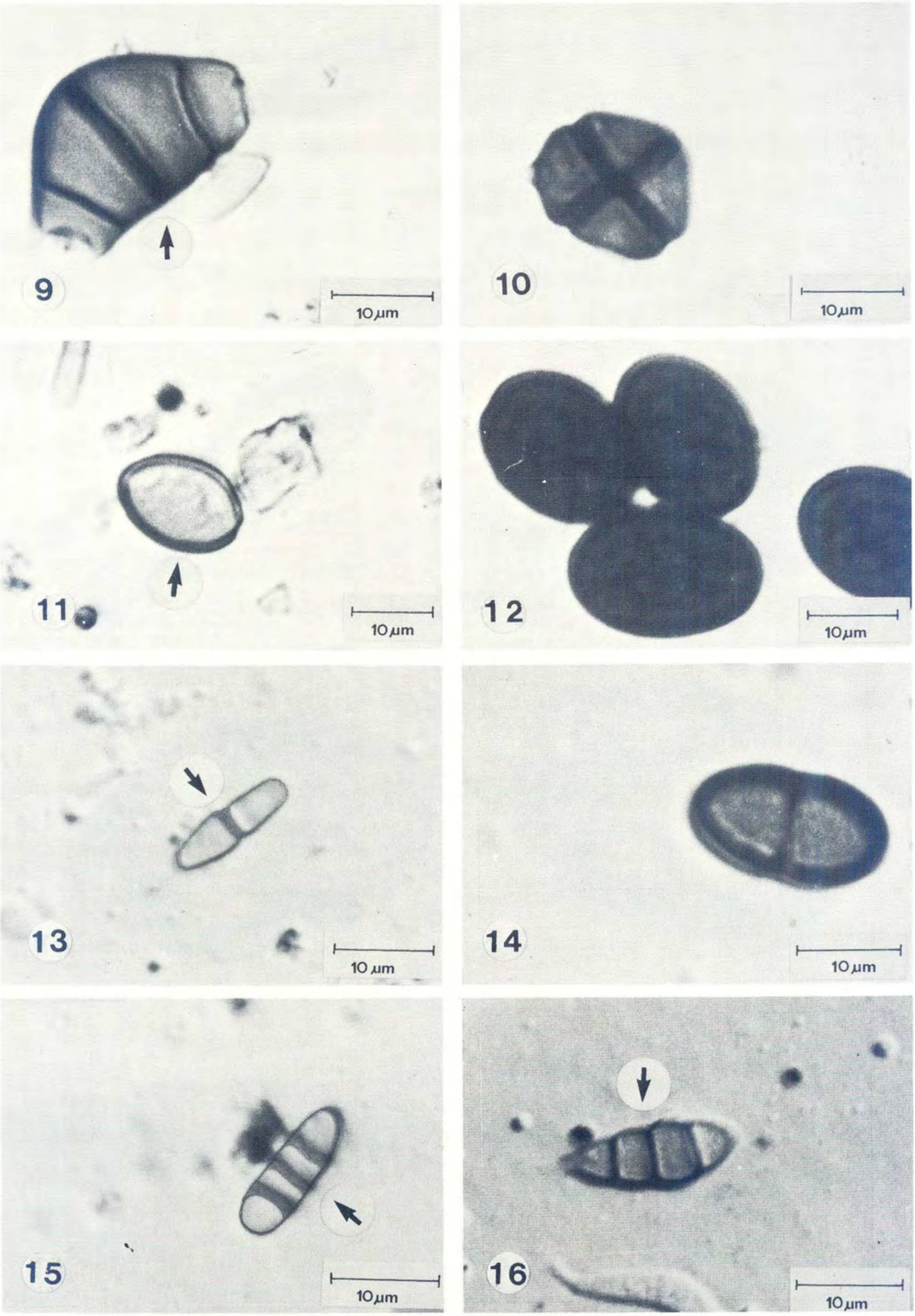

FIG. 9 *Curvularia;* FIG. 10 *Spegazzinia;* FIG. 11 Hyaline amerospore; FIG. 12 Dark amerospores; FIG. 13, 14 Didymospores; FIG. 15, 16 Four-celled phragmospores

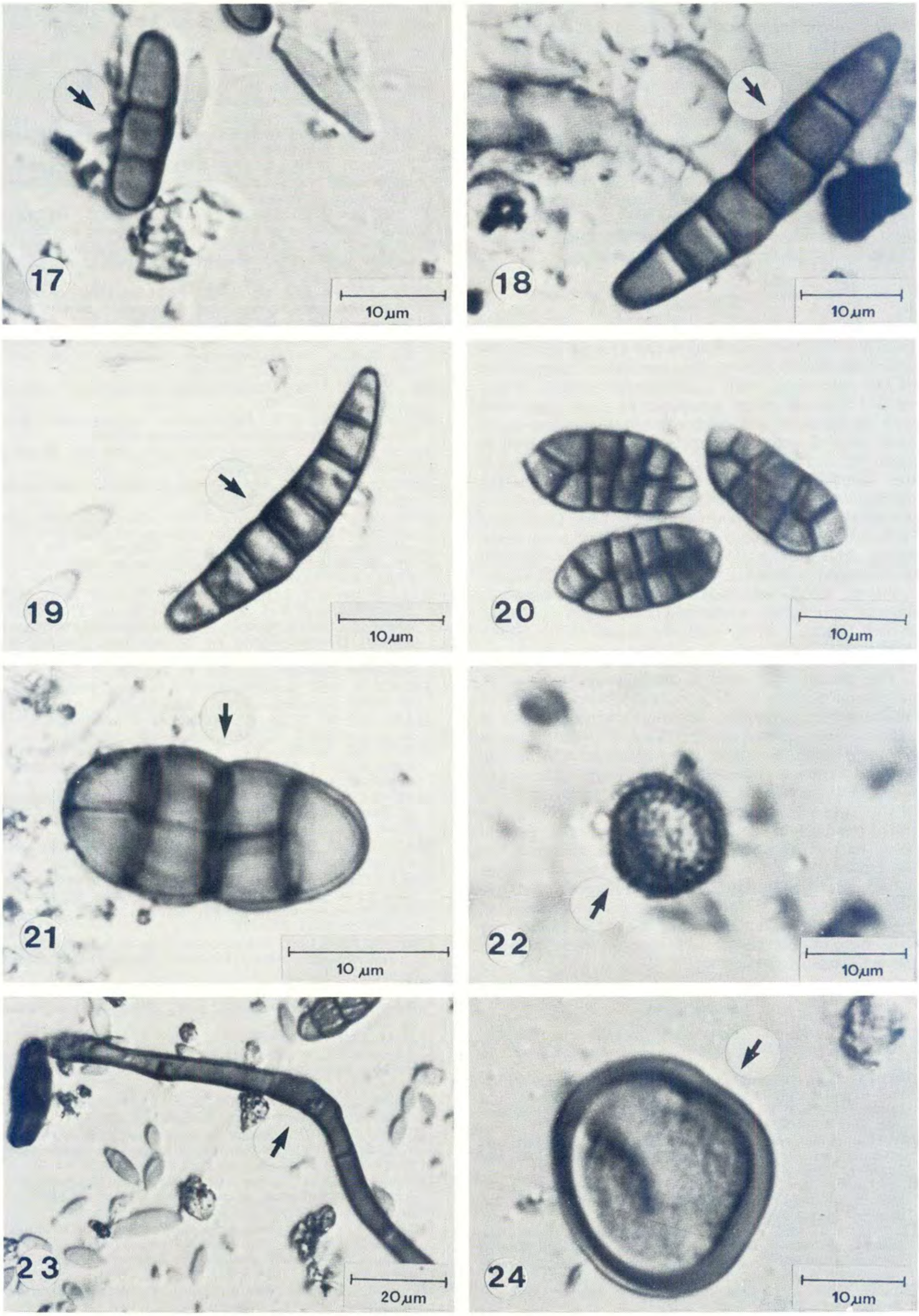

FIG. 17 Three-celled phragmospore; FIG. 18 Six-celled phragmospore; FIG. 19 Seven-celled phragmospore; FIG. 20, 21 Dictyospores, FIG. 22 Rust spore; FIG. 23 Hyphal fragment; FIG. 24 Pollen grain

Cladosporium was clearly the predominant component of the aerospora, comprising as it did 83,37% of the total number of entities counted. Hyaline amerospores were the second most important component (4,54%) of the aerospora. This group was so large because it was usually not possible to identify one-celled, hyaline spores to the generic level, and it included conidia of commonly-occurring fungi such as *Aspergillus*, *Penicillium*, *Trichoderma*, *Phoma*, etc. Other components that comprised more than 1% of the aerospora were four-celled phragmospores, hyphal fragments, didymospores, *Alternaria*, and dark amerospores. The remaining 13 minor components together made up only 3,66% of the total aerospora.

Discussion

Cladosporium was by far the most prevalent spore type (83,37%) encountered in the present qualitative and quantitative survey. The qualitative composition of the aerospora, with *Cladosporium* clearly predominant over all other components, is in agreement with the results of aerospora surveys of pastures where outbreaks of ovine photosensitivity had occurred in New Zealand (Brook, 1963; McKenzie, 1971) and in the United Kingdom (Lacey, 1975). Although *Cladosporium* is unconnected with photosensitivity, it is interesting to note that Ordman & Etter (1956) and Ordman (1963, 1970), using exposed plate techniques, found *Cladosporium* to be the dominant component of air-borne pathogens in cities. Conidia of *Tetraploa aristitata*, which were particularly abundant over one pasture in England associated with ovine photosensitivity (Lacey, 1975), were not found in the South African survey.

The presence of even a small proportion of *P. chartarum* conidia (0,29%) is noteworthy in view of the known causative role of conidia of this fungus in hepatogenous photosensitization. *P. chartarum* is normally only a minor component of the mould flora of pastures, and even during severe outbreaks of facial eczema in New Zealand it comprised only 1% of the total aerospora (Di Menna & Parle, 1970). No sheep were present on the pasture used in the present study and consequently it was not possible to relate the presence of *P. chartarum* conidia to outbreaks of dikoor. Further studies on the qualitative and quantitative changes of the aerospora of pastures associated with the onset of outbreaks of dikoor in sheep may shed more light on the possible role of fungal spores in the aetiology of the disease.

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