Occurrence of fungal spores in dust was reported by several workers although their role in causing allergic reactions and infection is a subject of controversey (Brown[4], Reyman and Schwartz[15]). However none of them dealt with thermophilic fungi. The presence of thermophilic fungi in the thin film of dust accumulated on books in libraries is of interest since the existence of elevated temperatures is not detectable. Thermophilic fungi have a wide range of temperature tolerance. The spores present in dust remain dormant until elevated temperatures necessary for germination and growth are available. It is also observed that they are ubiquitous in nature and can be isolated from almost all habitats notwithstanding the existence of elevated temperatures. Thermophilic fungi were reported to cause disease or allergic reactions in animals and man (Ainsworth and Austwick[1,2], Gregory and Lacey[10], Pore and Larxh[13],). However none of the isolates included in the present study were found to be pathogenic as the albino rats inoculated (I.P. route) with different concentrations of spore suspension prepared in saline showed no visible symptoms even after a month of inoculation. Thus the present investir gation confirms the view (Ainsworth and Austwick[1,2], Emerson[8]) that the medical implications of thermophilic fungi do not appear to have more than minor economic significance.

The author wishes to thank Dr. S. Ramachandran, Dr. K. S. Gopalkrishnan and Dr. V. H. Pawar.

Hindustan Antibiotics Research Centre, Pimpri, Poona 411 018, India, *April* 25, 1978.

A. SUBRAHAMANYAM.

1. Ainsworth, G. C. and Austwick, P. K. C., *Trans., Brit. Mycol. Soc.*, 1955, **38**, 369.
2. — and —, *Fungal Diseases of Animals*, Commonwealth Agricultural Bureaux, Farnham Royal Bucks, England, 1959, pp. 148.
3. Apinis, A. E. and Pugh, G. J. F., *Mycopathol. Mycol. Appl.*, 1967, **33**, 1.
4. Brown, G. T., *J. Allergy*, 1936, 7, 455.
5. Cooney, D. G. and Emerson, R., *Thermophilic Fungi. An Account af theiz Biology, Activities and Classification.* W. H. Freeman and Co., San Francisco, 1964, p. 188.
6. Crisan, E. V., " The Isolation and Identification of Thermophilic Fungi," *M.S. Thesis*, Purdue University, Lafayette, Indiana, 1959, pp. 107.
7. Eggins, H. O. W. and Malik, K. A., *Automic van Leenwenhoek. Ned.*, 1969, **35**, 178.
8. Emerson, R., "Thermophillis" in *The Fungi*, ed. Ainsworth and Sussman, 1968, **3**, 108.
9. Fergus, C. L., *Mycologia*, 1964, **56**, 267.
10. Gregory, P. H. and Lacey, M. E., *J. Gen. Microbiol.*, 1962, **30**, 75.
11. Kuster, E. and Locci, R., *Arch. Mikrobiol.*, 1964, **48**, 319.
12. Mulinge, S. K. and Apinis, A. E., *Trans. Brit. Mycol. Soc.*, 1969, **53**, 361.
13. Pore, R. S. and Larsh, H. W., *Mycologia*, 1967, **59**, 927.
14. Resz, A., *Hyg. 2 Abt.*, 1968, **122**, 597.
15. Reyman, F. and Schwartz, M., *Acto path. microbiol. Scand.*, 1947, **24**, 77.
16. Subrahmanyam, A., Studies on the morphology, culture characters and biological activities on some thermophilic fungi from Maharashtra, *Ph.D. Thesis,* Poona University, Poona, India, 1976.
17. Tansey, M. R., *Mycologia*, 1971, **63**, 537.
18. — and Brock, T. D., *Bacteriol. Proc.*, 1971, p. 36 (Abstr.).
19. Ward, J. E. Jr. and Cowley, G. T., *Mycologia*, 1972, **64**, 200.

ADDITIONAL DIAGNOSTIC SYMPTOMS OF COVERED SMUT OF BARLEY

COVERED smut of barley [*Ustilago hordei* (Pers.) Lagerh.] is a disease of world-wide distribution. It is normally characterized by conversion of spikelets of ear-head in black powdery mass covered by grayish-white persistent membrane. However, during the course of a survey of plant diseases in March 1978, at the University farm, Kanpur, some plants of huskless barley (Variety DL 281) were observed to show smut sori on top leaves and base of the peduncle as well. Since no smut fungus is reported to cause any symptom on parts other than earhead of barley, detailed studies were taken up.

The affected ears exhibited typical symptoms of covered smut of barley. In addition, smut sori were also observed on flag leaf and the leaf below in the form of grayish-white amphigenous streaks. In most cases the midrib was primarily involved, but leaf tissues on either side of the midrib were also replaced by smut sori. It was not uncommon to find two or three streaks running parallel side by side along long axis of leaf (Fig. 1A and 1B). Initially, the streaks were yellow, changing gradually to grayish-white with the formation of hard, peristent membrane of the sorus. It was remarkable to note that smut sori on flag leaves were evident even before emergence of the smutted earheads and in certain cases the smutted ear remained enclosed for long in the sheath whorl of three to four abnormally congregated leaves due to shortening of internodes, and the laminae of these leaves exhibited judicious development of smut sori (Fig. 1B). At maturity the leaves were torn away along the sori thus giving them a ragged appearance. Spherical to

subglobose sori at the base of peduncle were also found (Fig. 1C).

FIG. 1A. Smut sori on flag leaf and the leaf below it in different stages of development with partially emerged smutted earhead of barley. 1B. Congregated barley leaves enclosing smutted earhead in the sheath whorl and their laminae torn away along the smut sori. 1C. Smut sori at the base of peduncle.

The morphological characters and size of the chlamydospores, and their mode of germination agreed closely with *U. hordei* causing covered smut of barley.

This is the first report of the development of the symptoms of covered smut of barley on leaf and peduncle. These symptoms can be utilized in the diagnosis of the disease even before the emergence of smutted ears in the barley variety DL 281, and in early roguing out of the affected plants.

Department of Plant Pathology,
Chandra Shekar Azad University
of Agriculture and
Technology, Kanpur,
April 24, 1978.

D. V. SINGH.
L. S. CHAUHAN.
H. K. SAKSENA.

PHYSIOLOGICAL EVIDENCES FOR QUICK REGENERATION OF WALL ON ISOLATED PLANT PROTOPLASTS

WALL regeneration around isolated protoplasts of green plants was first reported by Pojnar *et al.*[1] and later supported by Mishra and Colvin[2]. A number of reports that have appeared in the last decade or so have also provided evidences for wall regeneration[3–12]. A careful examination of the time course of wall regeneration as reported in these studies reveal that generally the time scale corresponding to this process is in terms of days since isolation[1,3–12]. However, there have been a few reports where this process has been described to be much quicker, of the order of hours since isolation. While Mishra and Colvin[2] reported a complete wall regeneration between 4–6 hours of isolation in protoplasts from green tomato fruits, Williamson *et al.*[14] found that in *Vicia hajastana* suspension cultures, the microfibril deposition was initiated after 10–25 minutes and within 20 hours, the protoplasts were observed to be densely covered with microfibrils. However, both the reports of quick regeneration of wall drew their evidences from ultrastructural studies under the electron microscope, calcofluor staining or X-ray diffraction studies. Since the cell wall is a physical functional barrier around a plant cell, it was presumed that it should be possible to demonstrate the development of the same around the protoplasts through simple plasmolysis and osmolysis experiments.

The wall regeneration studies were carried out on protoplasts isolated from the following plant materials :

1. Placenta cells and the cells of inner wall of Simla pepper (*Capsicum annum*).
2. Placenta cells and the cells of inner wall of green tomato fruits (*Lycopersicon esculentum*).
3. Young leaves around the growing point in cabbage head.

The methods for isolation of protoplasts have been previously described[13]. In brief, it involved (i) incubation of tissue sections in 5–10% pectinase dissolved in 0·25 M sodium nitrate for 1½ hours, (ii) decanting the enzyme mixture and macerating the softened tissue with a glass rod, (iii) filtering the macerate through a thick layer of cotton wool and (iv) observing the isolated protoplasts in the incubation medium in a fixative coated cavity slide under an optical microscope.

For osmolytic studies of wall regeneration, the medium with the protoplast was diluted with drops of distilled water and the microscopic field was so adjusted as to observe the same protoplasts upon dilution. The maximum dilution was to the extent of 5 drops of distilled water to one drop of the incubation medium. Such dilutions were performed at frequent intervals on samples kept on different slides.