

## NANNIZZIA INCURVATA, STOCKDALE ISOLATED FROM SOIL SAMPLES

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**ABSTRACT.**—Seven of 30 soil samples collected from two sites in Urbana, Illinois, were induced to produce the perfect form of *Microsporium gypseum*, which was identified as *Nannizzia incurvata*, Stockdale, 1961. Best results were achieved by using nonsterile soil treated with an antibiotic solution (20 units of penicillin, 40 units of streptomycin and 0.5 mg of cycloheximide per ml) and baited with either child's hairs or horse hairs at 25°C. Cleistothecial formation was visible beginning from 20 to 25 days after soil samples were baited with hair.

Nannizzi (1927) was first to observe the perfect state of *Microsporium gypseum* to which he gave the name *Gymnascus gypseum*. However, his work came under severe criticism because the substrates used were not sterilized and the cultures were, therefore, impure. Subsequent workers assumed that Nannizzi was mistaken and as a result, very little progress was made for more than three decades, until Dawson and Gentles (1959) using the hair bait technique, reported the perfect state of *Keratinomyces ajelloi*. Soon thereafter, Griffin (1960), Szathmay and Herpay (1960), and Stockdale (1961) independently discovered perfect states of *M. gypseum*. This discovery was confirmed in the following years in India (Sarkar, 1962; Mohapatra and Gugnani, 1964) and in Argentina (Varsavsky, 1964).

The discoveries of sexual states in other species of dermatophytes and re-

lated keratinophilic fungi followed in quick succession. Dawson and Gentles (1961) described the perfect state of *Microsporium nanum* and *Trichophyton terrestre*, Georg et al. (1962) of *M. breuseghemii*, Ajello (1961) of *M. cookei*, Randhawa and Sandhu (1964) of *Keratinophyton terreum*, Stockdale et al. (1965) of *Trichophyton similis*, and Ajello and Cheng (1967) of *Trichophyton mentagrophytes*.

This information necessitated the reclassification of these fungi. Stockdale (1961), therefore, created a new genus *Nannizzia* to accommodate the perfect form of *M. gypseum*. She later classified the perfect state of *M. gypseum* in three different species of *Nannizzia*, namely, *N. incurvata*, *N. gypsea* and *N. fulva*. She included the perfect states of all species of *Microsporium* under the genus *Nannizzia* whereas those of *K. ajelloi* and *T. terrestre* were transferred to the genus *Arthroderma*. Comparatively recently, Dawson et al. (1964) studied the environmental conditions affecting sexual reproduction in the species belonging to these two genera.

The present work records for the first time the occurrence of *N. incurvata* in the United States. Various substrates, baits and growth temperatures were investigated to determine those most favorable for the growth of the perfect state.

### MATERIALS AND METHODS

Thirty samples of soil were cultured for keratinophilic fungi: 14 were col-

lected from a garden bed and 16 from a ploughed field in Urbana, Illinois. Soil was scraped from surface layers of the two sites. After bringing the samples to the laboratory, they were poured in sterile petri plates; the plates were half filled. Each soil sample was moistened with aqueous antibiotic solution containing 20 units of penicillin, 40 units of streptomycin and 0.5 mg cycloheximide (Actidione, The Upjohn Co.) per ml and baited with either autoclaved human (child's) hair, horse hair, dog hair or chicken feathers by pressing tufts of hair or feathers partially into the soil. The plates were kept at 25° C. and examined at regular intervals.

In another set of experiments, three hair samples (1496, G3120, G2791) positive for *M. gypseum* were cultured separately on Sabouraud's dextrose agar. When luxuriant growth was obtained, an aqueous spore suspension was prepared by adding about 5 ml of sterile 0.85 per cent sodium chloride to each culture tube (25 x 150 mm) and admixing the culture and water thoroughly. About 2 ml of the suspension was inoculated in 20 petri plates which were half filled with sterile soil and baited with sterile child hairs, horse hairs, dog hairs, or chicken feathers. The plates were kept at room temperature (25° C.) and examined for 3 months at weekly intervals.

#### RESULTS AND DISCUSSION

Of the 30 soil samples examined, seven yielded keratinophilic fungi that developed the perfect state; three from the garden bed and four from the ploughed field.

After 20 to 25 days of incubation at 25° C., cleistothecial formation was visible as small white tufts of hyphae which gradually enlarged, became pale-buff and finally yellowish-buff.

Cleistothecia were globose, pale to yellow-buff, measuring 255 to 500 $\mu$  in diameter, exclusive of appendages. Peridial hyphae were numerous, septate, verticillately branched, thick-walled and with constrictions. Free ends were numerous; they were straight tapering hyphae which were slender, smooth walled and septate or they were hyphae which were slender, smooth walled, septate and having loosely or tightly coiled spirals. Elliptical, rough walled macroconidia typical of *M. gypseum* were present.

On crushing the cleistothecium, the

peridial hyphae collapsed and asci were released. The asci were globose to ovate in shape and measured 5 to 6  $\mu$  in diameter. Each ascus contained 5 to 8 ascospores.

The ascospores were light yellow, smooth walled and measured 2 to 3  $\mu$  by 1.5 to 2.0  $\mu$ .

In studies to determine the factors affecting the formation of perfect forms on hair, it was observed that cleistothecia were formed at 25° C. but not at 22° C. Child hair and horse hair but not dog hair or chicken feathers appeared to be most suitable for perfect stage formation. The perfect forms were not produced on sterile soil but were formed on unsterile soil treated with antibiotic solution.

None of the hair samples in the second experiment produced the perfect state though all of them had the characteristic growth of *M. gypseum*, forming cinnamon-brown colonies with numerous and characteristic macroconidia and microconidia.

Stockdale (1961) proposed the genus *Nannizzia* for (*N. incurvata*) the perfect state of *M. gypseum*. She later (1963) found certain differences between *N. incurvata* and *Gymnoascus gypseum* originally described by Nannizzi (1927) and came to the conclusion that they represented two separate but closely related species. She (*loc. cit.*) therefore proposed the name *Nannizzia gypsea* for Nannizzi's form. Morphologic characters of cleistothecia encountered in the present study agreed with the description of *Nannizzia incurvata*, Stockdale, 1961, except that in very few cases asci were seen having five instead of eight ascospores. The present form can be differentiated from *N. gypsea* and *N. fulva* (closely related species of *Nannizzia*) in that the peridial hyphae curved away from the cleistothecium whereas in the latter they curve towards it. Moreover, the presence of straight hyphal appendages was a constant feature of the cleistothecia of *N. incurvata* which occurs less frequently in the other two species.

#### LITERATURE CITED

- AJELLO, L. 1961. The ascigerous state of *Microsporium cookei*. Sabouraudia, 1: 173-177.  
 AJELLO, L. and Shu-Lan Cheng. 1967. The perfect stage of *Trichophyton mentagrophytes*. Sabouraudia 5: 230-234.

