THE GENUS

Aspergillus

KENNETH B. RAPER

Professor, Departments of Bacteriology and Botany
University of Wisconsin
Madison, Wisconsin

DOROTHY I. FENNELL

Research Associate, Department of Bacteriology
University of Wisconsin
Madison, Wisconsin

WITH A CHAPTER ON PATHOGENICITY

Peter K. C. Austwick

Senior Research Officer, Central Veterinary Laboratory
Weybridge, Surrey
England

PLANT PATHOLOGY
INSTITUTE OF AGRICULTURE
UNIVERSITY OF MINNESOTA
ST. PAUL, MINNESOTA 55101

CHAPTER

XVI

Aspergillus niger Group

OUTSTANDING CHARACTERS

Conidial heads in some shade of black—greenish black, brownish black, purplish black, or carbon black; globose, radiate, or split into few to many irregular or well-defined columns of conidial chains

Conidiophores hyaline to brown, typically smooth but in a few species slightly granular or punctate, usually heavy walled, brittle, splitting longitudinally when crushed

Vesicles globose or nearly so, hyaline or colored in light to dark brown shades

Sterigmata in one or two series depending on the species, often deeply colored or even filled with pigment

Conidia globose, subglobose, elliptical, or horizontally flattened; smooth or nearly so, echinulate, verruculose, or with conspicuous longitudinal striations

Sclerotia globose to subglobose, cream colored when young, then buff to pinkish or grayish buff or brown

GENERAL CONSIDERATIONS

Included here are some of the most common of all saprophytic fungi—molds that are worldwide in distribution and capable of growing upon the

widest possible range of organic substrates. Because of the characteristic pigmentation of the conidial heads, members of the group are commonly referred to as "*Aspergillus niger*" without regard to morphology or other distinguishing criteria. This practice is not without some justification, for Van Tieghem's species, *A. niger*, is by far the most abundant member of the group, and the degree of intragroup variation is such that specific identifications often become quite difficult.

The black Aspergilli are not all alike, however, and contrasting differences in structural detail readily distinguish certain species within the group. At one end of the scale is *A. carbonarius* (Bain.) Thom with robust conidial structures, biseriate sterigmata, and very large multinucleate conidia. At the other end are the species *A. japonicus* Saito and *A. aculeatus* Iizuka, which possess only a single series of sterigmata and truly echinulate conidia. Intermediate between these, and centered upon *A. niger* Van Tieghem, are most of the recognized species, characterized by biseriate sterigmata and relatively small conidia. These vary in the shade of black pigmentation of their conidial heads and in the dimensions of conidiophores, vesicles, and sterigmata. They are most troublesome to classify and are, at the same time, the species about which numerous papers have been written.

The dimensions of the sterigmata and the surface markings of the conidia of these latter species are emphasized in the "Group Key" and in the species descriptions that follow. In making the necessary observations and measurements, care should be exercised to include mature conidial heads from different areas within the colonies; otherwise incomplete and sometimes misleading information may be recorded. The primary sterigmata not infrequently continue to lengthen after sporulation has begun, and the younger conidia in a chain often show slightly larger dimensions and less conspicuous and less diagnostic markings than do older conidia. The pigmentation of the conidial heads varies not only in different species, but sometimes within the same isolate, depending upon the substrate and the age of the culture; hence, color determinations should be based upon continued observations throughout the period of growth and sporulation.

We have attempted to evaluate original descriptions and current interpretations of species concepts, insofar as possible, and to recognize a limited number of species that we believe possess reasonably stable and distinguishing characteristics. In some cases the descriptions of these are drawn in rather broad terms, but to do otherwise could multiply the taxons *ad infinitum* without sufficient bases to separate one from another.

Mosseray in 1934 did subdivide the *A. niger* group into numerous species by establishing narrow limits. He studied (in tube cultures on "Raulin-Neutré Gelose") 63 strains of black Aspergilli previously assembled by

Professor Biourge and, from among these, recognized 35 species, of which 25 represented either new species or new combinations. Using other conditions of culture and vastly greater numbers of strains for comparative observations, other investigators, including ourselves, have found his classification to be too detailed and too often hinged upon individual cultures to be generally useful. Nevertheless, following our own Key to the *A. niger* group, we are appending a translation of Mosseray's Key, which presents the bases he employed for separating the species.

GROUP KEY

I. **Sterigmata in two series**

    A. **Colonies (conidial heads) on Czapek's agar appearing carbon black to the naked eye**

        1. Conidia 6 to 10 μ in diameter at maturity......... *A. carbonarius* (Bainier) Thom

        2. Conidia 5.0 μ or less in diameter at maturity

            a. Conidiophores not exceeding 4.0 mm. in length

                (1) Colonies spreading rapidly on Czapek's agar...................................... *A. ficuum* (Reich.) Hennings

                (2) Colonies growing more slowly on Czapek's agar

                    (a) Conidia at maturity horizontally flattened, mostly 3.0 to 3.5 μ in diameter, with longitudinal color bars or striations................................. *A. phoenicis* (Cda.) Thom

                    (b) Conidia at maturity globose, mostly 4.0 to 5.0 μ, irregularly roughened with conspicuous ridges and echinulations not arranged as longitudinal striations..... *A. niger* V. Tiegh.

            b. Conidiophores commonly exceeding 5 mm. and reaching 1 cm. but also commonly with shorter stalk bearing diminutive heads................. *A. pulverulentus* (McAlp.) Thom

    B. **Colonies (conidial heads) grayish olive brown or deep olive brown when young; usually becoming reddish brown to brownish black, but with olive or grayish colors often persistent.**

        1. Heads quickly dark black-brown or reddish brown

            a. Conidia under 5.0 μ in diameter, horizontally flattened, and appearing striate at maturity

                (1) Heads quickly dark black-brown; colony reverse uncolored; conidiophores mostly 2 to 3 mm. but up to 5.0 mm. long; conidia mostly 3.0 to 3.5 μ in diameter............ *A. tubingensis* (Schöber) Moss.

(2) Heads quickly reddish brown; colony re-
verse in similar shades; conidiophores
mostly 1.0 to 1.5 mm. long; conidia mostly
4.0 to 4.5 µ in diameter.................... *A. awamori* Nakazawa

b. Conidia 6.0 to 8.0 µ in diameter, globose to sub-
globose, coarsely tuberculate.................. *A. flavo-furcatis* Ba-
tista and Maia (see
A. flavus group)

2. Heads persistently dark grayish brown or olive-
brown

a. Conidia at maturity elliptical, conspicuously
echinulate, 5.0 to 5.5 µ by 3.3 to 3.8 µ.......... *A. ellipticus*, sp. nov.

b. Conidia at maturity globose or nearly so, some-
times elliptical when young

(1) Conidia at maturity conspicuously spinu-
lose...................................... *A. heteromorphus* Ba-
tista and Maia

(2) Conidia at maturity irregularly and finely
roughened

(a) Conidial heads generally small, in age
on malt agar splitting into fairly num-
erous compact divergent columns...... *A. foetidus* (Naka.)
Thom and Raper

(b) Conidial heads large, columns few and
poorly defined on malt agar

(1') Basal mycelium on malt agar un-
colored or only faintly yellow...... *A. foetidus* (Naka.)
T. & R. var. *pallidus*
N., S., & W.

(2') Basal mycelium on malt agar bright
golden yellow.................... *A. foetidus* (Naka.)
T. & R. var. *acidus*
N., S., & W.

II. Sterigmata uniseriate

A. Conidia globose to subglobose, conspicuously echi-
nulate; vesicles commonly 20 to 35 µ but ranging from
15 to 45 µ... *A. japonicus* Saito

B. Conidia subglobose to definitely elliptical, conspicu-
ously echinulate; vesicles commonly 60 to 80 µ, rang-
ing from 35 to 100 µ................................... *A. aculeatus* Iizuka

Mosseray's Synopsis *of the* Aspergillus niger *Group*

A. Conidia 6 to 10 µ in diameter, rough; vesicles sub-
globose; primary sterigmata often 100 µ or more in
length; colonies jet black

A. Sporulation more or less dense; heads normally
large; reverse fumose or very dark olive with my-
celium more or less wrinkled

Conidiophores 2 to 4, or even 6, mm. long; sclero-
tia present in "natural" media.......·..... *A. carbonarius* (Bain-
ier) Thom & Currie

Conidiophores 1 to 2 mm. long; sclerotia not reported... *A. pulchellus* (Speg.) Thom & Church

B. Sporulation less dense; heads small, extremely small fumigatiform heads on very short conidiophores also present; mycelium gray yellow; reverse dark reddish brown and mycelium much wrinkled.. *A. pseudo-carbonarius* (Bainier n.n.) Mosseray

B. Conidia 2.5 to 4 μ, even to 5 μ in diameter, mostly more or less rough and more or less colored but some smooth or nearly so; vesicles normally globose; primary sterigmata mostly 20 μ long or longer, sometimes up to 100 μ

A. Colonies deep purple-brown or clear purple-brown

　I. Conidiophores short (up to 2 mm. on an average)

　　α. Wrinkles in reverse shallow, transverse (in tubes)

　　　—Heads small; conidiophores short (up to 1 mm.), 8 to 15 μ in diameter, primary sterigmata 5 to 20 μ; reverse cream colored.............................. *A. microcephalus* Mosseray

　　　—Heads "medium;" conidiophores up to 2.5 μ; primary sterigmata up to 45 μ; reverse dark reddish brown, often spotted and becoming very dark.......... *A. longobasidia* (Bainier) Mosseray

　　β. Wrinkles in reverse of mycelium fairly numerous, occasionally anastomosing

　　　—Appearance "mealy," deep purple-brown; conidiophores very short up to 0.5 mm. and 7 to 13 μ in diameter; primary sterigmata 4 to 12 μ; reverse deep olive-brown..................... *A. pseudo-citricus* Mosseray

　　　—Appearance subgranular; brown-purple, at times clear brown to umber; conidiophores up to 1.5 mm. by 7 to 20 μ; conidia smooth or nearly so; growth rapid; reverse somewhat colored
　　　Reverse cream to pale brown; primary sterigmata 8 to 20 μ.............. *A. fuliginosus* Peck
　　　Reverse deeper brown; primary sterigmata up to 50 μ; sclerotia occasional........................... *A. sclerotifer* Mosseray

Reverse uncolored to citron yellow; primary sterigmata 8 to 20 μ....... *A. citrino-niger* Mosseray

γ. Wrinkles sharp and numerous (reticulated)

—Reverse becoming rapidly dark, almost black

Aspect mealy; color purple-brown; conidiophores up to 1 mm.; drops not colored; primary sterigmata 12 to 30 μ; conidia smooth or nearly so.............................. *A. densus* Mosseray

Aspect mealy to granulate, deep purple-brown; drops numerous, bronze; primary sterigmata 12 to 30 μ............................. *A. rutilans* Mosseray

Aspect subgranular, brown-purple; drops numerous, black, some of them very large; primary sterigmata 12 to 30 μ................... *A. guttifer* Mosseray

—Reverse not so dark, sometimes olive or colorless

Reverse deep olive, often spotted; primary sterigmata 15 to 30 μ..... *A. Buntingii* Mosseray

Reverse not colored; commonly showing areas sterile or free from spores; mycelium slightly yellow at first; primary sterigmata 20 to 50 μ..... *A. variegatus* Mosseray

II. Conidiophores up to 3 mm., rarely 4 mm., long

α. Sporulation normal

—Reverse uncolored or slightly olive; drops few or none; primary sterigmata 20 to 40 μ; mycelium often yellow at first.................................. *A. niger* Van Tieghem

—Reverse orange-brown or purple-brown; drops large, black; primary sterigmata 8 to 25 μ; mycelium colorless or rarely yellow...................... *A. Biourgei* Mosseray

—Reverse pale reddish brown; drops very few; heads very small; primary sterigmata 15 to 30 μ; mycelium colorless or sometimes rose; sporulation very slow. *A. Churchii* Mosseray

—Reverse golden yellow; drops none; primary sterigmata 20 to 40 μ; mycelium reddish yellow at first........... *A. luteo-niger* (Lutz) Thom & Church

—Reverse dark olive-brown; conidio-
phores up to 1 to 2 mm.; primary sterig-
mata very variable; vesicles subglobose *A. anomalus* Mosseray

—Reverse cream to brown, spotted
slightly, wrinkled with a dark band in
center, sporulation scattered, pale,
conidiophores up to 4 mm. long....... *A. tubingensis* (Schö-
ber) Mosseray

β. Sporulation abnormal
—Sporulation almost complete, absent in
patches or at the margin, with a granu-
lar appearance, and proliferation of
mycelium at the top of the slant ..... *A. granulatus* Moss-
eray

—Sporulation massed at the thin end of
the agar, less dense toward the bottom
of the tube
Mycelium wooly, gray-white or yellow-
ish gray, carrying on the thin areas
numerous simple heads ("fumiga-
tiformes") and some normal heads;
reverse cream, slightly wrinkled.. *A. velutinus* Mosseray
Mycelium less wooly, white; conidio-
phores 1 to 3 mm. long, abundant
at the point of inoculation, the re-
mainder often sterile; reverse
much wrinkled, cream........... *A. ficuum* (Reich.)
Hennings

III. Conidiophores tall, up to 1 cm.; sporulation
scanty, mostly toward the thin end of the
agar
—Heads large, splitting into divergent col-
umns; conidiophores up to 1 cm. by 30 μ;
primary sterigmata up to 100 μ; reverse
cream or slightly rose................... *A. elatior* Mosseray
—Heads small, mycelium gray-rose; conid-
iophores rarely up to 1 cm., more often
2 to 6 mm.; very numerous little fumigati-
form heads, on short conidiophores at the
surface; reverse clear rose or salmon..... *A. pseudo-elatior*
Mosseray

IV. Conidiophores very irregular from less than
1 mm. to 3 mm. with much larger heads; re-
verse clear reddish brown; mycelium yellow
at first then dark brownish red; sporulation
delayed................................. *A. pseudo-niger*
Mosseray

B. Colonies not purple-brown but clear brown;
morphology of *A. niger;* conidia mostly smooth

or nearly so

    I. Colonies clear brown or sepia; conidiophores up to 1 mm.; reverse olive or lighter; with reticulated wrinkles; vesicles 20 to 50 μ; primary sterigmata 12 to 50 μ; conidia smooth..................................... *A. olivaceo-fuscus* Mosseray

    II. Colonies umber; conidiophores 1 to 3 mm.; reverse reticulated dark, reddish brown; conidia smooth or nearly so............... *A. Schiemanni* Thom

    III. Colonies reddish salmon; conidiophores 1, 2, or even 3 mm.; reverse slightly wrinkled, uncolored, or pale cream; conidia smooth.. *A. cinnamomeus* Schiemann

C. Conidia 3 to 5 μ, globose, smooth, slightly colored; vesicles globose; conidiophores up to 1 mm., slender; primary sterigmata 12 to 20 μ; colonies appearing somewhat granular, deep brown; reverse olive passing to bronze; mycelium sulphur yellow at first.... *A. citricus* (Wehmer) Mosseray

D. Conidia 3 to 5 μ, globose or elliptical, smooth or slightly rough (or else 2.5 to 4.5 μ, round and smooth); colonies violaceous.

    A. Sterigmata in two series; vesicles globose; colonies purplish violet or mauve; reverse violet-brown; conidia 3 to 5 μ, globose, smooth, uncolored.. *A. Awamori* Usami

    B. Sterigmata in one series, short; vesicles subglobose; colonies in violaceous shades to mauve

      —Colonies mauve ("violet livide"), reverse uncolored, wrinkled; conidia globose or obovate, smooth 3 to 5 μ in long axis; sporulation slow; narrowly growing..................... *A. malvaceus* Mosseray

      —Colonies violaceous or dark violet-slate; reverse dark yellow or orange, slightly wrinkled; conidia globose and rough, 3 to 4.5 μ; sporulation very rapid, and colonies broadly spreading, with sclerotia common on rice or other "natural" substrata, rare upon sugar media.. *A. japonicus* Saito

      —Colonies violet-brown; reverse purplish brown, wrinkled; conidia globose, rough, 3 to 5 μ in long axis; sporulation slow and more or less incompletely covering the surface; dwarf heads abundant................................. *A. atro-violaceus* Mosseray

      —Colonies dark brown or carob brown, with a mealy or granular appearance, reverse dark brown, or olive at the margins, wrinkled;

conidia smooth, globose, 2.5 to 4 μ; vesicles
globose or pyriform; primary sterigmata 3.5
to 7.5 μ in diameter........................ *A. atro-fuscus* Moss-
eray

Aspergillus carbonarius (Bainier) Thom, in J. Agr. Research **7:** 12 (1916).

Synonyms

> *Sterigmatocystis carbonaria* Bainier, in Bull. soc. botan. France **27:** 27–28
> (1880).
>
> *Sterigmatocystis acini-uvae* Caballero, in Bol. soc. españ. hist. nacl., Madrid,
> **28:** 429 (1928).
>
> *Aspergillopsis pulchellus* Spegazzini, in Mycologia Argentini V, in Anales
> Museo Nacl. Buenos Aires, Ser. 3, **13:** 436 (1911).
>
> *Aspergillus pulchellus* (Speg.) Thom and Church, in The Aspergilli, p. 181
> (1926).
>
> *Sterigmatocystis fusca* Bainier, in Bull. soc. botan. France **27:** 29, Plate 1,
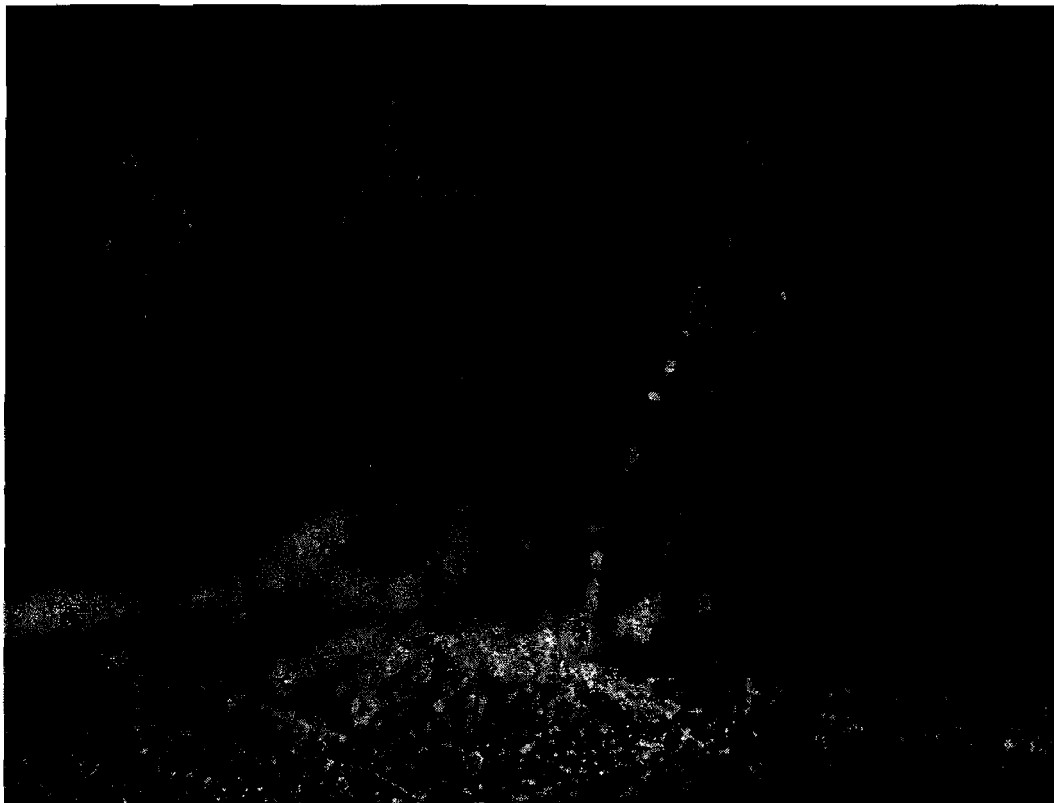> Fig. 5 (1880). Roumeguères Fungi Gallici Exsiccati No. 995. (Not *S.*

FIGURE 66. *Aspergillus niger.* Conidial heads showing the characteristic way in which the spore masses split into divergent columns, × 35. (Photograph by Edward Yuill.)

fusca Bain. *fide* Sartory and Jourde, in Compt. rend. soc. biol. **64:** 926–928 (1908).

Aspergillus fonsecaeus Thom and Raper, in A Manual of the Aspergilli, p. 227 (1945).

Aspergillus dipus Ferdinandsen and Winge, in Botan. Tidsskr. **30:** 220, Fig. 6 (1910).

Colonies on Czapek's solution agar (Fig. 67*A*) growing rather slowly at room temperature (24–26° C), 2.5 to 3.5 cm. in 10 days, basal mycelium white and moderately compact, somewhat raised and cushion-like with abrupt margins, sporulating areas carbon black from the massed large conidial heads borne on long conidiophores, which are produced abundantly throughout the colony except for the marginal 1 to 2 mm., more or less zonate; reverse white or slightly grayed, later becoming dirty yellowish to almost black at colony centers; exudate not conspicuous to the naked eye but at low magnifications appearing as small droplets attached to the conidial heads and beading the conidiophores, colorless at first, then dark brown; odor slight, not distinctive. *Conidial heads* globose up to 500 to 600 μ in diameter, then radiate and split into comparatively few poorly defined columns and eventually reaching 2.0 mm. or more in diameter; *conidiophores* up to 5 to 6 mm. long and 35 to 40 μ in diameter, hyaline below, progressively more colored toward the vesicle, often appearing internally granular and brown, with walls as much as 5.5 μ thick, brittle and splitting longitudinally; *vesicles* globose, commonly 60 to 80 μ broad but ranging up to 100 μ in diameter, rather heavy walled, pitted where sterigmata are detached, commonly filled with brown granular material, fertile over the entire surface; *sterigmata* in two series, brownish, primaries mostly 30 to 50 μ by 8.0 to 11.0 μ but up to 70 or 80 μ long, occasionally septate (Fig. 68*C*), secondaries few per primary, 8 to 15 μ by 6.0 to 8.0 μ; *conidia* globose with hyaline and often deciduous spinules when young, at maturity closely and very coarsely verruculose or warted (Fig. 69*A*), spore bodies 5.5 to 8.0 μ with roughenings projecting as much as 1.5 μ beyond the spore wall, surface ornamentation showing no tendency to be arranged in longitudinal lines. *Sclerotia* occasionally produced, more abundantly on Czapek's agar containing 1 per cent corn steep liquor, globose to subglobose, at first light buff, becoming pinkish to grayish brown in age, commonly measuring 1 mm. or more in diameter.

Colonies on malt extract agar rapidly growing 6 to 7 cm. in diameter in 1 week, basal mycelium thin, white, mostly submerged, giving rise to a continuous but uncrowded stand of very long-stalked, carbon black, globose to radiate, and sharply split large conidial heads which give the colonies their characteristic speckled appearance; reverse uncolored; conidiophores commonly reach lengths of 5 to 6 mm. and conidial heads diameters of 1
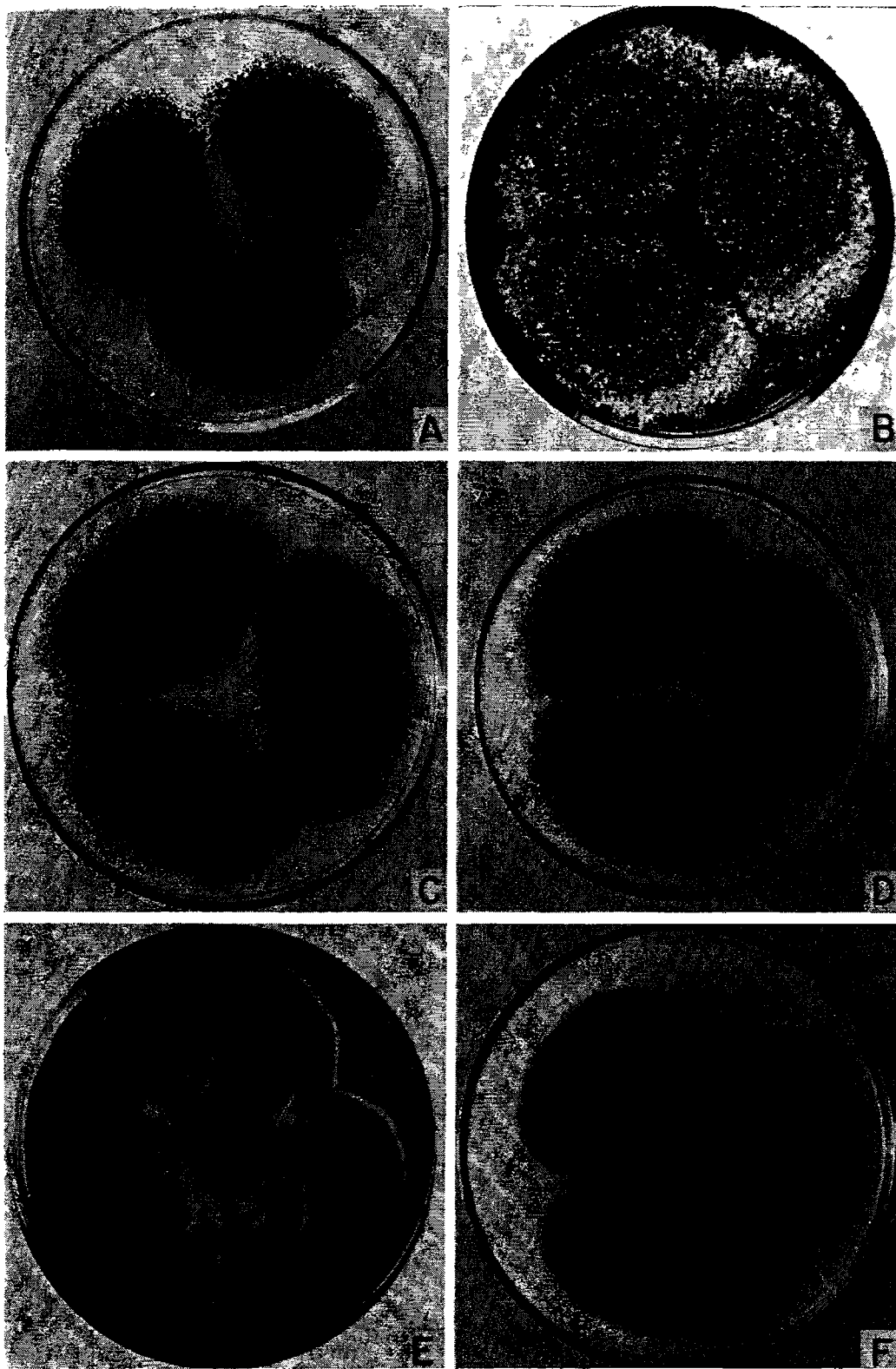
FIGURE 67. *Aspergillus niger* group. Cultures growing on Czapek agar at room temperature, 10 days. *A*, *A. carbonarius*, WB 369. *B*, *A. niger* WB 346 a strain producing abundant sclerotia. *C*, *A. niger* WB 326 representing the species in its more characteristic form. *D*, *A. phoenicis* WB 4757. *E*, *A. foetidus* WB 341. *F*, *A. japonicus* WB 1782.
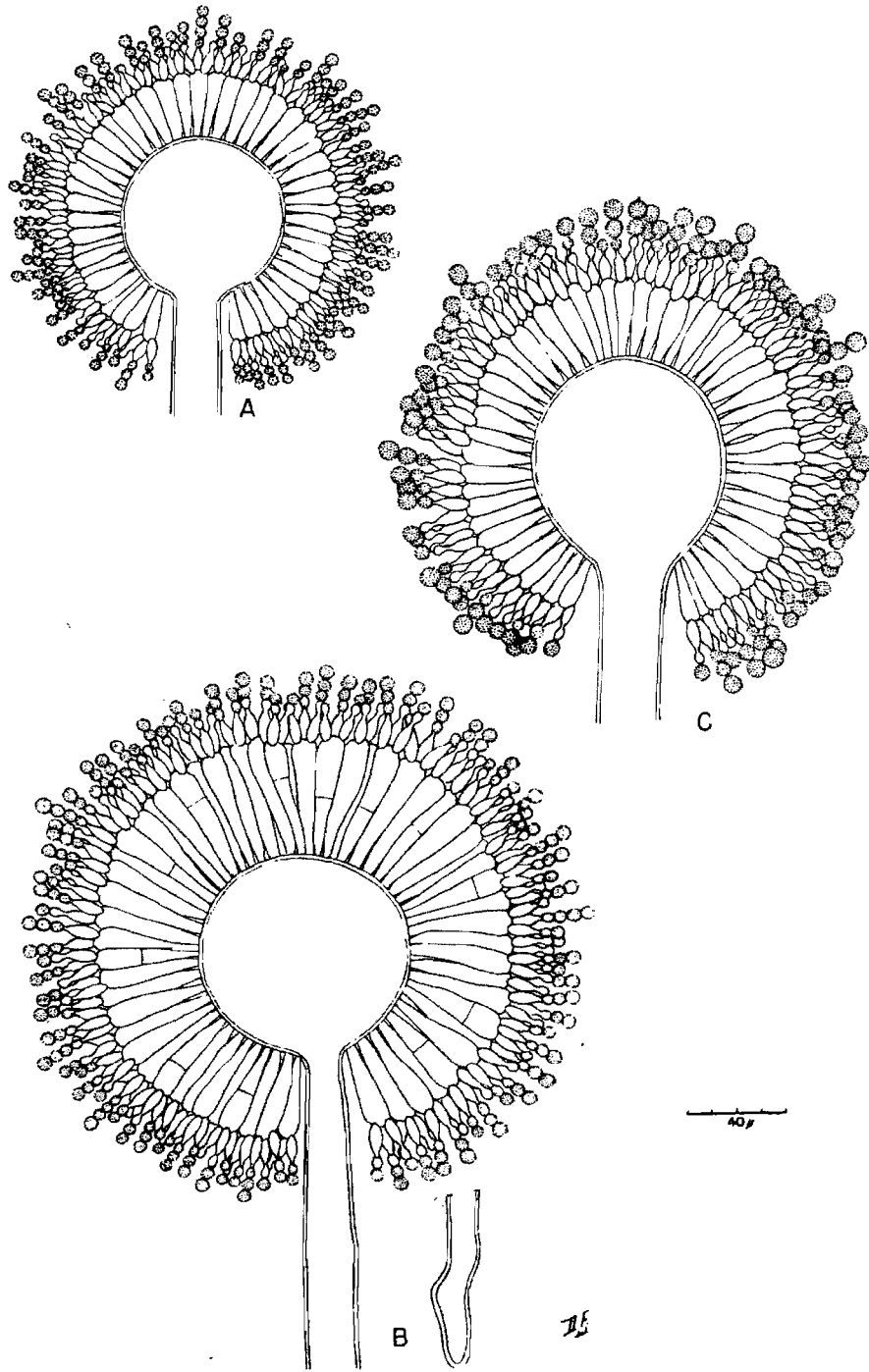
FIGURE 68. Conidial structures in *Aspergillus niger* group, × 500. *A*, *A. niger* WB 326, typical head showing globose vesicle and primary sterigmata about twice the length of secondaries; *B*, *A. phoenicis* WB 1956, characterized by long and occasionally septate primary sterigmata; *C*, *A. carbonarius* WB 369, characterized by large primary sterigmata and large conidia. (After Thom and Raper, 1945.)
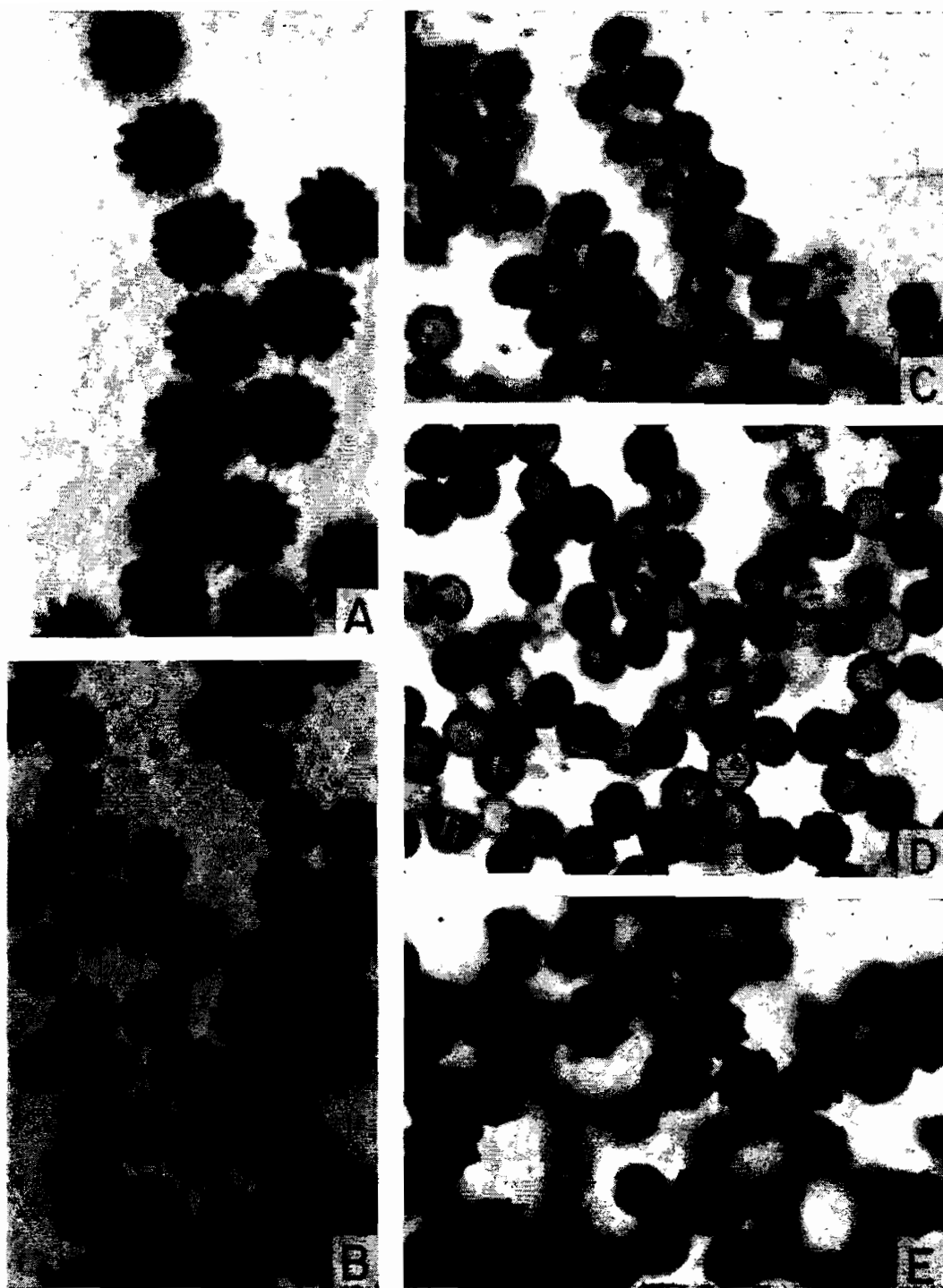
FIGURE 69. *Aspergillus niger* group: conidia of representative types. *A*, Very large, coarsely roughened conidia of *A. carbonarius*, $\times$ 1240. *B*, Conspicuously echinulate conidia of *A. japonicus*, $\times$ 1720. *C–E*, Conidia of other members of the *A. niger* group showing a transition from spores with slight longitudinal orientation of discontinuous color bars and ridges (*C*) to those with low color bars and ridges definitely arranged in a longitudinal pattern (*D*) to others with very heavy color bars and ridges similarly arranged (*E*), $\times$ 1720.

mm. in 1 week but may ultimately reach 2.0 to 2.5 mm. Other details of morphology as on Czapek's agar.

Species description based upon WB 369, cited as representative of this species by Thom and Raper (1945); WB 4849, contributed in 1962 by the CBS, where it was received as the type of *Sterigmatocystis acini-uvae* Caballero; WB 4871, received from the CBS in 1962 as Sainclivier's (1949) strain of *A. pulchellus* (Spegazzini) Thom; and WB 67, originally received from Da Fonseca as *Sterigmatocystis fusca* Bainier and subsequently used by Thom and Raper (1945) as the basis for *A. fonsecaeus* Thom and Raper.

Probable Synonyms

> *Aspergillus atropurpureus* Zimmerman, in Centr. Bakteriol. Parasitenk. Abt. II, **8**: 218 (1902), was described as having very short secondary sterigmata but with other characters that align it unmistakably with *A. carbonarius* (Bainier) Thom.
>
> *Sterigmatocystis castagnei, nomen nudum* Biourge, attached to an undescribed culture in the Biourge Collection, listed by him as near *A. carbonarius* in the *A. niger* group.

Aspergillus ficuum (Reich.) Hennings, in Hedwigia **34**: 86 (1895).

Synonyms

> *Ustilago ficuum* Reichardt, in Verhandl. Konigl. Zool. botan. Ges., Wien, **17**: 335 (1867).
>
> *Aspergillus batatae* Saito, in Centr. Bakteriol. Parasitenk., Abt. II, **18**: 34 (1907).

Colonies on Czapek's solution agar growing rapidly at room temperature (24–26° C), 5 to 6 cm. in diameter at 10 days to 2 weeks, plane, closely to loosely velutinous, thin white basal mycelium almost completely submerged, conidial structures in very dark purplish brown shades, appearing black *en masse* to the unaided eye, abundant and crowded throughout most of the colony but thinning at the margins to give a speckled appearance; reverse uncolored or very slightly yellow; faint ester odor; exudate inconspicuous, clear, in very small droplets. *Conidial heads* purple-black to brown-black, globose, sometimes remaining so but usually splitting into several well-defined columns, mostly 300 to 500 μ but up to 1 mm. in diameter; *conidiophores* erect or somewhat sinuous, commonly pigmented in brown shades, with walls up to 4.0 μ thick but most commonly 2 to 3 μ, variable in length from 500 μ to 2 mm. but mostly 1.0 to 1.5 mm. long, diameter throughout most of their length 16 to 22 μ but reduced just below the vesicle to 10 to 15 μ, *vesicles* globose, heavy walled, commonly pigmented, variable from 25 to 75 μ in diameter but mostly 40 to 60 μ, fertile over the entire surface or with a limited area around the conidiophore devoid of sterigmata; *sterigmata* in two series, brownish, primaries mostly

20 to 40 μ by 6.0 to 8.0 μ but ranging from *ca.* 10 μ by 4.0 μ to 75 μ by 10 μ, often in the same mount, secondaries mostly 7 to 10 μ by 3.0 to 3.5 μ, often crowded and difficult to measure; *conidia* globose and irregularly roughened when young, becoming horizontally flattened and longitudinally striate at maturity, spore bodies mostly 3.5 to 4.0 μ in transverse diameter with coarse striations which may extend beyond the cell wall as much as 1 μ.

Colonies on malt extract agar growing rapidly, 7 to 8 cm. in 10 days, with thin hyaline basal mycelium inconspicuous, surface smooth and closely velvety in some strains from the densely crowded conidial heads borne on stalks of uniform length, somewhat looser textured in others where less abundant conidial heads are borne on conidiophores of variable length, carbon black to the naked eye; reverse uncolored or faintly yellow with the black of conidial structures apparent through the vegetative mycelium; odor as on Czapek's agar. Conidial heads as described above but usually somewhat smaller, more quickly and uniformly split into divergent columns, and borne on somewhat shorter conidiophores.

Species description based upon WB 364, received in 1909 from Professor Westerdijk as *A. ficuum* (Reich.) Hennings; WB 320, received in 1922 from Da Fonseca as *St. pseudo-carbonaria, n.n.* Bainier (not *A. pseudo-carbonarius* (Bainier) Mosseray, *q.v.*); WB 4785, received in 1962 from the IFO as *A. batatae* Saito, possibly type; and WB 4541, received from R. M. Natour in 1960 as the causative agent of a soft rot of *Dracena*, subsequently published (Natour and Miller, 1960) as *A. niger* var. *floridanus* but not described.

Aspergillus phoenicis (Cda.) Thom, in The Aspergilli, p. 175 (1926); see also Thom and Raper, A Manual of the Aspergilli, pp. 222–223, Figs. 61*E*, 62*B* (1945).

· Synonyms

> *Ustilago phoenicis* Cda. in Icones Fungorum, IV, p. 9, Plate 3, Fig. 26 (1840).
> *Sterigmatocystis phoenicis* (Cda.) Pat. and Delacr. in Bull. soc. mycol. France 7: 119, Plate 9 (1891); also Thom and Currie, J. Agr. Research 7: 14 (1916).
> *Aspergillus ustilago* Beck, in H. Wawra, Itinera Principum S. Coburgi, II, p. 148, Wien (1888); Saccardo, Sylloge Fungorum, X, p. 526 (1892).
> *Aspergillus saitoi* Sakaguchi, Iizuka, and Yamazaki, J. Appl. Mycol. (Japan) 3(3): 68 (1949) and 3(4): 98–101 (1950). See also J. Agr. Chem. Soc. Japan 24: 138–142 (1951).
> *Aspergillus saitoi* var. *kagosima* Saka. *et al.*, J. Appl. Mycol. (Japan) 3(3): 68 (1949); as var. *kagoshima, ibid.* 3(4): 98–99 (1950); as var. *kagoshimaensis,* J. Agr. Chem. Soc. Japan 24: 140–141 (1951).
> *Aspergillus awamori* var. *hominis* Batista and Maia, in Anais soc. biol. Pernambuco 15(1): 186–188, Fig. 4 (1957).

Colonies on Czapek's solution agar (Fig. 67*D*) at room temperature (24–26° C) growing somewhat restrictedly, 3.0 to 4.5 cm. in diameter in 2 weeks, deeply velvety, lightly radially wrinkled, consisting of a fairly compact white basal mycelium which in most strains extends 0.5 to 1.0 cm. beyond the central area of abundant sporulation to produce a conspicuous white zone that is irregular at the margin and granular in texture; reverse white to slightly gray; exudate inconspicuous; odor slight. Sclerotia produced in some strains, white, globose to subglobose or discoid, commonly 1 to 2 mm. or more in diameter. *Conidial heads* produced abundantly in central colony area, when young globose and variable in color from light tan to black but splitting into few to many well-defined columns and soon becoming very dark brown-black, appearing carbon black to the naked eye, mostly 300 to 500 μ but up to 800 or even 1200 μ in diameter; *conidiophores* variable in length, usually ranging from 1.0 to 2.5 but up to 4.0 mm. long and from 10 to 20 μ in diameter, often slightly constricted just below the vesicle, splitting longitudinally when crushed, with walls smooth, 1 to 2 μ thick, and lightly colored near the vesicle; *vesicles* (Fig. 68*B*) globose or nearly so, mostly 45 to 65 μ but occasionally up to 80 or 85 μ in diameter; *sterigmata* in two series, brownish, primaries variable, in young sporulating heads from actively growing margins often from 20 to 35 μ and rarely exceeding 50 to 60 μ in length, in mature heads mostly 40 to 60 μ by 5.5 to 7.5 μ but sometimes septate and reaching 100 to 110 μ in large conidial structures, particularly those in which conidia remain compacted into globose masses rather than splitting into divergent conidial columns, secondaries 6.5 to 11.0 μ by 3.0 to 3.5 μ; *conidia* globose and from almost smooth to irregularly roughened when young, at maturity becoming horizontally flattened, mostly 3.0 to 3.5 μ but up to 4.0 μ in diameter and conspicuously roughened with separate bars oriented longitudinally or confluent to form striations.

Colonies on malt extract agar rapidly growing and broadly spreading, 6 to 7 cm. in diameter in 2 weeks, plane, velvety, heavily sporulating throughout, reverse white to cream or with the black of the conidial structure visible; conidial heads as described on Czapek but usually smaller, rarely exceeding 800 μ in diameter and borne on shorter stalks.

Species description centered upon WB 4757 and 4758, received in 1962 from the CBS as its strains "Sakaguchi" of *A. saitoi* Saka. *et al.* and *A. saitoi* var. *kagoshimaensis* Saka. *et al.*, respectively; WB 365, received in 1922 from Da Fonseca and identified by Thom as *A. phoenicis;* WB 1956, received in 1944 from the Chemical Warfare Service as an isolate from army equipment in the South Pacific and considered representative of the species by Thom and Raper (1945); WB 4740, the type culture of *A. awamori* var. *hominis* Batista and Maia, received from the ATCC in 1955;

and WB 4856, received from the CBS in 1962 as Shih's strain of *A. luchuensis* var. *rubeolus*.

Probable Synonyms

A. granulatus Mosseray, in La Cellule 43: 249–250, Plate III, Figs. 25–28 (1934), as described and as suggested by Mosseray, appears to have been closely related to *A. phoenicis*. WB 4855, received in 1962 from the CBS as Mosseray's strain is now a deeply floccose strain which produces only diminutive heads and bears little resemblance to Mosseray's description.

A. phaeocephalus Durieu and Montagne, in Flora Alger, p. 342 (1849). A member of the *A. niger* group probably approximating *A. phoenicis*.

Syn: *Sterigmatocystis phaeocephalus* (D. and M.) Saccardo, in Fungi Italici, Fig. 903 (1881), and No. 1244 in Saccardo, Mycologiae Veneta (1877).

A. velutinus Mosseray, in La Cellule 43: 252–253, Plate III, Figs. 38–41 (1934), was originally described as a flocculent species closely related to *A. phoenicis*, separated only by the common production of fumigatiform heads. Strain WB 4877, received from the CBS as Mosseray's strain of this species, conforms with the original description, but we feel that the distinction drawn is not sufficient for species recognition.

Aspergillus niger Van Tieghem, in Ann. sci. nat. Botan., Ser. 5, 8: 240 (1867). See also Thom and Currie, J. Agr. Research 7: 1–15 (1916); Thom and Church, The Aspergilli, pp. 167–170 (1926); Thom and Raper, A Manual of the Aspergilli, pp. 216–219, Figs. 61A, 62A (1945).

Synonym

Sterigmatocystis nigra V. Tieg., in Bull. soc. botan. France 24: 102–103 (1877).

Colonies on Czapek's solution agar (Fig. 67B) growing restrictedly, attaining a diameter of 2.5 to 3.0 cm. in 10 days to 2 weeks at room temperature (24–26° C), consisting of a compact to fairly loose white to faintly yellow basal mycelium that is largely submerged and bears abundant erect and usually crowded conidial structures, typically black near carbonaceous but sometimes deep brownish black, covering the entire colony except for a narrow growing margin; reverse usually colorless, occasionally pale yellow at colony center or even throughout; odor moldy, not distinctive; exudate lacking or limited to small droplets, colorless. *Conidial heads* (Fig. 66) typically large and black, at first globose, then radiate or in age often splitting into two or more loose to reasonably well-defined columns, commonly reaching 700 to 800 μ in diameter, smaller and atypical heads sometimes appearing brown or with peripheral conidia in larger heads sometimes not fully pigmented; *conidiophores* variable, commonly 1.5 to 3.0 mm by 15 to 20 μ, with walls smooth, comparatively thick (up to 2.0 to 2.5 μ), colorless or in brownish shades particularly in the upper half; *vesicles* (Fig.

68*A*) globose or nearly so, commonly 45 to 75 μ in diameter, often smaller and occasionally 80 μ; *sterigmata* in two series, brownish in color, primary sterigmata varying with the strain and with the age of the conidial heads, in most cultures 20 to 30 μ by 5 to 6 μ at the onset of sporulation but often reaching 60 to 70 μ (or even more) by 8 to 10 μ and sometimes septate at maturity, in other strains never exceeding 35 to 40 μ in length, secondary sterigmata more uniform, ranging usually from 7 to 10 μ by 3.0 to 3.5 μ; *conidia* typically globose at maturity, less often slightly discoid, somewhat variable in size in different strains and even in the same mount but mostly 4.0 to 5.0 μ in diameter, appearing brown with walls comparatively heavy and irregularly roughened or showing low to fairly prominent ridges and echinulations that are usually discontinuous rather than arranged as conspicuous longitudinal color bars.

Colonies on malt agar growing more rapidly, 5 to 6 cm. in 2 weeks at room temperature, plane or in occasional strains lightly flocculent, heavily sporulating throughout, in carbonaceous to very deep brownish black shades; reverse uncolored or faintly yellow; exudate lacking; odor slight, not distinctive. Conidial heads much as on Czapek's agar but typically borne on somewhat shorter conidiophores and showing an increased tendency to form divergent spore columns in age, otherwise as described above. Sclerotia produced in some strains not in others, occasionally dominating the colony appearance (Fig. 67*B*), globose to subglobose, commonly 0.8 to 1.2 mm. diameter, at first cream to buff then shifting to vinaceous buff in age.

Species description based upon numerous isolates from a variety of sources, of which the following may be regarded as representative: WB 326, isolated from a tannin-gallic acid fermentation (Connecticut) in 1913; WB 334 (Thom's 4247), the object of R. A. Steinberg's intensive studies on the mineral nutrition of *A. niger*; WB 330, received from Eastman Kodak Company in 1915 as an isolate from Chinese galls; WB 367, received from the Bainier Collection (France) in 1922 under the name *Sterigmatocystis nigra* var. *Barthelat* (*nomen nudum?*) and WB 4361, received in 1957 from Professor R. Misra, Banares Hindu University. WB 5121, isolated at Wisconsin in 1962 from Costa Rican soil by Kyung-Joo Kwon, is representative of strains producing predominantly sclerotial colonies.

Variations may, of course, be expected within a ubiquitous species such as *Aspergillus niger*. Of the aforementioned strains, WB 367 produces conidia that usually fall near the lower end of the range cited, whereas those of WB 330 average about 5.0 μ. This latter strain was identified by Sakaguchi and Iizuka (J. Agr. Chem. Soc. Japan **27**: 324–329 (1952)) as *A. saitoi* var. *kagoshimaensis*. It fails to agree either culturally or morphologically with WB 4758 received as the Sakaguchi type of this variety (see p. 307).

Probable Synonyms

St. antacustica Cramer, in Vierteljahresschrift Naturforsch. Ges. Zurich **4:** 325 (1859), was based upon a black *Aspergillus*, isolated from the external ear, which showed a double series of sterigmata and provided the basis for his erection of the genus *Sterigmatocystis*. It has been considered by different authors as probably representing either *A. niger* or *A. phoenecis*.

A. fuliginosus Peck, *fide* Mosseray (La Cellule **43:** 229–231, Plate IV, Figs. 96–98 (1934)), as represented by two of Mosseray's cultures received in 1962 from the CBS, cannot be distinguished from *A. niger* V. Tiegh. In his description of *A. fuliginosus* Peck, Mosseray listed *Sterigmatocystis fuliginosa* Bainier (Bull. soc. botan. France **28:** 78 (1881)) and *A. praecox* · Mosseray (Ann. soc. sci. Bruxelles, Ser. B, **54:** 79 (1934)) as synonyms.

A. fumaricus (Wehmer) Thom and Church, in The Aspergilli, p. 181 (1926). This name was first used as a *nomen nudum* by Wehmer (Ber. deut. chem. Ges. 51(14): 1663–1664 (1918)) to designate a culture of the *A. niger* group which produced fumaric acid primarily. A culture subsequently received from Neuberg under this name was used by Thom and Church as the basis of their species description. It was apparently an aberrant culture with yellow-brown conidial heads, proliferating sterigmata, and barred conidia commonly 5 μ but occasionally 8 μ in diameter. In the absence of this culture positive identification is impossible, but it would appear to have represented some variant or mutant strain of *A. niger*.

A. longobasidia, *nomen nudum* Bainier. Name attached to a culture (WB 366) received by Thom from the Bainier Collection in 1922 (also sent to Biourge) that showed the general characteristics of *A. niger* V. Tieghem, which are still retained. Subsequently studied by Mosseray of Biourge's Laboratory and cited by him as *A. Bainieri* (Ann. soc. sci. Bruxelles, Ser. B, **54:** 79 (1934)) and in the same year recognized by him in a second publication · (La Cellule **43:** 227–228 (1934)) as *A. longobasidia* (Bainier) Mosseray. Mosseray's culture, WB 4857, received from the CBS in 1962, now appears to be a lightly sporulating strain of *A. awamori*.

A. nigricans Wreden, in Compt. rend. **65:** 368–370 (1867), has been considered as *A. niger* by Wilhelm (1877), Siebenmann (1883), and Wehmer (1907). The absence of an adequate description or identifiable material justifies rejection of this name and acceptance of Van Tieghem's specific name.

A. nigriceps Berk. and Curtis in the Curtis Collection (Wright No. 927, cited by Cooke, M. C., in Grevillea **17:** 21 (1888)). A slide from this material in the Harvard Collection shows a member of the *A. niger* group, inseparable from *A. niger* Van Tieghem.

A. pyri English *n. n.*, in doctoral thesis, State College of Washington, Pullman, Washington, pp. 76–78, 1940; cited in abstract as a uniseriate new species in the *A. niger* group, but subsequent discovery of double sterigmata (personal correspondence, June 1943) led English to withdraw the name.

A. Welwitschiae (Bresadola) Hennings was discussed by Wehmer in Centr. Bakteriol. Parasitenk. Abt. II, **18:** 394–395 (1907). He examined Hennings'

material but found no basis for separating it from *A. niger*. Examination of a strain received from the CBS as *A. Welwitschiae* (Bres.) Henn., contributed by Swart in South Africa, likewise affords no basis for separation.

Syn: *Ustilago Welwitschiae* Bresadola.

More than half a century ago, Elizabeth Schiemann (1912) subjected a culture identified as *Aspergillus niger* to high concentrations of various metallic salts and described four mutants that differed substantially from the parent strain. These were referred to interchangeably as *A. niger* mut. *cinnamomeus*, mut. *fuscus*, mut. *altipes*, and mut. *proteus*, or as *A. cinnamomeus*, *A. fuscus*, *A. altipes*, and *A. proteus*. Three of the four cultures were preserved and distributed. In 1916, the first two were listed by Thom and Currie as *A. cinnamomeus* Schiemann and *A. Schiemanni* (Schiemann) Thom, the latter a new combination necessitated by prior use of the specific epithet *fuscus* by both Bonorden and Bainier. This treatment was repeated by Thom and Church (1926). In 1945, Thom and Raper returned both specific names to mutant status but retained the epithet *Schiemanni* for the mutant *fuscus*. In the present study we have re-examined these mutants with the exception of *proteus*, for which no culture is available, and as in the case of other mutants of known origin, we believe that those of Schiemann, in spite of their historical interest, should not be afforded taxonomic recognition.

At least two species have been described which we consider to have been based upon naturally occurring mutants of *A. niger*.

> *Aspergillus hennebergi* Blochwitz, in Ann. Mycol. **33**: 238-239 (1935), was described as closely related to the smaller members of the *A. niger* group in morphological characters and to *A. wentii* in color but with sclerotia at first bright yellow then red. Neill (1939) listed this species as a synonym of *A. ochraceus* Wilhelm. Strain WB 4883, received in 1962 from the CBS as Blochwitz's culture upon which the species was based, conforms reasonably well with the author's description but fails to produce sclerotia. Comparison of this strain with members of both the *A. niger* and *A. wentii* groups leads us to conclude that it represents a mutant of *A. niger*. In common with most strains of known mutant origin in this group, it shows relatively small vesicles on disproportionately coarse stalks and spore chains containing both smooth normal-appearing and shrunken aberrant conidia.

> *Aspergillus kawachii* Kitahara and Yoshida, in J. Fermentation Technol. **27**: 162 (1942). We have been unable to locate this reference, but from the limited information provided by Nehira and Nomi (J. Fermentation Technol. **34**: 423-428 (1956)) and from examination of strain WB 4886, received in 1962 from Osaka University Faculty of Engineering, we believe this species may best be regarded as a very lightly sporulating, tan mutant of *A. niger*.

Aspergillus pulverulentus (McAlpine) Thom, in J. Agr. Research **7**: 10–11 (1916); see also Thom and Church, The Aspergilli, p. 179 (1926).

Synonyms

> *Sterigmatocystis pulverulenta* McAlpine, in Agr. Gaz. N. S. Wales **7**: 302 [1896]; (1897).
>
> *Aspergillus (Sterigmatocystis) strychni* Lindau, in Hedwigia **43**: 306–307 (1904).
>
> *Aspergillus elatior* Mosseray, in La Cellule **43**: 253–255, Plate II, Fig. 13; Plate III, Figs. 29–32 (1934).

Colonies on Czapek's solution agar growing somewhat restrictedly at room temperature (24–26° C), 3 to 4 cm. in 10 days to 2 weeks, somewhat raised, white, flocculent, lightly sporulating and with scattered large black conidial heads developing in submarginal area, subsequently producing abundant small atypical conidial structures in central colony area to become grayish black, with few large carbon black heads on long stalks projecting above the still white mycelium in the outer 1.0 to 1.5 cm.; reverse white; odor slight, not distinctive; exudate limited, in small droplets at the mycelial surface, clear. *Conidial heads* very variable in form and size, ranging from globose to radiate or split into few irregular columns and up to 600 to 700 μ in diameter when borne on long stalks, to very small with few and tangled chains of conidia when borne on short stalks from the loose mycelial felt; *conidiophores* bearing large heads commonly up to 7 to 8 mm. but reaching 1 cm. or more in length and up to 20 μ in diameter, smooth, colorless below but lightly tinted in brown shades above, walls 1.5 to 4.0 μ thick; *vesicles* globose or nearly so, fertile over the whole surface, ranging up to 70 to 80 μ in largest heads; *sterigmata* in two series, brownish, primaries in young heads up to 30 to 40 μ, but later commonly reaching 60 to 75 μ and up to 120 μ by 12 to 15 μ, sometimes septate, secondaries numerous, mostly 8.0 to 12.0 μ by 3.0 to 3.5 μ in largest heads but nearer 6 to 8 μ long in smaller structures; *conidia* variable, at maturity globose to subglobose, ranging from 3.5 to 4.0 μ up to 5.0 μ and from almost smooth to definitely verruculose with low but conspicuous granules and bars sometimes oriented in discontinuous longitudinal lines but only rarely confluent to form true striations.

Colonies on malt agar growing rapidly, attaining diameters of 6 to 7 cm. in 2 weeks, white and loosely flocculent, lightly sporulating with scattered large conidial heads borne on long stalks projecting several millimeters above the mycelial felt and with smaller, fractional heads abundant at the colony surface; reverse in cream to peach shades. Conidial structures as on Czapek's but with heads more commonly splitting into compact columns.

Species description based upon WB 4851, received in 1962 from the CBS as Mosseray's strain of *A. elatior* Mosseray. This culture was obtained by Mosseray from the Biourge Collection as No. 694a, *Aspergillus pulveru-*

lentus, and was used by him as the basis of *A. elatior* n.sp., although he considered it to be closely related to *A. pulverulentus* and *A. phoenicis*.

As originally described from a seed pod, *A. pulverulentus* showed vesicles up to 170 μ in diameter and primary sterigmata up to 144 μ. We have encountered no *Aspergillus* in laboratory cultures that attains these dimensions, but on grains and other plant materials members of the *A. candidus*, *A. ochraceus*, and *A. niger* groups are known to attain gigantic proportions.

Possible Synonym

    A. giganteus (Mattlet) Dodge, in Medical Mycology, p. 629 (1935). Described as a black-brown species with conidiophores 2 to 6 mm. long and rough conidia 4 to 5 μ in diameter. May have represented a form near *A. pulverulentus*.

      Syn: *Sterigmatocystis giganteus* Mattlet, in Ann. soc. belge méd. trop. **6**: 31–32 (1926).

      Aspergillus Mattleti Hendrickx, in Publ. inst. natl. étude agron. Congo Belge, sér. tech., **35**: 7 (1948).

Aspergillus tubingensis (Schöber) Mosseray, in La Cellule **43**: 245–247, Plate 3, Figs. 58–60 (1934).

Synonym

    Aspergillus niger forma *Tuebingen*, *nomen nudum* by Schöber, in Jahrb. wiss. Botan. **72**: 1 (1930).

Colonies on Czapek's solution agar at room temperature (24–26° C) rather slowly growing, attaining diameters of 4.0 to 5.0 cm. in 10 days, deeply velvety, plane or nearly so, somewhat zonate, consisting of a fairly compact white basal mycelium which may extend 2 or 3 mm. beyond the central area of abundant sporulation and remains white even in age at contiguous margins of adjacent colonies, conidial heads in slightly grayish black-brown shades, borne on long conidiophores which commonly have a metallic sheen at low magnifications; reverse white; exudate and odor lacking. *Conidial heads* globose to radiate, mostly 200 to 300 μ in diameter but ranging up to 500 μ, globose heads of a single strain examined at low magnifications show considerable variation in color from light gray-tan to chocolate brown, and radiate heads often show spores of lighter color terminal in the chains; *conidiophores* smooth, long and coarse, commonly 2 to 3 mm. but up to 5 to 6 mm. high with diameters that reach 30 μ but are most commonly 15 to 20 μ, usually lightly colored in brown shades, comparatively thin walled with wall thickness most commonly 1 μ and only rarely reaching 2 μ; *vesicles* globose, rather variable in size, most commonly 40 to 60 μ in diameter but ranging from *ca.* 20 μ up to 80 μ, fertile over their entire surface; *sterigmata* in two series, primaries mostly 15 to 30 μ long but ranging up to 50 μ, secondaries numerous, 7.5 to 10.0 μ by 2.8 to 3.3 μ; *conidia* globose

with hyaline echinulations when first formed, becoming progressively darker and rougher and finally appearing longitudinally striate from conspicuous bars of coloring material, mature conidia somewhat horizontally flattened, 3.0 to 3.5 μ in diameter. *Sclerotia* produced in some strains, occasionally dominating the colony appearance, globose to subglobose, cream colored at first then pinkish buff and sometimes darkening to almost black in age, commonly 500 to 800 μ in diameter.

Colonies on malt extract agar at room temperature growing rapidly, reaching 6 cm. in diameter in 10 days but showing no further increase in age, thin, plane, velvety, indistinctly zonate, heavily sporulating in very dark brown shades but often showing a thin surface development of white mycelium which later becomes more conspicuous; reverse uncolored. Conidial heads usually somewhat smaller and borne on considerably shorter stalks than on Czapek's but with other dimensions similar to those described above.

Species description based upon WB 4866 and 4875, both received in 1962 from the CBS, the former as strain "Oelkers" of *A. niger* f. *Tuebingen* and the latter as Mosseray's strain of *A. tubingensis* (Schöber) Mosseray. Both are believed to have stemmed from Schöber's original isolate. Strain WB 4700, received in 1962 from J. N. Rai, Lucknow, India, is representative of strains producing abundant sclerotia.

Possible Synonym

> *A. atropurpureus* Blochwitz, in Ann. Mycol. **32**(1/2): 86 (1934). Blochwitz proposed the name *A. atropurpureus* for the purple-brown members of the *A. niger* group as he obtained them from the tropics. He gave conidial measurements as 2.5 to 3.5 μ in diameter, which would suggest relationship with *A. tubingensis* (Schöber) Mosseray.
>> Not: *A. atropurpureus* Zimmerman, in Centr. Bakteriol. Parasitenk., Abt. 2, **8**(5/7): 218 (1902).

Aspergillus awamori Nakazawa, in Inst. Govt. Research Formosa, Rept. No. 4 (1915); *nomen nudum* Nakazawa in *ibid.* Rept. No. 1 (1907) and No. 2 (1912); see also Nakazawa, Simo, and Watanabe, J. Agr. Chem. Soc. Japan, **12**: 931–974, Fig. 3 (1936).

Synonyms

> *Aspergillus* sp. in awamori-koji, in K. Usami, Centr. Mykol. 4: 194, Fig. 1 (1914).
>
> *Aspergillus awamori* Usami, in Blochwitz, Ann. Mycol. **31**: 76–77 (1933); also in Mosseray, La Cellule **43**: 264–265 (1934).
>
> *Aspergillus pseudo-citricus* Mosseray, in La Cellule **43**: 228–229, Plate IV, Figs. 103–104 (1934).
>
> *Aspergillus pseudo-niger* Mosseray, in La Cellule **43**: 256–257, Plate IV, Figs. 113–117 (1934).

Aspergillus pseudo-niger (C. and L.) Sainclivier, in Thèse doctorale, Univ. Rennes, I, Contributions à l'etude de quelques Aspergilles, pp. 97–103, Plate II (1949).

Sterigmatocystis pseudo-nigra Cost. and Lucet, in Bull. soc. mycol. France **19**: 38 (1903).

Aspergillus miyakoensis Nakazawa *et al.*, in J. Agr. Chem. Soc. Japan **12**: 931–974, Fig. 12 (1936).

Colonies on Czapek's solution agar growing rapidly at room temperature (24–26° C), 5 to 7 cm. in 10 days, consisting of a slightly radially furrowed, tough, usually white but sometimes yellow basal mycelium typically bearing abundant conidial structures throughout, but in some strains remaining essentially sterile in central colony areas while tardily becoming floccose at the margins and sporulating normally only in these areas, conidial surface somewhat irregular from the variability in conidiophore lengths, occasionally slightly zonate, heavily sporulating at first in very dark greenish brown shades later becoming very dark chocolate brown (R., Plate XXVIII); reverse uncolored or irregularly mottled or zonate in clear yellow shades, shifting to brown or reddish brown in age; exudate lacking or limited in amount; odor faint or sometimes pronounced and suggestive of mushrooms. *Conidial heads* globose then loosely radiate or split into ill-defined columns, variable in size within a single strain, ranging from 100 μ to 500 μ but mostly 200 to 300 μ in diameter, individually appearing first olive-brown then Natal brown (R., Plate XL); *conidiophores* thick walled (up to 2.0 μ), smooth, brittle, usually somewhat colored in terminal areas, variable in length from 200 to 300 μ to 2 to 3 mm. and correspondingly variable in diameter from 5 to 20 μ, the majority between 1.0 and 1.5 mm. high; *vesicles* globose or nearly so, usually light brown, variable in size, most commonly between 25 and 50 μ in diameter but ranging from 10 to 70 or 80 μ, fertile over their entire surface except on smaller vesicles; *sterigmata* in two series but in small heads (and perhaps in young structures) often developing only one secondary per primary and giving the appearance of a single series of septate sterigmata, often darkly pigmented, primaries mostly 10 to 20 μ by 4.5 to 7.0 μ but ranging from <5 to 40 μ by 3.5 to 10.0 μ and not correlated with vesicle size; secondaries 5.5 to 10.0 μ by 3.0 to 4.0 μ; *conidia* in random mounts variable, many globose to subglobose, 3.5 to 5.0 or even 6.0 μ in diameter and ranging from almost smooth to definitely roughened with coarse echinulations or irregular low ridges and bars, others horizontally flattened, mostly 4.0 to 4.5 μ in transverse diameter, and showing almost continuous longitudinal bars of color that may protrude as much as 1 μ. In mounts made from carefully selected material, the former are found to be predominant in young or persistently compact heads and the latter in mature heads that are loosely radiate or split into divergent columns.

Colonies on malt extract agar at room temperature rapidly growing, broadly spreading, 7 to 8 cm. in diameter at 10 days, plane, usually low, fine textured and velvety, but occasionally deeper and looser, deep chocolate brown, in some strains showing a thin but conspicuous weft of white aerial mycelium. Conidial heads as described on Czapek's but more frequently attaining diameters of 400 to 500 μ and in occasional strains splitting into well-defined columns.

Species description based primarily upon WB 4948 and 4951, received in 1962 from the Instituto Ozwaldo Cruz as unidentified strains of *Aspergillus*, and upon WB 4795, received in 1962 from the IFO as *A. aureus* var. *murinus* Naka *et al.* Additional named cultures regarded as inseparable from *A. awamori* as described include: WB 4958, received from the CBS in 1962 as Sainclivier's (1949) strain of *A. pseudo-niger* (C. and L.) Sainclivier; WB 4869 and 4870, also from the CBS, as Mosseray's strains of *A. pseudo-citricus* Mosseray and *A. pseudo-niger* Mosseray, respectively. Of these latter strains, WB 4870 appears to be somewhat intermediate between this species and *A. tubingensis* because of its smaller conidia.

WB 4859 and 4860, received in 1962 from the CBS as its strains "Kominami" and "Ciferri," respectively, of *A. miyakoensis* N., S., and W., are morphologically inseparable from the cultures cited as representative of *A. awamori* and are the strains in which normal sporulation on Czapek's agar occurs only at the margins.

The name *A. awamori* is used here for a variable group of blackish brown, biseriate Aspergilli that we believe to represent the concept of this species as it was originally reported in the Japanese literature. It was first applied by Nakazawa to a species of *Aspergillus* found to be most favorable for the koji of the awamori alcoholic fermentation. Considerable confusion has been created by the cultures distributed to Western investigators by various Japanese workers, and one senses that their use of the name depends more upon the source and application than upon the cultural and morphological characters. To further complicate the nomenclature, Inui in 1901 described the favorable black aspergillus of the awamori koji as a uniseriate species, *Aspergillus luchuensis*. Cultures subsequently distributed from Japan under this name proved to be either biseriate or to show both double and single sterigmata in the same conidial head. The name has since been accepted by Japanese workers as applying to a biseriate species, and it was cited by Nehira (1945) as a synonym of *A. awamori*. It is now impossible to draw a distinction between the two species. The earlier name, *A. luchuensis*, may be rejected because of the apparent error in the original description and the name *A. awamori* appropriately applied to the blackish-brown Aspergilli under consideration.

Probable Synonyms:

> *A. aureus* var. *murinus* Nakazawa, Simo, and Watanabe, in J. Agr. Chem. Soc. Japan **12**: 959–960, Fig. 7 (1936), as represented by WB 4795, received in 1962 from the IFO as Nakazawa's strain of this variety, is wholly representative of *A. awamori* as here considered.
>
> *A. awamori* var. *fuscus* Nakazawa, Simo, and Watanabe, in J. Agr. Chem. Soc. Japan **12**: 959, Fig. 6 (1936), by description and as represented by WB 4844, received in 1962 from the CBS as Ciferri's strain of this variety, is believed to be synonymous with *A. awamori*.
>
> *A. awamori* var. *minimus* Nakazawa, Simo, and Watanabe, in J. Agr. Chem. Soc. Japan **12**: 955, 956, Fig. 1 (1936), as described and as represented by WB 4845, is believed to be synonymous with *A. awamori* Nakazawa.
>
> *A. awamori* var. *piceus* Nakazawa, Simo, and Watanabe, in J. Agr. Chem. Soc. Japan **12**: 956–957, Fig. 2 (1936), as described and illustrated, and as represented by WB 4846 from the CBS as Ciferri's culture of this variety, is regarded as inseparable from the species.
>
> *A. inuii* Sakaguchi, Iizuka, and Yamazaki, in J. Appl. Microbiol. (Japan) **3**(4): 97–104 (1950). We were unable to obtain a culture of this fungus despite repeated attempts. However, according to Sakaguchi's description, it differs from his concept of *A. luchuensis* Inui in producing conidial heads in which both single and double series of sterigmata are seen. It may, in fact, represent *A. luchuensis* as it has been widely distributed by various Oriental investigators since Inui described it as a uniseriate species.
>
> *A. luchuensis* Inui, in J. Coll. Sci. Imp. Univ., Tokyo **15**: 469, Plate 22, Figs. 1–8 (1901). As originally described and illustrated this name was apparently applied to a favorable organism in the awamori alcoholic fermentation with brown-black conidial heads and uniseriate sterigmata. The text, however, particularly as it relates to a comparison with *A. wentii* Wehmer, raises some question as to whether Inui might not have had a fungus with two series of sterigmata. Subsequent to this, other authors including Blochwitz (1933) and Mosseray (1934) obviously considered the name for biseriate strains. Sakaguchi, Okazaki, and Iwasaki (1950) clearly state that this species is biseriate. Shih (1936) must have regarded *A. luchuensis* as a biseriate species since the type culture of his *A. luchuensis* var. *rubeolus* shows quite prominent primary sterigmata; and Nehira (1945) in his treatment of the black Aspergilli lists this species as a synonym of *A. awamori* Nakazawa. The correct disposition of *A. luchuensis* Inui must, therefore remain in doubt.
>
> *A. luchuensis* var. *rubeolus* Shih, in Lingnan Sci. J. **15**(3): 374 (1936), was described as differing from the species by becoming chocolate brown rather than black and was considered by Thom and Raper as a possible synonym of *A. japonicus* Saito. WB 4856, received from the CBS in 1962 as Shih's isolate, has proved to be a strain of *A. phoenicis* (Cda) Thom as presently considered.
>
> *A. niger* var. *fermentarius* Nakazawa, Takeda, Shimo, and Okada, in J. Agr. Chem. Soc. Japan **10**(2): 171–172; 184 (1934). Isolated from fermented

tobacco and found to produce higher yields of alcohol from sugar than other strains examined. Described in terms which seem to relate it to *A. awamori*.

A. niger mut. *fusca* Blochwitz, in Ann. Mycol. **32:** 87–88 (1934), was separated from *A. niger* because of its different pigmentation. Strain WB 4864, received in 1962 from the CBS as Blochwitz's culture is considered to be a strain of *A. awamori* Nakazawa.

A. usamii Saka., Iizuka, and Yamazaki, in J. Appl. Microbiol. (Japan) **3(4):** 97–104 (1950), as represented by WB 4760, received from the CBS in 1962 as their strain "Sakaguchi" of this species, is inseparable from *A. awamori*. *A. usamii* mut. *shirousamii* Saka. *et al.*, in J. Agr. Chem. Soc. Japan **28(12):** 972 (1954), was described as an "albino type" ultraviolet-induced mutant of *A. usamii*. Interestingly enough their culture, WB 4889, received in 1962 from Osaka University, bears a striking resemblance to Schiemann's mut. *cinnamomeus, q.v.*

Aspergillus ellipticus, sp. nov.

Coloniae in agaro Czapekii 2.0–2.5 cm. in dia. decem diebus 24–26 C crescentes; submersum mycelium tenue, planum, album et producens album vel leviter flavum, leviter floccosum, basale reticulum et multa, sed non densa, crassa, cinereo-brunnea (e caryophyllo vel ex osse brunnea, R., Tabula XL) capitula conidica in longissimis conidiophoris; reverso albo in primo, deinde obscure subfusco-flammeo in maturitate; exudato non multo, claro; leve odore escarii fungi. Capitula conidica globosa vel radiantia vel inaequaliter divisa, plerumque 400–700 µ in dia. decem diebus sed usque 1.5 mm. in maturitate; conidiophorae erectae, 0.5–1.0 cm. × 12–20 µ in dia., leviter constrictae sub vesiculā, incolores vel leviter coloratae, enodes vel eleganter sparseque punctatae, membranis plerumque 3.5–4.5 µ crassis; vesiculae globosae vel leviter subglobosae, plerumque 75–100 µ in dia., in totā superficie fertiles; sterigmata duoseriata, primaria plerumque 20–40 × 5.5–7.5 µ, secundaria 9–11 × 3.5–4.5 µ; conidia elliptica in maturitate, plerumque 5.0–5.5 × 3.3–3.8 µ, conspicue echinulata cum moderatis et aequaliter distantibus spinis; enodia vel eleganter echinulata, imperfecte differentiata conidia, pauca, 7.5–9.0 × 2.5–3.0 µ. Pseudo-sclerotia tarde formata et pauca (plura in agaro Czapekii cum 20% saccharo), obscure fulva in primo, brunnea in maturitate, discoida, 500–800 µ in dia., formata in turbinatis hypharum massis, usque 1.0–1.5 mm., quae rigidas, septatas, in apice circinatas vel implicatas, 300–500 µ longas et 6.0–8.0 µ in dia., appendiculas, hyalinas in primo deinde ex aureo flavas et ad extremum brunneas, producent. Nulla ascoporica reproductio observata est.

Typica cultura, WB 5120, in anno 1962 e Costa Rica solo isolata.

Colonies on Czapek's solution agar (Fig. 70A) growing slowly at room temperature (24–26° C), 2.0 to 2.5 cm. in diameter at 10 days, submerged mycelium thin, plane, white, developing a white or faintly yellow somewhat cottony basal felt through which the very long conidiophores arise, conidial heads in somewhat grayish brown shades near clove brown ((R., Plate XL) when young, later becoming bone brown (R., Plate XL), numerous but uncrowded at 10 days, sometimes developing conidial structures on shorter
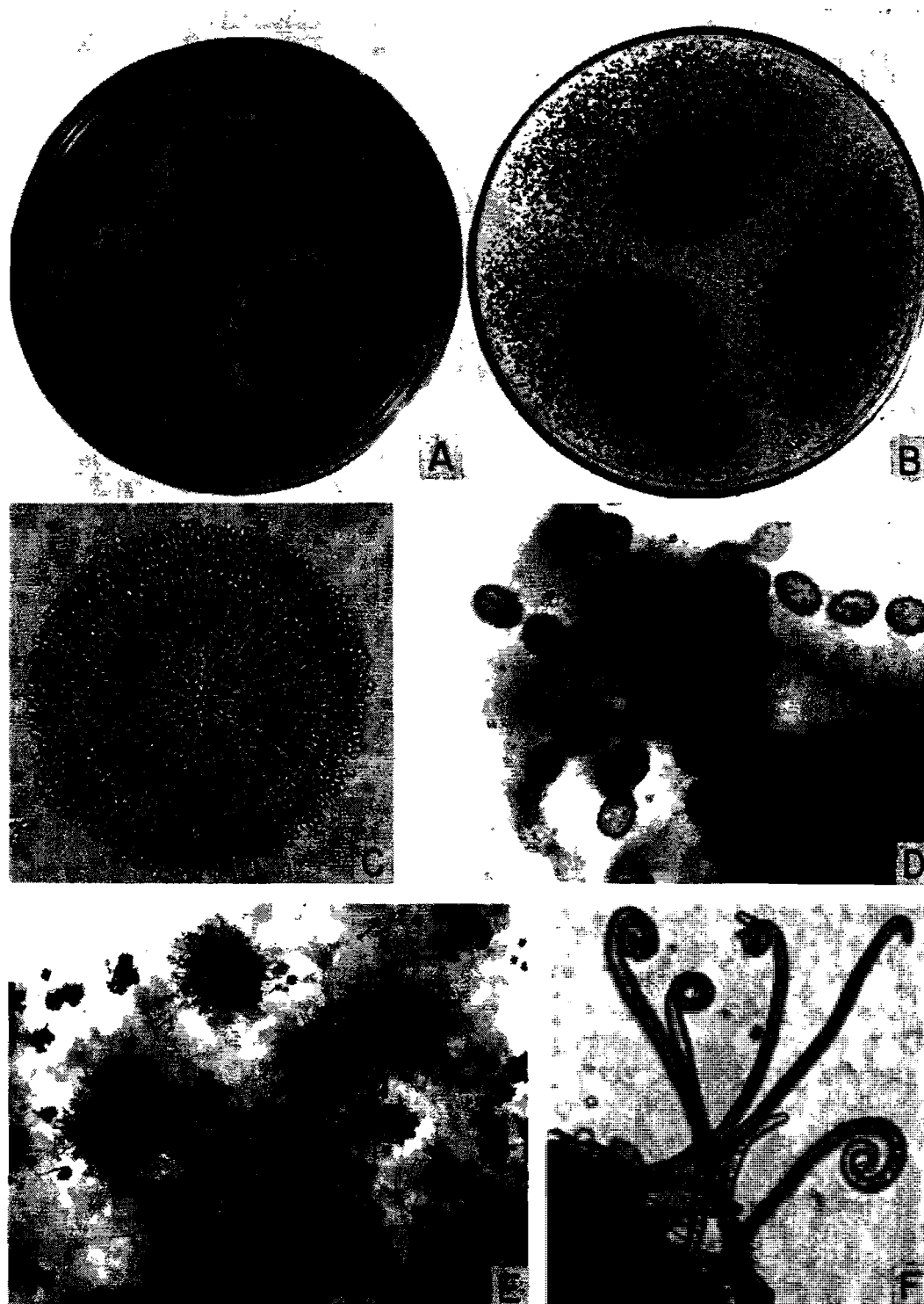
FIGURE 70. *Aspergillus ellipticus*, n.sp., WB 5120. *A* and *B*, Colonies on Czapek and malt agars, respectively; 10 days' incubation at room temperature. *C*, Typical conidial head, × 180. *D*, Characteristic elliptical spinulose conidia, × 1090. *E*, Colony margin showing sclerotia enveloped by loose mycelia from which project heavy-walled hyphae with circinate tips, × 14. *F*, Detail of such hyphae, which are found only in this species, × 295.

stalks at colony centers; reverse white and unwrinkled, becoming dull brownish orange in age; exudate very limited, clear; slight odor of edible mushrooms. *Conidial heads* globose to radiate or irregularly split, rarely forming discrete columns of conidia, large, commonly 400 to 700 μ but up to 1 mm. in diameter at 10 days, and as much as 1.5 mm. in age; *conidiophores* erect, mostly 5 to 8 mm. but not uncommonly 1 cm. in length, 12 to 20 μ in diameter, slightly constricted just below the vesicle, uncolored or only faintly colored, smooth or delicately and sparsely punctate, with walls commonly 3.5 to 4.5 μ thick; *vesicles* (Fig. 70*C*) globose or slightly subglobose, large, commonly 75 to 100 μ in diameter, fertile over their entire surface; *sterigmata* in two series, primaries mostly 20 to 40 μ by 5.5 to 7.5 μ but sometimes longer or shorter, secondaries 9 to 11 μ by 3.5 to 4.5 μ; *conidia* mostly pyriform with hyaline echinulations when young, becoming elliptical (Fig. 70*D*), mostly 5.0 to 5.5 μ by 3.3 to 3.8 μ, often appearing globose when not in chains, conspicuously echinulate at maturity, with spines discrete and evenly spaced, smooth or delicately echinulate conidia which appear to be imperfectly differentiated are occasionally seen and may reach dimensions of 7.5 to 9.0 μ by 2.5 to 3.0 μ.

Sclerotium-like structures (Fig. 70*E*) are developed late and in limited numbers on Czapek's agar and somewhat more abundantly on Czapek with 20 per cent sucrose. These, however, differ in origin and in pattern from the almost naked sclerotia normally produced by members of the *A. niger* group. The sclerotioid bodies are dull yellowish when young becoming brown in age, discoid in form, 500 to 800 μ in diameter, and borne within turbinate masses up to 1.0 to 1.5 mm. in diameter that consist of enveloping peridial hyphae from which arise stiff, septate, terminally circinate or coiled appendages (Fig. 70*F*) 300 to 500 μ in length and 6.0 to 8.0 μ in diameter. The appendages are at first hyaline, then golden yellow, and finally brown; they are reminiscent of the ornamentation that characterizes the ascocarps of *Myxotrichum*. No evidence of an ascosporic stage has been found, but the singular structures described are under continuing observation.

Colonies on malt extract agar (Fig. 70*B*) rapidly growing, 7 to 8 cm. in 10 days, basal mycelium thin and plane, zonate from alternating production of long-stalked and short-stalked conidal structures, conidial heads produced abundantly in dark black-brown shades; reverse uncolored but with black of conidial heads visible through the mycelium. Conidial heads as described on Czapek's agar, but dimensions of all structural elements except the conidia somewhat reduced.

Species description based upon WB 5120 as type, isolated locally in 1962 by Kyung Joo Kwon from Costa Rican soil.

Aspergillus heteromorphus Batista and Maia, in Anais soc. biol. Pernambuco
   15(1): 200–201, Figs. 11–20 (1957).

Colonies on Czapek's solution agar growing slowly at room temperature
(24–26° C), 3 cm. in diameter in 2 weeks and showing no further radial
growth at 5 weeks, radially furrowed, narrowly and conspicuously zonate,
margins compact and entire, basal mycelium largely submerged, compara-
tively thin and tearing easily, surface appearing close textured and velvety
from the dense stand of short-stalked conidial heads in shades at first near
ecru-olive or buffy olive (R., Plate XXX) rather slowly becoming dark
brownish black near fuscous black (R., Plate XLVI) but with the lighter
shades often persistent and fading to deep or dark olive-buff (R., Plate XL)
in central area or submarginal zone; reverse at first yellow or greenish yel-
low darkening in age to very dark greenish brown, with agar similarly
colored; exudate becoming conspicuous in age, brown; odor slight, somewhat
unpleasant. *Conidial heads* globose to loosely radiate, small, commonly 100
to 200 μ in diameter but in central colony area occasionally reaching 400 μ;
conidiophores smooth, uncolored, mostly less than 500 μ by 4.5 to 6.5 μ but
ranging up to 800 μ long; *vesicles* globose to subglobose, mostly 15 to 30 μ
in diameter, fertile on the upper half to three-fourths; *sterigmata* in two
series, primaries mostly about 10 to 15 μ by 6.5 to 8.0 μ but irregular and
sometimes much swollen with diameters reaching 15 μ and with both large
and small primaries sometimes seen in the same head; secondaries errati-
cally produced, often only one or two per primary, 4.5 to 6.5 μ by 2.5 to 3.5 μ;
conidia globose, variable when young from smooth to echinulate and from
3.0 to 5.0 μ in diameter but when mature conspicuously spinulose and mostly
3.5 to 4.0 μ in diameter. *Sclerotia* reported as numerous on Czapek's agar
with 1 per cent corn steep liquor, globose, white when young, 300 to 600 μ
in diameter.

Colonies on malt extract agar rapidly growing, 7 to 8 cm. in diameter at
2 weeks, basal mycelium hyaline, thin and submerged, conidial heads in
shades near Chaetura black (R., Plate XLVI) to true black produced
abundantly in alternating zones of large heads on long stalks and smaller
heads on short stalks to give an uneven surface; reverse uncolored or slightly
grayed; exudate lacking; odor faintly moldy. Conidial structures as on
Czapek.

Species description centered upon WB 4747, received in 1955 from the
ATCC as the type strain, No. 269, isolated from a culture of *Trichophyton*
at the Institute of Mycology, University of Recife, Brazil. Although this
strain no longer shows the heteromorphism emphasized by the authors and
used as the basis for the name, the strain is recognized as representing a
valid species and the name is retained for it.

Disparities between our description and that of the authors may result

from continued cultivation of this strain in pure culture, or from differences in incubation temperature and maintenance techniques.

Aspergillus foetidus (Naka.) Thom and Raper, in A Manual of the Aspergilli, pp. 219–220, Fig. 61*C* (1945).

Synonym

    A. aureus Nakazawa, in Inst. Govt. Research Formosa, Rept., I (1907).

    Not: *A. aureus* Berkeley, in English Flora, V, p. 346 (1836).

        S. aurea Greco, in Origine des tumeurs et mycoses argentines, Buenos Aires, pp. 671–694, Figs. 418–428 (1916).

Colonies on Czapek's solution agar (Fig. 67*E*) growing rather slowly at room temperature (24–26° C), attaining a diameter of 3.5 to 4.5 cm. in 10 days to 2 weeks, with vegetative mycelium in white or yellowish shades, largely submerged or forming a rather compact and comparatively tough surface felt, plane or radially furrowed, azonate or weakly zonate, in some strains bearing abundant olive-brown to brownish black conidial heads throughout except at the growing margin, in others sporulating tardily and less abundantly; exudate lacking or inconspicuous; colony reverse in yellow to orange shades, becoming reddish brown in age; odor very strong, penetrating, actinomycete-like. *Conidial heads* at first small, globose to radiate, remaining so in crowded central colony areas, others near colony margin becoming irregularly split into several rather well-defined columns, commonly 200 to 300 μ in overall diameter but in some strains reaching 500 μ; *conidiophores* mostly 500 to 800 μ by 7 to 10 μ, but up to 1 mm. in length by 12 μ in diameter, walls smooth and 1.0 to 2.0 μ thick, colorless or in age becoming brownish in terminal areas; *vesicles* small, subglobose or slightly elongate, mostly 25 to 35 μ in diameter but reaching 40 to 50 μ in some strains, fertile over the entire surface in larger heads or the upper three-fourths on smaller vesicles; *sterigmata* in two series, pigmented in brown shades, primaries somewhat variable, mostly 7 to 12 μ by 3.0 to 5.0 μ, occasionally longer, secondaries mostly 7 to 8 μ by 2.5 to 3.0 μ; *conidia* globose or nearly so, with walls brown, often appearing almost smooth but when mature irregularly and finely roughened, mostly 4.0 to 4.5 μ in diameter, borne in chains without obvious disjunctors.

Colonies on malt agar growing somewhat more rapidly, 5 to 6 cm. in 2 weeks, plane, velvety, with vegetative mycelium submerged and colorless or only slightly yellow, heavily sporing throughout, blackish brown shades, azonate or inconspicuously so; reverse in light yellow shades to almost colorless; odor not pronounced. Conidial heads usually splitting into numerous conspicuously divergent and compact columns and reaching 300 to 350 μ in diameter in most strains but up to 600 to 800 μ in others; conidia more

uniformly echinulate than on Czapek agar. Structural details of conidial heads as described above.

Species description based primarily upon WB 341, received in 1930 from Yakoyama as *A. aureus* Nakazawa and used (but not cited) by Thom and Raper (1945) as the basis of *A. foetidus, nom. nov.*, and upon WB 4784, received from the IFO in 1962 as *A. foetidus* T. and R. (Nakazawa's strain of *A. aureus* Nakazawa), which probably stems from the same original source but now differs from WB 341 in its reduced sporulation. WB 4843, received in 1962 from the CBS as Ciferri's strain of *A. awamori* var. *fumeus* Nakazawa, Simo, and Watanabe differs from these strains only in the more olive-brown color of its conidial heads. WB 337, received in 1923 from Neuberg in Berlin as Wehmer's strain of *A. niger citricus, n.n.*, and subsequently described by Mosseray (1934) as *A. citricus* (Wehmer) n. comb. is also included. The dimensions of conidial structures in this strain, but not the conidia, average somewhat larger than the other cultures cited.

In the awamori fermentation as studied by Nakazawa (1907), *A. aureus* Nak. was β, the unfavorable organism which gave a yellow color to the "koji" used.

Probable Synonyms

A. aureus var. *brevius* Nakazawa, Simo, and Watanabe, in J. Agr. Chem. Soc. Japan **12:** 962–963, Fig. 11 (1936), was described as having shorter stalks, longer primary sterigmata, and lighter colored conidia than the species. However, NRRL 337, considered by Iizuka (*ibid.* **27:** 524–529 (1953)) as representative of the variety, is included here as a strain of *A. foetidus* Th. and R. On the other hand, WB 4841, received from the CBS in 1962 as Ciferri's strain of var. *brevius* is believed to be a strain of *A. awamori*.

A. awamori var. *ferrugineus* Naka., Simo, and Watanabe in J. Agr. Chem. Soc. Japan **12:** 957–958, Fig. 4 (1936). No culture could be obtained, but relationship to *A. foetidus* is suggested by the brown colonies, olive heads, short conidiophores, vesicles of intermediate size, and globose finely echinulate conidia.

St. luteo-nigra Lutz, in Bull. soc botan. France **53:** 48–52 (1907), was described with yellow mycelium and fuscous smooth conidia 5.0 μ in diameter. These characters suggest relationship to *A. foetidus*.

A. luteo-niger V. Luijk. Mistake in citation in a Biourge manuscript for the "Van Luijk" strain of *A. luteo-niger* (Lutz) Thom and Church received from the CBS.

A. perniciosus Inui, in J. Coll. Sci. Imp. Univ. Tokyo **15:** 473, Plate 21, Figs. 9–12 (1901), illustrated as uniseriate and described as a yellow-brown form intermediate between *A. wentii* and *A. luchuensis* that was unfavorable for the awamori alcoholic fermentation. The disposition of this species remains in doubt, but close relationship with *A. foetidus* is suggested.

Aspergillus foetidus var. *pallidus*, Naka., Simo, and Wat.

Synonym

> *Aspergillus aureus* var. *pallidus* Nakazawa, Simo, and Watanabe, in J. Agr. Chem. Soc. Japan **12**: 961–962, Fig. 10 (1936).

Colonies on Czapek's solution agar growing rather restrictedly, attaining a diameter of 2.0 to 2.5 cm. in 10 days to 2 weeks at room temperature (24–26° C), plane or very lightly furrowed, consisting of a compact basal mycelium, nonsporulating and white or yellowish at the margin but otherwise bearing crowded conidial heads in dull grayish olive to olive-brown shades approximating dark olive (R., Plate XL) to Chaetura or olivaceous black (R., Plate XLVI); reverse at first colorless, then yellowish, becoming dark yellowish brown in age; odor less pronounced than in the species, not diagnostic. *Conidial heads* globose to radiate, up to 500 to 600 μ in diameter, usually splitting into few and ill-defined columns; *conidiophores* smooth, colorless or in brownish tints, commonly about 1 mm. long by 8 to 16 μ in width, occasionally longer; *vesicles* globose or nearly so, up to 50 to 60 μ in diameter in largest heads, fertile over the entire surface; *sterigmata* in two series, brownish, primaries commonly 10 to 15 μ by 3.5 to 5.0 μ when young, but up to 30 to 40 μ in older heads, secondaries mostly 7 to 10 μ by 3.0 to 4.0 μ; *conidia* at first elliptical to ovate and smooth or nearly so, becoming globose or subglobose, 3.5 to 4.5 μ in diameter and delicately roughened, adherent in fluid mounts but with connectives not evident.

Colonies on malt growing somewhat more rapidly, plane, usually velvety and heavily sporing throughout, in dark olive-black shades. Conidial structures up to 700 to 800 μ in diameter, essentially as on Czapek's agar, but with mature conidia 3.0 to 3.5 μ, globose, echinulate and with a suggestion of longitudinal orientation of surface markings.

Varietal description based upon WB 4797, received in 1962 from the IFO as Nakazawa's culture of *A. aureus* var. *pallidus* N., S., and W. (1936) and WB 4842, from the CBS in 1962 as Ciferri's strain of the same variety. WB 4794, received in 1962 from the IFO as Nakazawa's strain of *A. aureus* var. *minor* is regarded as inseparable from the preceding cultures.

The variety differs from the species primarily in its more restricted growth on Czapek's agar, the larger dimensions and more olive pigmentation of its conidial structures, and the absence of definite divergent columns of conidia in mature heads on malt agar.

Probable Synonym

> *A. subfuscus* Johan-Olsen, in Medd. Naturhist. Fören. Kristiania, 1885. Reported as pathogenic to rabbits when injected intravenously, and described as showing smooth globose spores 3.0 to 3.5 μ and a single series

of sterigmata up to 20 μ in length, but with some secondary sterigmata seen in the original material. Colonies were olive-brown with a greenish shade.

Aspergillus foetidus var. *acidus*, Naka., Simo, and Wat.

Synonym

> *Aspergillus aureus* var. *acidus* Nakazawa, Simo, and Watanabe, in J. Agr. Chem. Soc. Japan **12:** 960–961, Fig. 8 (1936).

Colonies on Czapek's solution agar growing rather slowly, 4.0 to 5.0 cm. in 10 to 14 days at room temperature (24–26° C), at first flocculent and near white to pale yellowish, lightly sporulating, later producing relatively few globose to radiate, brownish black conidial heads in marginal and submarginal areas; reverse in yellow shades turning dull yellow-brown in age; odor not pronounced; *conidial heads* comparatively large, 350 to 400 μ in diameter, not splitting into distinct columns; *conidiophores* relatively short and wide, commonly 600 to 800 μ by 20 to 30 μ, rarely 1 mm. in length, *vesicles* globose or nearly so, up to 80 to 85 μ in diameter, fertile over the entire surface; *sterigmata* biseriate, brownish in color, primaries 20 to 40 μ by 4.6 μ, secondaries 6 to 10 μ by 2.5 to 3.5 μ; *conidia* globose to somewhat flattened, brown, 4.0 to 4.5 μ in diameter, comparatively heavy walled, appearing smooth or with surface slightly irregular but not echinulate or rugulose.

Colonies on malt growing more rapidly and sporulating irregularly within 10 days, broadly zonate, plane or closely wrinkled, with vegetative mycelium largely submerged and bright golden yellow; conidial heads borne on short conidiophores as above but somewhat larger than on Czapek's, reaching diameters of 500 to 600 μ and showing numerous ill-defined columns of conidia.

Varietal description centered upon WB 4796, received in 1962 from the IFO as Nakazawa's strain of *A. aureus* var. *acidus* N., S., and W. A culture, WB 4840, received in 1962 from the CBS as Ciferri's strain of this variety fails to fit the description and is believed to represent *A. awamori* Nakazawa.

The variety differs from the species in its more lightly sporulating colonies on Czapek's and malt agars, its larger conidial heads and structural parts, its relatively short and wider conidiophores, and especially its bright yellow mycelium on malt agar.

Probable Synonym

> *A. nakazawai* Sakaguchi, Iizuka, and Yamazaki, *nomen nudum*, in J. Appl. Microbiol. (Japan) **3(3):** 65–72 (1949); described in *ibid.* **3(4):** 97–104 (1950). From the description and from examination of WB 4750, received

in 1962 from the CBS as Sakaguchi's strain, we regard this species as representing an aberrant and very poorly sporulating strain of *A. foetidus* var. *acidus*.

Aspergillus japonicus Saito, in Botan. Mag. (Tokyo) **20**: 61–63 (1906).

Synonyms

Aspergillus japonicus var. *atrofuscus* Iizuka, in J. Agr. Chem. Soc. Japan **27**: 807, Fig. 4 (1953).

Aspergillus brunneo-violaceus Batista and Maia, in Anais soc. biol. Pernambuco **13**: 91–93, Figs. 1, 2 (1955).

Aspergillus japonicus var. *capillatus* Nakazawa, Takeda, and Suematsu, in J. Agr. Chem. Soc. Japan **8**: 12–17, Fig. 5 (legend with Fig. 4) (1932).

Aspergillus atro-violaceus Mosseray, in La Cellule **43**: 268–269, Plate IV, Figs. 122–125 (1934).

Colonies on Czapek's solution agar (Fig. 67*F*) growing rapidly at room temperature (24–26° C), in most strains 5.0 to 6.0 cm. in diameter in 10 days, but in occasional strains less, consisting of a dense, white, irregularly wrinkled basal mycelium which tardily gives rise to a dense stand of conidial structures in purple-brown or purple-black shades, occasional strains producing abundant white to cream-colored globose sclerotia in central colony areas; reverse at first uncolored but later becoming purple drab and occasionally with a slight yellow-green tinge; exudate lacking; odor sometimes quite strong but not distinctive. *Conidial heads* variable, small, radiate or split into few indistinct columns, rarely exceeding 300 μ in diameter at 10 days but in age sometimes distinctly columnar and up to 600 to 700 μ long or split into two divergent columns of similar length; *conidiophores* smooth or with a limited surface granulation, colorless or slightly pigmented particularly just below the vesicle, sinuous, mostly 500 to 1000 μ by 5 to 10 μ but varying greatly in these dimensions; *vesicles* somewhat colored in brownish yellow shades, often somewhat elongate but in older or larger heads more nearly globose, mostly 20 to 30 μ by 25 to 35 μ but ranging from less than 15 μ to 45 μ in diameter, in normal heads fertile over most of their surface but in small heads at the apex only; *sterigmata* uniseriate, 5.5 to 8.0 μ by 3.0 to 4.5 μ, rarely swollen to double their normal size; *conidia* mostly globose, occasionally subglobose, strongly echinulate, with echines discrete and regularly spaced (Fig. 69*B*), commonly 0.5 μ long, occasionally longer, spore bodies mostly 3.0 to 3.5 μ. *Sclerotia* produced abundantly but tardily by some strains, white to cream, globose, up to 500 μ in diameter.

Colonies on malt extract agar growing rapidly, 7 to 8 cm. in diameter in 10 days at room temperature, more quickly and heavily sporulating than on Czapek's; conidial heads usually larger than on Czapek's and split into

conspicuous columnar masses, commonly reaching diameters of 500 μ in 10 days and showing a narrower range of vesicle and stalk measurements; sterigmata and conidia as described above.

Species description based upon WB 1782, isolated in 1941 from Panama soil; WB 2053, received in 1946 from the Philadelphia Quartermaster Depot as an active cellulose destroyer; WB 4912, the type of *A. brunneo-violaceus* Batista and Maia (1955), received from the authors in 1962; WB 4786, received from the IFO as Nakazawa's strain of *A. japonicus* var. *capillatus* Nakazawa, Takeda, and Suematsu; WB 4839, received in 1962 from the CBS as Mosseray's strain of *A. atroviolaceus* Mosseray; and WB 5117, isolated in 1962 by Kyung Joo Kwon from Costa Rican soil.

Although no authentic culture of *A. japonicus* was available for the current study, this specific name is accepted since it was clearly based upon a uniseriate member of the *A. niger* group with relatively small vesicles and globose echinulate conidia and its description predates those of other species and varieties here included.

Probable Synonyms

A. japonicus var. or mut. *grisea* was cited by Blochwitz in Ann. Mycol. **33**: 240 (1935) as the appropriate designation for *A. malvaceus* Mosseray, which was described as having mauve colonies and globose or obovate smooth conidia 3.0 to 5.0 μ in long axis. Although WB 360, cited and illustrated by Thom and Raper (1945) as *A. violaceo-fuscus* Gasperini, may represent Mosseray's species since it shows both the colony color and the type of conidia described, we hesitate to recognize *A. malvaceus* on the basis of this isolate, which we believe to represent an aberrant strain of *A. japonicus*.

A. nanus Montagne, in Sylloge Generum Specierumque Cryptogamarum, p. 300, No. 112, Paris (1856); Saccardo, Sylloge Fungorum, IV, p. 71 (1886). Species reported as dark brown with sterigmata about 15 μ in length and spores 3 μ in diameter. Although the sterigmata are unusually long, this may have approximated *A. japonicus*.

A. yezoensis Sasaki, in J. Fac. Agr. Hokkaido Univ. **49**: 144, Plate IX, Fig. 18 (1950), as illustrated and described appears to have been based upon a strain of *A. japonicus*. However, WB 4819, received in 1962 from the IFO as Sasaki's strain, is biseriate and fails to satisfy the author's description; it duplicates WB 4797 and is here considered as *A. foetidus* var. *pallidus*.

Aspergillus aculeatus Iizuka, in J. Agr. Chem. Soc. Japan **27**: 806, Figs. 1–2 (1953).

Synonym

Aspergillus japonicus var. *viridiflavus* Iizuka, *ibid.*, p. 807.

Colonies on Czapek's solution agar growing rapidly at room temperature (24–26° C), 5 to 6 cm. in diameter in 12 days, plane, producing a dense

stand of conidial structures, heavily sporing throughout in purple-brown or purple-black shades often with a slight gray-tan surface "bloom"; reverse uncolored or in fairly conspicuous yellow shades to near black at colony center, yellow pigment diffusible; exudate and odor lacking; white to cream-colored sclerotia produced by occasional strains, most abundant at colony centers and at contiguous margins. *Conidial heads* globose at first, then splitting into relatively few compact divergent columns, reaching diameters up to 1 mm. but commonly 500 to 700 μ, shattering easily with columns deciduous, individual heads often variable in color with conidia nearest the vesicles light tan; *conidiophores* uncolored or slightly brownish below the vesicles, usually 1.0 to 2.0 mm. by 9 to 13 μ but up to 2.5 mm. long and 18 to 29 μ in diameter with walls up to 2.0 to 2.5 μ thick, smooth or occasionally showing a limited deposit of granular material; *vesicles* (Fig. 71*A*) often somewhat elongate when young, globose or nearly so when fully developed, heavy walled, commonly pigmented in brown shades and 60 to 80 μ in diameter but ranging from 35 to 100 μ, fertile over the entire surface; *sterigmata* in a single series, closely packed, 6.5 to 10.0 μ by 3.0 to 4.4 μ; *conidia* (Fig. 71*B*) ranging from definitely elliptical to globose or nearly so, varying with the strain or within a single strain, mostly 3.5 to 4.0 μ by 4.5 to 5.0 μ, but with occasional cells measuring as much as 4 by 7 μ, in mounts showing a purplish tinge, conspicuously echinulate with echines discrete and rather widely spaced.
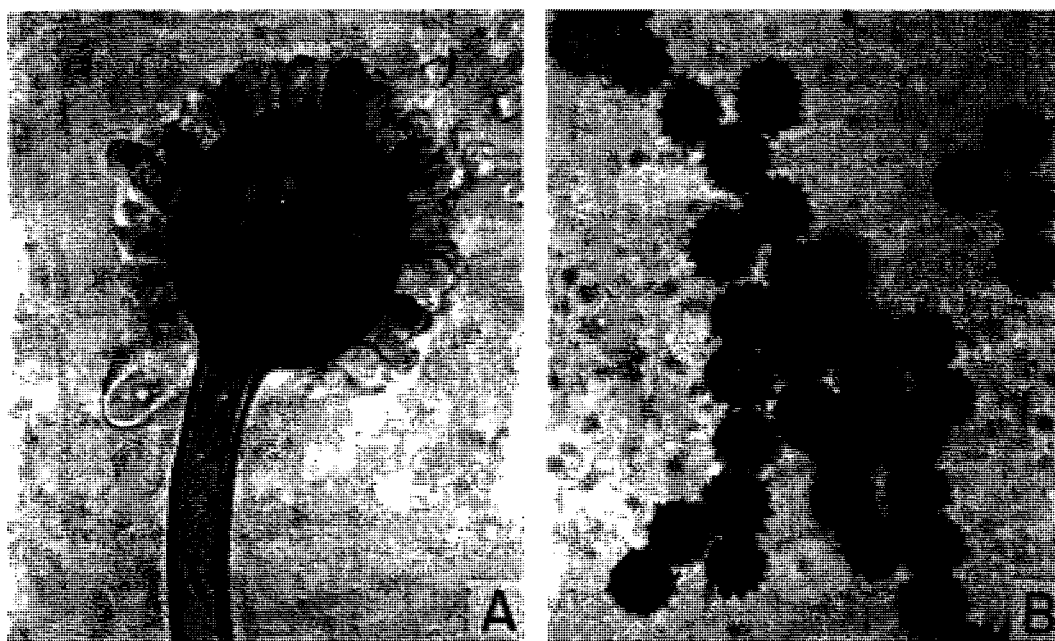

FIGURE 71. *Aspergillus aculeatus*, WB 5094. *A*, Conidial heads showing pigmented vesicle and uniseriate sterigmata, × 740. *B*, Globose to somewhat elliptical conidia that are strongly echinulate, × 1485.

Colonies on malt extract agar growing as on Czapek's, 5 to 6 cm. in diameter in 12 days, plane, heavily sporing in purple-brown or purple-black shades; reverse uncolored or slightly yellow; odor faintly moldy. Conidial structures as described on Czapek's.

Species description based upon WB 5094, 5118, and 5119 isolated in 1962 in our laboratories from tropical soils. WB 358, received from Blakeslee in 1915 and cited by Thom and Raper (1945) as representative of *A. japonicus* Saito, is also included here because of its elliptical conidia and its large vesicles. Although we were unable to obtain a culture of *A. aculeatus* Iizuka from the author, the preceding strains are assigned to this species since it is the first member of the *A. niger* group described as uniseriate with large vesicles and predominantly elliptical conidia.

Possible Synonyms:

> *A. violaceo-fuscus* Gasperini, in Atti soc. toscana sci. nat. Pisa, Mem. 8, Fasc. 2, 326–328 (1887); was described with elliptical verruculose conidia and biseriate sterigmata. The latter were reported to consist of basidia 6 to 8 μ by 3.0 μ and "simple" cylindrical to pyriform sterigmata 2 to 4 μ long. Since we know of no black *Aspergillus* with single secondary sterigmata this small, we suspect that Gasperini misinterpreted partially developed conidia for these structures. If we knew this interpretation to be correct, since other measurements conform reasonably well, *A. violaceo-fuscus* would be the valid name for cultures here assigned to *A. aculeatus* Iizuka. The only species of black Aspergilli known to have a double series of sterigmata and elliptical conidia is *A. ellipticus* n. sp. (p. *111*), but this differs so markedly that consideration of Gasperini's species with it is precluded.

> *A. violaceo-fuscens* mut. *grisea* Blochwitz (intended as *A. violaceo-fuscus?*), in Ann. Mycol. 32: 87 (1934). Reported as differing from the species in the pigmentation of its conidial heads. WB 4880, received in 1962 from the CBS as Blochwitz's strain of *A. violaceo-fuscus* mut. *grisea*, shows the elliptical echinulate conidia here considered as diagnostic of *A. aculeatus* but vesicles which fail to reach the dimensions described for this species, none seen exceeding 45 μ in diameter.

Probable Group Synonyms

> *A. barbae* Castellani, cited by A. Sartory, in Champignons parasites de l'homme et des animaux, p. 595 (1922), as having been isolated from the beards of natives of Uganda and Ceylon. Only conidia described: brownish, spherical, 4.5 μ in diameter. Some member of the *A. niger* group.

> *S. castanea* Patterson, in Bull. Torrey Botan. Club 27: 284 (1900). The type appears to have been lost, but examination of material determined by Mrs. Patterson under this name in 1901 in the Pathological Collections of the Bureau of Plant Industry at Washington shows a strain of the *A. niger* group with conidia mostly 3.5 to 4 μ but occasionally 4.5 μ in diameter.

A. cookei Sacc., in Sylloge Fungorum, IV, p. 71 (1886). Bibliographic name change for *A. mucoroideus* Cooke.

    Syn: *A. mucoroideus* Cooke, in Grevillea **12:** 9 (1883). Author's diagnosis suggests some member of the *A. niger* group.

A. gallomyces Calmette, in German Patent No. 129,164 (Verfahren zur Umwandlung von Tannin in Gallusaüre) (1902). Apparently a variant of *A. niger* associated with the gallic acid fermentation.

Aspergillopsis intermedia Spegazzini, in Anales museo nacl. Buenos Aires, Ser. 3, **13:** 435 (1911). A member of the *A. niger* group with primary sterigmata 40 to 50 μ in length and conidia 4.0 to 4.5 μ in diameter.

A. javanicus cited by Takahashi and Sakaguchi, in J. Agr. Chem. Soc. Japan **1**(10): 100 (1925), only in parenthesis after *A. fumaricus* Wehmer and apparently deemed a synonym. In *A. niger* group.

A. Macfiei Dodge, in Medical Mycology, pp. 629–630 (1935). An unidentifiable member of the *A. niger* group isolated from a case of otomycosis in man.

    Syn: *Sterigmatocystis* sp. in Macfie, Am. Trop. Med. Parasitol. **15:** 279–281 (1921).

Alliospora sapucaya Pim, in Proc. Roy. Indian Acad. (1883) and in J. Botany, p. 234 (1883). Some member of the *A. niger* group causing rot of alliaceous bulbs.

OCCURRENCE AND SIGNIFICANCE

The black aspergilli are probably more common than any other group within the genus. They are worldwide in distribution and occur in and upon the greatest variety of substrata, including grains, forage products, spoiled fruits and vegetables, exposed cotton textiles and fabrics, leather, dairy products and other protein-rich substrata, and decaying vegetation in the field. They are abundant in all soils examined and appear to be particularly abundant in soils from tropical and subtropical areas. With the possible exception of the *A. flavus* group, which is of great economic importance in the Orient, the black aspergilli are undoubtedly more widely used in industry than any other group of molds.

Because of their cosmopolitan distribution and their roles in natural processes of decomposition, because of their extensive use in physiological and nutritional studies of the fungi, and even more because of their important applications in industry, the number of papers that have been published relating to the black aspergilli is in excess of one thousand since 1945. Obviously, we cannot, in this volume, present anything approaching a complete coverage of this vast literature. We shall attempt to provide the reader with summaries of developments in certain key areas of special interest and sufficient references to provide an introduction to the relevant literature. The vast majority of published reports refer only to "*Aspergillus niger*," and it is often impossible to correlate metabolic activity with species identity as determined by cultural and morphological criteria.

Acids

GALLIC ACID. The production of gallic acid by the fermentation of gall nuts, representing the oldest acid fermentation, was first reported by Scheele in 1787, and it was further investigated by Robiquet in 1852. Van Tieghem in 1867 named and described the responsible organism as *Aspergillus niger*. A few additional studies were made during the next half century, notably those of Knudsen (1913a, 1913b). The biochemistry of the fermentation was considered in some detail by Dalvi (1931), and more recently by Nishira and Mugibayashi (1953, 1955) and by Lippitsch (1961).

CITRIC ACID. Wehmer (1893) reported the production of citric acid from glucose by molds designated as species of a new genus *Citromyces*, since recognized as monoverticillate Penicillia. It was not until Thom and Currie (1916) and Currie (1917) published their papers on citric acid production that this fermentation became identified with the *A. niger* group and led to the establishment of the first, and still important, industrial mold fermentation. Subsequent to this, investigators in the United States and abroad made many additional and important contributions. Among these the works of Molliard (1919, 1924); Bernhauer and co-workers (1926–1941); Challenger, Subramaniam, and Walker (1927); the United States Department of Agriculture group, including Wells, May, Moyer, Herrick, and Porges (1932 *et seq.*); and Doelger and Prescott (1934) are considered to be outstanding for the period prior to 1945. (See Thom and Raper (1945, pp. 290–293) for a more complete listing and Perlman (1949) for a review of the citric acid fermentation up to 1949.)

Since that time many papers relating to the mechanism of citric acid formation have been published, including those by Carson *et al.* (1951), Bomstein and Johnson (1952), Ramakrishnan, Steel, and Lentz (1955), Shu, Funk, and Neish (1954), Cleland and Johnson (1954), and Joshi and Ramakrishnan (1960). At the same time, important advances have been made in the conduct of the fermentation: (1) less highly refined substrates may be used as the carbon source; and (2) the acid can be produced in large scale submerged cultures.

Since the citric fermentation is strongly influenced by trace elements (Shu and Johnson, 1948; Tomlinson *et al.*, 1950, 1951), various techniques have been developed to remove these metallic ions when they are present in excess, including treatment of the sugar or molasses with ferrocyanide (Gerhardt *et al.*, 1946; Clement, 1952; S. M. Martin, 1955) or with cationic resins (Perlman *et al.*, 1946; Litýnski, Jurkowska, and Zak, 1952). Alternatively, it is now possible to minimize or circumvent the deleterious effect of such mineral elements by the addition of alcohols to the fermentation medium (Moyer, 1953a, 1953b). Application of submerged-culture techniques to the citric acid fermentation was first reported by Szücs (1944, 1948) and Karow and Waksman (1947) (also Waksman, 1946; Waksman

and Karow, 1946) and subsequently by Clement (1952), S. M. Martin and Waters (1952), Moyer (1953a, 1953b), Shu (1956), and S. M. Martin (1957). Unlike the other studies, which hinged upon purification of the substrates, Moyer's success was based upon the addition of alcohols, particularly methanol, which by "poisoning" the fermentation counteracted the effects of metallic ions and other impurities, thus permitting the use of crude substrates such as molasses and hydrolyzed starch. Additional papers relating to the alcohol effect have been published by Springer and Gernet (1956), Taha and El-Zainy (1959), Ciegler and Raper (1960), and Usami and Taketomi (1961). Considerable information regarding actual practice in industry, traditionally secret, has been provided by a series of British Patents issued to the Miles Laboratories, Elkhart, Indiana (1956, 1961, 1962).

In all mold fermentations, and especially in the production of citric acid, the use of carefully selected strains is essential. As might be expected, cultures optimal for surface production are not employed, as a rule, for the submerged fermentation and *vice versa*. Mutant strains have been used advantageously (Perlman *et al.*, 1946; Imshenetskii *et al.*, 1960), but attempts to utilize heterokaryons have proved unsuccessful (J. L. Yuill, 1951; Ciegler and Raper, 1957).

GLUCONIC ACID. Selected strains of *A. niger* are used industrially for the production of gluconic acid, which has been investigated by many workers. Outstanding contributions have been made by Molliard (1922), Bernhauer (1926), Amelung (1927), and the United States Department of Agriculture group, including Herrick, May, Wells, Moyer, Gastrock, Porges, and others (1927 *et seq.*). Beginning with studies on *Pencillium purpurogenum* var. *rubrisclerotium* (May, Herrick, Thom, and Church, 1927) and with *P. chrysogenum* (Moyer, May, and Herrick, 1936), basic problems relating to mold growth and acid production in submerged culture were worked out on laboratory and semi-plant scales. Attention was then directed to *Aspergillus niger*, a more vigorous and more productive species. The acid was recovered as the Ca salt, CaCO₃ being added to the fermentation to neutralize excess acid. Maximum yields were attained with a very high rate of aeration, which was achieved by conducting the fermentation under increased air pressure, and a boron-tolerant strain that permitted fermentation of 25 per cent glucose solutions. In 1952 a method for the direct production of Na-gluconate on a pilot plant scale was reported by Blom *et al.* of the Northern Regional Research Laboratory. The fermentation has been studied outside the United States by Ikeda, Hishimaki, *et al.* (1959), Elías (1956), Carlson (1960), and Taha, Gad, and Abbasy (1960). Important by-products of the fermentation as it is now conducted in industry are the enzymes, glucose oxidase (Goldsmith *et al.*, 1960) and catalase.

FUMARIC ACID. Certain strains of the *A. niger* group produce appreciable

amounts of fumaric acid, and it was to one of these forms that Wehmer applied the name *A. fumaricus* (1918). More recently, however, species of *Rhizopus* rather than strains of the black aspergilli are generally employed when fumaric acid is produced in industry by fermentation (Foster and Waksman, 1939). The work of Rhodes, Moyer, *et al.* (1959) is of special interest since these authors report enhanced yields of fumaric acid with *Rhizopus arrhizus* by the addition of methanol.

OXALIC ACID. Under certain conditions some strains of *A. niger* produce appreciable quantities of oxalic acid. While it is usually avoided rather than encouraged, this fermentation has been investigated by Wehmer (1891), Thom and Currie (1916), Raistrick and Clark (1919), Jaquot (1938), and others. More recently, Cleland and Johnson (1956) and Müller (1960) have investigated conditions leading to the formation of this acid, and throughout the literature relating to the citric fermentation the reader will encounter frequent reference to nutritional and cultural conditions that favor the production of oxalic acid.

Enzymes

AMYLOLYTIC. The production of amylolytic enzymes by members of the *Aspergillus niger* group represents a recent development, in contrast to the age-old use of *A. oryzae* for this purpose. Work at the Northern Regional Research Laboratory resulted in a series of papers by Corman, Langlykke, Le Mense, Tsuchiya, and others (1946–1950) on the production of amylolytic enzymes in submerged cultures by selected strains of *A. niger*. The primary objective was to secure a malt substitute suitable for converting grain in the ethanol fermentation. The effectiveness of fungal amylases for this purpose was demonstrated by Erb *et al.* (1948) and fully confirmed on an industrial scale by Hanson, Bailey, *et al.* (1955). Additional investigations have been reported by workers outside the United States, including Shu and Blackwood (1951) and Shu (1952) in Canada, Drews *et al.*, (1952, 1953) in Germany, Pathak (1952) in India, Beran and co-workers (1956, 1957) in Prague, and Vyatkin (1959) in Russia. Many studies have been reported from Japan, but space permits citation of only a few: Minoda (1952), Asai and Minoda (1952), Tanabe *et al.* (1952, 1953), Ono and Tanaka (1954), Murota *et al.* (1954), and Minoda and Asai (1960). Starchy substrates other than grains successfully converted for ethanol production have included white potatoes (Beran *et al.*), sweet potatoes (Tanabe *et al.*), and cassava (Teixeira *et al.*, 1950). A German patent by Malsch (1951) covers the use of *A. niger* for the fermentation of inulin to ethanol. In much of this work, it is emphasized that the black Aspergilli, as a rule, produce relatively more maltase (Roy and Underkofler, 1951), or maltase and limit dextrinase, and less α-amylase than do the amylolytic strains of *A. oryzae*.

Amylolytic enzymes obtained from black Aspergilli, designated *A.*

Usamii (Komaki *et al.*, 1956–1957) and *A. phoenicis* (Rentshler *et al.*, 1962), have been used successfully for the production of crystalline glucose from starch; while in a Russian patent *A. awamori* (Feniksova and Shilova, 1960) is said to be superior to *A. oryzae* as a source of enzymes for producing glucose syrup from starch.

It is of interest that the black Aspergilli studied by the Japanese are generally designated as *A. awamori*, *A. usamii*, or some species other than *A. niger*, and Kitahara and Kurushima (1950) have proposed a classification of the black Aspergilli upon the basis of their saccharifying "curves" that is in agreement with the morphological classification proposed by Nehira in 1945.

In contrast to results reported for the citric acid fermentation (see above), Johanides and Alačević-Grlić (1959) reported increased amylolytic activity by heterokaryons formed by selected strains of *A. niger*.

PROTEOLYTIC. Selected strains of *Aspergillus oryzae* have been used traditionally to produce proteolytic enzymes for use in food fermentations such as soy sauce, and to a lesser degree as an agent for the chillproofing of beer. These are mainly alkaline proteases. During the past decade certain of the black Aspergilli, usually designated *A. saitoi*, or less frequently *A. shirousamii*,* have been studied intensively by Yoshida and co-workers (1955–1962) because of their capacity to produce proteolytic enzymes with optimal activity at pH 2.7 to 3.0. Additional studies on acid proteases from these fungi have been reported by Sakamoto *et al.* (1957, 1958) and by Gabeloteau and Desnuelle (1960).

PECTOLYTIC. Some members of the *A. niger* group have been investigated as a source of pectolytic enzymes although these products are usually produced commercially by selected species and strains of Penicillia. Reid (1950a) reported good yields of pectic enzymes from *A. foetidus*, which has been further investigated by Ayres *et al.* (1952) and Reid and Brooks (1955). Kontio (1950) studied the formation of extracellular pectic enzymes of *A. niger*, as did also Hauptmann (1952), Fujii *et al.* (1954), Martakov (1954), H. Saito (1955), Tuttobello and Mill (1961), and Kogan and Shavruk (1962). Much of this work relates to the properties of the enzymes and their use in the clarification of fruit juices, wines, *etc.* There are other applications as well. Isimaru and Toyama (1950) reported good results with a culture of *A. niger* in the retting of ramie fibers, while Asai and co-workers used crude enzyme preparations from the same species for the degumming (retting) of Manila hemp (1950) as well as ramie (1951). Gaponenkov (1953) found *A. niger* to substantially reduce the pectin content of sugar beets during storage, and Gaumann and Bohni (1947) found that *A. niger* produced pectase, as an adaptive enzyme, and pectinase, as a constitutive enzyme.

LIPOLYTIC. Members of the *Aspergillus niger* group have been isolated on

* Probably *A. usamii* mut. *shirousamii* Sakaguchi, Iizuka, and Yamazaki.

numerous occasions from vegetable oils and oil-bearing seeds, and experimental studies with these molds, while not extensive, indicate that some strains are capable of producing substantial amounts of lipolytic enzymes. Ramakrishnan and Banerjee (1952a) isolated A. niger along with several other Aspergilli from peanuts, and Ramakrishnan (1954) subsequently studied the influence of culture media on the lipase and esterase activity of A. niger. In parallel papers the same investigators (1951a, 1952b) studied the lipolytic activity of A. niger isolated from, and grown upon, castor beans and of A. niger, A. flavus, and A. oryzae grown on coconut (1951b). Coursey (1960) and Coursey and Eggins (1961a, 1961b) found A. niger to be one of the most common and most active fungi implicated in the lipolysis of palm oil during storage. Gonzales (1957) reported A. niger to be a common mold but to show relatively little lipolytic activity in stored olives. Vishwanathan et al. (1957, 1961) found A. awamori when grown on peanut oil to be more actively lipolytic than either A. flavus or A. fumigatus.

Shipe (1951) compared the lipases formed by A. niger and by Penicillium roqueforti, while Chandan et al. (1962) found lipase synthesis by A. niger to be inhibited by the antibiotics pimaricin, nystatin, and penicillin.

MISCELLANEOUS. Many additional enzymes are produced by Aspergillus niger, of which the following may be noted briefly. Catalase, mentioned as a by-product of the gluconic acid fermentation, has been studied by Scott and Hammer (1960) and by Bhatnagar and Krishnan (1960) and is reported by the former investigators to have greater stability at low pH than catalase from beef liver. Pokrovskaya et al. (1962) have described the production of glucose oxidase, also mentioned in connection with the gluconic acid fermentation. Lamaire and Brunel (1951) have reported the production of an adaptive enzyme, cyanamidase, when Sterigmatocystis nigra is grown in the presence of calcium cyanamide. Chitinolytic enzymes were found by Otakara (1961, 1962) that would liquefy glycolchitin, and Koh et al. (1958) reported A. flavus and A. niger to elaborate keratinolytic enzymes when grown on keratin as the sole source of carbon. Tsuchiya, Jeanes, et al. (1952) reported dextran-degrading enzymes to be present in the fungal amylase preparations produced for grain conversion (see above), while Whistler and Masak (1955) obtained preparations from A. foetidus that would hydrolyze the xylan of corn cobs to xylobiose.

Phytopathogenicity

The black Aspergilli grow normally as saprophytes on dead plant tissues and other organic materials, but they may become pathogenic under certain circumstances and on some crop plants. The most serious disease caused by A. niger is crown rot of peanuts (groundnuts). The fungus attacks the plant soon after germination and apparently exerts its deleterious effect in substantial part by the production of oxalic acid, as reported by Gibson (1953a,

1953*b*), who studied the disease in East Africa. Infection occurs by invasion of the hypocotyl and is most severe at temperatures of 30° C and above. Other investigators who have studied crown rot include Clinton (1960, 1962) in the Sudan, Morwood (1953) and Purss (1960) in Queensland, Jain and Nema (1952) and Nema *et al.* (1955) in India, and Jackson (1962) in Georgia.

Other diseases reported to be caused by *A. niger* include a boll rot of cotton (Ray, 1946); a seedling blight of sorghum (Leukel and Martin, 1943), a bole rot of sisal (Wallace, 1952; Lock, 1962); the black mold of onions (Venkatarayan and Delvi, 1951; El-Helaly *et al.*, 1962), garlic (Mathur and Mathur, 1958), and shallots (Aycock and Jenkins, 1960); a stem rot of *Dracena* (Natour and Miller, 1960); chlorosis of almond trees (Sibilia, 1948); the spoilage of dates (Bliss, 1946; Almandil, 1961); and a root-stalk rot of *Sansevieria* (Alvarez and Diaz, 1949). *A. carbonarius*, a large-spored member of the *A. niger* group, has been reported to cause grape rot in India, (S. L. Gupta, 1956).

Nutrition and Physiology

Aspergillus niger has been used more extensively than any other fungus in investigations on mold nutrition and physiology. As early as 1909 Latham studied nitrogen assimilation by *A. niger* (*S. nigra*); he was followed in 1911 by Dox, who studied its phosphorus assimilation. Beginning in 1918 and continuing up to 1956, Steinberg published a succession of papers on the physiology of *A. niger*, with special reference to the role of heavy metals in its nutrition. A single strain of *A. niger*, which is carried in our collection as WB 334 (Thom No. 4247), was used throughout these investigations. Studies of a somewhat similar character have been conducted by Bortels (1927), Levy (1932), Gollmick (1936), and others. The following references should provide entrance to the vast literature of the field.

Bertrand, with the aid of De Wolf after 1955, has published an extensive series of papers on the mineral nutrition of *A. niger*. The importance of vanadium was reported in 1941; the role of gallium was considered in 1954; and the partial replacement of zinc by cadmium was described in 1955. These workers have reported zinc to be essential for the synthesis of nucleic acids (1961*a*) and of the amino acids, tryptophan, tyrosine, and phenylalanine (1961*b*), and also to be a specific coenzyme of aldoase (1958*a*); but it was not required for the synthesis of invertase (1958*b*). Sulphur metabolism of *A. niger* has been studied by Steinberg (1941) and by Weissman and Trelease (1955).

The uptake of soluble silica compounds by *A. niger* has been demonstrated by Holzapfel and Engel (1954) and of quartz by Holzapfel (1955). Levels of fluorine above 0.0006 per cent were reported to inhibit growth and sporulation, apparently by interfering with potassium assimilation

(Litynski, Jurkowska, and Pieniak, 1956). Acetic acid was reported by Fencl and Leopold (1957) to inhibit spore germination; CO_2 at 3 per cent had the same effect but at 0.5 per cent was stimulatory (Vakil et al., 1961). Barinova (1962) regarded CO_2 at low levels as a catalyst that accelerated penetration of nutrients into the conidia. Fleury (1948) reported thiourea to have a fungistatic effect against A. niger, and Behal and Eakin (1959) found that different purine and pyrimidine analogs interfered with particular but different stages of development. Jefferson and Sisco (1961) reported microgram quantities of various steroids to enhance growth of A. niger. Shaffer et al. (1957) added deuterium to Czapek's medium and observed that the growth rate of A. fonsecaeus was inversely proportional to the deuterium content, but that the fungus sporulated well even with 99 atom per cent of deuterium. Jerebzoff (1963) found isoleucine, but not related amino acids, to induce an endogenous rhythm in dark-grown cultures that was expressed as alternate sterile and sporulating zones. Bonner (1948) has reported on the interrelationship of temperature and humidity upon the growth of A. niger, while Miller and Anderson (1961) have demonstrated the excessive proliferation of conidiophores and intrahyphal hyphae under nearly anaerobic conditions.

Caldwell (1963) studied the effect of high partial pressures of oxygen and found A. niger to be the least sensitive of the several fungi tested. Küster and Gielessen (1958) exposed spores of A. niger to very high pressures (up to 10,000 atmospheres). Many spores were killed; cultures from treated spores had a lower chitin content; and by repeated passage, it was possible to isolate pressure-resistant spores. Küster and Theismann (1949) reported that growth from spores exposed to moderate sonic vibrations was accelerated, while that from spores exposed for longer times was retarded.

The growth of A. niger, like that of most other fungi, is inhibited by the antibiotic actidione, 100 p.p.m. in solid media being sufficient to preclude growth of this fungus (Jeffers, 1954). Plumericin ("Plumericin," 1951) is also reported to be an effective antibiotic against A. niger.

Berk (1952, 1953) has studied the biological effects of ionizing radiation of radium and polonium on A. niger and has noted that growth and sporulation become progressively abnormal as dosage increases, but removal of the source permits the fungus to recover except at levels above 1000 particles per spore. Modrone (1948) and Usami, Koike, and Taketomi (1960) have described the production of mutations resulting from exposure to nitrogen mustards and have discussed their altered metabolism and morphology.

Soil Testing

By making use of the extreme sensitivity of Aspergillus niger to mineral nutrients, selected strains may be employed successfully as assay organisms for determining mineral deficiency of soils. They were first used to detect

deficiencies in phosphorus and potassium, but with refinements in methods, their use has been extended to estimation of other elements, including a variety of trace metals. Papers on the so-called *A. niger* method of soil testing date back to the work of Kiessling and Schmidt (1932) and to that of Schlots, Smith, and Brown in the same year, soon followed by the more elaborate studies of Stock (1933) and of Niklas, Poschenrieder, and Trischler (1933). Many additional references to this method have appeared during the past 30 years, of which only a limited number can be included here. Generally speaking, the method compares quite favorably with available chemical methods and in many cases is more convenient for routine laboratory use. In most tests, results are interpreted as dry weight of fungus growth at different levels of the metal under test, but in some cases (*e.g.*, determinations of copper) a difference in pigmentation of the conidia is utilized as well (Velasco *et al.*, 1960).

Rosselet (1953) employed *A. niger* for measuring available potassium in Lowveld soils in South Africa; Eno and Reuzer (1955) measured the availability of the same element from biotite, muscovite, green sand, and microcline in Florida and concluded that the fungus was active in releasing potassium from these minerals. *A. niger* has been used for determining phosphorus in soils (Jensen, 1953; Cunha *et al.*, 1960), magnesium in rocks and soils (Toursel, 1941; M. I. C. Martínez, 1952), and magnesium in plant tissues (Gorski *et al.*, 1961). Tucker *et al.* (1953, 1955) employed *A. niger* for determining the availability of zinc in Illinois soils and found it to compare favorably with the best chemical methods. Levels of copper in soils were similarly determined by Dole (1952) and by Simonart and Huygh (1953); Henkens (1961) compared the *A. niger* method with chemical methods and found that one of the latter gave the best correlation between crop yield and copper content. Niklas and Toursel (1941) and Wetter (1954) employed the method for the detection of manganese in soil. Mulder (1948) used the *A. niger* test to estimate copper, magnesium, and more particularly molybdenum in soil and as a plant nutrient, and subsequently extended its usefulness (1954); the Mulder (M) strain of *A. niger* has, because of its sensitivity, become widely used by other investigators. The same general technique was used by Hewitt and Hallas (1951) as a test for the presence of molybdenum as a plant nutrient, and in 1960 Fernandez and Childers used the *A. niger* bioassay for measuring molybdenum deficiences in apple trees characterized by a uniform chlorosis of young leaves.

Papers on the detection of multiple trace elements include those of Devi (1954) and Stapp and Wetter (1953), as well as a series of reports by Nicholas and Fielding (1947, 1951) and Nicholas (1949, 1953) in which assay curves are reported to be satisfactory through the following ranges (in milligrams per 50 ml. of culture solution): Mg, 25 to 500; Cu, 0.05 to 2.0; Zn, 0.1 to 5.0; Fe, 0.1 to 5.0; Mn, 0.01 to 2.0; and Mo, 0.0001 to 0.02.

Fungicide Evaluation

Aspergillus niger, as represented by Thom 215–4247 (NRRL 334), has probably been used more widely in testing the efficacy of fungicides and fungus-proofing methods than any other fungus, with the possible exception of *Chaetomium globosum*. It is employed in United States Government specification tests of mildew resistance treatments of textiles, leather, paint, varnishes and lacquers, paper, and electrical equipment. In general, *Aspergillus niger* has little activity in the actual degradation of these substrates *per se*, and the reasons for its inclusion in such tests are often obscure. The frequency with which strains of *A. niger* are isolated from materials undergoing microbial damage reflects not so much the activity as the ubiquity of the species. With the exception of strains referred to as members of the *A. luchuensis* series (see Thom and Raper, 1945), members of the *A. niger* group have been found to be inactive on both cotton and wool (W. L. White, Darby, *et al.*, 1948; Reese and Downing, 1951). The truly black members of the group will, however, reduce the tensile strength of cotton strips if the mineral salts test solution is fortified with 0.5 per cent glucose (Simpson and Marsh, 1959; Gams, 1960) or even in the absence of a supplementary carbon compound, if the initial pH of the medium is adjusted to *ca.* 3.0 (Simpson and Marsh, 1964). Strains identified as *A. niger* have been used as the source of cellulase for studies by Walseth (1952) and Cayle (1962) on the optimum conditions for enzymatic hydrolysis and on the mechanism of action of this enzyme. *Aspergillus niger* is, however, tolerant to copper and provides a satisfactory superficial organism for visual testing of the evenness of deposition of a copper-containing fungicide, the amount of fungicide applied, and the effects of leaching (Marsh, Greathouse, *et al.*, 1944; Bayley and Weatherburn, 1945). *A. niger* has also been utilized in plate assays of copper-containing fungicides and in studies on the mechanism of their action by Block (1956), Vicklund, Manowitz, and Bagdon (1954), and Sijpesteijn and Janssen (1958). *Aspergillus niger* and *A. japonicus* were found to be among the predominant fungi on jute rotproofed with copper compounds or pentachlorphenol (Bhattacharyya and Bose, 1954). Jurkowska (1952) showed that *A. niger* could be adapted to growth in a medium saturated with $CuSO_4$.

Aspergillus niger is likewise one of the organisms used for testing the efficiency of various fungicides in finished paper products and in the control of slime formation in the pulping process during its manufacture (Appling and McCoy, 1945; Buckman, 1949; Appling *et al.*, 1949; G. A. Cruikshank, 1949; Barail, 1950; Malivánková, 1956; Zabel and O'Neil, 1957; Conkey and Carlson, 1960).

The ability of *A. niger* to grow on oils, paints, lacquers, resins, plastics, and synthetic fibers has been investigated extensively and is exploited in

specification testing of the fungicidal efficiency of these products. Many of these have been reported in internal government and industry reports, but see Vicklund and Manowitz (1947); Ruggieri (1950); Reese, Cravatz, and Mandels (1955); Berk, Ebert, and Teitell (1957); Shapiro (1958); Cooke (1954); Žlnay and Medrická (1951); and Flerov *et al.* (1963). *A. niger* has been used as a test organism in studies of the efficiency of some of these materials when used as waterproofing agents of electrical insulating material by Titus (1945); Berk and Teitell (1951); Teitell, Berk, and Kravitz (1955); and Al'Bitskaya and Shaposhnikova (1960). *A. niger* is known to cause extensive loss of waterproofness of cotton duck (Wade, 1947*a*) and has been used for testing the efficacy of waterproofing treatments (Wade, 1947*b*).

The mycology of the leather industry was reviewed by Musgrave (1948), and members of the *A. niger* group were listed as among the most common fungi occurring on this substrate. While it does not damage the collagen matrix of leather (Barghoorn, 1950), it has been found to cause loss of tensile strength, increased stiffness, and weakening of the grain surface of both chrome- and vegetable-tanned leathers by attacking the greases and other substances incorporated during tanning and subsequent treatment (Kanagy *et al.*, 1949; H. R. Wilson *et al.*, 1954; Mitton and Turner, 1955; Dahl and Kaplan, 1956).

Although *A. niger* does not decompose rubber hydrocarbons but grows on rubber presumably at the expense of organic admixtures (Nette *et al.*, 1959), it was one of four organisms tested by Heinisch *et al.* (1962) against fungicides used for the preservation of sheet rubber.

Aspergillus niger, commonly isolated from the glass surfaces of optical instruments (Turner *et al.*, 1946) and used in testing the efficacy of control measures (Vicklund, 1946), has failed to grow on clean optical glass in experimental trials (Richards, 1949) and is probably not significant in the etching of lenses (see *A. restrictus* and *A. glaucus* groups).

Antibiotics

A limited number of antibiotics, all of minor importance, are reported to be produced by members of the *Aspergillus niger* group. Krasilnikov and Korenyako (1945) found three of eight strains of *A. niger* to produce a substance resembling penicillin, named *aspergillin*, that was, however, active against both Gram-positive and Gram-negative bacteria. The same antibiotic, apparently, was discussed three years later by Kovalev (1948), who reported it effective against anthrax when used subcutaneously. Radetzky (1948) described *aspergin*, a wax degrading substance isolated from *A. niger*, as strongly active against skin tuberculosis of guinea pigs when injected subcutaneously. Tedeschi (1948) reported an ethyl

ether-soluble fluorescent pigment (unnamed) to possess antiprotozoic activity, particularly against flagellates. Ramon et al. (1948) found a filtrate of A. niger to inactivate tobacco mosaic virus; and Mirić and Dunegan (1953) reported a thermostable substance (unnamed) from A. niger to be toxic to Xanthomonas pruni. Akatsu (1952) and Yoshida, Onishi, and Akatsu (1952) have studied an antibiotic, produced by A. japonicus, that inhibits the growth of a white pellicle-forming yeast harmful in soy sauce manufacture. Vasudeva and Govindaswamy (1953) obtained filtrates from A. niger that inhibited the growth of Fusarium udum, the fungus causing wilt in pigeon peas.

Several papers have been published by Curtis and co-workers relating to a peptide produced by A. niger, named malformin and now characterized, that causes thickening and tumescence of stems and petioles of bean seedlings and root curvature in maize (R. W. Curtis, 1958, 1961; Postlethwait and Curtis, 1959; Nobutaka and Curtis, 1961; and Marumo and Curtis, 1962).

L. P. Miller (1962) isolated from A. niger spores a substance which, at 5 mg. per ml. in a favorable nutrient solution, precluded spore germination in several fungi but was stimulatory at lower concentrations.

Biosynthesis and Biological Conversions

Synthesis of the following vitamins has been reported: biotin (Eakin, 1942); thiamine (Rossi and Jacoli, 1949); and riboflavine (Vokral, 1958; Zajic and Kuehn, 1962).

Nickerson and Thimann (1941) record that species of Zygosaccharomyces showed greatly increased conjugation when grown in the presence of A. niger or when an alcohol extract of the mold was added to the yeast cultures.

J. L. Yuill (1948) reported a strain of A. niger used for citric acid production to produce i-erythritol as well. He subsequently (1952) reported a culture of the same species, but not a good citric strain, to produce large amounts of mycodextran, a product previously reported by Dox in 1915. Glucosan was reported by Van Sumere and Shu (1957) to be synthesized by A. niger from pentoses. Pinck and Allison (1944) reported A. niger to be one of several fungi producing lignin-like complexes in limited amounts.

Sastry and Sarma (1957) report increased yields of ascorbic acid from A. niger by the addition of increasing amounts of the precursor, glucuronic acid. Homogentisic acid in 15.7 per cent theoretical yield was obtained by Kluyver and Van Zijp (1951) from phenylacetic acid by a replacement culture technique.

Aspergillus niger has been used effectively in a number of biological conversions. Challenger, Lisle, and Dransfield, in their extensive studies

on biological methylation of arsenic and selenium compounds, have shown (1954) that *A. niger* is capable of effecting such transformations. Prema and Bhattacharyya (1962*a*) demonstrated the transformation of terpenes, specifically the hydroxylation of α-pinene, and in a second paper (1962*b*), extended their studies to meno- and sesquiterpenes. A number of papers relating to the conversion of steroids have been published. Hydroxylation of steroids, primarily progesterone, at the 11α position has been shown to be a common conversion in strains of *A. niger*, *A. awamori*, *A. usamii* and its mutant, and *A. saitoi* (Fried, Thoma, Gerke, *et al.*, 1952; Iizuka *et al.*, 1958; Hanč *et al.*, 1959; Shirasaka *et al.*, 1960; and Timofeeva *et al.*, 1961). 6β,11α-Dihydroxyprogesterone has been reported as a secondary product during this conversion by Fried and Iizuka and their colleagues. Progesterone and its derivatives have been converted by *A. niger* to their equivalent corticosterones through hydroxylation at the 21 position (Zaffaroni *et al.*, 1955; Wix, Weisz, and Bodansky, 1957; Weisenborn and Laskin, 1962). An ultraviolet-induced mutant of the strain used by Wix was found to be lacking the 21-hydrogenase enzyme (Wix, Natonek, and Kovács, 1959). Traces of progesterone were obtained from pregnenolone by Perlman (1952).

Aspergillus niger has been shown to synthesize considerable amounts of sterols with the use of fatty acids, in part derived from autolysis of the mycelium, as the raw material (Vanghelovici and Felicia, 1941). A tetraethanoid sterol, 14-dehydroergosterol, occurring as a minor component in admixture with ergosterol, was isolated from the nonsaponifiable matter of a strain of *A. niger* by Barton and Bruun (1951).

Pigments

The early work of Linossier (1910), Quilico (1933), and Quilico and Di Capua (1933) on aspergilline, the readily soluble black pigment found in the conidia of *A. niger*, has been noted elsewhere (Chapter III). The name *aspergillin*, misapplied to several antibiotics (Tobie, 1946), should be reserved for this pigment. As seen in growing colonies of *A. niger*, pigment production has been shown to be depressed or chemically changed by the action of an adjacent colony of a degenerate, yeastlike *Mucor* (Verdcourt, 1957). Various yellow pigments have been extracted from the mycelia of members of the *A. niger* group. Oxford (1950) obtained an unidentified water-soluble yellow or orange pigment from 10 of 100 strains of black aspergilli in his collection. Reid (1950*b*) found two principal pigments in a variety of black strains (including the type culture of *A. foetidus*). Neither was identified, but they appeared not to be flavins. One, extractable with chloroform, gave a pale yellow fluorescence under ultraviolet light; the other, insoluble in chloroform but extractable with butyl alcohol,

fluoresced deep blue. *Asperxanthone*, $C_{16}H_{14}O_5$, was isolated from the mycelium of *Aspergillus niger* by Lund *et al.* (1953). Astill and Roberts (1953) and Davies, King, and Roberts (1954) reported the isolation and structure of *flaviolin*, a napthaquinone pigment, from *A. citricus* (Wehmer) Moss. (here considered synonymous with *A. foetidus*). Zajic and Kuehn (1962) reported the synthesis of three pigments by *A. niger:* (1) riboflavine; (2) an acidic carotenoid named *asperxanthin*, which appeared to be related to neurosporoxanthin; and (3) a pterinoid compound. *Fonsecin*, a yellow naphthapyrone pigment, was isolated in high yield from a yellow *Cladosarum*-like ultraviolet mutant of *Aspergillus fonsecaeus* by Galmarini *et al.* (1962).

Pathogenicity and Toxicity

See Chapter VII.