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Methods Used by Dr. R. K. Benjamin, and Other Mycologists, to Isolate Zygomycetes

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THE METHODS USED BY DR. R. K. BENJAMIN, AND OTHER MYCOLOGISTS, TO ISOLATE ZYGOMYCETES

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ABSTRACT

The methods that Dr. Richard K. Benjamin used to isolate Zygomycetes are discussed. These processes involved the following five steps: (1) collection, (2) plating, (3) isolation, (4) culture, and (5) maintenance. Additional methods, materials and modifications used to isolate Zygomycetes are summarized. The author considers the flattening of the aerial hyphae onto the substrate of the faster- and higher-growing Mucorales for several consecutive days to be the critical step in isolating species of *Coemansia*, *Piptocephalis*, *Syncephalis*, and Dimargaritales. The methods used by other scholars to isolate, culture, and study many taxa in Zygomycetes also are discussed.

Key words: *Dimargaris*, *Dispira*, fungi, Kickxellales, mycoparasite, *Tieghemiomyces*, Zoopagales.

INTRODUCTION

When I arrived at the Garden in February 1969 to start my Ph.D. studies, I became the third student of Dr. Richard K. Benjamin. At that time, he studied both Laboulbeniales and Zygomycetes. His methods used for the study of Laboulbeniales were discussed previously (Benjamin 1971, 1986, 1993). Therefore, this paper will be confined to Zygomycetes.

In February 1969, there were approximately 1900 fungi accessioned into the mycological culture collection of Rancho Santa Ana Botanic Garden (RSA). The general fungal collection contained species of *Chaetomium* Kunze, Eurotiales G.W.Martin ex Benny & Kimbrough, Onygenales Cif. ex Benny & Kimbrough (Ascomycetes), a few isolates of species of *Absidia*, *Circinella*, *Mucor*, *Rhizopus*, and other Mucorales (Zygomycetes), and some other fungi used in teaching the laboratory component for the mycology class. There was never a large collection of *Mucor* because the species were hard to identify, and the members of the genus were so numerous in nature that, if they were all isolated, the RSA culture collection would have been two or three times larger.

A majority of Zygomycetes in the RSA culture collection was separated into four collections: (1) species of *Coemansia* and other Kickxellales; (2) Dimargaritales (most species of *Dimargaris*, and all species of *Dispira* and *Tieghemiomyces*); (3) *Kuzuhaea*, species of *Piptocephalis*, and a few species of *Syncephalis*; and (4) Thamnidiaceae s.l. (Mucorales). These collections were all well represented in the RSA mycological herbarium, as was an extensive collection of Onygenales (Benjamin 1956; cited in Currah 1985) and Myxomycetes (Benjamin 1949).

The thamnidiaceous Mucorales in the RSA mycological culture collection were studied by the author (Benny 1973) for his Ph.D. dissertation, and later the work was formally published (Benny 1992, 1995*a*,*b*; Benny and Benjamin 1975, 1976, 1991, 1993; Benny et al. 1985; Benny and Schipper 1992). Benjamin (1959, 1961, 1963, 1965) published several papers on Dimargaritales, but unidentified cultures of six isolates of *Dimargaris* and two isolates of *Dispira* remain to be studied. Benjamin (1959, 1966) reviewed *Piptocephalis*, describing one species, and renaming two other taxa. He made drawings of most of the *Piptocephalis* species that he considered valid and these were included in a study of the genus (Gräfenhan 1998). A *Piptocephalis*-like monotypic genus, *Kuzuhaea* (*K. moniliformis* R.K.Benjamin [1985*a*]), also was described and illustrated. Several species of *Syncephalis* are in culture but this genus was never monographed. Benjamin (1959, 1966, 1979) illustrated several species of *Syncephalis* and later described *S. hypogena* R.K.Benjamin (Benjamin 1985*b*). The initial eight genera (*Coemansia*, *Dipsacomyces*, *Kickxella*, *Linderina*, *Martensiomyces*, *Martensella*, *Spiromyces*, and *Spirodactylon*) of Kickxellales were discussed and illustrated by Benjamin (1958, 1959, 1961, 1963); an additional three taxa (*Mycoëmilia*, *Myconymphaea*, *Ramicandelaber*) were described recently (Kurihara et al. 2001, 2004; Ogawa et al. 2001). A large culture collection of over 160 isolates of *Coemansia* spp. should be studied, and at least 80 other isolates not in culture exist only as herbarium specimens that also need to be examined. Several undescribed species are probably present in this *Coemansia* collection. Benjamin (1958) described one new species, *C. mojavensis* R.K.Benjamin, and redescribed *C. aciculifera* Linder. Benjamin (1960, 1962, 1978, 1985*a*; Benjamin and Mehrotra 1963) described other Zygomycetes during this period.

Usually, Dr. Benjamin made at least one herbarium specimen of each isolate deposited in the RSA fungal culture collection. These specimens were all glued into boxes and, therefore, they are in excellent condition for study. Cultures as well as herbarium specimens are available for the majority of the fungi mentioned above. Not many cultures of *Syncephalis* spp., however, are available in the RSA culture collection. The majority of *Syncephalis* collections are available only as herbarium specimens.

Many of the taxa that are discussed here were described before Dick Benjamin studied Zygomycetes (ca. 1955–1985). Often these fungi were elevated in rank, for instance from species to genus or from family to order. Few new taxa have been described in the past two decades since he published his last single-authored papers on Zygomycetes (Benjamin 1985*a*,*b*).

The taxonomic scheme of Zygomycetes that was recognized in 1969, when I became Dr. Benjamin's graduate student, included three orders, Entomophthorales, Mucorales, and

Zoopagales (Ainsworth et al. 1973). At that time, the majority of the fungi studied by Dr. Benjamin were included in Mucorales (Hesseltine 1955; Benjamin 1959; Hesseltine and Ellis 1973). Benjamin (1979) added four orders, Dimargaritales, Endogonales, Harpellales, and Kickxellales, to Zygomycetes. Most mycologists, however, maintained Harpellales in Trichomycetes (Lichtwardt 1986; Benny 2001). Recent molecular studies indicate that Harpellales do belong in Zygomycetes, whereas Amoebidiales and Eccrinales should be excluded (Tanabe et al. 2005). Asellariales probably are related, based on septal morphology; no molecular studies have been published. Morton and Benny (1990) described Glomerales for the arbuscular mycorrhizal fungi, then in Endogonales. Finally, Cavalier-Smith (1998) described Basidiobolales, Geosiphonales, and Mortierellales (Benny et al. 2001). Schüssler et al. (2001) transferred Geosiphonales and Glomerales to Glomeromycota. This left eight orders—Basidiobolales, Dimargaritales, Endogonales, Entomophthorales, Kickxellales, Mortierellales, Mucorales, and Zoopagales—in Zygomycetes.

Dimargaritales, Endogonales, Kickxellales, and Mortierellales were originally families of Mucorales (Hesseltine and Ellis 1973). Benjamin (1979) transferred two mucoralean families, Helicocephalidaceae and Piptocephalidaceae, to Zoopagales. Sigmoideomycetaceae (Benny et al. 1992) were originally included in Mucorales but later transferred to Zoopagales based on morphological and molecular evidence (Chien 2000; Tanabe et al. 2000). The synonymy of most traditionally recognized mucoralean families (Alexopoulos et al. 1996) with Mucoraceae is based on the phylogenetic studies of O'Donnell et al. (2001). Umbelopsidaceae (Mucorales) was proposed recently for the single genus *Umbelopsis* (Meyer and Gams 2003). The current classification of Zygomycetes recognized here is presented in Table I. Current literature to the taxa in Table I can be found in Kirk et al. (2001).

All aforementioned fungi were isolated using techniques that will be discussed here; other methods for isolating fungi from dung and soil are discussed by Bills et al. (2004) and Krug et al. (2004). Benjamin never used antibiotics, although later the author found their use could facilitate the isolation of some Zygomycetes. The culture media and techniques developed by other scholars for Zygomycetes (Endogonales, saprobic and predaceous Entomophthorales, Mortierellales, predaceous Zoopagales) are also surveyed.

Table 1. The current classification of the Zygomycetes.

Zygomycota R.H.Whittaker, nomen nudum, without a Latin diagnosis
ZYGOMYCETES G.Winter
BASIDIOBOLALES Cavalier-Smiths
  Basidiobolaceae Claussen
    Basidiobolus Eidam
DIMARGARITALES R.K.Benjamin
  Dimargaritaceae R.K.Benjamin
    Dimargaris van Tieghem
    Dispira van Tieghem
    Spinalia Vuillemin
    Tieghemiomyces R.K.Benjamin
ENDOGONALES R.K.Benjamin
  Endogonaceae Paoletti
    Endogone Link: Fr.
    Pteridiospora C.-G.Wu & Lin
    Sclerogone Warcup
    Youngiomyces Y.J.Yao et al.
Genus of unknown affinities:
    Densospora P.A.McGee
ENTOMOPHTHORALES Schröter
  Ancylistaceae Fischer
    Ancylistes Pfitzer
    Conidiobolus Brefeld
    Macrobiophthora Reukauf
  Completoriaceae Humber
    Completoria Lodhe
  Entomophthoraceae Warming
    Batkoa Humber
    Entomophaga Batko
    Entomophthora Fresenius
    Erynia (Batko) Remaudière & Hennebert
    Eryniopsis (Batko) Humber
    Furia Humber
    Massospora Peck
    Orthomyces Steinkraus, Humber & Oliver
    Pandora Humber
    Strongwellsea Batko & Weiser
    Tarichium Cohn
    Zoophthora Batko
  Meristacraceae Humber
    Ballocephala Drechsler
    Meristacrum Drechsler
    Zygnemomyces Miura
HARPELLALES Lichtwardt & Manier
  Harpellaceae Léger & Duboscq
    Carouxella Manier Riux & Whisler ex Manier & Lichtwardt
    Harpella Léger & Duboscq
    Harpellomyces Lichtwardt & Moss
    Stachylina Léger & Gauthier
    Stachylinoides Ferrington, Lichtwardt & López-Lastra
  Legeriomycetaceae Pouzar
    Allantomyces Williams & Lichtwardt
    Austrosmittium Lichtwardt & Williams
    Baetimyces L.G.Valle & Santamaria
    Barbatospora White, Siri & Lichtwardt
    Bojamyces Longcore
    Capniomyces Peterson & Lichtwardt
    Caudomyces Lichtwardt, Kobayasi & Indoh
    Coleopteromyces Ferrington, Lichtwardt & López-Lastra
    Ejectosporus Peterson, Lichtwardt & Williams
    Ephemerellomyces White & Lichtwardt
    Furculomyces Lichtwardt & Williams
    Gauthieromyces Lichtwardt
    Genistelloides Peterson, Lichtwardt & Horn

ZYGOMYCETES GROWING ON DUNG AND SOIL

The selection and preservation of Zygomycetes (Dimargaritales, Kickxellales, Mucorales, Zoopagales [Piptocephalidaceae, Sigmoideomycetaceae]) from the field is done in several steps: (1) collection, (2) plating, (3) isolation, (4) culture, and (5) maintenance and preservation. These steps are discussed in detail in the following text.

Collection

Dung.—The collection of dung is an important first step in the isolation of many species of Dimargaritales, Kickxellales, *Piptocephalis*, *Syncephalis*, the sporangiolar Mucorales, and possibly other Zygomycetes as well. In the southern California desert, dung is found where rodents dig for food, along their runs or trails, or in places where they congregate; it often will

Table 1. Continued.

Genistellospora Lichtwardt
Glotzia Gauthier ex Manier & Lichtwardt
Graminella Léger & Gauthier
Graminelloides Lichtwardt
Lancisporomyces Santamaria
Legeriodes White
Legeriomyces Pouzar
Legeriosimilis Williams, Lichtwardt, White & Misra
Orphella Léger & Gauthier
Pennella Manier ex Manier
Plecopteromyces Lichtwardt, Ferrington & López-Lastra
Pseudoharpella Ferrington, White & Lichtwardt
Pteromaktron Whisler
Simuliomyces Lichtwardt
Smittium Poisson
Spartiella Tuzet & Manier ex Manier
Stipella Léger & Gauthier
Tectimyces L.G.Valle & Santamaria
Trichozygospora Lichtwardt
Zygopolaris Moss, Lichtwardt & Manier
KICKXELLALES R.K.Benjamin
Kickxellaceae Linder
Coemansia van Tieghem & Le Monnier
Dipsacomyces R.K.Benjamin
Kickxella Coemans
Linderina Raper & Fennell
Martensella Coemans
Martensiomyces Meyer
Mycoëmilia Kurihara et al.
Myconymphaea Kurihara et al.
Ramicandelaber Ogawa et al.
Spirodactylon R.K.Benjamin
Spiromyces R.K.Benjamin
MORTIERELLALES Cavalier-Smith
Aquamortierella Embree & Indoh
Dissophora Thaxter
Lobosporangium M.Blackwell & Benny
Modicella Kanouse
Mortierella Coemans
MUCORALES Schröter
Mucoraceae Dumortier
Absidia van Tieghem
Actinomucor Schostakowitsch
Amylomyces Calmette
Apophysomyces Misra
Backusella Hesseltine & J.J.Ellis
Benjaminiella von Arx
Blakeslea Thaxter
Chaetocladium Fresenius
Chlamydoabsidia Hesseltine & J.J.Ellis
Choanephora Currey
Circinella van Tieghem & Le Monnier
Circinomucor von Arx
Cokeromyces Shanor
Cunninghamella Matruchot
Dichotomocladium Benny & R.K.Benjamin
Dicranophora Schröter
Ellisomyces Benny & R.K.Benjamin
Fennellomyces Benny & R.K.Benjamin
Gilbertella Hesseltine
Gongronella Ribaldi
Halteromyces Shipton & Schipper
Helicostylum Corda
Hesseltinella Upadhyay

Table 1. Continued.

Hyphomucor Schipper & Lunn
Kirkomyces Benny
Mucor Micheli ex L.: Fr.
Mycocladus Beauverie
Mycotypha Fenner
Parasitella Bainier
Phascolomyces Boedijn
Pirella Bainier
Rhizomucor (Lucet & Cost.) Wehmer ex Vuillemin
Phycomyces Kunze: Fr.
Pilaira van Tieghem
Pilobolus Tode: Fr.
Poitrasia P.M.Kirk
Protomycocladus Schipper & Samson
Radiomyces Embree
Rhizopodopsis Boedijn
Rhizopus Ehrenberg: Fr.
Saksenaea Saksena
Spinellus van Tieghem
Sporodiniella Boedijn
Syncephalastrum Schröter
Syzygites Ehrenberg: Fr.
Thamnidium Link
Thamnostylum von Arx & Upadhyay
Thermomucor Subrahamanyam et al.
Utharomyces Boedijn
Zychaea Benny & R.K.Benjamin
Zygorhynchus Vuillemin
Umbelopsidaceae Meyer & W.Gams
Umbelopsis Amos & Barnett
ZOOPAGALES R.K.Benjamin
Cochlonemataceae Duddington
Amoebophilus Dangeard
Aplectosoma Drechsler
Bdellospora Drechsler
Cochlonema Drechsler
Endocochlus Drechsler
Euryancale Drechsler
Helicocephalidaceae Boedijn
Brachymyces Barron
Helicocephalum Thaxter
Rhopalomyces Corda
Piptocephalidaceae Schröter
Kuzuhaea R.K.Benjamin
Piptocephalis de Bary
Syncephalis van Tieghem & Le Monnier
Sigmoideomycetaceae Benny et al.
Reticulocephalis Benny et al.
Sigmoideomyces Thaxter
Thamnocephalis Blakeslee
Zoopagaceae Drechsler emend. Duddington
Acaulopage Drechsler
Cystopage Drechsler
Stylopage Drechsler
Zoopage Drechsler
Zoopagus Sommerstorff
Probable member of the Zygomycetes based on septal morphology:
ASELLARIALES Manier ex Manier & Lichtwardt
Asellariaceae Manier ex Manier & Lichtwardt
Asellaria Poisson
Orchesellaria Manier ex Manier & Lichtwardt
Genus of unknown affinity:
Baltomyces Cafaro

accumulate in a low place if animals have used a higher trail for a long period of time. Benjamin never liked to collect the dung of herbivores because it often was a source of mites; it also was not very productive for the types of Zygomycetes in which he was interested. Over a period of many years it was noted that Kickxellales, and the other Zygomycetes listed above, were most often collected on the dung of mice and omnivorous rats (Fig. 1). Some rats in California that eat only vegetation (pack rats) are a better source for Ascomycetes and Deuteromycetes than for Zygomycetes. However, as many types of dung as can be found should be collected and evaluated for diversity; some species of fungi may be restricted to a certain animal, geographic region, or time of year. At an institution with a mammalogist or herpetologist, it may be possible to get dung from animals that they have collected in live traps. Zoologists might also know where to collect dung, because they will know where to find rodents, bats, rabbits, deer, and other animals.

Dung should be collected using forceps and rubber gloves. I never used gloves myself, but a Hanta virus was reported in the southwestern United States in dung and urine of some of the same rodent species that I often collected in southern California. Also, watch for insects, snakes, other animals, or plants before putting your hands or feet where they can be bitten or impaled. The dung that Dr. Benjamin collected was usually dry after being in the air a short time. If the collections are to be processed immediately, then you can put them in a tightly closed container, such as a zip-lock bag or vial, but for long-term storage it is best to put the dung in paper bags. Mark each bag with good collecting data, such as place, collector, date, animal source if known, and GPS (Global Positioning System) coordinates, if possible. Dr. Benjamin marked each bag with a collection number preceded by a ''D'' (for dung) or an ''S'' (for soil). These collecting numbers and data were then written in a collecting book, and then included in the label information (Fig. 2). Fungi to be included in the RSA fungal culture collection were later assigned a RSA number.

Soil.—You can collect soil using a small trowel or spoon and then place it in a sterile plastic collecting bag or test tube. Include soil characteristics (soil type, water or moisture level, pH) and ecological information, if possible. Mark the container with collecting data as discussed above. Substrates collected for isolation of Zygomycetes will remain viable longer if refrigerated.

Plating

Dung.—Do not plate out too much dung at one time because it will be hard to observe more than about 20 plates when the fungi start to grow. For dung from small animals such as rats and mice, a 25 × 100 mm plastic Petri dish makes a good moist chamber (Krug 2004). You should put one or two layers of filter paper or paper towel, cut to shape, in the bottom of the dish; wet the paper with distilled water; pour off the excess water. Moist chambers should be moist but not contain standing water. Too much water promotes excessive bacterial growth, which inhibits fungi. Van Tieghem (van Tieghem and Le Monnier 1873; van Tieghem 1875, 1878) incubated dung, especially horse dung, in moist chambers with standing water and species of *Syncephalis* often would sporulate on the water surface and the wall of the incubation vessel.

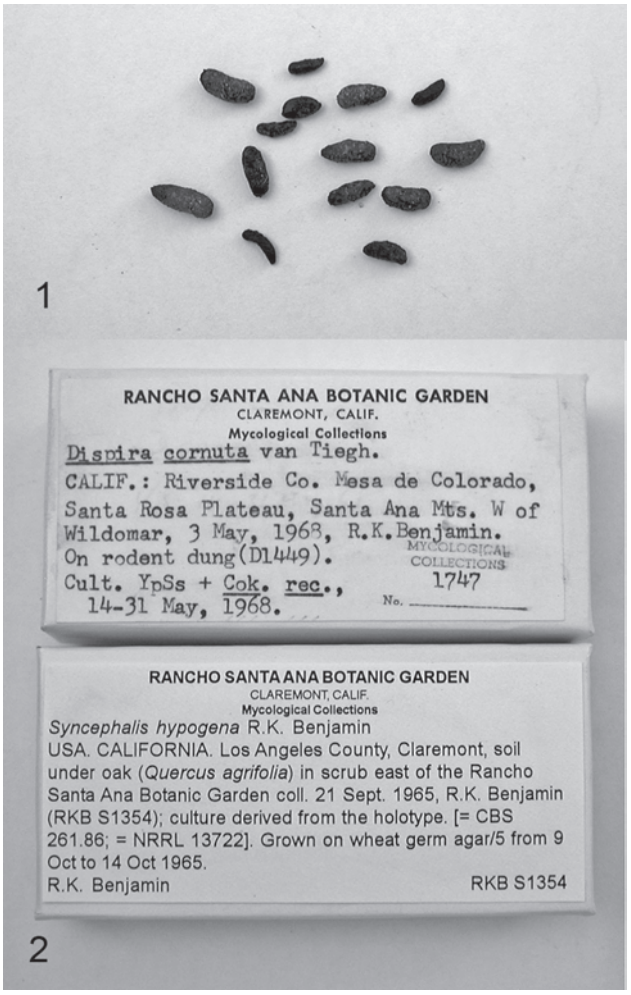

Fig. 1, 2. Rodent dung and herbarium labels.—1. Rodent dung showing relative size of mouse (small) and rat (larger) dung.—2. Labels on two herbarium specimens showing a collection from dung (D1449) and soil (S1354). (Fig. 1, 2, ×0.8).

Mouse dung should be placed about 1 cm from the outer edge of the dish, and pieces of dung should be separated from each other by at least 1 cm in this outer ring, which can contain seven or eight pieces of dung; three pieces of dung then can be placed 1 cm apart in the center of the plate. Pieces of rat dung should be placed ca. 1.5 cm from one another and 1.5 cm from the wall of the Petri dish; one dung pellet might be placed in the center if space is available (Fig. 3). You should be able to observe the first fungi, probably some species of *Mucor*, in 3–5 days. Other common Zygomycetes that can often be observed in these cultures are species of *Absidia*, *Cunninghamella* Matruchot, and possibly *Syncephalastrum racemosum* Cohn ex J.Schröter, and *Thamnostylum piriforme* (Bainier) von Arx & Upadhyay during the warmer parts of the year. During the cooler months you might encounter species of *Phycomyces*, *Thamnidium elegans* Link, *Chaetocladium brefeldii* van Tieghem & Le Monnier, and *Pirella circinans* Bainier.

If Zygomycetes are present, you can isolate them by transferring mature spores from merosporangia or sporangia on sporangiophores growing from the dung to Petri dishes containing the appropriate culture media (see Appendix I) or you can push these fungi over with a sterile inoculating tool.

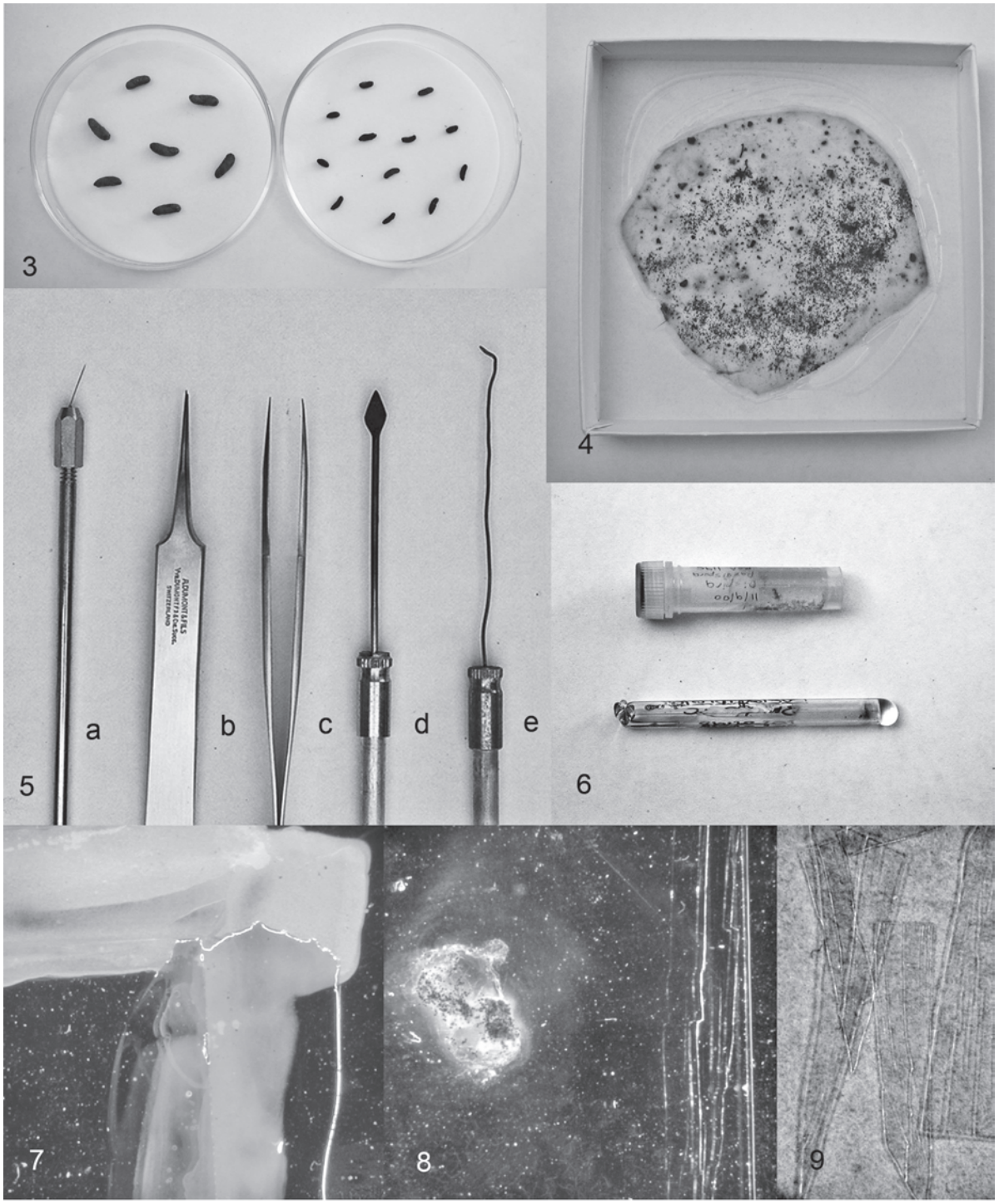

Fig. 3–9. Illustration of selected techniques used in the isolation and culture of Zygomycetes.—3. Recommended distribution of rat (left) and mouse (right) dung in Petri dishes 100 mm in diameter.—4. Herbarium specimen of a portion of the agar from a Petri dish 100 mm in diameter showing the distribution of sandy soil.—5. Inoculating tools and forceps used to isolate and transfer Zygomycetes. Handles equipped with a stainless steel minuten insect pin (*a*), a full spear point for cutting agar (*d*), and a stiff nichrome wire for transferring spore-bearing aerial hyphae (*e*). Number 5 stainless steel watchmakers forceps as viewed from above (*b*) and laterally (*c*).—6. A cryovial (above) and lyophil (below) used for culture storage.—7. The upper right corner of an 18 mm^2 coverglass sealed with paraffin and partially sealed with clear fingernail polish.—8. An unsealed 18 mm^2 coverglass elevated with a glass shard (right edge).—9. Several glass shards made from a 18 mm^2 coverglass. (Fig. 3, ×0.4; Fig. 4, 6, ×0.8; Fig. 5, ×0.9; Fig. 7, ×6.5; Fig. 8, ×5; Fig. 9, ×8).

This is a very important part of isolating any of the slower growing Zygomycetes, or the parasites, because they are often not as tall or fast growing as those that first appear. Do this several days in a row after determining whether you want to isolate any members of Mucorales. The parasites, and other "merosporangiferous Mucorales" (Benjamin 1958, 1959, 1961, 1963, 1965, 1966), usually do not appear as frequently as *Mucor* spp. and the other Mucorales. Again, you should find many species of *Mucor*, and one or more species of the following mucoralean genera: *Absidia*, *Cunninghamella*, *Thamnostylum*, *Syncephalastrum*, and possibly *Rhizopus*. I recommend isolating these early appearing fungi to get the practice that you will need when the "merosporangiferous Mucorales" start to appear. These fungi are now classified in Dimargaritales, Kickxellales, Mucorales (*Syncephalastrum*), and Zoopagales (*Piptocephalis*, *Syncephalis*) (Benjamin 1979). These Dimargaritales, Kickxellales, Mucorales, and Zoopagales should appear about 3–7 or even 10 days after the first Mucorales. From my experience the most common of the merosporangiferous Mucorales will be species of *Coemansia*, *Piptocephalis*, and *Syncephalis*.

Soil.—Benjamin (1985*b*) recommended sprinkling soil over the surface of WgB agar (Fig. 4); this is the method he used to isolate *Syncephalis hypogena* (Appendix 1). I have used CH and Wg5 agars for the same purpose. Hunter and Butler (1975) sprinkled soil on PDA plates, whereas Ho (2000, 2001, 2002) placed 2–3 mg of soil on CM agar plates. Indoh (1962) put 2–3 mg of soil in a Petri dish, poured cool carrot agar in the dish, and then swirled the plates to evenly disperse the soil; no formula was given for carrot agar but perhaps other media (CA, CaA, CJA, PCA) could be substituted.

Isolation

Zygomycetes transferred to primary isolation plates from moist chambers and Petri dishes containing nutrient media.—The isolation of species of *Coemansia*, *Piptocephalis*, or any other of the merosporangerous taxa from dung, soil, or other substrates, requires the use of either watchmakers' forceps, like those used by electron microscopists, or very fine needles, such as stainless steel minuten insect pins, full spears, and inoculating wire (Fig. 5), but some medical or dental probes might also work well. The fine pins can be used to transfer spores from wet structures, while the forceps are best for either wet or dry sporulating heads. Inoculating tools are sterilized using a Bunsen burner or alcohol lamp and they are then cooled in the sterile agar to be inoculated (minuten insect pins) or in 95% ethanol in a Copeland jar or other secure container (forceps) and then rapidly passed through the flame to burn off the excess alcohol. The sterile forceps are then used to transfer fungi to fresh media.

You can use almost any culture medium for the Zygomycetes, but I have found that a clear medium that is high in nutrients works best for the isolation of these fungi. I usually use MEYE medium for this purpose, if the ingredients are available (Appendix 1). Emerson's YpSs agar can also be used as a primary isolation medium. Germinating spores and growth of hyphae are readily observed on MEYE using a dissecting microscope if the light comes from below the stage.

Transfer of spores of *Coemansia* spp., which are released in a spore droplet when mature, can be done with either the small needle or the forceps. It is often easiest to mark the bottom of the agar plate with five or six small circles about 1 cm from the edge of the dish. This circumscribes the possible points of inoculation and makes them easier to find when observing them with the dissecting microscope, especially since only the tips of the inoculating tool should touch the agar. If possible, I would suggest that agar plates be incubated at 18°C. Some of these fungi, especially species of *Piptocephalis*, will only grow below 20°C. Some *Coemansia* spp. may also have this requirement; those species that can grow above 20°C will grow a little slower at the lower temperature, but their morphology will not be altered.

Similarly, isolation of Kickxellales and other merosporangiferous Mucorales will be facilitated if early-appearing, tall members of Mucorales are pressed down each day. *Coemansia* spp. will not be found in every collection of dung that you bring into the laboratory, and you will not be able to isolate every *Coemansia* that appears. Some isolates may be contaminated with other fungi that can be pushed over using a sterile inoculating tool to expose the fungi of interest.

Kurihara et al. (2001, 2004) isolated new genera of Kickxellales from moist soil sprinkled on the surface of MA (Ogawa et al. 2001), incubated in a moist chamber that is baited with sterile dried edible shrimp or reared mealworms similar to the procedure of Degawa and Tokumasu (1998*b*), soil enriched with a 5% aqueous solution of one of the following: peptone, soytone, or yeast extract (Kurihara 2002), and the soil-plate method of Warcup (1950) using 10% carrot extract agar (CaA); cultures were transferred to ShA (Degawa and Tokumasu 1998*a*,*b*) or the isolates were grown on one-half strength MEYE/2 (based on Benjamin 1959).

Benjamin (1985*a*) isolated *Syncephalis hypogena* by touching the tip of a sterile stainless steel minuten insect pin to a spore head and then transferring the spores to several premarked spots on a plate of YpSs agar (Appendix 1). A small piece of host inoculum was added and those colonies that were not contaminated were transferred to fresh YpSs plates. Ho (2000, 2001, 2002) transferred host + *Syncephalis* sp. together after 1 week to fresh CM plates. When the *Syncephalis* sporangiophores matured, spores were transferred to premarked spots on new CM plates. Spores of the host were added 24 hr later.

Isolation of Piptocephalis *and* Syncephalis *using yeast cells* of Cokeromyces recurvatus *Poitras*.—Spores of a species of *Piptocephalis* or *Syncephalis* are transferred to Petri dishes containing MEYE agar. After germination of the parasite spores (2–3 days) the *C. recurvatus* yeast cells are placed on the germlings. Jeffries and Kirk (1976) grew *C. recurvatus* in 200 mL of PGB liquid medium at 25°C in a 250 mL Erlenmeyer flask, and after 1 or 2 days yeast cells were present in the bottom of the flask. The parasites should sporulate in 1 week or less.

Isolation of Coemansia, Piptocephalis *and* Syncephalis *using benomyl*.—An isolation procedure for Zygomycota from soil was tried recently that appears to be excellent for *Coemansia*, the mycoparasites *Piptocephalis* and *Syncephalis*, and members of Mucorales and Mortierellales. I isolated these Zygomycetes from soil using CH3 and Wg5 agars (Appendix I) supplemented, after autoclaving and cooling, with sterile chlortetracycline hydrochloride (50 p.p.m.) and streptomycin sulfate (100 p.p.m.) to restrict bacteria, and benomyl (10 p.p.m.) to

prevent the growth of many undersired fungi. Wg5 agar was the preferred medium because it allows observation of the agar surface for *Syncephalis* sporangiophores and is effective in inducing sporulation of *Coemansia*. Wg5 agar supplemented with benomyl at concentrations of up to 20 p.p.m. could be added without inhibiting most Zygomycetes, except a few species of *Mortierella* (Strauss et al. 2000). Benomyl (20 p.p.m.) and the antibiotics chloramphenicol (200 p.p.m.) or novobiocin (100 p.p.m.) can be added to the culture medium prior to autoclaving (Edward E. Butler pers. comm. 1993; Strauss et al. 2000).

Culture

The majority of Zygomycetes in Mucorales, Dimargaritales, Kickxellales, and Piptocephalidaceae grow well, either alone or with the appropriate host, on any number of culture media. Dr. Benjamin often used MEYE agar and Emerson's YpSs agar for routine culture (Appendix 1). *Spiromyces minutus* R.K.Benjamin and most host-free Dimargaritaceae can be grown on a medium containing glycerol (YGCH).

The zygomycetous obligate biotrophic mycoparasites in Dimargaritaceae (Dimargaritales) and Piptocephalidaceae (Zoopagales) require a host (Gams et al. 2004). Dr. Benjamin usually used *Cokeromyces recurvatus*, which is low-growing and dark, but some species require other hosts, including *Umbelopsis ramannianus* (A.Møller) W.Gams, a species of *Mortierella* Coemans, or a *Chaetomium* sp. for two species of Dimargaritaceae (*Dispira simplex* R.K.Benjamin [Benjamin 1961] and *D. implicata* Misra & Lata [Misra and Lata 1979]). Dr. Benjamin always kept a culture of *C. recurvatus* growing and would transfer it to a fresh plate of YpSs agar a few days before he looked through moist chambers just in case a *Piptocephalis* sp. or a member of the Dimargaritaceae might be present. The transferred spores of the parasite were stabbed into the edge of the new *C. recurvatus* colony, and the parasite often started to appear after a few days. One of the best culture media for Dimargaritales, especially *Dimargaris* spp., is V8 (Appendix 1). *Syncephalis* spp., however, often only sporulate well on the original host and, therefore, a *Mucor* sp. or other natural host needs to be transferred with the *Syncephalis* sp.

Bawcutt (1983) grew *Syncephalis leadbeateri* Bawcutt on CSLA or PCA agar (Appendix 1). Hunter and Butler (1975) maintained *S. californica* on PDA in a cycle of 12 hr dark and 12 hr light at 24°C. Hunter and Butler (1975) inoculated plates of PDA with *Rhizopus oryzae* Went. & Prinsen Geerl. and after 1 or 2 days sprinkled 0.1 g of orchard soil on these plates. *Syncephalis californica* could be detected as a result of the multilobed vesicles that are produced by the mycoparasite on the *R. oryzae* hyphae. When grown on Wg5 the multilobed vesicles were the site of sporangiophore formation in aging cultures. Sporangiophores also form when a small piece of agar bearing the infected host was transferred to WA (Hunter et al. 1977).

Maintenance

Cultures should be transferred every 6–12 mo and then stored in the refrigerator. If mite infestations occur, cultures can be stored in test tubes with the lid sealed with parafilm or another mechanical barrier, or under oil or in sterile water. Several procedures, including the use of miticides, were discussed by Smith and Onions (1983). Guo and Michaelides (1998) stored cultures of *Mucor piriformis* A.Fischer in sterile distilled water kept at 3–5°C (Boesewinkel 1976). *Coemansia* spp. can be lyophilized, frozen in 25–50% glycerol in a cryovial (Fig. 6), or stored under nitrogen or even frozen at −185°C if the facilities are available. Berny and Hennebert (1991) recommended lyophilizing cultures using a 3°C/min cooling rate in 10% skim milk containing 5% sodium glutamate plus one of the following: 5 or 10% honey, 10% raffinose, or 10% trehalose. Other cryoprotectants include dimethylsulfoxide (DMSO) or 10% glycerol (Smith and Onions 1983). I used 20% skim milk as a cryoprotectant when lyophilizing Zygomycetes. After 3–4 yr, cultures of all five species of *Dichotomocladium* Benny & R.K.Benjamin and *Syzygites megalocarpus* Ehrenberg: Fries were no longer viable. The method proposed by Berny and Hennebert (1991) might extend the viability of these and other taxa.

Freezing in 10–25% glycerol or 15% DMSO in water at −85°C are other good methods to maintain Zygomycetes for long periods. Kitamoto et al. (2002) grew cultures over SDM (65% moisture W/W) with 10% glycerol as the cryoprotectant and then rapidly froze them at −85°C and tested viability by inoculating on PDA; several zygomycete cultures remained viable for 20 mo. These and other methods are discussed in detail by Fennell (1960), Jong and Birmingham (2001), and Nakasone et al. (2004).

Making Microscope Slides

Microscope slides of culturable Zygomycetes are made by placing small amounts of the fungus on a microscope slide and then covering the material with a drop of 95% ethanol to facilitate removal of air bubbles. Tilt the slide to allow the excess alcohol to drain off, blot it away using a piece of filter paper, and then add a drop (or more) of 2% KOH to the specimen to cause the fungus to swell to its natural shape. KOH-phloxine (Martin 1952) consists of one drop of 2% KOH and 1 drop of water containing a small amount of phloxine dye added to the water to stain the specimen, or one drop of water without the dye can be used. Seal these slides with paraffin, and then cover the cool paraffin with clear fingernail polish (Fig. 7). A thicker slide can be made by placing small slivers of coverslip under the coverglass (18 mm × 18 mm No. 1 cover glasses work best) and then seal the slide with paraffin (Fig. 8, 9). I apply the paraffin with a large paper clasp that has been opened to expose one straight side (Fig. 10, 11) that can be applied to the edge of the cover glass (Benny and Blackwell 2004; Krug et al. 2004). This microscope slide lasts only 3–7 days but it is the best method to make specimens for drawings or photographs. More permanent slides can be made using other mounting agents, such as lacto-fuchsin (Carmichael 1955), lacto-phenol cotton blue (Stevens 1974; Dhingra and Sinclair 1995), or lacto-phenol trypan blue (Phillips and Hayman 1970). Benjamin (1959) recommended mounting zygospores in Hoyer's solution (Alexopoulos and Beneke 1952; Thirumalachar and Narasimhan 1953) to observe the ornamentation of the zygosporangial wall. Dr. Thaxter mounted his Zygomycetes in 50% glycerol containing a small amount of eosin; some of these slides have survived for over 100 yr (Fig. 12).

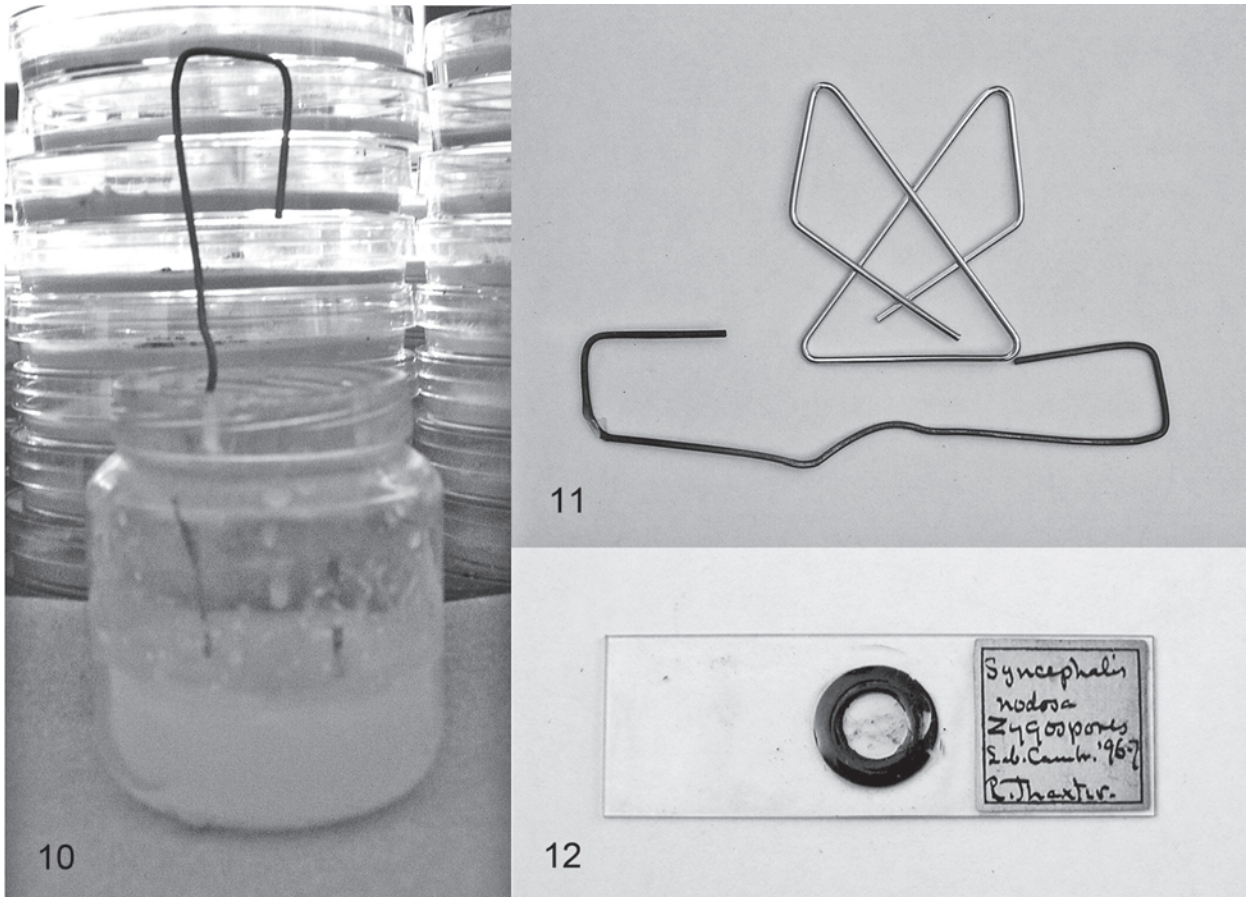

Fig. 10–12. Tools used to seal slides with paraffin and a slide of *Syncephalis nodosa* zygospores.—10. A jar containing paraffin and the paraffin applicator.—11. An Acco No. 1 paper clasp (above) and an unfolded paper clasp (below) used to transfer melted paraffin to the edge of a coverglass.—12. A microscope slide of *S. nodosa* zygospores mounted in 50% glycerol and eosin, made by Dr. R. Thaxter in 1897, ringed with King's cement. (Fig. 10, ×0.8; Fig. 11, ×0.65; Fig. 12, ×1).

Culture Media used for Monographic Studies of the Mucorales

The methods outlined above can be used to isolate Zygomycetes, and their parasites, from dung and soil. Species of *Coemansia* (Fig. 13, 14), *Dispira cornuta* van Tieghem (Fig. 15, 16) and other Dimargaritales, *Piptocephalis* (Fig. 17), and *Syncephalis* (Fig. 18–22) can be encountered using these techniques. Other taxa often present include species of *Absidia* (Fig. 23–25), *Blakeslea*, *Cunninghamella* (Fig. 26), *Rhizopus* (Fig. 27, 28), *Zygorhynchus* (Fig. 29), and *Umbelopsis* (Fig. 30) of Mucorales, and *Mortierella* (Fig. 31, 32) of Mortierellales. Many of the taxa in Mucorales and Mortierellales are hosts for the mycoparasitic Zygomycetes.

Media for Optimal Asexual and Sexual Reproduction of Selected Species in Mucorales

Many mucoralean species sporulate on the media listed below but several taxa should be treated as described in the discussions in the following parts of the paper. *Cokeromyces* (26°C, 12 hr light/12 hr dark) produces sporangia on 2% ME + 0.5% YE, CM, MEYE, SMA, V8, and WSH, and zygospores on MEYE, TPO, V8, YpD, and YpSs. The remaining thamnidiaceous Mucorales produce sporangia on 2% MEA, CM, MSMA, PCA, PDA, SMA, WortG, WSH, and YpSs, and zygospores on DCA, LYE, MEYE, MMTDD, MSMA, RFA, V8, Wg, Whey, WSHDD, YpD, and YpSs.

Dr. A. F. Blakeslee's studies of zygospore formation.—Blakeslee studied zygospore formation in Mucorales (Blakeslee 1904; Blakeslee et al. 1921, 1927; Blakeslee and Cartledge 1927) early in the 20th century. He devised methods to handle large numbers of cultures during this time including those that were dry-spored and could readily contaminate the laboratory, such as species of *Cunninghamella*, *Rhizopus*, and *Syncephalastrum* (Blakeslee et al. 1921). Blakeslee (Blakeslee et al. 1921, 1927; Blakeslee and Cartledge 1927) used several culture media (B230, B360, B362, B388, B391). Blakeslee et al. (1927) recommended B362 for crossing experiments because growth of aerial hyphae was sparse and the zygospores could be readily observed. I have found that adding 95% ethanol to a culture also facilitates the observation of zygospores using a dissecting microscope with substage lighting. Povah (1917) used B230 in his study of *Mucor* and Christenberry (1940) used a modification of this medium, C230, in his survey of Mucorales in the southeastern United States.

Studies by various mycologists on nonfastidious Mucorales.—Studies on Mucorales conducted at the Centraalbureau voor

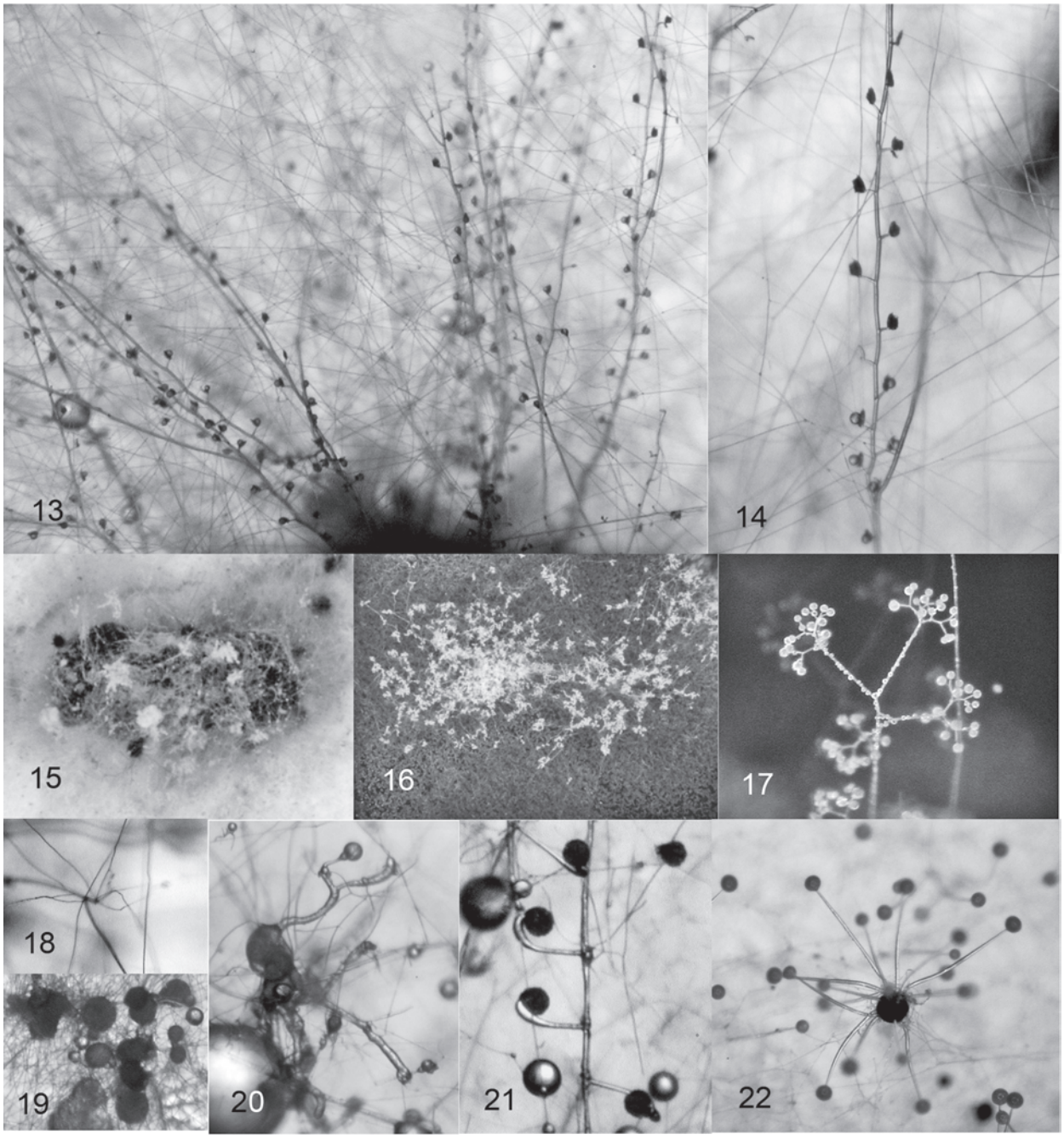

Fig. 13–22. Selected ''merosporangiferous Mucorales'' that can be encountered on soil and dung.—13, 14. *Coemansia* sp.—13. A *Coemansia* colony growing from a clump of soil.—14. A fertile hypha showing the spore drops on the lower sporocladia.—15, 16. *Dispira cornuta*.—15. A few sporulating heads (white) of *D. cornuta* on mouse dung.—16. A colony of *D. cornuta* growing on *Cokeromyces recurvatus*.—17. Typical dichotomous branching of *Piptocephalis freseniana*.—18–22. *Syncephalis* spp. from soil.—18. Relatively thin, aerial hyphae typical of most species of *Syncephalis*.—19. Lobate galls produced by an undescribed species of *Syncephalis*.—20. Long, sinuous galls formed by *S. basibulbosa*.—21. Sporangiophores of *S. cornu* attached to a *Mucor* hypha.—22. Sporangiophores of *S. sphaerica* arising from the *Rhizopus* sporangium. (Fig. 13, 22, ×6; Fig. 14, 18, ×9; Fig. 15, 17, ×10; Fig. 16, ×5; Fig. 19, ×7; Fig. 20, 21, ×17).

Schimmelcultures (Utrecht, The Netherlands) utilized several culture media not used elsewhere including beerwort agar (BWA), cherry decoction agar (CDA), prune decoction agar (Prune), and whey agar (Whey); PDA was also used (Schipper 1967, 1969, 1973). These media, especially BWA, were used in the monographs of *Mucor*, *Rhizomucor*, *Parasitella*, and *Thermomucor*, especially for culture descriptions, whereas zygospores were produced on BWA, CDA, PDA, Prune, and Whey (Schipper 1973, 1975, 1976, 1978*a*,*b*, 1979). They were also used in the studies that resulted in the reviews of *Absidia* and *Zygorhynchus* (Schipper 1986*b*, 1990). Raper and Thom (1949) reported that beerwort is complex chemically and batches are not uniform in composition. They recommended a malt extract agar (B230 with 25 g agar) as a substitute for

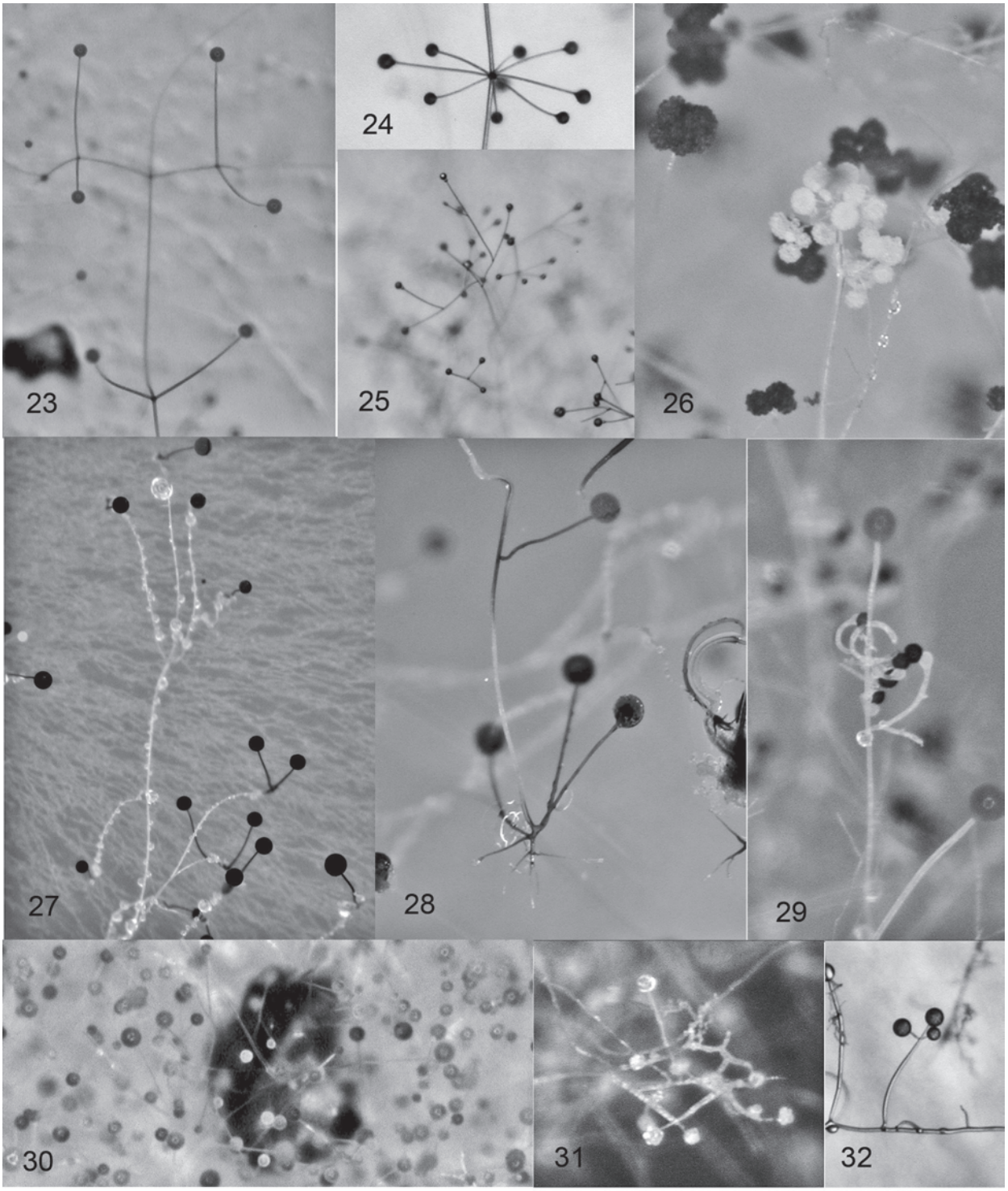

Fig. 23–32. Selected species of the Mucorales and Mortierellales that can be isolated from soil observed through a dissecting microscope.—23–25. Branching typical of several species of *Absidia*.—26. Sporulating heads of *Blakeslea trispora* (dark) and *Cunninghamella* sp. (light) growing on a plate inoculated with soil.—27, 28. Branching typical of *Rhizopus*.—29. A sporangium and zygospores of a species of *Zygorhynchus*.—30. A species of *Umbelopsis*.—31, 32. Two unidentified species of *Mortierella*. (Fig. 23, ×5; Fig. 24, 26, 32, ×10; Fig. 25, 28, ×8; Fig. 27, ×3; Fig. 29, 31, ×14; Fig. 30, ×6).

culture media that use brewing wort such as BWA (Raper and Thom 1949). In the papers on *Halteromyces*, *Hyphomucor*, *Protomycocladus*, and *Rhizopus*, and on species of *Mucor* and *Rhizopus*, the fungi were described on MEA, OMA, and SMA, and zygospores of *Rhizopus* were produced on CDA, MEAS, PDA, and YEA (Shipton and Schipper 1975; Schipper 1984, 1986*a*; Schipper and Stalpers 1984; Schipper and Samson 1994).

Several genera (*Absidia*, *Actinomucor*, *Amylomyces*, *Chlamydoabsidia*, *Circinella*, *Gongronella*, *Phycomyces*, *Zygorhynchus*) of Mucorales were monographed at the Northern Regional Research Laboratory (NRRL, now NCAUR), Peoria, Illinois. These fungi were grown on SMA (Hesseltine 1954) in order to describe the colony characteristics and the asexual stage, and most taxa were transferred to PDA (Benjamin 1958, 1959) in order to induce zygospore formation, with CZA and MEA also being used (Hesseltine and Fennell 1955; Benjamin and Hesseltine 1957, 1959; Hesseltine et al. 1959; Hesseltine and Ellis 1961, 1964, 1966; Ellis and Hesseltine 1965, 1966; Ellis et al. 1976). Váňová (1968, 1971) and Zheng (2002) have also used PDA and SMA when describing new species of *Absidia* and *Zygorhynchus*, respectively, and in the monograph of *Cunninghamella* (Zheng and Chen 2001).

The thamnidiaceous Mucorales (*Backusella*, *Dichotomocladium*, *Ellisomyces*, *Fennellomyces*, *Helicostylum*, *Kirkomyces*, *Phascolomyces*, *Pirella*, *Thamnidium*, *Thamnostylum*, and *Zychaea*) were grown on MSMA at 26°C for description of the colony and asexual production, whereas zygospores, except in *Helicostylum*, *Pirella*, and *Thamnidium*, formed optimally on several media including 2% ME, LYE, MSMA, MEYE, TPO, YPD, YpSs, Whey, and WSH. Species of *Helicostylum*, *Pirella*, and *Thamnidium* produce zygospores at a range of 7 to 20°C, with optima at 15°C (*Pirella*) or 3–10°C (*Helicostylum*, *Thamnidium*) on 2% ME + 0.5% YE, LYE, MEYE, PDA, TPO, Whey, WSHDD, YpD, and YpSs (Benny and Benjamin 1975, 1976, 1991, 1993; Benny 1995*a*,*b*; Benny and Schipper 1992). Many species of the following thamnidiaceous genera—*Chaetocladium*, those taxa that produce yeast cells readily on MEYE (*Benjaminiella*, *Cokeromyces*, and *Mycotypha*), and *Radiomyces*—do not grow well on MSMA. Therefore, these fungi were described on MEYE or YpSs, and zygospores were induced on LYE, MEYE, TPO, V8, Whey, YpD, and YpSs. Species of *Radiomyces* also reproduce well asexually on V8, Wg, YpSs, and WortG (Benny and Benjamin 1976, 1991; Benny et al. 1985). The response of each species to specific culture media can vary considerably, for instance sporangia of *Thamnostylum repens* (van Tieghem) Upadhyay usually form only on WortG and the zygospores of *Backusella ctenidia* (Durrell & Fleming) Pidoplichko & Mil'ko were produced only on LYE, but most taxa reproduce asexually and sexually on several media. The reader is encouraged to try several culture media when growing a member of Mucorales, especially when trying to induce zygospores.

Isolation and sporulation of choanephoraceous Mucorales.—*Blakeslea*, *Choanephora*, *Gilbertella*, and *Poitrasia*, are characterized by the formation of subglobose to ellipsoid, smooth or striate sporangiospores with apical, hyaline appendages, a persistent-walled sporangium with a preformed dehiscence line, and zygospores with opposed (*Gilbertella*) or apposed (*Blakeslea*, *Choanephora*, *Poitrasia*) suspensors. Sporangiola of choanephoraceous Mucorales are borne on sporangiophores separate from those bearing sporangia; in *Blakeslea* the sporangiolar walls have a dehiscence zone and the spores are readily released, but in *Choanephora* the zone is lacking and the spores and sporangiolum are not separable (O'Donnell 1979; Kirk 1984; Benny 1991); species of *Gilbertella* and *Poitrasia* do not produce sporangiola. *Blakeslea trispora* Thaxter, *Choanephora cucurbitarum* (Berkley & Ravenel) Thaxter, and *Poitrasia circinans* (Naganishi & Kawakami) P.M.Kirk do not sporulate readily on most ordinary laboratory culture media. In Florida, all three taxa can be isolated in the summer from soil, and *B. trispora* and *C. cucurbitarum* have been found on dung and on plates exposed to the air. *Choanephora cucurbitarum* is a plant parasite that causes wet rot of summer squash and string beans and affects several other crops; the fungus sporulates on the infected plants. Spores from sporangia or sporangiola can be transferred using watchmakers' forceps to an appropriate culture medium (CH, CH3, Wg5). Asexual reproduction, usually as sporangiola in *B. trispora* and *C. cucurbitarum* but only as sporangia in *P. circinans*, probably will start in 3–4 days when grown between 25 and 30°C, especially near the outer edge of the culture in a Petri dish. Accumulation of carbon dioxide represses sporulation and, therefore, it is recommended that the cultures not be grown in test tubes or in sealed Petri dishes. Zygospore formation can be induced in *B. trispora*, *C. cucurbitarum*, and *P. circinans*, if both the "+" and "−" mating types are crossed, on CH, CH3, or Wg5 agars. Crossing the mating types of these fungi, and *Gilbertella persicaria* (Eddy) Hesseltine, on relatively nutrient-rich culture media, for instance APDA, MEYE, PDA, V8, or YpSs, will result in the production of a readily visible dark and sometimes wide line composed of tightly packed zygospores.

Preservation of cultures of *B. trispora*, *C. cucurbitarum*, and *P. circinans* is important because these fungi often lose their ability to sporulate after a few transfers. *Gilbertella persicaria*, however, usually does not lose its ability to sporulate after repeated transfer and this species does not require a nutrient-poor culture medium in order to induce asexual reproduction (Benny 1991). The cultures can be lyophilized or frozen at −85°C or −195°C using cryoprotectants.

Isolation and culture of Dicranophora*,* Spinellus*,* Sporodiniella*, and* Syzygites.—*Dicranophora* is a rare mucoralean fungus whose only species, *D. fulva* J. Schröter, is found in nature as a facultative parasite of *Boletus* Fries, *Gomphidius* Fries, *Paxillus* Fries, and *Suillus* Gray (Zycha et al. 1969; Voglmayr and Krisai-Greilhuber 1996). *Dicranophora* has been found in Europe and the United States (Zycha et al. 1969; Voglmayr and Krisai-Greilhuber 1996; R. D. Goos pers. comm. 1996). *Dicranophora fulva* is a homothallic, psychrotolerant fungus that was grown on 2% MEA at 15°C (Voglmayr and Krisai-Greilhuber, 1996). I have grown *D. fulva* (NRRL 22204) at 17°C on several culture media including MEYE, V8, Wg5, and YpSs in the dark except when taken out into a light room for observation. After 3 weeks sporangia, sporangiola, and zygospores formed on all media, but V8 was the best medium for asexual reproduction.

Spinellus is also a facultative parasite of mushrooms, especially species of *Mycena* (Pers.) Roussel that must be grown below 20°C. Ellis and Hesseltine (1962) isolated *S. fusiger* (Link) van Tieghem from a parasitized *Mycena* by placing infected mushroom tissue on PMG agar and incubating the plates at 15°C. *Spinellus fusiger* is homothallic but usually only sporangia are produced in culture. Leadbeater and Richardson (1963) devised two culture media, BPM and CSS, that promote excellent sporangia formation and zygospores also were produced. I grew *S. fusiger* (NRRL 22323) at

17°C on MEYE, V8, Wg5, and YpSs agars with the best sporangial formation occurring on MEYE and YpSs.

Sporodiniella is a mesophilic fungus that can be grown at 23–25°C. It occurs in the tropics (Ecuador, Indonesia, Taiwan) as a facultative parasite of insect larva (Evans and Samson 1977; Chien and Hwang 1997). Evans and Samson (1977) described *S. umbellata* Boedijn from cultures growing on 2% MEA or MWA. Chien and Hwang (1997) grew *S. umbellata* on CA, MEA, and a mealworm larva placed in the center of a CM plate at 20°C; good asexual reproduction was observed on all media. Zygospores have been observed on larvae collected in the field and they might be produced on mealworm larvae in culture under optimal conditions, for instance fluctuating temperatures or drying of the host.

Syzygites (Hesseltine 1957) and its only species, *S. megalocarpus* is found in nature as a facultative parasite of several mushroom taxa, and also on Ascomycetes and Gasteromycetes (Kovacs and Sundberg 1999). *Syzygites megalocarpus* is homothallic and zygospores are produced on zygophores that are up to 0.5 cm high. Sporangia are formed on separate fertile hyphae that are 4–5 cm long; the name applied to the asexual reproductive phase is *Sporodinia grandis* Link. Hesseltine (1957) said that the synonymy of *Syzygites* and *Sporodinia* Link was noted in the middle of the 19th century and that *Syzygites* is the correct name because of priority and more importantly since it was applied to the teleomorph. I isolated *S. megalocarpus* on MEYE from mushrooms collected in the summer in Gainesville, Florida, because it can grow from 5–30°C (Wenger and Lilly 1966). The anamorph and teleomorph both appeared in the same plate without me knowingly having manipulated the growth conditions. Kaplan and Goos (1982) found that sporangial formation was promoted by a moist substrate whereas zygospores formed when less water was available.

Isolation and culture of Chaetocladium *and* Parasitella*, and* Absidia parricida *Renner & Muskat ex Hesseltine & J.J.Ellis*.—*Parasitella* and probably *Chaetocladium* are fusion parasites (Gams et al. 2004). *Chaetocladium* Fresenius (Benny and Benjamin 1976) is a psychrotolerant, facultative parasite of other Mucorales in nature that arises from galls (Jeffries 1985). It was encountered on mucoralean hosts growing on dung collected in winter in southern California. *Chaetocladium brefeldii*, the most common of the two known species, is heterothallic and grows from 7°C to 20–25°C with the species description completed on cultures grown without a host at 21°C (Benny and Benjamin 1976). Asexual and sexual reproduction of *C. brefeldii* occurs equally well on MEYE and YpSs. Zygospores form best at 15°C.

Parasitella is heterothallic and the only known species, *P. parasitica* (Bainier) Sydow, is a facultative, gall-forming parasite of other Mucorales in nature. *Parasitella parasitica* grows and sporulates well between 15°C and 30°C, and zygospores form at 20°C (Schipper 1978*b*). In nature, *P. parasitica* parasitizes only hosts of the opposite mating type, inducing a transfer of genetic information in the process (Kellner et al. 1991; Wöstemeyer et al. 1995). The galls of *P. parasitica* were unwittingly illustrated before the fungus was described (Thaxter 1895; Blakeslee 1904). Satina and Blakeslee (1926) observed that *Chaetocladium* and *Parasitella* could become hosts for one another and that the galls formed were characteristic of both taxa. Both *C. brefeldii* and *P. parasitica* were isolated in southern California (Richard K. Benjamin pers. comm. 1972). *Parasitella* can be grown on ordinary culture media without a host.

Absidia parricida is also a parasite of several taxa in the Mucorales. Some swollen regions are present at the contact point of the host and *A. parricida*. The hyphae of *Rhizopus oligosporus* Saito collapse and concurrently sporulation is suppressed when it is parasitized by *A. parricida* (Hesseltine and Ellis 1966).

A method to induce sporulation in Apophysomyces elegans *Misra et al. and* Saksenaea vasiformis *Saksena*.—*Apophysomyces elegans* and *S. vasiformis* are monotypic organisms that cause systemic mycoses in humans and these fungi are notoriously difficult to get to sporulate in culture. Padhye and Ajello (1988) recommended adding three drops of 10% YE solution to Petri dishes containing 20 mL sterile distilled water. Add 2 pieces of SABD agar 1 cm^2 with actively growing colonies of *A. elegans* or *S. vasiformis*, and incubate at 37°C for 10–12 days. The optimum number of sporangia of these two fungi will form under these conditions.

Isolation, culture, and sporulation of species of Pilobolus *Tode*.—*Pilobolus* (Grove 1934; Hu et al. 1989) is coprophilous. It is the only taxon in Mucorales that forcibly discharges its sporangium and has an obligate requirement for the addition of dung extract or a siderophore (Renshaw et al. 2002) to the culture medium in order to permit growth and induce sporulation. The two genera *Pilaira* and *Utharomyces* (Zycha et al. 1969; Kirk and Benny 1980), often classified with *Pilobolus*, sporulate on ordinary laboratory culture media such as YpSs (see Appendix 1).

Singh and Webster (1976) found that decoctions of horse or rabbit dung provide the best sporangial formation of *Pilobolus* spp.; Hu et al. (1989) made a dung agar, DEA, from the excrement of these animals. Hesseltine et al. (1953) noted that coprogen, which is present in dung, can replace dung decoction in media. Nutrient media devoid of dung extract include BEA, IAM, PBM, and SHM; the latter culture medium is the simplest to make and produces excellent sporulation of *Pilobolus*.

The author has used culture media containing either dung decoction or SHM (Levetin and Caroselli 1976), which is a simplified formula based on PBM (Page 1960) to isolate and culture *Pilobolus* spp. Mature black sporangia are transferred from the sporangiophore apex or from the walls of the moist chamber using watchmakers' forceps and then placed on the surface of the culture medium. The Petri dish is placed in a 37°C incubator overnight to stimulate spore germination and the next morning it is placed at room temperature to induce germ tube formation (Bourret and Keierleber 1980). Bourret (1982) devised a culture medium (IAM) in which ferric sulfate and ascorbic acid replaced hemin in the growth medium. *Pilobolus* requires light to produce trophocysts, from which the sporangia arise. The sporangiophores are phototropic (Page 1962).

Fungi in Umbelopsis *(Mucorales)*.—Gams (1977) said that the preferred culture medium for *Umbelopsis* is MEA. Sugiyama et al. (2003) used both MA and MEA in their study of *Umbelopsis*. Wg5 containing antibiotics and benomyl also is a good culture medium for species of *Umbelopsis*.

Fungi in the Mortierellales.—Members of Mortierellales, especially *Mortierella*, are common components of soils that also contain other Zygomycetes. Gams (1977) recognized 64 species of *Mortierella* and ten additional taxa have been described (Veerkamp and Gams 1983; Gams and Carreiro 1989; Gams 1991; Chen 1992; Pfenning and Gams 1993; Degawa and Tokumasu 1998*a*; Degawa and Gams 2004). Gams (1977) said that best culture media for *Mortierella* are PCA and SEA. Kuhlman (1969) recommended using TSM to identify *Mortierella* spp. Zygospores have been induced in several species of *Mortierella* using CDY, CM, dHSA, HIA, HSA, LMEA, MA, MEA, OMA, PABA, PCA, RS, SABD, ShA, TSM, and WA (Linnemann 1958; Gams and Williams 1963; Williams et al. 1965; Gams et al. 1972; Kuhlman 1972; Chien et al. 1974; Degawa and Tokumasu 1997, 1998*a*,*b*). *Mortierella multidivaricata* R.K.Benjamin (1978) was grown on MEYE, PYED, and YpSs; this species was later transferred to *Gamsiella* (R.K.Benjamin) Benny & M.Blackwell (Benny and Blackwell 2004). Carreiro and Koske (1992) isolated species of *Mortierella* on refrigerated MYP plates; it was later determined that one of these fungi was *Dissophora decumbens* Thaxter (Gams and Carreiro 1989). Zak and Wildman (2004) discuss isolation of psychrophiles and other fungi from stressful environments. *Lobosporangium transversale* (Malloch) M.Blackwell & Benny (Benny and Blackwell 2004) produces sporangia on CM, CZA, HSA, LA, MEA, PAB, ShA, and TSM. Another medium for isolating and growing species of *Mortierella* is Wg5 with antibiotics and benomyl.

Isolation of Fungi in Zoopagales

Dr. Charles Drechsler described the majority of the known species of Cochlonemataceae and Zoopagaceae, three species of Helicocephalidaceae (Zoopagales), and many taxa of *Basidiobolus* and *Conidiobolus* (Entomophthorales) (see references in Lumsden 1987). Members of three zoopagalean families (Cochlonemataceae, Helicocephalidaceae, Zoopagaceae) are obligate parasites of small invertebrates (amoebae, nematodes, rotifers, or eggs of the latter two). Isolation of the fungi, therefore, requires the presence of the appropriate host and their food source, for instance bacteria and fungal spores.

Drechsler (1929, 1936) devised a procedure to isolate members of Cochlonemataceae and Zoopagaceae, described below, that simulated natural conditions to provide both the host and parasite in the same Petri dishes. *Helicocephalum*, *Rhopalomyces*, and some species of *Syncephalis* (Zoopagales), and *Ballocephala* and *Meristacrum* (Entomophthorales) also were encountered using the same method (Drechsler 1934, 1940, 1943, 1951*b*, 1955, 1961*a*).

Cochlonemataceae and Zoopagaceae.—Drechsler's procedure for isolating members of Cochlonemataceae and Zoopagaceae involved the double inoculation of plates of CM. The first phase required putrescent plant material that probably was infected with species of *Pythium* Pringsheim, *Phytophthora* de Bary, or other related organisms. These specimens were washed in several changes of sterile distilled water (10–15 mL each) until the liquid was no longer turbid, blotted dry, and then placed on one spot on the agar surface. When the infecting organism was observed growing from the sample it was removed leaving the chromistan, often a species of *Pythium*, some bacteria, and usually some of the desired host invertebrates in the plate (Drechsler 1929). Drechsler (1935*b*) said that Zoopagaceae would not be present in sufficient numbers in a culture until the host invertebrates also had reached the necessary population density. The initial inoculum often yielded a few host invertebrates that required several days or a few weeks to grow and reproduce, a process dependent on the concurrent growth of bacteria and the availability of fungal spores that can be used for food. Drechsler (1935*b*) said that fungi that produce dense or dry aerial hyphae retard the growth of bacteria and physically prevent the movement of host animals. Inoculation of isolation plates with diseased plant samples that have been in contact with moist soil contain invertebrates and bacteria and, as a result, the aerial hyphae of most fungi will collapse in the bacterial lawn, leaving room for the movement of amoebae, nematodes, and rotifers. The hyphae of predaceous zoopagalean fungi are not degraded in the bacterial layer as are many other fungi. These plates could be scanned for the desired zoopagalean parasites when they were a few weeks old and, later, Drechsler (1936) recommended adding a small amount of decaying plant material (for example, leaf mold, grass clippings, compost) to each plate to increase the variety of both the hosts and the parasites. Drechsler (1959) kept his plates in a battery jar in order to keep the agar moist which is essential for the optimal development of the host during the long incubation period of several weeks to a few months; plastic bags or other closed containers can be used instead. Dung and moss can also be good sources of Cochlonemataceae and Zoopagaceae (Blackwell and Malloch 1991; Duddington 1951).

The high summer temperature in Drechsler's laboratory in Washington, D.C., (1935*b*) that presumably lacked air conditioning was unfavorable for the development of most host invertebrates and, therefore, the growth of predaceous fungi. *Stylopage cephalote* Drechsler (1938), however, grew out in May and July when it was too warm for most zoopagalean fungi to appear in laboratory culture (Drechsler 1951*a*). The host amoebae of *Cochlonema agamum* Drechsler (1946) did well at 23°C but the infection reduced the cytoplasm mass to 20–25% of its original volume and the nucleus also changed its appearance. Storage of the plate at 15°C overnight revitalized the host but *C. agamum* ceased growing and underwent cytoplasmic changes.

Drechsler (1935*a*) noted that more asexual spores are formed when the host expires on the substrate surface, whereas zygospore formation is promoted when the host dies further below the surface. Drechsler (1935*b*) also recommended using culture media with 15 to 20 grams of agar per liter so the host invertebrates do not burrow but stay on the surface where they can be more readily studied.

Cochlonema bactrosporum Drechsler (1939) can be detected because it produces vertical chains of aerial spores. Mature, intact chains readily break up and the spores become scattered on the agar surface. In species that produce abundant chains of asexual spores, such as *Cochlonema symplocum* Drechsler (1941), *C. agamum* (Drechsler 1946), and *C. eryblastum* Drechsler (1942), these are visible, using lateral illumination, as white tufts randomly scattered over the surface of the culture medium. The branched aerial spore chains of *Zoopage pachyblasta* Drechsler (1947*b*) are observed as thin tufts. In *Cochlonema megaspirema* Drechsler (1937) the aerial asexual

reproductive hyphae grow more or less horizontally usually for several millimeters, and in *Zoopage mitospora* Drechsler (1938) the area of infection is restricted to a circle 15 mm in diameter; asexual reproduction was observed after placing a coverslip over the area of sporulation. *Cystopage lateralis* Drechsler (1941) asexual spores are formed in the agar or on its surface. The single spores of *Acaulopage rhicnospora* Drechsler (1935*b*) were vertical when observed using a dry objective. *Stylopage hadra* Drechsler (1935*c*) appears in plates in 5–15 days and can be observed with the naked eye but when growth is minimal it can be detected only using the low power dry microscope objective. The more or less erect chain of spores produced by *Endocochlus asteroides* Drechsler (1935*b*) can be observed using a low or medium power dry objective.

Saikawa and Sato (1991) isolated *Cochlonema odontosperma* Drechsler (1935*b*) by placing leaf mold on the surface of WA and incubating the plates for 2–3 weeks at room temperature. Saikawa and Kadowaki (2002) found two species of *Acaulopage* by placing a sample consisting of fallen leaves, paper, and wood in 9 cm Petri dishes containing 10 mL of distilled water; spores of the fungus appeared on the surface of the water. The amoeba host was maintained on SA agar, and WA was used to maintain the fungal culture (Saikawa and Kadowaki 2002).

Microscope observations have been made on members of Cochlonemataceae and Zoopagaceae by (1) placing an agar block on a slide, adding a drop of lactophenol-cotton blue, and then a coverslip (Dayal and Srivastava 1979), and (2) fixing the material with glutaraldehyde, post-fixing with osmium tetroxide, embedding in Spurr's resin, and presumably making 0.5 μm-thick sections and mounting them on microscope slides (Saikawa and Sato 1991).

Barron (1977) recommended placing a drop of infected nematodes on relatively dry agar so that it is absorbed in half a day, and the infected nematodes will swim out on the agar. When the fungus sporulates, mounts are made of the infected nematodes by transferring the animal on a small piece of agar, gently placing it on a slide, fixing with glacial acetic acid : absolute ethanol (1 : 3), and then adding lactophenol or lactophenol-cotton blue, covering the specimen with a coverslip, and heating over an alcohol burner to melt the agar. This results in very little disturbance of the host or the fungus.

Keys to the taxa of Cochlonemataceae and Zoopagaceae parasitizing amoebae, nematodes, and rotifers have been published by Barron (2004), Cooke and Godfrey (1964), and Dayal (1973/1974). Barron (2004) presents detailed procedures that he has used to isolate and propagate nematodes and rotifers and their fungal hosts, and Barron (1977) compared Drechsler's technique with methods he used to isolate nematophagous fungi.

Helicocephalidaceae.—Ellis and Hesseltine (1962), and Ellis (1963), devised a method that supported germination and growth of *Rhopalomyces elegans* Corda in culture. *Bacillus cereus* Frankland & Frankland var. *mycoides* Flügge was grown in glass Petri dishes on TKY agar at 25°C for 20 hr. After the plate was autoclaved 20 min and allowed to cool and resolidify, the spores of *R. elegans* from a fresh culture were streaked on the agar surface. Up to 90% of the *R. elegans* spores will germinate but the culture will not grow. A piece of the TKY agar containing germinating *R. elegans* spores is then transferred to LFK agar and inoculated on or next to the liver. The inoculated LFK plates are incubated at 25°C in light, and in 4–6 days *R. elegans* will produce sporangiophores on the lamb fat and begin to sporulate. The original cultures of *R. elegans* were isolated from HIA or WA plates sprinkled with soil and debris and incubated at 25°C for up to 6 weeks (Ellis 1963). Dr. R. K. Benjamin (pers. comm. 1970) reported that this procedure was not effective in inducing spore germination in a species of *Helicocephalum*.

Sigmoideomycetaceae.—*Thamnocephalis sphaerospora* R.K. Benjamin & Benny (Benny et al. 1992) was grown on 2% ME + 0.5% YE, MEYE, and YpSs at 26°C under 12 hr light/12 hr dark using *Cokeromyces recurvatus* as the host. *Microascus doguetii* Moreau also served as a host for *T. sphaerospora* which was originally isolated from frog dung (Benny et al. 1992). Chien (1992, 2000) later reported that *Thamnocephalis quadrupedata* Blakeslee was isolated from frog dung and that it was a haustorial parasite of *Basidiobolus ranarum* Eidam. Chien (2000) grew cultures of *T. quadrupedata*, using *B. ranarum* as the host, on CM and MEA.

Fungi in Endogonales

Germination of zygospores, and zygospore and sporocarp formation in Endogone pisiformis *Link*.—Sporocarps of *E. pisiformis* were surface-sterilized in bleach (0.4% sodium hypochlorite), washed several times in sterile distilled water, and inoculated on MMNs and YpSs agars. The culture grew in 7–14 days at 22°C. These cultures also could be grown on CM (Difco), LYE, and PDA (Berch and Fortin 1983*a*,*b*).

Zygospore-containing sporocarps of *E. pisiformis* were produced after growing a culture of *E. pisiformis* in 200 mL of MMN broth, 3 mo at 22°C in the dark, in glass bottles containing pieces of broken glass to macerate the mycelium. Large test tubes containing a mixture of 10 mL peat and 100 mL vermiculite (thoroughly mixed) and MMN solution with 10 g/L glucose, were inoculated with the unwashed mycelial suspenson (Berch and Castellano 1986). These tubes were incubated for 3 mo in a 18°C water bath, and illuminated with fluorescent and incandescent light (Molina and Palmer 1982). The temperature of the tubes above the level of the water in the water bath was 23°C. The sporocarps formed first at the water line and then up to 5 cm above the surface of the substrate. Plants were not required for sporocarp formation (Berch and Castellano 1986). Dalpé (1990) later reported that *E. pisiformis* grew on several media and that thiamine HCl was required to maintain the viability of the cultures.

Growth of Sclerogone *and* Densospora.—Warcup (1975) reported growing a fungus in culture that he later described as *Sclerogone eucalypti* Warcup (1990). The cultures of *S. eucalypti* resembled those formed by *E. pisiformis* and they sometimes could be subcultured (Warcup 1975). Warcup (1985) reported slow growth of *Glomus tubaeformis* Tandy in culture. This species was later transferred to *Densospora* (McGee 1996). *Densospora* contains four species that form ectomycorrhizae and sporocarps that contain blastospores (''chlamydospores'' of some authors). Blastospore formation is a characteristic of Glomales whereas the formation of ectomycorrhizae occurs in Endogonales (McGee 1996). The

affinities of *Densospora* will depend on the results of future research.

Fungi in Entomophthorales

The saprophytes.—Drechsler (1947*a*) initially isolated *Basidiobolus* using the same technique that he used for Cochlonemataceae and Zoopagaceae (Zoopagales). Later, Drechsler (1952) devised a canopy technique that was very productive for the isolation of *Basidiobolus* and *Conidiobolus* (Entomophthorales). This procedure requires the application of soft agar in a circle in the center of the Petri dish lid leaving a 15 mm-wide zone around the edge of the lid; fine leaf mold is mixed into the soft agar so that it is fastened to the lid and also moistened. This agar-leaf mold zone can be mite-proofed by applying a narrow circular band of heavy mineral oil or soft Vaseline between the soft agar and the vertical wall of the lid. The next year, Drechsler (1953) further refined the canopy method. Callahan (2004) also used this technique but suggested methods of sampling and homogenizing the material to be used to make the canopy. Drechsler (1956) suggested a canopy method for isolating species of *Basidiobolus* from frog dung in which he placed frog(s) in a glass jar containing 25–50 mL of distilled water for 10–15 hr. The next day the frog was returned to its natural habitat, the dung was filtered from the water using a small piece of filter paper and the moist paper is attached to the Petri dish lid soiled side down; any *Basidiobolus* present would shoot spores onto the agar below. Usually, at least one species of *Basidiobolus* can be isolated on the CM. Colonies of *Conidiobolus* growing in Petri dishes are shown by Drechsler (1961*b*). Species of *Basidiobolus* and *Conidiobolus* have been grown and studied on several culture media: CM, GGY, MEA, MEYE, MP5, NsCM, PDA, SMA, and YpSs (Couch 1939; Drechsler 1947*a*, 1953, 1956, 1961*b*; Benjamin 1962; Srinivasan and Thirumalachar 1967; King 1977; Callahan 2004).

The pathogens.—Methods for the isolation, culture, identification, and preservation of the entomopathogenic Entomophthorales have been published by Humber (1997*a*, 1997*b*) and Papierok and Hajek (1997). The non-entomogenous Entomophthorales (Tucker 1981) *Ballocephalla*, *Macrobiothophora*, *Meristacrum*, and *Zygnemomyces* have been described and illustrated (Reukauf 1912; Drechsler 1940, 1951*b*; Miura 1973). Barron (2004) presents procedures to grow the parasites of nematodes and rotifers in culture. *Ballocephala sphaerospora* Drechsler and *B. verrucospora* Richardson have been examined ultrastructurally (Saikawa 1989; Saikawa and Oyama 1992; Saikawa and Sakuramata 1992). Tucker (1981) published keys to the non-entomogenous Entomophthorales.

Recognition of Merosporangiferous Mucorales

Dimargaritales.—The majority of species are found on dung, especially of rodents, although a few isolates of *Dimargaris* have been made from soil. The sporangiophores are white when young but become brownish with age. With the exception of a few wet-spored species of *Dimargaris*, most taxa are dry spored. You are most likely to encounter species of *Dispira* or *Tieghemiomyces* (Benjamin 1959, 1961, 1963, 1965, 1966). These fungi are low growing, and they often form dense colonies in culture and possibly on dung, as well. Wet-spored *Dimargaris* spp. often appear somewhat like *Absidia* spp. in their branching pattern, but the young fruiting structures are dry, like in species of *Aspergillus* Link, and in some taxa the older ones are wet; in *D. arida* R.K.Benjamin and *D. xerosporica* (B.S.Mehrotra and Baijal) R.K.Benjamin the spores are dry at maturity. Benjamin and Tucker (1978*b*) provide information on culturing *Dimargaris cristalligena* van Tieghem so that both asexual and sexual reproductive structures can be studied.

Dimargaritales can be grown in the laboratory at 25°C on the bench with natural daylight from a south window; some isolates of *D. cristalligena* may need to be cultivated at 18–21°C for typical growth. When grown on a fungal host on MEYE, YpSs, or V8, the parasite will form a typical colony. Sporulation of Dimargaritales is optimal when the cultures are grown on V8. When grown without a host, YGCH is the only medium that can be used because Dimargaritales lack the ability to use six-carbon sugars (Barnett 1970; Binder and Pierce 1976).

Kickxellales.—These fungi are either white or some shade of yellow. All known genera, except *Spiromyces* and *Spirodactylon* R.K.Benjamin, are wet-spored at maturity (Benjamin 1958, 1959, 1961, 1963, 1966; O'Donnell et al. 1998; Kurihara et al. 2000, 2001, 2004; Ogawa et al. 2001). Most are low-growing and grow readily on MEYE, MEYE/2, YpSs, or YGCH (Appendix 1). *Coemansia* spp. are often encountered in nature. Benjamin and Tucker (1978*a*) provide information on culturing *Coemansia mojavensis* so that both asexual and sexual reproductive structures can be studied; this procedure should also work for other *Coemansia* spp. Benjamin (1958) said that the optimal culture medium (2% ME, 0.5% YE, CaA, CM, CM-DD, CMPY, CM-S, ME-P, MEYE, MEYE/2, MMT, PAB, PAB-DEX, PCA, PG, PYED, PYEDS, SDY, V8, Wg, WgDD, Wg-S, WSHDD, YGCH, or YpSs) depends on the strain of *Coemansia* being cultured.

Mucorales.—The only genus of Mucorales that produces a merosporangium is *Syncephalastrum* (Benjamin 1959, 1966; Benjamin and Tucker 1978*d*). The merosporangia usually contain 4–12 sporangiospores. Zygospores are like those of *Mucor* (suspensors opposed; zygosporangium dark and ornamented). One species, *S. racemosum* Cohn ex Schröter, is common worldwide. *Syncephalastrum racemosum* will grow and sporulate on most culture media. Benjamin (1959) used YpSs to produce zygospores.

Piptocephalidaceae (Zoopagales).—Piptocephalidaceae contain three genera: *Kuzuhaea*, *Piptocephalis*, and *Syncephalis*. Only the latter two taxa will be encountered on dung or soil; *Kuzuhaea* is known only from the original description (Benjamin 1985*a*).

Piptocephalis *and* Kuzuhaea.—The sporangiospores of *Piptocephalis* spp. are either wet- or dry-spored when mature and the sporangiophores are usually light brown in color. These fungi are readily recognized by their spore-bearing branches that form three-dimensional dichotomies. Many species grow 0.5–1 cm high, and they are easily isolated using the appropriate host, usually *Cokeromyces recurvatus* or *Umbelopsis ramannianus*. Some species must be grown at 18°C, and you may find species with lower temperature growth require-

ments during the winter (Benjamin 1959, 1966). Benjamin (1959, 1966) used YpSs agar to grow *P. lepidula* (Marchal) R.K.Benjamin and *P. unispora* R.K.Benjamin. Benjamin and Tucker (1978*c*) described culture methods that will make it relatively easy to study both asexual and sexual production in species of *Piptocephalis*. *Kuzuhaea moniliformis* and *Piptocephalis* spp. can be grown on MEA, PDA, YpD, YpSs, and YpSs/5.

Syncephalis.—Members of genus *Syncephalis* are very low growing, usually simple, and they produce a sporangiophore with basal rhizoids and an apical vesicle that bears uni- or multispored merosporangia (Benjamin 1959, 1966; Ho 2000, 2001, 2002, 2003). The merosporangia are dry-spored when young (they appear somewhat like an *Aspergillus* sp.) but they are wet-spored when mature. You may find *Syncephalis* spp. on a mucoralean host and some species form galls or gall-like structures (Hunter and Butler 1975; Hunter et al. 1977). *Syncephalis* spp. can be observed on the filter paper around the dung pellets, especially when you see the young (dry) and mature (wet) sporangiophores together. You can also find *Syncephalis* on soil cultures using PNB agar. Hunter et al. (1977) isolated *S. californica* Hunter & E.E.Butler when ground *R. oryzae* mycelium was added to a dilute soil mixture. Sporangiophores were formed readily when infected *R. oryzae* was transferred to WA (Hunter et al. 1977). *Syncephalis* spp. usually need to be cultured on the host species you find them on in the original isolation plate. Study them soon, however, because some *Syncephalis* spp. may cease sporulating in culture after a few transfers. Many *Syncephalis* spp. will survive several transfers when grown on Wg5 agar. Benjamin and Tucker (1978*e*) described culture methods that will make it relatively easy to study both asexual and sexual production in species of *Syncephalis*.

Ellis (1966) grew several isolates of *Syncephalis* without a host on a liver medium (SLM). *Syncephalis* spp. have been isolated from CH and Wg3 plates grown in the laboratory at 25°C on the bench with natural daylight from a south window; Wg5 is a good medium to grow cultures of most isolates of *Syncephalis*. Species of *Syncephalis* can be grown on any culture medium that will support grown of the host. Good reproduction occurs on CH and Wg5. Kuzuha (1980) used 5GY to induce zygospore production in *S. sphaerica* van Tieghem. I was able to induce zygospore formation after crossing compatible strains of *S. sphaerica* on Wg5.

Several taxa of *Syncephalis* were transferred to relatively slow-growing hosts (*Cokeromyces recurvatus*, *Umbelopsis ramannianus*) in order to determine if the parasite would sporulate and grow beyond the margin of the host colony. Several species of *Syncephalis*, *S. cornu*, *S. depressa*, *S. nodosa*, *S. plumigaleata*, and *S. sphaerica*, were grown on six culture media, BPM, CSS, MEYE, V8, YpSs, and YpSs/5. The only parasite to sporulate on *U. ramannianus* was *S. depressa*; V8, YpSs, and YpSs/5 yielded good sporulation. All aforementioned *Syncephalis* spp. except *S. nodosa* grow and sporulate on *C. recurvatus*: CSS, MEYE, YpSs, and YpSs/5 were all good media for at least a few taxa. The vegetative hyphae of *Syncephalis* grew 2–40 mm beyond the margin of the colony of *C. recurvatus*; the vegetative hyphae then could be transferred to other hosts or used for molecular studies.

DISCUSSION

The five-step procedure (collection, plating, isolation, culture, maintenance) devised by Dr. Benjamin to isolate merosporangiferous Mucorales and other Zygomycetes uses materials that are available in most mycological laboratories. This technique is reproducible and relatively easy to learn. One of the most important elements promoting isolation of Zygomycetes is flattening the aerial mycelium of the faster-growing fungi for several consecutive days. This can be done in moist chambers containing dung or on soil plates, and makes the isolation of slower-growing Zygomycetes much easier.

Other methods likely to be useful in isolating Zygomycetes should either promote the growth of Zygomycetes, selectively inhibit specific fungi, or restrict the growth of most, if not all, fungi in a Petri dish.

A selective medium (MYAc) for the isolation of *Mucor* spp. has been devised by Bärtschi et al. (1991). MYAc contains ketoconazol at 50 µg/mL to inhibit anamorphic and teleomorphic Ascomycetes. *Actinomucor elegans* (Eidam) C.R.Benjamin & Hesseltine, *Mucor* spp., and *Rhizomucor pusillus* (Lindt) Schipper grew, but *Rhizopus stolonifer* (Ehrenberg: Fries) Vuillemin did not grow on MYAc; *Mortierella* and *Umbelopsis* were not tested. Strauss et al. (2000) have tested selective media for mucoralean fungi that contain benomyl and chloramphenicol. Hunter et al. (1977) used BMA to isolate Mucorales, and Dr. Edward E. Butler (pers. comm. 1993) recommended the use of PNB agar to isolate Mucorales from soil (Appendix 1). If a species of *Trichoderma* is present in the soil, it will often overgrow any mucoralean host and the merosporangiferous Mucorales if they are present. Selective inhibition of some of the faster growing fungi, such as species of *Trichoderma*, can be done using lithium chloride (Wildman 1991) or benomyl (20 mg/L). Surfactants (Steiner and Watson 1965), such as Tergitol NPX (NP-10), have been used to suppress rapidly growing fungi and appear to work better than either Oxgall or Rose Bengal. It is unknown, however, what effect lithium chloride or Tergitol NPX has on the growth of Zygomycetes, especially the merosporangiferous Mucorales. Benomyl does not appear to inhibit the species of *Piptocephalis* or *Syncephalis*, or the host fungi of the Mucorales, that have been encountered in the soil plates examined at a range of 5–20 p.p.m. Thiophanate-methyl is the current substitute for benomyl and it might prove to be a viable replacement for benomyl in selective culture media.

Many selective media for the isolation of fungi have been evaluated (Tsao 1970; Dhingra and Sinclair 1995). Especially useful are those media that contain antibiotics to inhibit bacteria. The author has used streptomycin sulfate (100 p.p.m.) and chlortetracycline hydrochloride (50 p.p.m.) routinely, and this formulation appears to be effective against most soil bacteria. Kannwischer and Mitchell (1981) used the antibiotics ampicillin and rifampicin to isolate *Phytophthora parasitica* Dastur from the roots and stems of plants. Other antibiotics or combinations of antibiotics may come into use in the future. Another culture medium used to inhibit bacterial growth is APDA (Tuite 1969; Guo and Michealides 1998); the author has found that APDA will promote zygospore formation in some cases.

Many other suitable culture media for isolation, growth, and sporulation of Zygomycetes are presented in publications that

are at least partially devoted to listing such formulations (Rawlins 1933; Raper and Thom 1949; Farrow 1954; Miller et al. 1957; Pridham et al. 1957; Tuite 1969; Tsao 1970; Stevens 1974; O'Donnell 1979; Johnston and Booth 1983; Smith and Onions 1983; Atlas 1993; Dhingra and Sinclair 1995; de Hoog et al. 2000; Samson et al. 2000; Bills and Foster 2004). Media formulations are also found in printed editions of the ATCC Fungi and Yeast Catalogue (Jong and Gantt 1987) or in the ATCC Media Handbook (Cote et al. 1984). The reader also is encouraged to make culture media from locally available chemicals and fruits and vegetables as done by Sideris (1931).

When the author's studies are complete on Dr. Benjamin's collections of *Coemansia*, *Syncephalis*, and Dimargaritales they will be mailed to the Farlow Herbarium, Harvard University. The remainder of Dr. Benjamin's fungal herbarium, including the *Piptocephalis* collection and the thamnidiaceous Mucorales, are already housed at Farlow Herbarium.

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APPENDIX 1.

Culture media used by R. K. Benjamin, G. L. Benny, and others.

2% ME + 0.5% YE—Malt-yeast agar: malt extract, 20 g; yeast extract, 5 g; agar, 15 g; distilled water, 1 L (Benny et al. 1992; Benny and Schipper 1992).

2% MEA—2% Malt extract agar: malt extract, 20 g; agar, 20 g; distilled water, 1 L (Benny and Benjamin 1975).

5GY—5 Glucose-yeast extract agar: glucose, 5 g; yeast extract, 5 g; K_2HPO_4, 4 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; agar, 20 g; distilled water, 1 L (Kuzuha 1980).

10% YE—10% Yeast extract solution, filter sterilized, and stored at 4°C (Padhye and Ajello 1988).

APDA—Acetic potato dextrose agar: PDA [below] or PDA [Difco] + 3 to 5 drops of 25% lactic acid per L (based on Tuite 1969), or PDA [Difco] + 2.6 mL of 25% lactic acid (Guo and Michaelides 1998). I use PDA (Difco) + 6 drops of 50% lactic acid per L.

B230—Blakeslee's No. 230 agar: malt extract, 20 g; dextrose, 20 g; peptone, 1 g; agar, 20 g [30 g used for culture maintenance—Blakeslee et al. 1921]; distilled water, 1 L (Blakeslee et al. 1927).

B360—Blakeslee's No. 360 agar: B230 + replace water with dung decoction made as follows: horse dung [dried], 5 g—soak in 500 mL distilled water, filter, make volume to 1 L (Blakeslee et al. 1927).

B362—Blakeslee's No. 362 agar: whey powder, 20 g; dextrose, 10 g; agar, 20 g; distilled water, 1 L (Blakeslee et al. 1921, 1927; Blakeslee and Cartledge 1927).

B388—Blakeslee's No. 388 agar: malt extract, 60 g; dextrose, 10 g; agar, 20 g; distilled water, 1 L (Blakeslee et al. 1927).

B391—Blakeslee's No. 391 agar: dextrose, 30 g; agar, 20 g; dung decoction made as follows: horse dung [dried], 10 g—soak in 500 mL distilled water, filter, make volume to 1 L (Blakeslee et al. 1927).

BEA—Beef extract agar: beef, 210 g; water, 1 L—boil the meat until thoroughly cooked, filter, and make volume of broth to 1 L; agar, 15 g (Swartz 1934).

BMA—Benomyl-malt agar: benomyl, 25 mg; Blue Ribbon malt extract, 30 g; dextrose, 10 g; agar, 20 g; distilled water, 1 L (Hunter et al. 1977).

BPM—Bread-paper-malt extract agar: bread, 20 g; filter paper or powdered cellulose, 10 g; malt extract, 10 g; agar, 20 g; distilled water, 1 L; pH 5.5 (Leadbeater and Richardson 1963).

BWA—Beerwort agar: beerwort, diluted to ca. 3.5% sugars; distilled water, to make volume to 1 L; agar, 15 g; pH 7.0 (Schipper 1969).

C230—Christenberry's #230 agar: glucose, 20 g; maltose, 20 g; peptone, 1 g; agar, 30 g; distilled water, 1 L (Christenberry 1940).

CA—Carrot agar: carrots, 200 g—blended, boil 20 min in 500 mL distilled water, filter, make volume to 1 L; agar, 16 g (Chien and Hwang 1997).

CaA—Carrot extract agar: carrots peeled and thinly sliced, 100 g—heat 3 min in 300 mL distilled water in a microwave oven, filter, take supernatant and add distilled water to make 1 L; agar, 15 g (idea from Kurihara et al. 2000).

CDA—Cherry decoction agar: cherries, 200 g—wash, remove seeds, chop, boil in 500 mL distilled water, make supernatant to 1 L; agar, 15 g; pH 3.8–4.6 [pH should be above 5.0 for agar to solidify] (Schipper 1969).

CDY—Czapek-Dox agar [CZA, Difco] + yeast extract: CZA + yeast extract, 6.5 g; pH 5.0—adjust with HCl (Gams and Williams 1963).

CH—*Choanephora* agar: dextrose, 3 g; casamino acids [Difco], 2 g; KH_2PO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; thiamine HCl, 25 mg; agar, 20 g; distilled water, 1 L; pH 6.0 (based on Barnett and Lilly 1950, 1955).

CH3—*Choanephora* agar one-third strength: dextrose, 1 g; casamino acids [Difco], 1 g; KH_2PO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; thiamine HCl, 25 mg; agar, 20 g; distilled water, 1 L; pH 6.0 (based on Barnett and Lilly 1950, 1955).

CJA—Carrot juice agar: carrots, 10 g—blended and made into juice, filter, save supernatant; distilled water, to bring total volume to 1 L; agar, 17 g (Gruhn and Petzold 1991).

CM—Corn meal agar: yellow corn meal, 20 g—boil 10 min in 700 mL distilled water, filter and add distilled water to make 1 L, dextrose, 10 g; agar, 15 g; adjust pH to 6.0 (Benjamin 1958, 1959).

CMDD—Corn meal agar with dung decoction: CM + DD.

CMPY—Corn meal-peptone-yeast extract agar: CM + peptone, 10 g; yeast extract, 4 g (Benjamin 1958).

CM-S—Corn meal-steep agar: CM + corn steep liquor (Sigma), 5 mL [adjust pH to 6.0 with 1N NaOH] (Benny et al. 1992).

CSLA—Corn-steep liquor agar: corn steep liquor, 7.5 mL; sucrose, 10 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; KH_2PO_4, 4 g; $(NH_4)_2HPO_4$, 2 g; agar, 20 g; tap water, 1 L—adjust pH to 6.0 with 1N NaOH (Bawcutt 1983).

CSS—Corn-steep-sucrose agar: corn steep liquor, 7.5 mL; sucrose, 10 g; $(NH_4)_2HPO_4$, 2 g; KH_2PO_4, 4 g; $MgSO_4 \cdot 7H_2O$, 2.5 g; agar, 30 g; distilled water, 1 L; pH 5.5 (Leadbeater and Richardson 1963).

CZA—Czapek-Dox agar [Difco].

DCA—Dog Chow agar: Purina Puppy Chow, 10 g—boil 15 min in 100 mL distilled water, filter, and make supernatant to 1 L; maltose, 5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; K_2HPO_4, 0.25 g; peptone, 0.1 g; agar, 15 g.

DD—Dung decoction: horse dung [moist], 125 g; tap water, 1 L—soak 2–3 hrs, filter, make volume to 1 L; pH 6.0 or above 7.0 for *Pilobolus*—use in place of water for media needing DD) (Benny and Schipper 1992).

DEA—Dung extract agar: horse or rabbit dung [dry], 200 g—soak in 1 L of distilled water, filter, make supernatant to 1 L; agar, 13 g (idea from Hu et al. 1989).

dHSA—Defatted hemp seed agar: hemp seed, 40%, blended, boil in distilled water 30 min, filter through 8 layers of cheese cloth, centrifuge 5 min, remove oil in a separating funnel, dilute extract 20:1 with distilled water, 1 L; agar, 20 g (Gams et al. 1972).

GGY—Glycerol-glucose-yeast extract agar: glycerol, 10 g; glucose, 10 g; yeast extract, 5 g; KH_2PO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; agar, 20 g; pH adjusted to 6.8–7.0 (Srinivasan and Thirumalachar 1967).

HIA—Hay infusion agar: hay, 50 g; distilled water, 1 L, autoclave 30 min, filter, make supernatant to 1 L, agar, 15 g; pH 6.2—adjust with K_2HPO_4 (Gams et al. 1975).

HSA—Hemp seed agar: hemp seed, 10 g, blended in 100 mL distilled water, autoclave 10 min, filter through cheese cloth; add 100 mL filtrate to 900 mL distilled water; agar, 20 g (Kuhlman 1972).

IAM—Iron-ascorbate medium: sodium acetate, 10 g; $(NH_4)_2SO_4$, 0.66 g; K_2HPO_4, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; thiamine HCl, 10 mg; $CaCl_2$, 1 mg; $ZnSO_4$, 200 µg; $CuSO_4$, 100 µg; H_3BO_3, 100 µg; MoO_3, 100 µg; $MnCl_2$, 100 µg; distilled water, 1 L—pH 7.7 using 0.1 M NaOH before autoclaving; agar, 20 g; filter sterilize the following and add after media is cool enough to be poured—$FeSO_4$, 0.1 mmol; ascorbic acid, 2 mmol (Bourret 1982).

LA—Leonian's agar: malt extract, 6.25 g; peptone, 0.625 g; maltose, 6.25 g; KH_2PO_4, 1.25 g; $MgSO_4 \cdot 7H_2O$, 1.25 g; agar, 15 g; distilled water, 1 L (Leonian 1924; Benny and Blackwell 2004).

LFK—Liver agar: per 100 × 15 mm glass Petri dish—water agar with K_2HPO_4. 0.4%; and agar, 2%, 25 mL; 10% KOH, 2 drops; hot lamb fat, 2 drops; sterile baby beef liver, 5 mm^3 piece in middle of plate; pH 7.5–8.0 (Ellis 1963; Ellis and Hesseltine 1962).

LMEA—Linnemann's malt extract agar: malt extract, 30 g; agar, 18 g; distilled water, 1 L (Linnemann 1958).

LYE—Leonian's agar + yeast extract: malt extract, 6 g; yeast extract, 1 g; peptone, 0.6 g; maltose, 6 g; KH_2PO_4, 1.2 g; $MgSO_4 \cdot 7H_2O$, 1.2 g; agar, 15 g; distilled water, 1 L (Malloch and Cain 1971; Benny and Benjamin 1975).

MA—Miura agar: glucose, 1 g; KH_2PO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; KCl, 0.2 g; $NaNO_3$, 2 g; yeast extract, 0.2 g; agar, 15 g; distilled water 1 L (Degawa and Tokumasu 1998*b*; Sugiyama et al. 2003—no KH_2PO_4; yeast extract, 2 g; agar, 13 g).

MEA—Malt extract agar: malt extract, 20 g; agar, 20 g; distilled water, 1 L (Sugiyama et al. 2003).

MEAS—Schipper's malt extract agar: malt extract, 20 g; glucose, 35 g; agar, 15 g; distilled water, 1 L; pH 7.0 (Schipper 1984).

ME-P—Malt extract-peptone agar: malt extract, 20 g; peptone, 5 g, agar, 15 g; distilled water, 1 L (Richard K. Benjamin pers. comm. 1988).

MEYE—Malt extract-yeast extract agar: malt extract, 3 g; yeast extract, 3 g; peptone, 5 g; dextrose, 10 g; agar, 15 or 20 g; distilled water, 1 L (Benjamin 1958, 1959; Benny and Benjamin 1975).

MEYE/2—Malt extract-yeast extract agar one-half strength: malt extract, 1.5 g; yeast extract, 1.5 g; peptone, 2.5 g; dextrose, 5 g; agar, 15 g; distilled water, 1 L (Kurihara et al. 2001).

MMN—MMN agar of Marx [1969] without malt extract; glucose, 2.5 g; thiamine HCl, 25 µg; micronutrient solution, 2 mL [$Fe(NO_3)_3 \cdot 9H_2O$, 1.45 mg; $ZnSO_4 \cdot H_2O$, 0.88 mg; $MnSO_4 \cdot H_2O$, 0.41 mg—dissolve in 700 mL distilled water, clarify with H_2SO_4, make volume to 1 L]; no supplements (Molina and Palmer 1982).

MMNs—Modified Melin Norkrans [Marx 1969] + supplements: malt extract, 3 g; d-glucose, 10 g; $(NH_4)_2PO_4$, 0.25 g; KH_2PO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.15 g; $CaCl_2$, 0.05 g; $FeCl_3$ 1% solution, 1.2 mL [or sequestrene, 0.02 g]; NaCl, 0.025 g; distilled water, to make 1 L; pH 5.5–5.7 after autoclaving; supplemented with: biotin, 25 µg; thiamine HCl, 100 µg; n-inositol, 10 µg; agar, 15 g (Berch and Fortin 1983*a*).

MMT agar—Moyer's multiple treat agar: potato infusion, 500 mL [boil 200 g peeled Russet potatoes in 500 mL distilled water, filter, and adjust volume to 500 mL]; molasses (unsulfured), 5 g; peptone, 1 g; white Karo syrup, 20 g; M salts, 10 mL [$MgSO_4$, 0.25 g/L; KH_2PO_4, 0.30 g/L; NH_4NO_3, 2.25 g/L]; Fe tartrate solution, 2 mL [concentration: 5 mg/mL]; Zn stock, 0.5 mL [$ZnSO_4 \cdot 7H_2O$, 22 mg = 5 mg Zn++/mL]; $CaCO_3$ [powdered], 4 g; agar, 20 g; distilled water, 1 L (Cutler and Swatek 1969).

MMTDD—MMT-dung decoction agar: MMT agar with distilled water, 500 mL + DD—made with distilled water, 500 mL.

MP5—Maltose-peptone agar No. 5: maltose, 3 g; meat peptone, 1 g; agar, 20 g; distilled water, 1 L (Couch 1939).

MSMA—Modified synthetic *Mucor* agar: dextrose, 10 g; $NaNO_3$, 4 g; K_2HPO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; thiamine HCl, 0.5 mg; agar, 15 g; distilled water, 1 L (Benny and Benjamin 1975).

MYAc—Malt-yeast agar with chloramphenicol: malt extract, 20 g; yeast extract, 2 g; agar, 15 g; distilled water, 1 L; chloramphenicol, 500 µg/mL; ketoconazole, 50 µg/mL—1% w/v in 95% ethanol [filter sterilize] (Bärtschi et al. 1991).

MYP—Malt extract-yeast extract-peptone agar: malt extract, 7 g; yeast extract, 0.5 g; peptone, 1 g; distilled water, 1 L; agar, 15 g; penicillin G, 0.5 g; streptomycin sulfate, 0.5 g (Carreiro and Koske 1992).

MWA—Mealworm agar: dried, ground, mealworms (*Tenebrio molitor*), 200 g—boil 3 hr in 500 mL distilled water, filter, make supernatant to 1 L; agar, 20 g (Samson 1974).

NsCM—Nonsupplemented corn meal agar: yellow corn meal, 25 g, cook 10 min in 700 mL distilled water; filter, keep supernatant and make to 1 L of distilled water; agar, 20 g (Benjamin 1962).

OMA—Oat meal agar: rolled oats, 30 g; distilled water, 1 L—heat to boiling and simmer 2 hr, filter, and bring volume to 1 L; agar, 15 g (Gams et al. 1975).

PAB—Pablum agar: pablum, 50 g—boil in 700 mL distilled water, filter, adjust final volume to 1 L; agar, 15 g (Benjamin 1959).

PABA—Dilute Pablum agar: Pablum cereal, 12.5 g cooked 10 min in 175 mL distilled water, filter through cheese cloth; distilled water, to make 1 L; agar, 15 g (Kuhlman 1972).

PAB-DEX—Pablum-dextrose agar: PAB + dextrose, 10 g (Benjamin 1959).

PBM—Page's basal medium: L-asparagine, 7.55 g; sodium acetate anhydrous, 6.03 g; hemin, 10 mg—dissolve in 37.5 mL N/10 NaOH, thiamine HCl, 10 mg; KH_2PO_4, 1.0 g; K_2HPO_4, 1.2 g; $MgSO_4 \cdot 7H_2O$, 1.0 g; NaCl, 0.1 g; $CaCl_2$, 0.1 g; micronutrient solution, 1.0 mL; agar, 20 g; distilled water to bring volume to 1 L (Page 1960).

PCA—Potato-carrot agar: potatos, peeled and diced, 20 g; carrots, peeled and diced, 20 g—boil carrots and potatoes in 300 mL of tap water, filter, and add water to adjust volume to 1 L; agar, 20 g (Bawcutt 1983).

PDA—Potato dextrose agar: potatoes, peeled and cut, 200 g—boil extract 10 min in 700 mL distilled water, filter, adjust final volume to 1 L; dextrose, 20 g; agar, 15 g (Schipper 1969—pH 6.6; Benjamin 1958, 1959—pH not mentioned).

PG—Peptone-glucose agar: peptone [Difco], 10 g; dextrose, 20 g; agar, 20 g; distilled water, 1 L (Gauger 1961).

PGB—Peptone-glucose broth: peptone, 20 g; glucose, 20 g; KH_2PO_4, 1.0 g; $MgSO_4 \cdot 7H_2O$, 1.0 g; distilled water, 1 L (after Jeffries and Kirk 1976).

PMG—Peptone-malt extract-glucose agar: peptone, 1 g; malt extract, 20 g; glucose, 20 g; agar, 25 g; distilled water, 1 L; pH 4.0–5.0 (Ellis and Hesseltine 1962).

PNB—Potato-novobiocin-benomyl agar: potatoes, peeled and cut, 200 g—boil extract in 700 mL distilled water, filter, adjust final volume to 1 L; dextrose, 10 g; agar, 15 g; distilled water, 1 L; benomyl, 20 mg; novobiocin, 100 mg [latter two heat stable], pH 5.6–6.0 [critical]—put a small amount of soil near edge of Petri dish or use dilution plate method (Edward E. Butler pers. comm. 1993).

Prune—Prune decoction agar: prunes, 200 g—wash, remove seeds, chop, boil in 500 mL distilled water, make supernatant to 1 L; agar, 15 g; pH 3.8–4.6 [pH should be above 5.0 for agar to solidify] (Schipper 1969).

PYED—Peptone-yeast extract-dextrose agar: peptone, 1 g; yeast extract, 1 g; dextrose, 0.5 g; agar, 15 g; distilled water, 1 L; adjust pH to 6.5 (Benjamin 1978).

PYEDS—Peptone-yeast extract-dextrose-corn steep agar: PYED + corn steep liquor, 5 mL; adjust pH to 6.0 with 1N NaOH.

RFA—Rabbit food agar: rabbit ration, 25 g—boil in 1 L of distilled water and allow to sit 30 min, filter, save supernatant, make volume to 1 L; agar, 15 g (Roxon and Batra 1973).

RS—Rape seed agar: rape seed [*Brassica napus* L.], 100 g, thoroughly washed; distilled water, 1 L, boil seeds for 30 min, filter, and make volume to 1 L; agar, 20 g (Satour 1967).

SA—Sato and Aoki's agar: KNO_3, 0.2 g; $MgSO_4 \cdot 7H_2O$, 0.02 g; KH_2PO_4, 0.1 g; K_2HPO_4, 0.3 g; $NaHCO_3$, 0.02 g; Na_2SiO_3, 0.02 g; agar, 20 g; distilled water, make volume to 1 L; unadjusted pH 7.8 (Saikawa and Kadowaki 2002).

SABD—Sabauroud-dextrose agar (Difco)

SDM—Sawdust medium [in test tubes]: beech [*Fagus crenata* Blume] sawdust, 1.5 g; rice bran, 0.45 g; distilled water containing 10% glycerol, 3.5 mL, and adjust water content to 65%—inoculate sterile tubes with a 3 mm^3 piece of agar from a 7-day-old plate of PDA containing the culture, incubate the tubes at 25°C for 28 days (Kitamoto et al. 2002).

SDY—Soil-dextrose-yeast extract agar: loam soil extract, 100 g—boil 10 min in 800 mL distilled water, filter, make volume to 1 L; dextrose, 2 g; yeast extract, 1 g; K_2HPO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; agar, 15 g.

SEA—Soil extract agar: garden soil and distilled water, equal weights, autoclave 30 min, let soil settle to bottom of flask, filter; agar, 15 g to 1 L of soil extract (Gams et al. 1975).

ShA—Shrimp agar: dried ground edible shrimp, 3 g; agar, 15 g; distilled water, 1 L (Degawa and Tokumasu 1997, 1998*a*,*b*).

SHM—Simplified hemin medium: hemin, 10 mg—dissolve in 37.5 mL N/10 NaOH; sodium acetate [$CH_3COONa \cdot 3H_2O$], 10 g; thiamine HCl, 10 mg; $(NH_4)_2SO_4$, 0.66 g; K_2HPO_4, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; agar, 15 g; distilled water to bring volume to 1 L (Levetin and Caroselli 1976).

SLM—*Syncephalis* liver medium: baby beef liver, 0.5 cm^2—wash in 3 changes of distilled water, drain, sterilize and place in the center of a sterile 15 × 100 mm Petri dish; add 20 mL of sterile medium containing K_2HPO_4, 0.4%; tryptone [Difco], 0.1%; agar, 2% (Ellis 1966).

SMA—Synthetic *Mucor* agar: dextrose, 40 g; asparagine, 2 g; KH_2PO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; thiamine HCl, 0.5 mg; agar, 15 g; distilled water, 1 L (Hesseltine 1954).

TKY—Tryptone-potassium-yeast extract agar: tryptone, 5 g; K_2HPO_4, 3 g; yeast extract, 5 g; add KOH to bring pH to 9.0; distilled water, 1 L; agar, 20 g; autoclave, pour media in glass Petri dishes and when cool inoculate with *Bacillus cereus* var. *mycoides* and incubate for 20 hr at 25°C; autoclave this culture, cool and solidify, and then streak spores of *Rhopalomyces elegans* Corda (Ellis and Hesseltine 1962).

TPO—Tomato paste-oatmeal agar: tomato paste, 20 g; instant baby oatmeal, 20 g; agar, 15 g; distilled water, 1 L (Hesseltine 1960; Benny and Benjamin 1975).

TSM—Thorton's standardized medium: K_2HPO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $CaCl_2$, 0.1 g; NaCl, 0.1 g; $FeCl_3$, 0.002 g; KNO_3, 0.5 g; asparagine, 0.5 g; mannitol, 1 g; agar, 15 g; distilled water, 1 L (Thorton 1922; Benny and Blackwell 2004).

V8—V8 juice agar [modified]: V8 juice, 5.5 oz can; adjust volume to 1 L with distilled water; $CaCO_3$ [powdered], 3 g; agar, 15 g (Benny and Benjamin 1991).

WA—Water agar: distilled water, 1 L; agar, 20 g (Saikawa and Kadowaki 2002).

Wg5—One-fifth strength wheat germ agar: wheat germ, 3 g—heat in a microwave oven 3 min in 500 mL of distilled water and then filter through cheese cloth, take the supernatant and add distilled water to adjust volume to 1 L; dextrose, 1 g; agar, 15 g.

Wg—Wheat germ agar: wheat germ, 15 g—boil 10 min in 700 mL distilled water, filter, and adjust volume to 1 L; dextrose, 5 g; agar, 15 g (Benny 1972).

WgB—Benjamin's wheat germ agar: wheat germ, 10 g—bring to a boil in 800 mL of distilled water and then filter through cheese cloth, take the supernatant and add distilled water to adjust volume to 1 L; dextrose, 1 g; agar, 15 g (Benjamin 1985*b*).

WgDD—Wheat germ-dung decoction agar: Wg, 500 mL + DD made with distilled water, 500 mL.

Wg-S—Wheat germ-steep agar: Wg + corn steep liquor, 5 mL; adjust pH to 6.0 with 1N NaOH.

Whey—Whey agar: powdered whey, 20 g; dextrose, 10 g; agar, 15 g; distilled water, 1 L (Schipper 1969; Benny et al. 1985).

WortG—Wort-glucose agar: wort agar [Difco], 50 g; glucose, 35 g; distilled water, 1 L (Benny and Benjamin 1991).

WSH—Weitzman and Silva-Hutner medium: alphacel [powdered cellulose], 20 g; $MgSO_4 \cdot 7H_2O$, 1 g; KH_2PO_4, 1.5 g; $NaNO_3$, 1 g; tomato paste, 10 g; baby oatmeal, 10 g; agar, 18 g; distilled water, 1 L; pH 5.6 (Weitzman and Silva-Hutner 1967).

WSHDD—Weitzman and Silva-Hutner-dung decoction agar: WSH, 500 mL + DD made with distilled water, 500 mL.

YEA—Yeast extract agar: yeast extract, 4 g; malt extract, 10 g; glucose, 4 g; agar, 15 g; distilled water, 1 L; pH 7.3 (Schipper 1984).

YGCH—Yeast extract-glycerol-casein hydrolysate agar: yeast extract, 10 g; glycerol, 15 mL; casein hydrolysate, 15 g; K_2HPO_4, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; agar, 15 g; distilled water, 1 L (O'Donnell et al. 1998).

YpD—YpSs-dextrose agar; YpSs + dextrose, 5 g (Benny and Benjamin 1975).

YpSs—Emerson's yeast-phosphate-soluble starch agar: soluble starch, 15 g; yeast extract, 4 g; K_2HPO_4, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; agar, 20 g [15 g used later; Benny and Benjamin 1975]; distilled water, 1 L (Benjamin 1959).

YpSs/5—One-fifth strength YpSs agar: soluble starch, 3 g; yeast extract, 0.8 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KH_2PO_4, 0.5 g; K_2HPO_4, 0.5 g; agar, 15 g; distilled water, 1 L (Benjamin 1985*a*,*b*).