9-1-1973

Nematosporaceae (Hemiascomycetidae): Taxonomy, Pathogenicity, Distribution, and Vector Relations

Lekh R. Batra

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Technical Bulletin No. 1469

Agricultural Research Service

U.S. DEPARTMENT OF AGRICULTURE

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Washington, D.C. Issued September 1973

For sale by the Superintendent of Documents, U.S. Government Printing Office
Washington, D.C. 20402 - Price $1.15
Stock Number 0100-02880

PREFACE

Most of the Nematosporaceae are destructive phytopathogens, which at times have made it impossible to grow cotton in some parts of the world. They are restricted to warmer parts of the world, although their vectors are of worldwide distribution. Three species occur in isolated endemic areas of the United States where they attack cotton, citrus, pecans, soybeans, and tomatoes. This publication is designed to aid in timely recognition and identification of pathological material of U.S. origin and to facilitate its disposal. It may also be useful to Agricultural Quarantine inspectors when intercepting similar material from abroad.

Figure 1.—Distribution of *Ashbya gossypii, Nematospora phaseoli, N. coryli,* and *N. lycopersici* (based on literature, specimens examined, or both). (The status of *N. phaseoli* as a species is doubtful.)

ACKNOWLEDGMENTS

Many colleagues helped with specimens or provided useful information. I wish to gratefully acknowledge the assistance of the following: T. Barksdale, P. B. Marsh, and M. E. Simpson of this Division; W. A. Clark, The American Type Culture Collection; the Director and M. B. Ellis, Commonwealth Mycological Institute; D. I. Ebbels, Ministry of Agriculture, Food and Co-operatives, The United Republic of Tanzania; K. Hefernann, Walter Reed Hospital, Washington, D.C.; R. Heim, Museum of Natural History, Paris; R. P. Korf, Cornell University; J. W. Kimbrough, University of Florida; W. H. Wheeler (retired), Agricultural Quarantine Inspection Division, Agricultural Research Service; and S. A. Wingard, Virginia Polytechnic Institute. R. C. Froeschner of the Smithsonian Institution checked names of hemipterous vectors and P. Diehl, my colleague, prepared table 1 and the distribution map (fig. 1).

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NEMATOSPORACEAE (HEMIASCOMYCETIDAE): Taxonomy, Pathogenicity, Distribution, and Vector Relations

By Lekh R. Batra, *research mycologist, Plant Protection Institute, Northeastern Region, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Md.*

INTRODUCTION

For almost a century *(42, 139)*[1] certain yeast, or yeastlike parasites of fruits and seeds have been known. They occur on a wide variety of crop plants and cause such diseases as "stigmatomycosis," "yeast-spot," "eye spot," and "internal rot." They seemingly represent a phylogenetic unit. They have cylindrical or pyriform asci, and the hyaline ascospores often are characteristically arranged in two fascicles lying end to end. All have acicular ascospores. (See figs. 2, *a-c*; 5, *c*; 11, *h* and *i*; 12, *A*; 13, *c-h*; 15, *j-k*.) They have similar habitats, and several species may simultaneously occur in the same fruit or seed. The majority are associated with punctures made by insects having piercing-sucking mouthparts. These fungi, along with some recently described *(122, 123, 143)* parasites of Crustacea, are now assembled in the family Nematosporaceae Novak and Zsolt.

The comparative morphology and taxonomy of the Nematosporaceae have been neglected in the past. They were placed in the catchall family Endomycetaceae Schröter *(45, 111, 159)* or Saccharomycetaceae *(37)* of the order Endomycetales. Gäumann *(64-66)* included them in the family Spermophthoraceae Guilliermond along with the monotypic *Spermophthora* Ashby and Nowell.

As proposed here, the family consists of five genera: *Ashbya* Guilliermond, *Eremothecium* Borzi, *Metschnikowia* Kamienski, *Nematospora* Peglion, and *Coccidiascus* Chatton (see a key to the genera on p. 8). The last-named genus is of doubtful affinity with the other members of the family. Except for its original description, very little is known about it.

The purpose of this paper is to characterize the family Nematosporaceae and to present diagnoses of the genera and species encountered on plants important to agriculture. I shall also discuss symptoms caused by species

[1] Italic numbers in parentheses refer to Literature Cited, p. 61.

pathogenic to beans, citrus, cotton, and tomatoes and evaluate their actual and potential danger in relation to their host range, geographic distribution, and their complex interrelations with the insect vectors. Two *Metschnikowia* spp., often found in floral nectar and isolated by insect microbiologists, are also treated.

SYSTEMATIC POSITION OF THE NEMATOSPORACEAE

The blastosporic, or cellular, nature of the thallus of some isolates of *Nematospora, Metschnikowia,* and *Coccidiascus* permitted their inclusion along with other ascosporogenous yeasts. Stelling-Dekker *(159)* was the first to bring together these fungi in the subfamily Nematosporoideae (Endomycetales: Endomycetaceae). This was accepted by Lodder and Kreger-van Rij *(112)* and Lodder *(111).* The genera *Ashbya* and *Eremothecium* found no place in their treatment of the cellular Hemiascomycetes because of their ". . .plurinucleate states. . ."*(98).*

Gäumann *(64-66),* considering the Hemiascomycetes to embrace the most primitively simple Ascomycetes, included *Nematospora, Ashbya,* and *Eremothecium* in the family Spermophthoraceae (Endomycetales) along with the monotypic *Spermophthora.* Three other families, Dipodascaceae, Endomycetaceae, and Saccharomycetaceae were also recognized for the order. He made no mention of *Coccidiascus.*

Spermophthora gossypii is a rare fungus that has thrice been reported to occur in association with the Nematosporaceae causing stigmatomycosis. Presumably it is also transmitted by hemipterous insects although definite proof for this assertion is lacking. Guilliermond *(71, 72)* investigated the species in detail and found it to be merogamous and diplobiontic. The gametophyte is a coenocyte, but the ascigerous thallus, or the sporophyte, is septate and has uninucleate cells. Guilliermond maintained the fungus in pure culture for many years, but soon after isolation it lost its mating ability. According to him *(72),* the gametes germinated without fusion and gave rise directly to gametophyte. These characteristics lead me to believe that *Spermophthora* is well placed as the sole member of the Spermophtoraceae, as adapted by Bessey *(37),* and that the yeastlike fungi with nematosporic ascospores should not be included in the family, as undertaken by Gäumann *(64-66).*

Bessey *(37)* believed the Hemiascomycetes to represent ". . .The ultimate degree of simplification in the Class Ascomycetes." He placed all the Nematosporaceae, as recognized here, in the Saccharomycetaceae, order Saccharomycetales *(Endomycetales sensu* Gäumann *(64-65)).* This is the only order he recognized for the entire group Hemiascomycetes. In addition to the Saccharomycetaceae, he recognized three additional ascosporogenous families: Endomycetaceae, Spermophthoraceae (monotypic), and Pericystaceae (monotypic). The last-named family includes the well-known "chalk-brood" fungus, Ascosphaera, which now has been shown to have ascogenous hypae and thus is a member of the Euascomycetes.

On the basis of comparative morphological, caryological, and nutritional studies of several genera of the Hemiascomycetes, the writer recently published *(33)* an abstract of a classification of the group. To correlate the position of Nematosporaceae with the other taxa of the group, the writer has reproduced here the substance of this classification, with minor changes. Some of the genera, families, and orders presented here undoubtedly include anomalous taxa within their limits because of gaps in knowledge about their biology. Moreover, the true relation of the order Taphrinales to the other Hemiascomycetes is still somewhat debatable.

Order Spermophthorales

Merogamous, diplobiontic, the two thalli filamentous and usually alternating with each other, fermentation negative.

There is but one family, the Spermophthoraceae Guilliermond [Revue Gen. Bot. 40:414. 1928] with the monotypic genus *Spermophthora (27, 71, 72).*

Order Dipodascales

Haplobiontic but with a characteristic uninucleate diploid mycelium formed as a result of gametangial copulation and remaining attached to the parent hypha, asci borne in metulae on distinct ascophores, asexual reproduction by blastoconidia, fermentation absent.

There are two families in this order: (1) The Dipodascaceae Engler and Gilg [Syllabus der Pflanzenfamilien, p. 59, 1924] which includes *Dipodascus* Lagerheim *(30), Endomyces* Reess *(112),* and *Schizosaccharomyces* Lindner *(111, 112).* (2) The Eremascaceae Engler and Gilg [Syllabus der Pflanzenfamilien, p. 59, 1924] with *Eremascus* Eidam and a new genus yet to be proposed.

Order Cephaloascales

Haplobiontic or diplobiontic, thallus filamentous or cellular, asexual reproduction by thin- or thick-walled blastoconidia, sexual reproduction by ascosporic or somatic copulation, gametangia occasionally present.

There is but one family, the Cephaloascaceae Batra[2] with the sole representative *Cephaloascus fragrans* Hanawa *(55, 155).*

Order Ascoideales

Haplobiontic, plants usually haploid, rarely diploid (for example, *Endomyces decipiens* Reess), thallus filamentous or cellular, asexual reproduction by arthroconidia or lacking, sexual reproduction by gametangial copulation, fermentation positive or negative.

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[2] Cephaloascaceae Batra, fam. nov. (Cephaloascales): Hemiascomycetidae. Mycelium septatum, cellulis uninucleatis; reproductio agamica e blastoconidiis madidis; reproductio sexualis gametangiogamica, cellula conjungente in ascophorum filamentosum fasciculos ascorum ad apicem ferens inflata; asci 1-4 spori, in metulis enati. Typus *Cephaloascus* Hanawa, Japan. J. Dermatol. Urol. 20: 14. 1920.

There are three families in this order: (1) Ascoideaceae, with the genus *Ascoidea* Brefeld and Lindau *(31, 32, 34, 35).* (2) Nematosporaceae, with the genera *Nematospora (45), Ashbya (27, 71, 146), Metschnikowia (97, 122, 123, 143), Eremothecium (41, 72-74),* and *Coccidiascus (46).* (3) Saccharomycetaceae, which includes most of the ascosporogenous yeasts characterized by multipolar or bipolar buddings (*98, 111, 112*).

ECONOMIC IMPORTANCE

Two of the five genera of the Nematosporaceae, *Ashbya* and *Eremothecium,* are major sources of commercial riboflavin. They also produce other flavinoids. However, these two genera and *Nematospora* spp. are also dangerous pathogens of cotton. They make it virtually impossible to grow this crop in certain parts of the world *(93).*

Nematosporaceae also attack the seeds and young fruits of other important crops such as beans, citrus, coffee, and tomatoes. They attack many other plants visited by their vectors, either simultaneously or during the periods when the aforementioned crops are out of season. A complete host range of the Nematosporaceae is given in table 1, p. 44. Recent estimates of damage are not known. However, a few examples of the extent and nature of losses are reviewed in the literature.

Peglion *(139, 140)* reported heavy spoilage of hazelnuts and in one case in Italy 25 percent of these nuts were destroyed by *Nematospora coryli* Peglion. It has been observed *(26, 27, 130-133)* that losses to cotton caused by *Ashbya, Nematospora,* and *Eremothecium* in the British West Indies ran as high as 20 percent for the early picking season and increased to almost 100 percent for the late picking.

Similar severe losses to the cotton crop were annually reported between 1927 and 1944 from many African countries *(2, 6, 9, 19, 76, 104).* Stigmatomycosis remained the most important cotton disease in Uganda and Rhodesia *(2, 76)* for several years.

Severely damaged bolls or locks are not picked, and the bolls with stained lint are picked separately and sold at a much reduced price. Since stigmatomycosis fungi enter the embryo *(50),* attacked seeds have low rate of germination *(88).* In India and Pakistan I observed that sites of stigmatomycosis in locks serve as nuclei for damage by secondary cellulose-decomposing fungi. Heavy losses in cotton due to Nematosporaceae have been reported also from Iraq *(23, 137),* Rhodesia *(93),* and other parts of the world.

Some Nematosporaceae are pathogens of invertebrates. Two species of *Metschnikowia* kill *Daphnia magna* (see p. 34), but two other species are apparently beneficial to the bees and serve as a source of nutrients. *Nematospora* (?) *coryli* was recently isolated at the National Communicable Disease Center, Kansas City, Mo., from the sputum of a patient from Texas (personal communication, J. W. Brandsberg). This is the only report of the fungus having been isolated from any warmblooded animal. Durleux *(58)* equates *Sargentella* sp., a pathogen of human urinary tract, with *Ashbya*

gossypii, but the evidence presented to support the conclusion is inadequate. His report has little merit from the etiological point of view.

MATERIAL AND METHODS

Pathogenic or plant-inhabiting saprophytic Nematosporaceae were readily isolated from the aseptically removed diseased tissues of fruits and seeds previously disinfested in 0.5 percent aqueous sodium hypochlorite or 95 percent ethanol. A cell suspension from treated macerated tissue was streaked on Difco malt extract agar containing 0.3 percent yeast extract. *Nematospora, Eremothecium,* and *Metschnikowia* were isolated in this manner.

To discourage bacterial contamination it was occasionally necessary to add to the previously autoclaved medium a mixture of penicillin and streptomycin, to make a final concentration of 6 to 10 p.p.m. of the antibiotics. Since the Nematosporaceae can tolerate low pH values, bacterial contamination was also reduced or eliminated by adjusting the pH of the medium to between 4 and 4.5.

Sources of Living Cultures

1. *Ashbya gossypii* (Ashby and Nowell) Guill. Isolated on November 11, 1968, (2,295-Batra) from *Citrus nobilis* Lour. having originated in Florida.
2. _____. Isolated on October 26, 1970, (2,553-Batra) from *Gossypium hirsutum* L., mailed by Mrs. T. H. Bracken from Charleston, S.C.
3. _____. Received from American Type Culture Collection (ATCC-8,717), who in turn received it from W. J. Robbins on September 2, 1942, isolated from cotton, (?) from the United States.
4. _____. ATCC-10,895, from W. J. Robbins, source unknown.
5. *Eremothecium ashbyii* (Guill.) Batra. Isolated on April 10, 1970, from *Citrus sinensis* (L.) Osbeck, cultivar Jaffa, supposedly having originated in Israel.
6. _____. ATCC-6,747 and ATCC-12,995, information on host or locality for the two cultures unknown to the donor.
7. *Nematospora coryli* Peglion. Isolated on November 17, 1966, (2,067-Batra) from osage orange, *Maclura pomifera* (Raf.) Schneid. infested with Nitidulidae. Lawrence, Kans.
8. _____. Isolated on August 25, 1968, (2,557-Batra) from soybean, *Glycine max* (L.) Merr., Beltsville, Md.
9. _____. Isolated on December 10, 1969, (2,558-Batra) from *G. max,* Gainesville, Fla.
10. _____. ATCC-10,648, originally named as *N. nagpuri* Dastur and Singh and isolated by the authors in 1929 from cotton growing in Nagpur, India.
11. _____. ATCC-10,661, source unknown, initially determined as *N. phaseoli* Wingard.
12. _____. ATCC-10,647 (=CBS-2,608), original source unknown.

13. *N. lycopersici* Schneider. Isolated on September 20, 1970, (2,518-Batra) from tomatoes, *Lycopersicum esculentum* Mill., cultivar Roma, Plant Introduction No. 272,636, collected during late June 1970 by T. Barksdale, Charleston County, S.C.

14._____. Isolated August 25, 1968, (2,560-Batra) from *G. max*, Beltsville, Md.

15. *Metchnikowia pulcherrima* Pitt and Miller. Isolated on August 14, 1969, (2412A-Batra) from floral nectar of alfalfa, *Medicago sativa* L., Murtaugh, Idaho.

16._____. ATCC-18,406, a subisolate of the type, isolated from *Vitis labrusca* L., Walnut Creek, Calif.

17. *M. reukaufii* Pitt and Miller. Isolated on August 5, 1968, (2,296-Batra) from spoiled pollen balls of the subterranean bee *Nomia melanderi* Cockrell, Logan, Utah.

18._____. ATCC-18, 407, subisolate of the type, isolated from a flower of *Epilobium angustifolium* L., Fort Smith, North West Territories, Canada.

Maintenance and Storage of Cultures

Most of the cultures were maintained on yeast extract malt extract agar slants stored at 4° to 5° C., and their nutritional characteristics were investigated soon after the initial isolations. They were transferred to fresh medium about every 6 months. The cultures were also freeze dried under vacuum and stored at 4° to 5° C. to minimize loss or impairment of biological characteristics. Each of the new isolates is being deposited with the American Type Culture Collection and with Centraalbureau voor Schimelcultures.

Determination of Nutritional Characteristics

Morphological and nutritional characteristics of the cultures were studied principally by the methods described by Wickerham *(193)* and Lodder and Kreger-van Rij *(112)*. All cultures, unless otherwise indicated, were incubated at 25° C. (± 0.5) in a well-ventilated, thermostatically controlled incubator. The incubator was not illuminated, and the relative humidity was unregulated.

Colony characteristics were observed in plastic petri dishes containing about 25 ml. of yeast extract malt extract agar. The seed inoculum consisted of one loopful of a mixture of cell and ascospore suspension spread in the middle of the dish over a circular area 4 to 5 mm. in diameter. Growth measurements of such colonies are linear and no consideration was given to thickness or height. All isolates sporulated readily on yeast extract malt extract agar, except *Metschnikowia* spp. for which V-8 agar and Difco cornmeal agar were used (*123*).

Fermentation, carbon and nitrogen assimilation, and vitamin utilization tests were conducted on Difco basal media, by the techniques of Wickerham *(193)*. The splitting of arbutin was tested according to the method of Lodder and Kreger-van Rij *(112)*.

Inoculation Experiments

Fruits (beans, citrus, cotton, and tomatoes) to be inoculated were disinfested either with 95 percent ethanol or with 0.5 percent aqueous sodium hypochlorite. Several small drops of inoculum suspension (ascospores, blastoconidia, or yeast cells) were aseptically deposited on the disinfested surfaces. The fruits were then punctured through the inoculum with a fine sterile glass needle. When the needle was removed, some inoculum was apparently sucked into the fruits. Nearly mature tomato fruits when punctured often exuded sap, which mixed with the drop of inoculum. The liquid dried within 30 minutes to an hour and left a shiny, somewhat sticky surface. Except for beans, the fruits were also inoculated with a 26-gage hypodermic needle. Appropriate controls—sterile distilled water was used in place of inoculum—were maintained.

Inoculated fruits and the controls were incubated at room temperature 25° C. (± 3) or in incubators maintained at 25 and 30. The beans were incubated in a moist chamber. The stalks of detached cotton bolls were kept immersed in tubes containing water; whereas, citrus and tomatoes were in containers without any accessory source of moisture. The age, source, and other characteristics of the fruits used in the experiments are discussed elsewhere in this paper.

Microscopic Examination and Measurements

Considerable information on the morphology, host range, and geographic distribution of the Nematosporaceae is based on herbarium specimens and some symptoms are also inferred from such specimens. The herbarium specimens to be examined microscopically were soaked in 95 percent ethanol for about a minute to remove air and then soaked for 5 to 10 minutes in 3 percent aqueous KOH. They were rinsed twice in distilled water and stained in lactophenol containing cotton blue or acid fuchsin.

Fresh specimens were directly transferred to the staining solution, that is, without treatment with ethanol and KOH.

Asci, ascospores, and vegetative cells of fresh specimens were measured in distilled water. The herbarium specimens were likewise measured in water after the ethanol and KOH treatments.

Abbreviations of Herbaria

The names of herbaria are abbreviated according to Lanjouw and Stafleu (*101*). The herbaria frequently mentioned in the text are: ATCC, The American Type Culture Collections, Rockville, Md.; BPI, The National Fungus Collections, U.S.D.A., Beltsville, Md.; CBS, Centraalbureau voor Schimmelcultures, Baarn, Netherlands; CMI, Commonwealth Mycological Institute, Kew, England; FLAS, Hebarium, Agricultural Experiment Station, Gainesville, Fla.

TAXONOMY AND LIFE CYCLE OF NEMATOSPORACEAE NOVAK AND ZSOLT

Thallus cellular or filamentous, uninucleate or multinucleate; asexual reproduction by blastosporic cells, thick-walled, resting or nonresting chlamydospores also present; asci terminal or intercalary and arising from thallus or from proasci, one- to many-spored, usually deliquescent in the middle; ascospores elongate, pointed at one or both ends, hyaline, with or without a flagellate appendage.

Transmitted by hemipterous insects, parasitic on plants, on crustacea or saprophytes. Type genus: *Nematospora* Peglion nec. *Nematospora* Tassi.

The genera of the family Nematosporaceae are distinguished on the basis of the shape of ascospores, the presence or the absence of proasci, and the behavior of the conjugant cell after caryogamy.

A Key to the Nematosporaceae on Crop Plants of Economic Importance

1. Ascospores needle-shaped 2
1. Ascospores sickle-shaped or bent ..... *Eremothecium* p. 10
   2. Thallus filamentous, coenocytic, sprout cells absent or rare, asci intercalary ..... *Ashbya* p. 17
   2. Thallus cellular-colonial or occasionally filamentous and septate, sprout cells present, asci free-floating or terminal 3
3. Ascospores with a flagellum-like cytoplasmic appendage and with two distinct uninucleate protoplasts, proasci thin-walled, non-refractive ..... *Nematospora* p. 22
3. Ascospores without an appendage and with 1 uninucleate protoplast, proasci thin- or thick-walled, highly refractive. . *Metschnikowia* p. 33[3]

Life Cycle

The Thallus

The genera *Ashbya* and *Eremothecium* are filamentous. Both are sparingly septate, and the hyphal cells (stained with haematoxylin) are multinucleate. Both also form blastospores under nutritional stress. *Ashbya gossypii* (CMI-26,138) from cotton and *E. ashbyii* from Jaffa orange produced blastosporic cells in their respective hosts. Both these fungi produce copious quantities of yellow pigmentation associated with riboflavin and

[3] *Coccidiascus* Chatton, for which no material was available, keys out along with *Metschnikowia* in lead 3. However, the former fungus has flattened rather than cylindrical ascospores.

some related compounds. These compounds are present in hyphal cells as aggregates of long, amorphous crystals.

The remaining three genera *(Nematospora, Metschnikowia,* and *Coccidiascus)* are usually cellular and may be considered as true ascosporogenous yeasts. However, true hyphae and pseudomycelium are often accompanied by a cellular thallus in *Nematospora.* Some isolates routinely produce filaments, and others are predominantly cellular. Vegetative cells of *Nematospora* (stained with haematoxylin) may have 1 to 10 nuclei, but those of *Metschnikowia (123, 124)* have only one nucleus. Colonies of *Metschnikowia pulcherrima* are reddish brown, but all other species of this genus and *Nematospora* are white, cream, or pale yellow.

Asexual Reproduction

The blastosporic cells are formed in all the five genera of the family. In addition to these spores, thick-walled, nonresting "chlamydospores" (proasci) are also present in *Metschnikowia (122, 123, 194).* Both types of reproductive structures are somewhat glutinous and presumably are suited for dissemination by contact rather than by wind.

Sexual Reproduction

The sexual reproduction is apomictic, and distinct gametes or gametangia are lacking in the Nematosporaceae. In *Nematospora* and *Ashbya* the ascospores act as gametospores. They are morphologically and functionally similar in these two genera. They are spindle-shaped and have a long, fine, flagellate cytoplasmic appendage of variable length at one end. This end has been traditionally referred to *(69, 70)* as the posterior end even though the appendage is nonmotile. The ascospores are binucleate, each nucleus usually lying toward the pole.

In *Nematospora* each of the two nuclei resides in a separate protoplast but without an intervening wall between them. Most of the anterior protoplast migrates into the now enlarging posterior half of the ascospore, the two nuclei fuse, and a zygotic wall is laid around the conjugant protoplast (see figs. 11, *b* and 13, *g*). Without any apparent resting period the zygote forms buds, or proasci. It may form filamentous thallus or may directly enlarge into an ascus.

The two nuclei in the ascospore of *Ashbya* are also contained in two separate protoplasts. As in *Nematospora,* the nuclei migrate to the center, fuse, and a zygotic wall is laid around the conjugant protoplast. The zygote soon enlarges and germinates by a germ tube (see fig. 7, *f*), with the awllike remnants of the spindle-shaped ascospores still attached to the developing vegetative hyphae.

Metchnikowia species may be heterothallic or homothallic *(123, 193).* Presumably mating takes place between two blastosporic cells. The asci originate as buds of thick-walled, globose or ellipsoid proasci or from similar thin-walled, presumably diploid cells. The nuclear cycle and the origin of the proasci are not well understood.

The intercalary sporiferous cells of *Eremothecium ashbyii* and similar terminal cells of *E. cymbalariae* are referred to as sporangia by some *(71)* and asci by others. The nuclear cycle of these fungi is still enigmatic. Neither the nuclear fusion nor meiosis has been observed in either species. The rather definitive number of spores, the mode of their delimitation by free cell formation, and their characteristic aggregation in two fascicles, or bundles, (simulating *Nematospora* spp.) lead one to interpret the sporiferous sacs as hemiasci (see also p. 17).

Eremothecium

Eremothecium Borzi, Nuovo Gior. Bot. Ital. 20:455. 1888.
= *Crebrothecium* Routien, Mycologia 41:183. 1949.

Vegetative cells multinucleate, sausage-shaped, and constricted near septa; conidia, or spout cells, present or absent; asci terminal or intercalary, cylindrical or flask-shaped, many-spored; ascospores acicular or semilunate-shaped (that is, lunate with one end blunt), uninucleate, with or without a cytoplasmic appendage, usually germinating in the middle. The ascospores are liberated by deliquescence of the equatorial region of the ascus.

Type species.—*E. cymbalariae* Borzi.

There are only two species known in the genus, they can be separated by the following key:

A Key to the Species of Eremothecium

Asci terminal, fusiform, with 30 to 60 or more ascospores per ascus, ascospores acicular, 19μ-25μ long . . . . . *E. cymbalariae* p.10
Asci intercalary, cylindrical, with (4-) 16-32 ascospores per ascus; ascospores semilunate, 16μ-25μ long . . . . . *E. ashbyii* (Guill.) Batra p.13

Eremothecium cymbalariae
(Figs. 2 and 3)

Eremothecium cymbalariae Borzi, Soc. Bot. Ital. Bul. in Nouvo Giorn. Bot. Ital. 20: 452. 1888.

Vegetative Characters

According to Ashby and Nowell *(27)*, the colonies on potato dextrose or sucrose agar are aerial and after 2 weeks turn gray. But Boedijn describes 1-month-old colonies on "toge agar" as white or "dirty white," 6 cm. in diameter [other growth conditions not given] circular, finely radiate, concentrically zonate, fluffy in the middle, and with a ". . . .rather sharp, weakly undulating border," *(41)*; mycelium well-developed, dichotomously branched, undulating, sparingly septate, and with many small oil drops in the cells, cells measure 2μ-6μ in diameter, according to Borzi *(42)*; the hyphae are constricted at septa and swollen in the middle, and the cells stained in haematoxylin show 8 to 12 nuclei each.

Asexual Reproduction

No conidia or yeast cells observed.

Sexual Reproduction

Gametangial or somatogamic copulation not observed; asci terminal but provided with a rostrum-like apical appendage, fusiform, solitary, hyaline, with 30 to 60 ascospores per ascus, 34μ-51μ X 12μ-25μ *(41)*, dehisce in the middle, presumably by gradual deliquescence; ascospores wedge-shaped, slightly bent, arranged in two characteristic symmetrical bundles that dovetail with the broad ends to form a fusiform mass (fig. 2, *c*), hyaline, elongate, with one end obtuse but the other gradually attenuated into a fine point, uninucleate, 19μ-25μ X 1.5μ-3.0μ.

Nutritional Characters

Not observed.

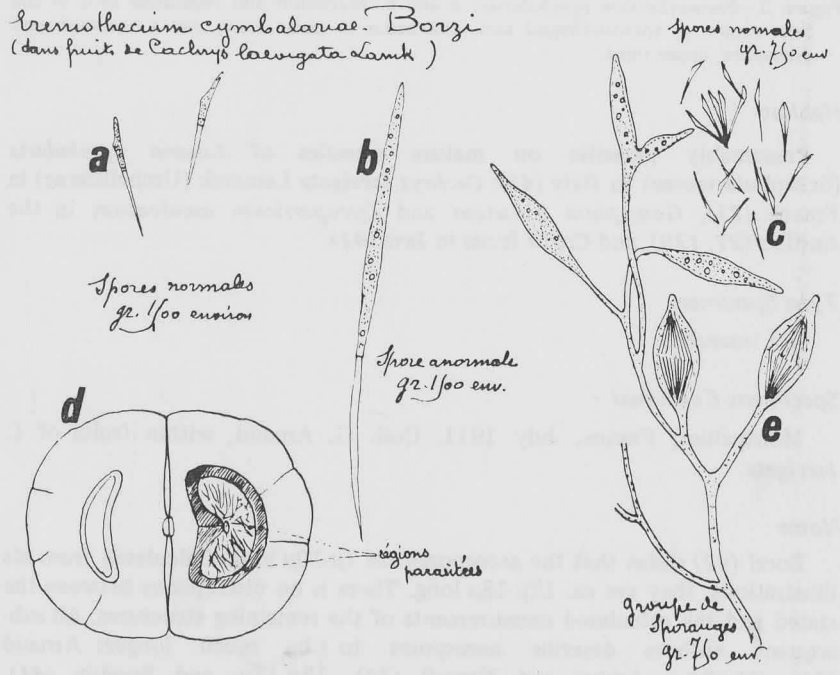

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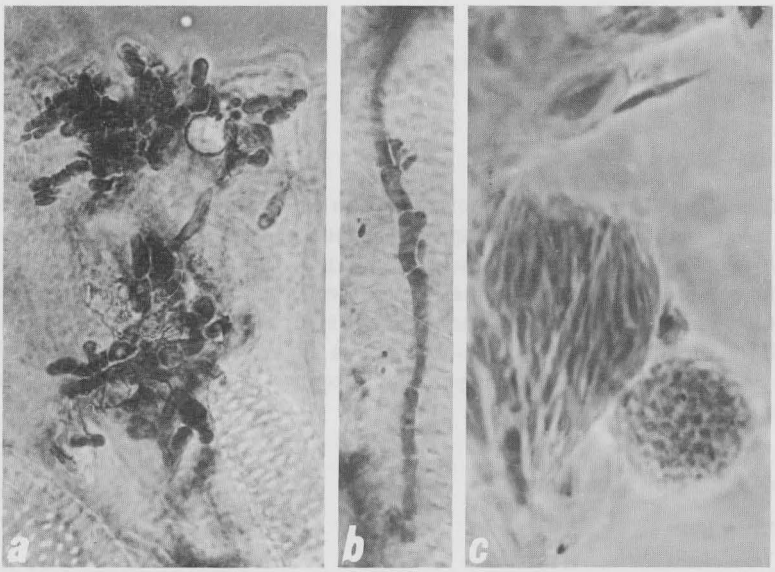
Figure 2.—*Eremothecium cymbalariae*: *a*, *b*, and *c*, ascospores; *d*, parasitized zone of a fruit of *Cachrys laevigata*; *e*, thallus bearing terminal asci. Approximate magnifications: *a*, *b*, X 1,100; *c*, *e*, X 750; *d*, X 3.



PN-2518

Figure 3.—*Eremothecium cymbalariae: a* and *b*, Mycelium and vegetative cells in the host tissue; *c*, spindle-shaped asci, one ascus in polar view, and a needle-shaped ascospore, upper right.

Habitat

Presumably parasitic on mature capsules of *Linaria cymbalaria* (Scrophulariaceae) in Italy *(42)*, *Cachrys laevigata* Lamarck (Umbelliferae) in France *(25)*, *Gossypium hirsutum* and *Lycopersicum esculentum* in the Antilles *(27, 120)*, and *Citrus* fruits in Java *(41)*.

Type Specimen

Not traceable.

Specimens Examined

Montpellier, France, July 1911, Coll. G. Arnaud, within fruits of *C. laevigata.*

Notes

Borzi *(42)* states that the ascospores are 7μ-10μ long. Calculated from his illustrations, they are ca. 12μ-18μ long. There is no discrepancy between the stated and the calculated measurements of the remaining structures. All subsequent authors describe ascospores to be much longer: Arnaud (*25*), 24μ-26μ; Ashby and Nowell (*27*), 15μ-17μ; and Boedijn (*41*), 19μ-25μ. Ascospores of Arnaud's collection are 13μ-19.5μ long. In all other respects the fungus matches the descriptions and the illustrations given by Borzi *(42)* and by others *(25, 27, 41)*. The smaller dimensions of the ascospore in Borzi's text, therefore, may be an error.

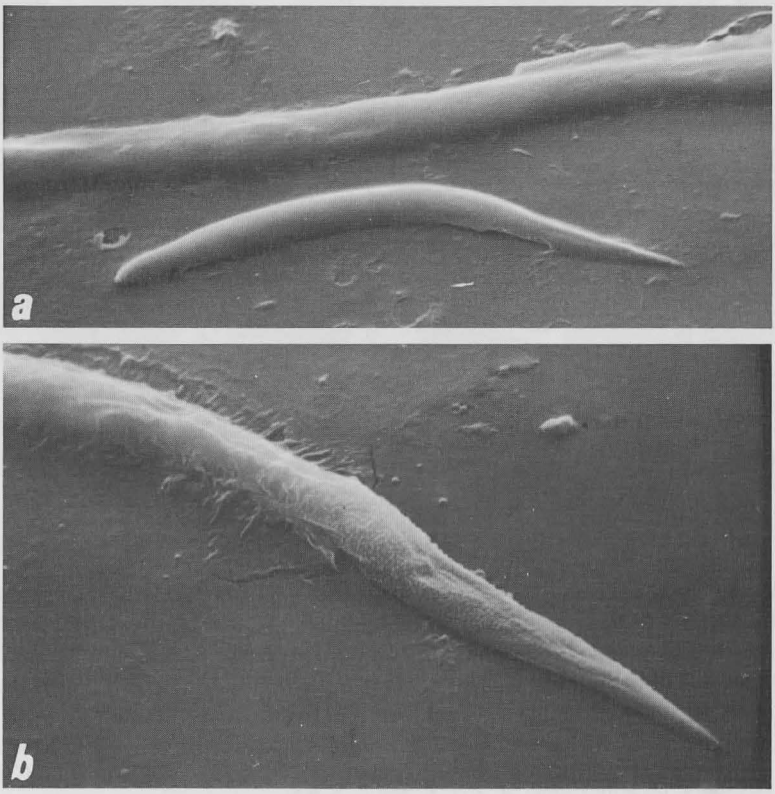
Eremothecium cymbalariae is a rare fungus. It is apparently transmitted by hemipterous insects. Asbhy and Nowell *(27)* found the fungus in 89 to 95 percent of cotton bolls in Montserrat and Tortola islands along with *Nematospora, Spermophthora,* and *Ashbya.* It may be of interest to note that the cylindrical, four-spored structures, which Arnaud *(25)* described as "macrosporangia" of *E. cymbalariae* may indeed be the asci of *Nematospora (27, 197).* The writer observed the ascospores of *Nematospora,* but not the asci, in Arnaud's collection from Montpellier. Boedijn *(41)* also found the two fungi in *Citrus* fruits, but he does not specifically mention about their occurrence in the same fruit.

Eremothecium ashbyii
(Figs. 4 and 5)

Eremothecium ashbyii (Guilliermond) Batra, comb. nov.

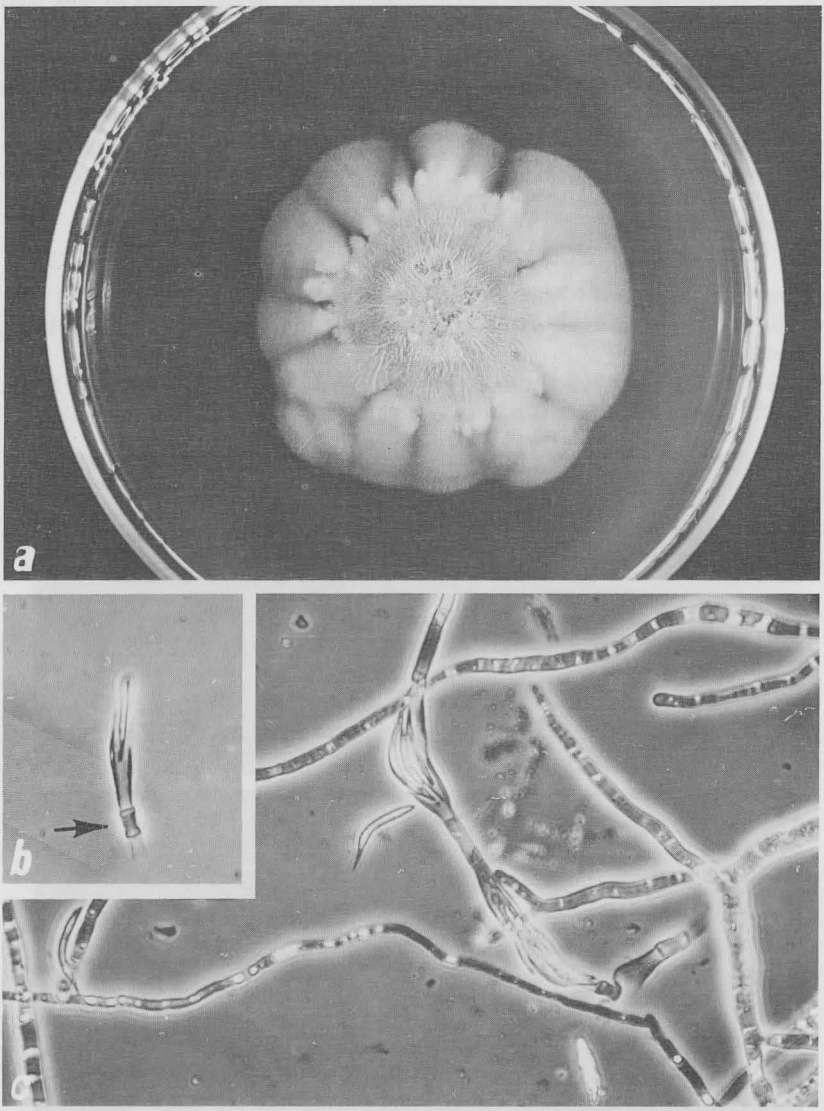
=*Crebrothecium ashbyii* (Guill.) Routien, Mycologia 41: 185. 1949, basonym.

=*Eremothecium ashbyii* Guill., [Paris] Acad. des Sci. Compt. Rend. 200: 1556. 1935 [name not validly published, no Latin diagnosis].



PN-2519

Figure 4.—Ascospores of *Eremothecium ashbyii.* Magnifications; *a,* X 2,800; *b,* X 6,500.



PN-2520

Figure 5.—*Eremothecium ashbyii*: *a*, 3-week-old colony on yeast extract malt extract agar (note the intramatrical mycelium toward the edge); *b*, callose plug (arrowed) adjacent to a dehisced ascus with an ascospore; *c*, hyphae, intercalary asci and an ascospore. Magnifications: *a*, X 1; *b*, and *c*, X 500.

Vegetable Characters

Colonies on yeast extract malt extract agar have the following characteristics. Initially white or tan, effuse. Three or four days after seeding becoming yellow, the pigment dispersing in the medium. After 2 weeks 5 cm. in diameter, wet, dull, compact, wrinkled, with a distinct substratal margin; mycelium dichotomously branched, sparingly septate, cells multinucleate, containing needlelike yellow crystals of flavinoid material; hyphae 2μ-6μ wide, containing wide callose plugs at intervals (fig. 5, *b*).

Asexual Reproduction

Conidia of any kind absent, but bulbils (hyphal fragments with a secondary wall) give rise to yeastlike cells that may enlarge into a thallus.

Sexual Reproduction

Neither Guilliermond *(72,73)* nor Routien in 1949 observed sexual fusion of what are assumed to be ascospores. Twice the writer observed fusion of two ascospores in slide cultures, but the nuclear condition of the conjugant cell was not ascertained. Single ascospore cultures reproduce asci within 36 to 48 hours, and *E. ashbyii* appears to be homothallic. Asci intercalary, usually in long chains, sometimes solitary and lateral, ellipsoid, with truncate or rounded ends, initially multinucleate but ultimately one nucleus stains deeply and conspicuously (? sexual caryogamy), on maturity deliquescent in the middle (fig. 5, *c*); 50μ-110μ × 10μ-18μ; ascospores usually 8 or 16 per ascus, occasionally more or less, hyaline, smooth or minutely verrucose, 1-celled, curved or bowed, with one end rounded and the other end acicular, without a cytoplasmic appendage, nearly all ascospores germinating by a germ tube to give rise to the thallus, uninucleate, (18μ-) 20μ-24μ (-31μ) × (1.5μ-) 2.0μ-3.5μ (-4.5μ) exclusive of the finely pointed, noncytoplasmic "tail," which is 2μ-10μ long.

Nutritional Characters

Fermentation positive for galactose, glucose, sucrose, and raffinose.

Assimilation of carbon compounds—

glucose	+	raffinose	+
galactose	—	melizitose	—
L-sorbose	—	inulin	+
maltose	+	soluble starch	+
sucrose	+	D-xylose	—
cellobiose	—	L-arabinose	—
trehalose	+	D-arabinose	—
lactose	—	D-ribose	+
melibiose	—	L-rhamnose	—

D-glucosamine	−	c-methy glucoside	−
ethanol	+	salicin	−
glycerol	+	lactic acid	−
erythritol	−	succinic acid	−
adonitol	−	citric acid	−
dulcitol	−	ethyl aceto-acetate	−
D-mannitol	−	i-inositol	−
D-sorbitol	−		

Nitrate reduction positive; growth in vitamin-free medium negative; gelatin liquefaction negative; splitting of arbutin negative; production of "esters" negative; growth at 37° C. positive; growth on 50 percent (w./w.) glucose yeast extract agar negative.

Habitat

A weak parasite of cotton and probably of *Citrus*, a relatively rare fungus.

Type

Routien in 1949 validated the species and designated Guilliermond's *(73, figs. 1, 6, 7, 13, 18, and 19)* as the type of the fungus (see notes below).

Specimens Examined

Sudan: CMI-14,782, a microscope preparation from a subisolate of the type, grown on malt extract agar from Sept. 1, 1948, to Sept. 8, 1948. The packet bears the following information: "Host: *Gossypium* bolls; loc. Sudan, Berber; Coll. R. E. Massey Ashby Guilliermond CBS -Bisby NCTC (7,041)" [punctuation provided]; (?) Israel: BPI, 2511-Batra, *Citrus sinensis*, cultivar Jaffa.

Notes

Eremothecium ashbyii is a rare fungus. It was described on the basis of R. E. Massey's (CMI-14,783) cultures forwarded to Professor Guilliermond by Dr. Ashby. This is the type collection. This isolate was widely distributed to many laboratories of the world, including such well-known culture banks as ATCC, CBS, NCTC, and NRRL. Many authors refer to "riboflavin" and "riboflavinless" strains, but most of the strains are morphologically similar and can be traced to Massey's original source. Mukerji *(126)* reports it from South Africa and the United States, but material from these countries was unavailable to the writer. It was isolated in 1970, at Beltsville from two allegedly imported Jaffa oranges that were exceptionally bitter, with the flesh relatively more yellowish than usual. When experimentally inoculated with a hypodermic needle into the common sweet oranges from Florida, Jaffa oranges, and green, unopened bolls of *G. hirsutum*, the fungus caused no detectable symptoms after 2 to 4 weeks. It did not survive in the growing

cotton bolls, but it was readily isolated from the oranges after the end of the experiment.

Guilliermond *(72-74)* expressed doubts about the nature of "sporiferous sacs" or asci and speculated that the initially plurinucleate cells may indeed be similar to the sporangia of *Spermophthora gossypii* (see p. 10). The writer attempted hybridizing the orange isolate and ATCC-6747 and 12,995 but found no plasmogamy or any new structures that could give further clues to the nature of the "sporiferous sacs." I assume these to be the hemiasci because: (1) at one stage they do have a single, deeply staining central nucleus, which may be the fusion nucleus, (2) there is a rather uniform number of spores per cell, and (3) of their close similarity to the hemiasci of *Ashbya gossypii* where caryogamy of the two ascosporic nuclei and the subsequent ascus development from the conjugant cell is known.

Ashbya

Ashbya Guilliermond, Rev. Gen. Bot. 40:562. 1928.

Type species.—Monotypic, *A. gossypii* (see below).

In 1928 Guilliermond *(71)* established the genus *Ashbya* with *Nematospora gossypii* Ashby and Nowell *(27)* as the type species. He separated this species from *Nematospora* because it was filamentous, produced riboflavin, did not form functional blastoconidia, and the asci were intercalary. Two years later Farries and Bell (*59*) demonstrated that *A. gossypii* and *N. coryli* (Nowell's isolate from the West Indies) exhibited nutritional properties that were similar. Judged from ecological, morphological, nutritional, and caryological points of view, the two organisms are very closely related to each other and their separation into two genera is purely a matter of choice. The writer continues to use the name *"Ashbya,"* for it has been widely accepted during the past 30 years or so.

Ashbya gossypii
(Figs. 6; 7; 8, *A*)

Ashbya gossypii (Ashby and Nowell) Guilliermond, Rev. Gen. Bot. 40:562. 1928.

=*Nematospora gossypii* Ashby and Nowell, Ann. Bot. 40:74. 1926.

=*Ashbia gossypii* (Ashby and Nowell) Ciferri and Fragoso, in Fragoso and Ciferri, Bol. Roy. Soc. Españ. Hist. Nat. 28:379. 1928.

Vegetative Characters

Colonies on yeast extract malt extract agar have the following characteristics. Initially white to tan. Three days after plating riboflavin or its derivatives usually appearing in some isolates as small droplets or needlelike crystals in the hyphae. After 2 weeks at 25° C., 4.5 cm. in diameter, pale lemon yellow, finely floccose, wet, even or wrinkled and raised in the middle,

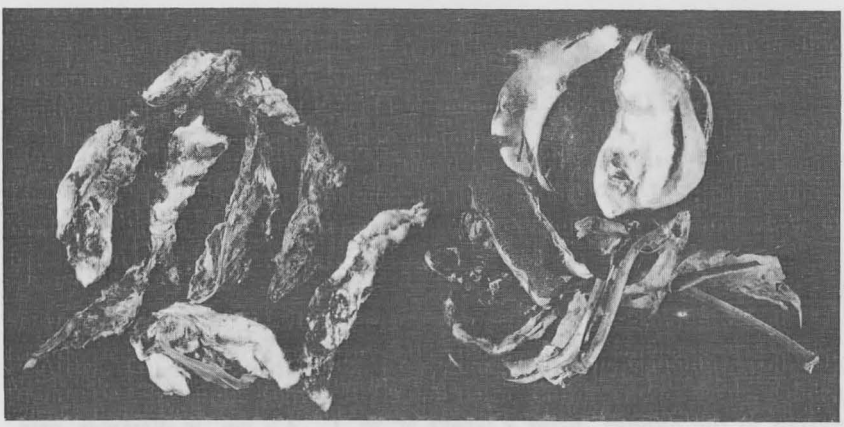
compact, with a distinct, effuse, filamentous, white margin; mycelium mostly extramatrical, branching dichotomously, branches soon attaining a perpendicular position, granular, septate, septa relatively thick but of low refringency, 3-day-old colonies usually producing 2 or 3 nuclei per cell and 2-week-old colonies producing 6 to 10 nuclei, often highly vacuolated, apical cells more than twice as long as intercalary, 6μ-10μ in diamter, often constricted near the septa.

Asexual Reproduction

Ashby and Nowell *(27)* originally reported yeastlike cells, after detachment, germinating to give rise to mycelium. Subsequent authors *(7, 146)* observed blastosporic sprout cells, solitary or in chains, terminal or lateral, but never observed them to detach and germinate. Sprout cells 4.5μ-7.0μ X 2.4μ-3.2μ, germinating in situ by a germ tube; hyphae regularly breaking up into cylindrical bulbils capable of giving rise to new colonies; relatively thick-walled, pyriform, chlamydospore-like structures also observed but their function unknown.

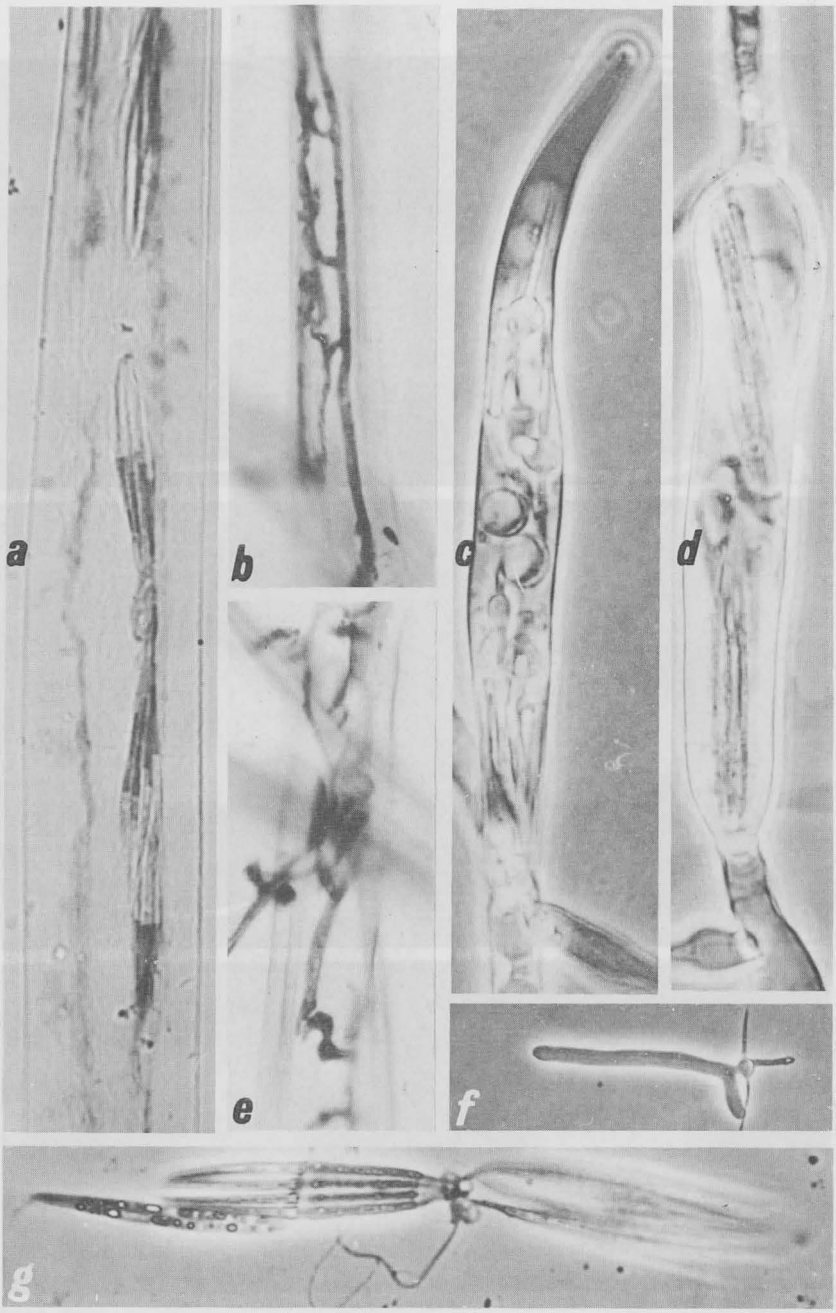
Sexual Reproduction

Homothallic, gametangia absent, occasional apomictic fusion between two lateral branches giving appearance of a clamp connection observed but their sexual significance unknown; the two uninucleate halves of the ascospore gametic in function, the nucleus from the upper half migrating into the middle of the cell where it fuses with its counterpart from the lower half, the conjugant cell or zygote germinating and giving rise to multinucleate mycelium; asci almost always delimited behind the growing tip but separated from it by a septum, soon appearing intercalary because of subsequent apical growth, rarely terminal, sometimes, because of a lateral branch having transformed into an ascus, may appear like letter 'V', usually in chains, sometimes



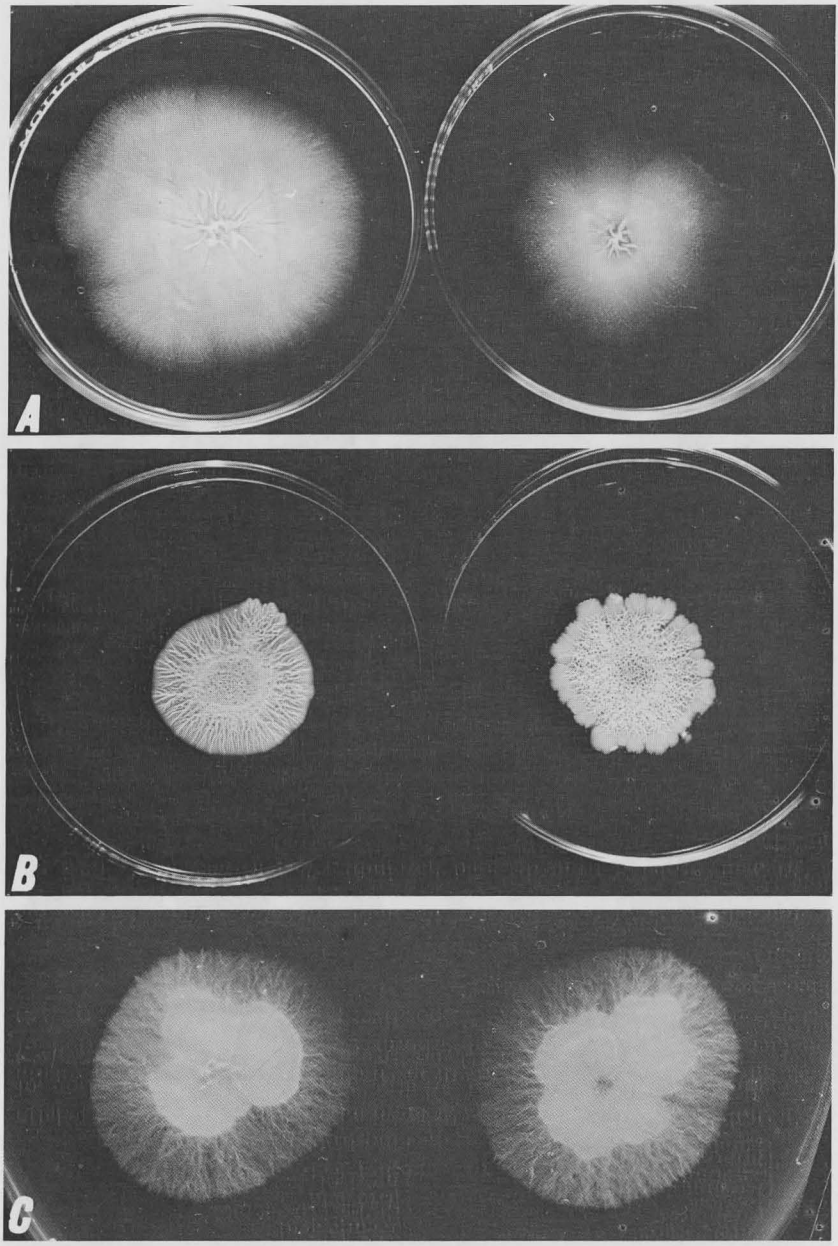
PN-2521

Figure 6.—Discolored tight locks of *Gossypium hirsutum* damaged by *Asbbya gossypii: Left,* CMI-53,757; *right,* CMI-26,136. Approximately X 1.



PN-2522

Figure 7.—*Ashbya gossypii: a*, Asci in chains within a cotton fiber, CMI-26,138; *b* and *e*, mycelium anastomosing within cotton fibers, CMI-26,138; *c*, terminal ascus with germinating zygotes; *d*, intercalary ascus with two spore bundles; *f*, germinating zygote; *g*, two bundles of four ascospores each (one out of focus) with their appendages intertwined in the middle. Magnifications: *a-e* and *g*, X 500; *f*, X 140.



PN-2523

Figure 8.—*a*, *Ashbya gossypii*, and *b*, *Nematospora coryli*: *Left* two plates on Difco yeast morphology agar; *right* two plates on yeast extract malt extract agra. *c*, *N. lycopersici* on yeast extract malt extract agar. All colonies 3 weeks old, grown at 25° C. in an incubator without light. All approximately X 1/2.

solitary, clavate, cylindrical, or fusiform, rarely pyriform, young asci appear striated and with abundant glycogen, hyaline, usually 12- or 16- spored, rarely 32-spored, (44μ-) 67μ-75μ (-85μ) X 11μ-13μ, 100μ-200μ X 10μ-20μ, according to Pridham and Raper (*146*) septa between asci split toward maturity, the free ends round off and the ascus wall, upon disintegration, is sticky and granular; ascospores arranged in two to four fascicles lying end to end, rarely the two bundles of ascospores in 8- or 12-spored asci arranged side by side or even clumped together, needle- or spindle-shaped, equipped with a characteristic appendage on the posterior end, hyaline, appearing septate after caryogamy, "septum" median, the fusion nucleus located just below the septum, (24μ-) 30μ-33μ X 2.0μ-4.5μ exclusive of appendage; ascosporic appendage flagelliform, nonmotile, appearing to be adhesive in function, 2 to 4 times the length of ascospores, sometimes smaller, originating from a cytoplasmic centrum and remaining attached to it, thus all the ascospores (some germinated) from an ascus string together after their release; ascospore germination by one or two germ tubes arising just below the septum from the posterior half of the ascospore, forking and attaining a considerable length before becoming septate, the appendage at this stage unrecognizable, the acutely pointed anterior end unstainable with common dyes but because of its refractivity, easily distinguishable even after hyphae well developed (fig. 9, *f* and *g*) and bearing asci, sometimes germinating by blastosporic buds that do not detach but rather elongate into hyphae, some spores germinating while still within intact asci.

Nutritional Characters

Fermentation negative.

Assimilation of carbon compounds—

glucose	+	L-rhamnose	—
galactose	—	D-glucosamine	—
L-sorbose	—	ethanol	+
maltose	+	glycerol	+
sucrose	+	erythritol	—
cellobiose	+	adonitol	—
trehalose	+	dulcitol	—
lactose	—	D-mannitol	—
melibiose	—	D-sorbitol	—
raffinose	+	c-methyl glucoside	—
melizitose	—	salicin	—
inulin	+	lactic acid	—
soluble starch	+	succinic acid	+
D-xylose	—	citric acid	—
L-arabinose	—	ethyl aceto-acetate	—
D-arabinose	—	i-inositol	—
D-ribose	—		

Nitrate reduction negative; growth in vitamin-free medium positive, addition of biotin gives increased growth *(63);* gelatin liquefaction negative; splitting of arbutin negative; production of "esters" negative; growth at 37° C. positive; growth on 50 percent (w./w.) glucose yeast extract agar positive.

Habitat

Parasitic on *Gossypium* and other hosts (see table 1).

Type Specimen

A dried subculture from the original isolate by Ashby and Nowell from cotton and received at Beltsville in 1930 from Dr. Westerdijk is hereby designated as the lectotype.

Specimens Examined

Africa: Malawi—CMI-53,757, *Gossypium* bolls, Maperena, Coll. E. L. Drab, no date; Republic of South Africa—CMI-16,151 (National Collection of Type Cultures, London, No. 2,660), *Gossypium* bolls, Coll. S. F. Ashby, no date; Tanzania—CMI: 26,136, *G. hirsutum* bolls, Morogoro, Coll. G. W. Wallace (his No. 1319), Dec. 9, 1928; 26,138, *Gossypium* lint, G. B. Wallace, 4. V. 1926; Uganda—26,137, cultivated *Gossypium,* Coll. J. D. Snowden (No. 1039), Mar. 14, 1927; 26,139, cultivated cotton, Bukalosa, Coll. J. D. Snowden, det. S. F. Ashby, no date.

Caribbean: Jamaica—CMI-62,196, cotton bolls, Coll. H. C. James, no date.

North America: California—*Citrus reticulata* (tangerine), Coll. Mrs. Kempton, Jan. 5, 1933, a microscope slide, BPI (Shear collection); Florida—FLAS, *Lycopersicum esculentum,* experimentally inoculated at Gainesville with an isolate from Satsuma orange, Coll. G. W. Weber, Mar. 16, 1932; BPI, *Citrus sinensis,* Coll. G. F. Weber (No. 7), Gainesville, Jan. 12, 1934; 2295-Batra, *C. nobilis,* presumably from Florida, isolated Nov. 11, 1968; South Carolina—*G. hirsutum,* 2553-Batra, Coll. Mrs. T. H. Bracken, Charleston, Oct. 26, 1970.

Source unknown: CMI-31,279, ex CBS (a dried culture).

Nematospora

Nematospora Peglion, Atti R. Accad. Naz. dei Lineci, Ser. 5, 6:278. 1897.

Vegetative Characters

Colonies yeastlike or mycelial; mycelium septate, pseudomycelium often present, cells hyaline, uninucleate or multinucleate, single cells oval, round, or cylindrical.

Asexual and Vegetative Reproduction

By blastosporic buds (sprout cells), thick-walled chlamydospore-like cells also present, distinct conidiophores lacking.

Sexual Reproduction

Ascospores act as gametospores, homothallic or (?) heterothallic; asci usually 8-spored, cylindrical, with rounded ends, often compressed in the middle, ascus wall deliquescent; ascospores spindle- or needle-shaped, with a flagellumlike appendage on one end and the other end bluntly pointed, with a refractive cytoplasmic zone in the middle, acting as gametospores and the resultant zygote germinating by forming a sprout cell (proascus) or a germ tube or directly giving rise to an ascus.

Nutritional Characters

Nitrate reduction absent.

Type Species

Nematospora coryli Peglion.

Habitat

Plant parasites or saprophytes, usually associated with insects.

The most distinguishing characteristic of the genus is the formation of zygote and its subsequent developmental behavior. Initially the two uninucleate protoplasts of the ascospore are distinct, each with its own protoplasmic membrane but without an intervening wall. In *N. coryli* and *N. lycopersici* the upper protoplast migrates, usually in *toto* but sometimes leaving behind cytoplasmic debris, and mingles with the lower one. The two nuclei fuse and a zygotic wall of varying thickness is laid within the existing ascosporic wall. The upper half of the ascospore now appears highly refractive and stains weakly, or not at all, with cytoplasmic dyes. The zygotic wall is thickest in the median and the "ascospore" thus appears septate. The refractivity of the upper half of the "ascospore" and its "septate" appearance were noted by many authors *(70, 71, 126, 156, 157, 196, 197)* but their significance was not fully realized. The zygote enlarges and usually gives rise to proasci or vegetative cells, but may rarely become an ascus itself (fig. 13). The writer did not observe conjugation between vegetative cells as reported by Manuel *(116)* for *N. coryli* and by Schneider *(156, 157)* for *N. lycopersici.*

Species Concept in Nematospora

Peglion (*139*) investigated the type species thoroughly, and one of his cultures (CBS-2,608) is maintained as the 'type' strain. His initial characterization, in spite of the absence of data on assimilation of carbon compounds and fermentation of sugars, is lucid and leaves no doubt about the identity of the

species. Three additional species, excluding *N. gossypii* Ashby and Nowell which is treated as *Ashbya*, were added to the genus: (1) *N. lycopersici* Schneider *(157)*; (2) *N. phaseoli* Wingard *(197)*; and (3) *N. nagpuri* Dastur and J. Singh *(50)*. Soon after its publication the cultures of the last-named species were investigated by Stelling-Dekker *(159)* and later by Lodder and Kreger-van Rij *(112)*, Carmo-Sousa *(45)*, and myself. It is considered to be the same fungus as *N. coryli*.

The idenity of the other two species (*N. lycopersici* and *N. phaseoli)* in the absence of authentic living material, remains uncertain. There are two, possibly three, fungi found in the Americas and variously identified. They appear to have characteristic nutritional requirements and pathogenic properties (table 3). One of these is *N. coryli*, and the other one is *N. lycopersici*. These two species are separable on the basis of shape of sprout cells (blastoconidia), relative abundance of mycelium, and nutritional characters. In *N. coryli* the sprout cells are predominantly broadly ellipsoid, and most isolates form abundant mycelium. *N. lycopersici*, on the other hand, has mostly globose sprout cells and mycelium is rarely formed and only in the old cultures. *N. phaseoli* may or may not be a synonym of one of these two species. Schneider *(157)* stressed this distinction in his original description. However, subsequent authors, some doubtfully *(45, 112, 159)*, synonymized the two species, since spherical cells occasionally are found in old cultures of *N. coryli*. There is a possibility that the two species hybridize for they often occupy the same ecological niche. Work is being continued in our laboratories to elucidate this point.

Nematospora phaseoli, beautifully illustrated and investigated in detail by its author *(196, 197)*, may be a distinct species or synonymous with *N. coryli* or *N. lycopersici*. No type specimens exist for the species. Professor Wingard has kindly placed at my disposal a large number of photomicrographs of *N. phaseoli*. All show spherical vegetative cells and asci resembling *N. lycopersici*. He observed both round *(196, fig. 2, C, D)* and elliposid cells *(196, fig. 1, A, E)*, which were predominant in young cultures. He also observed "tennis racket" shaped cells, which I encountered in many collections of *N. coryli*, particularly from Africa. In the absence of authentic material it is difficult to separate *N. phaseoli* from *N. coryli* or *N. lycopersici*.

A Key to the Species of Nematospora

1. Sprout cells usually ellipsoid or cylindrical, dulcitol and mannitol not utilized . . . . . *N. coryli* p. 24
1. Sprout cells usually spherical, dulcitol and mannitol utilized . . . . . *N. lycopersici* p. 30

Nematospora coryli

(Figs. 8, *B*; 9-11; 12, *A*)

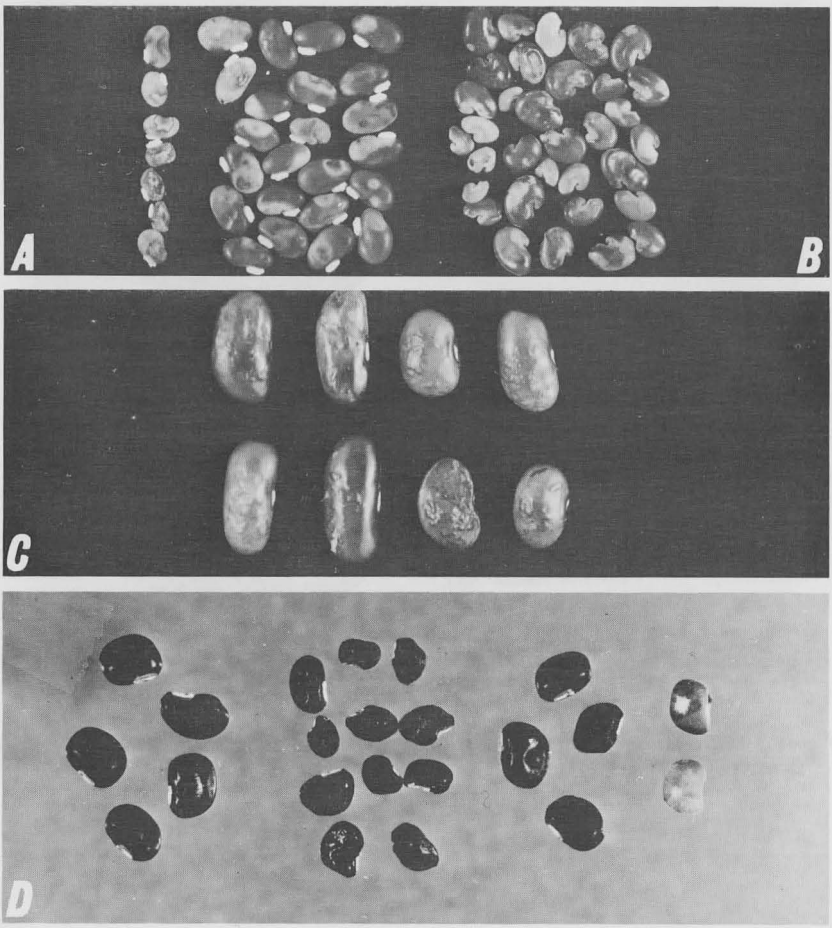
Nematospora coryli Peglion, Atti del, R. Accad. Naz. dei Lineci, Ser. 5, 6:278. 1897.

=*N. nagpuri* Dastur and Singh in Dastur, Ann. Mycol. 28:295. 1930.

?=*N. phaseoli* Wingard, Phytopathology 12:525-527. 1922.

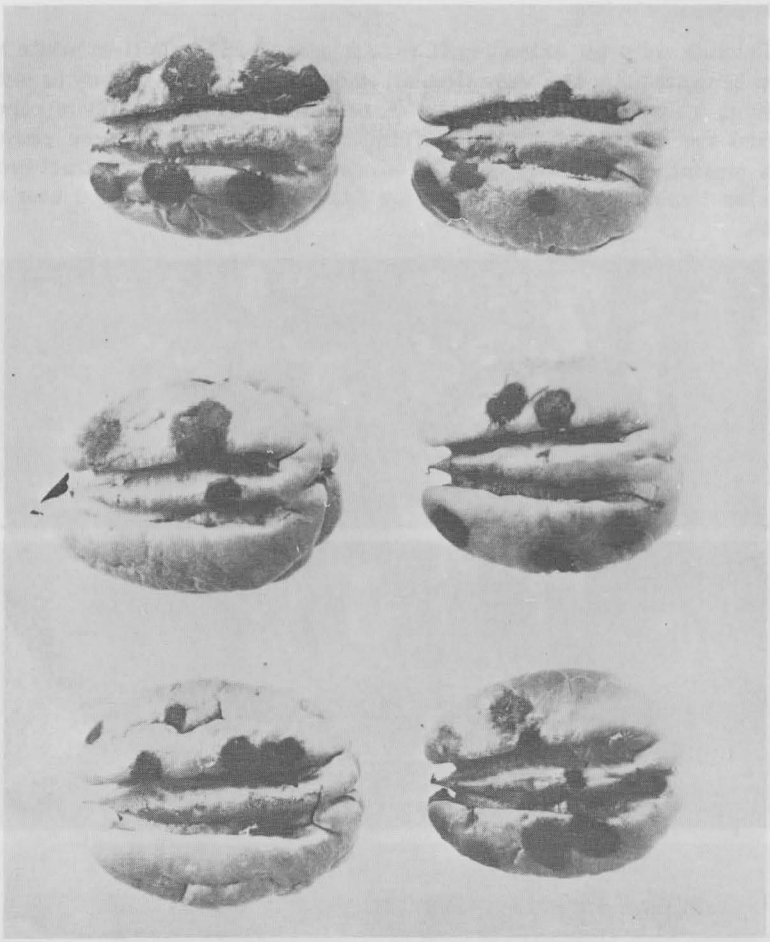
Vegetative Characters

Colonies on yeast extract malt extract agar at 25° C. initially white but soon becoming tan; at 7 days circular, smooth or wrinkled, creamy or pasty, without a distinct margin; 2-week-old or older colonies develop mycelium toward the margin, vegetative cells ellipsoid or oval, rarely globose, racquet cells present, hyaline. Old colonies on agar media or in yeast extract broth develop hyphae or pseudohyphae, few blastosporic cells attached near the septa.



PN-2524

Figure 9.—Seeds attacked by *Nematospora coryli*: *a*, Seeds of *Tephrosia* sp., *left row*, attacked when young, *right cluster*, presumably attacked toward maturity, lighter areas indicate fungus infections, CMI-26,133; *b*, seeds of *Crotalaria striata*, white blotches indicate fungus infection, CMI-121,729; *c*, seeds of *Phaseolus atropurpureus* (note blistering and small pimples over affected areas) CMI-121,923; *d*, *Vigna sinensis*, left five seeds apparently healthy, all others infected to varying degree, white sunken spots on two far right seeds (testa removed) show the fungus, CMI-26,130. All approximately X 1.

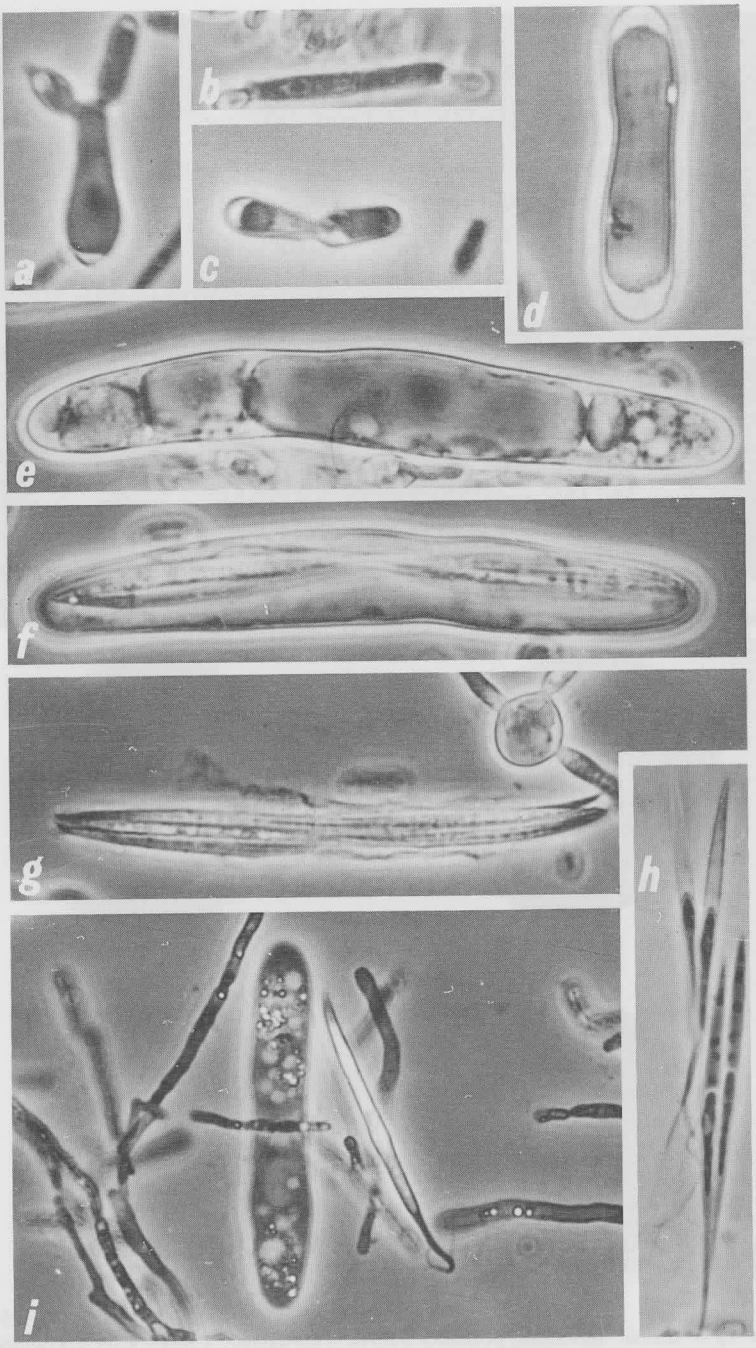


PN-2525

Figure 10.—*Nematospora coryli* on naturally attacked pecans. Approximately X 2. Courtesy G.F. Weber.

PN-2526 →

Figure 11.—*Nematospora coryli: a, b,* and *c,* Vegetative cells; *d* and *e,* developing asci; *f,* fully mature ascus; *g,* ascus with its wall partially dissolved away; *h,* ascospores, note the "septum" between the lower recipient and the donor protoplast, CMI-26,133 (Peglion's specimen); *i,* an ascus, highly refractive ascospore, and hyphae. Magnifications: *a, c, g,* and *i,* X 900; *b* and *h,* X 500.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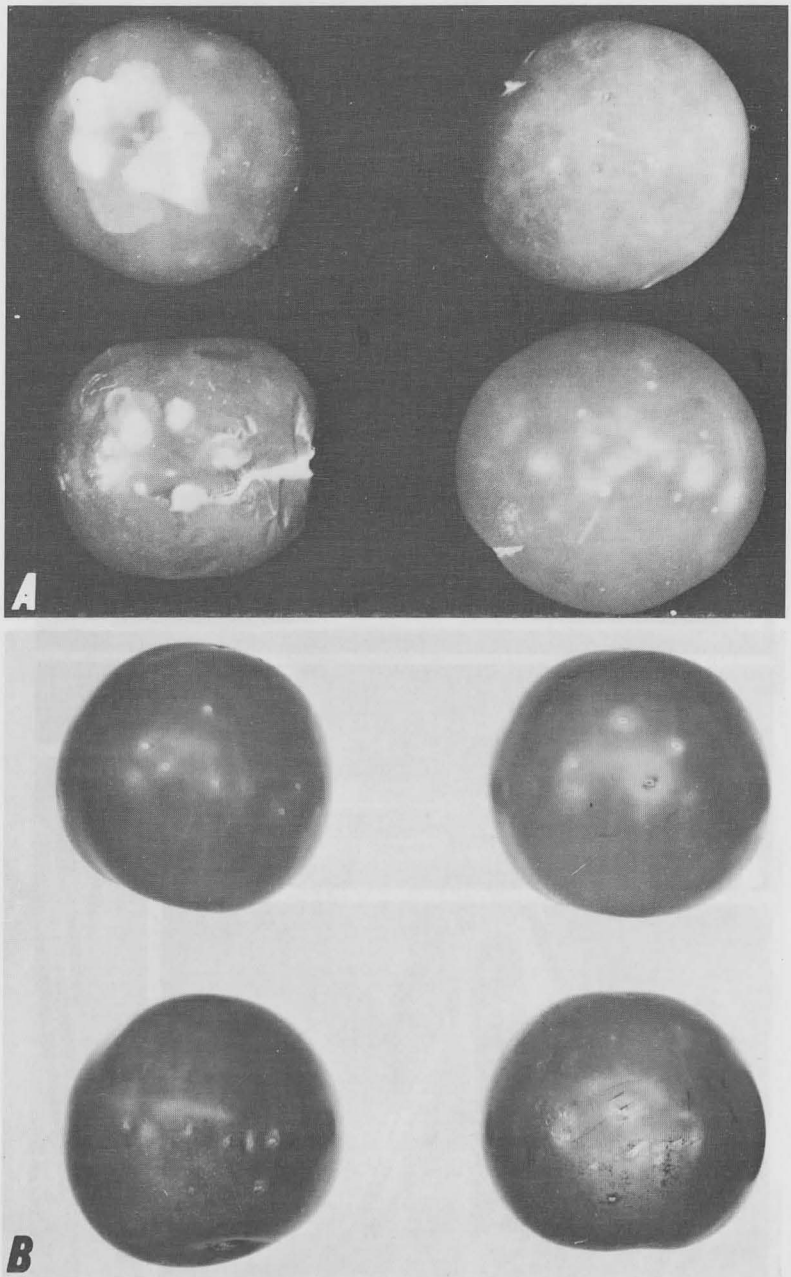

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Figure 12.—"Globe" tomatoes, probably cultivar Homestead, 1 week after inoculation. *a*, Inoculated with *Nematospora coryli* (note halo or discoloration near the puncture, now with callus, which indicates fungus within the tissue). *b*, Inoculated with *N. lycopersici* (tomatoes often burst open). Approximately natural size.

Asexual Reproduction

Distinct conidiophores lacking, reproduction mostly by ellipsoid, thin-walled sprout cells, 6μ-14μ X 3μ-8μ.

Sexual Reproduction

Homothallic, plasmogamy between two uninucleate sister protoplasts of the ascospore, zygote hyaline, unevenly thick-walled, sprouting proasci or becoming filamentous; asci mostly 8-spored but some 1-, 2-, 4-, or 16-spored, cylindrical, with rounded ends, hyaline, often naviculate, 60μ-70μ X 5μ-7.5μ (*140*), 63μ-114μ X 6.3μ-10μ (*50*); ascospores needle-shaped, usually 8 per ascus, shape, arrangement in ascus, and germination typical of the genus, 36μ-60μ X 2μ-3μ exclusive of the appendage, which is 20μ-50μ(-100μ) long.

Nutritional Characters

Fermentation weak for glucose and maltose, (weak) positive for sucrose and raffinose, negative for lactose.

Assimiliation of carbon compounds—

glucose	+	L-rhamnose	+
galactose	+	D-glucosamine	+ or −
L-sorbose	−	ethanol	+
maltose	+	glycerol	+
sucrose	+	erythritol	−
cellobiose	+	adonitol	−
trehalose	+	dulcitol	−
lactose	−	D-mannitol	−
melibiose	−	D-sorbitol	−
raffinose	+	c-methyl glucoside	−
melizitose	−	salicin	−
inulin	−	lactic acid	−
soluble starch	+	succinic acid	+
D-xylose	−	citric acid	−
L-arabinose	−	ethyl aceto-acetate	−
D-arabinose	−	i-inositol	−
D-ribose	−		

Nitrate reduction positive; growth in vitamin-free media negative; gelatin liquefaction negative; splitting of arbutin weakly positive; production of "esters" negative; growth at 37° C. positive; growth on 50 percent (w./w.) glucose yeast extract agar negative.

Habitat

Originally isolated from diseased hazelnuts, *Corylus avellana* L. (also see table 1).

Type Specimen

Not known. An authentic isolate from hazelnut was obtained from Peglion and studied by Lodder and Kreger-van Rij (*112*). A dried portion of a subisolate of this is located at BPI and is designated as lectotype.

Except for the ascospores, where characteristics include the entire range of variation, the above description is based on CBS-2608 isolate. This isolate (and ATCC-10,661) reduces nitrate, and my findings are in contrast to those of Carmo-Sousa *(45)* who reports negative assimilation. It grows well at 5°, 37°, and 40° C.

Specimens Examined

Africa: Gambia—CMI-51,682, *Gossypium hirsutum,* Gandum, Coll. D. Rhind, Dec. 5, 1952; Kenya—BPI-2,089-Batra, *Coffee arabica* seeds, Kitale, Mt. Elgan, Coll. Dr. Gothington, no date; Tanzania—CMI: 26,129, *Phaseolus vulgaris* bean, Coll. G. B. Wallace, no date; 26,130, *Vigna sinensis,* Morogoro, Coll. G. B. Wallace, July 29, 1930; 26,132, *Cajanus cajan* seeds, Shinyanga, Coll. G. B. Wallace, July 1930; 26,133, *Tephrosia* sp., Morogoro, Coll. G. B. Wallace, July 28, 1930; 53,292, *Anacardium occidentale,* Morogoro, Coll. G. B. Wallace, August 1953; 121,729, *Crotalaria striata* seed, Morogoro, Coll. Aug. 8, 1930; 121,730, *Cajanus cajan,* Coll. July 1930; 121,731, *Anacardium occidentale,* Morogoro, Coll. Mar. 19, 1953; 122,056, *Phaseolus lunatus,* Morogoro, Coll. Oct 15, 1929; Uganda—CMI-121,923, *Phaseolus atropurpureus,* Kempala, Coll. M. E. Parry, Comm. Sept. 17, 1966.

Asia: India—CBS, subisolate of the type of *N. nagpuri,* from cotton type; examined by the writer, from HC10.

North America: Florida—BPI: *Citrus paradisi,* cultures received from G. W. Weber on Jan. 12, 1934, (presumably isolated from his No. 8,016, Coll. E. F. De Busk, dated Nov. 14, 1932, from Palm Harbor, and on deposit at FLAS); *Carya illinoensis,* received from G. W. Weber on Jan. 1, 1934, (presumably isolated from his No. 8,155 dated Jan. 19, 1933, from Gainesville, and on deposit at FLAS); *Citrus sinensis,* culture received from G. W. Weber (his No. 8) on Jan. 12, 1934; FLAS-32,324 on immature pecan kernals, Clearwater, Pinellas County, Coll. J. H. Logan, Sept. 10, 1942; 11,112, *Citrus sinensis,* Sarasota, Coll. J. Gill, Jan. 29, 1936.

Caribbean: Jamaica—CMI-62,196, *Gossypium* sp. bolls, Coll. H. C. James, no date.

Nematospora lycopersici
(Figs. 8, *C*; 12, *B*; 13)

Nematospora lycopersici Schneider, Phytopathology 7:53. 1917.

Vegetative Characters

Colonies on yeast extract malt extract agar at 25° C., at 7 days yeastlike, smooth, butyraceous, white or tan, and without a distinct margin; at 21-days

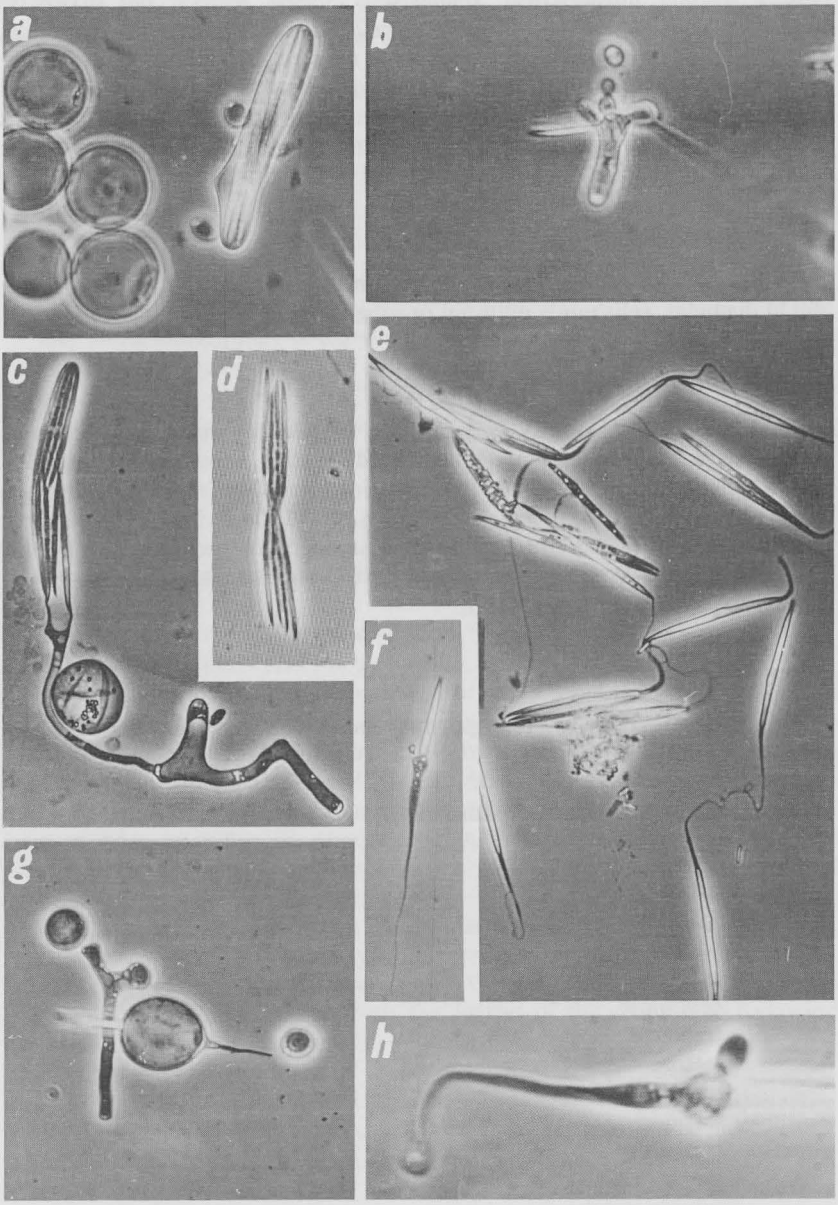

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Figure 13.—*Nematospora lycopersici: a*, Vegetative cells and an ascus (note the characteristic ridge); *b*, germinating zygote, with upper and lower halves of the ascospore still attached; *c*, a terminal and an intercalary ascus and a globose proascus or vegetative cell; *d*, ascospores with appendages locked to each other and ascus wall nearly dissolved away; *e*, ascospores (note the characteristic appendage and the ridge in the middle); *f*, a young zygote, the upper protoplast having recently migrated into the lower half of the ascospore; *g*, a zygote in preparation to form an ascus, or to vegetate; *h*, zygote with a bud. Magnifications: *a-g*, X 500; h, X 900.

have a distinct intramatrical, filamentous, lobate margin, but otherwise similar to younger colonies, vegetative cells usually globose or subglobose, 4μ-13μ in diameter, [Schneider *(156, 157)* stressed the cellular nature of the organism by such terms as "arthrospores" or spherical buds], mycelium and pseudomycelium present under nutritional stress conditions, racquet cells not observed. In yeast extract malt extract broth and other liquid media, the thallus usually cellular but otherwise similar to that on media with agar.

Asexual Reproduction

Typical of the genus, blastoconidia (sprout cells), usually globose, up to 35μ in diameter, cells rarely ellipsoid or subglobose.

Sexual Reproduction

Homothallic, plasmogamy between two uninucleate sister protoplasts of the ascospore, zygote unevenly thick-walled, hyaline, soon sprouting to give rise to spherical, thin-walled proasci, filament formation from the zygote not observed; asci 8-spored, cylindrical, hyaline, 60μ-100μ X 9μ-13μ; ascospores similar to those of *N. coryli*, with two uninucleate protoplasts, smooth-walled, and with a posterior flagellate cytoplasmic appendage, 33μ-43μ X 2.2μ (3.5μ) exclusive of appendage.

Nutritional Characters

Fermentation positive for glucose, galactose, sucrose, maltose, and raffinose, negative for lactose.

Assimilation of carbon compounds—

glucose	+	L-rhamnose	+
galactose	+	D-glucosamine	—
L-sorbose	+	ethanol	+
maltose	+	glycerol	+
sucrose	+	erythritol	—
cellobiose	+	adonitol	—
trehalose	+	dulcitol	+
lactose	—	D-mannitol	+
melibiose	—	D-sorbitol	—
raffinose	+	c-methyl glucoside	—
melizitose	—	salicin	—
inulin	+	lactic acid	—
soluble starch	+	succinic acid	—
D-xylose	—	citric acid	—
L-arabinose	+	ethyl aceto-acetate	—
D-arabinose	+	i-inositol	—
D-ribose	+		

Nitrate reduction positive; growth in vitamin-free medium positive; gelatin liquefaction negative; splitting of arbutin latent but positive; production of "esters" positive; growth at 37° C. positive; growth on 50 percent (w./w.) glucose yeast extract agar negative.

Type specimen

Not known.

Specimens Examined

The above description is based on LRB-2,518, an isolate from tomatoes collected in Sumter County, S.C. Additional specimens examined are: FLAS-32,323, *Phaseolus lunatus* seeds, Gainesville, Fla., Coll. Erdman West, June 12, 1943; CMI-77,422, *Phaseolus vulgaris*, Kampala, Uganda, Coll. C. Logan, July 16, 1959.

Metschnikowia

Metschnikowia Kamienski, Trav. Soc. Imp. Nat. St. Petersburg 30:363. 1899.

=*Monospora* Metschnikoff, Virchows Arch. 96: 178. 1884.
=*Monosporella* Keilin, Parasitology 12: 89. 1920.
=*Chlamydozyma* Wickerham, Mycologia 56: 257. 1964.

Cellular, pseudomycelium rare, true mycelium lacking; vegetative cells blastoconidial, spherical, ellipsoid, or cylindrical, thin- or thick-walled; sexual reproduction somatogamous; gametangia or gametes not known; asci formed from globose or ellipsoid, thick- or thin-walled proasci, 1- or 2-spored; ascospores needle-shaped, attenuated at one or both ends, without a cytoplasmic, flagellate appendage.

Saprophytes in floral nectar or weak parasites of aquatic invertebrates.

The genus has been recently monographed by Miller and van Uden *(123)*. They provide a complete review of the literature, a key to the species (see below), and descriptions of all the species of the genus. The following five species are recognized by them—

1. (a) *M. bicuspidata* (Metschnikoff) Kamienski var. *bicuspidata*, Kamienski, Trav. Soc. Imp. Nat. St. Petersburg 30: 363. 1899. This is the type of the genus and variety.
   (b) *M. bicuspidata* var. *australis* Fell and Hunter, Antonie van Leeuwenhoek; J. Microbiol. and Serol. 34: 369. 1968.
   (c) *M. biscuspidata* var. *californica* Pitt and Miller, Antonie van Leeuwenhoek; J. Microbiol. and Serol. 36: 365. 1920.
   (d) *M. biscuspidata* var. *chathamia* Fell and Pitt, J. Bact. 98: 853. 1969.

2. *M. krissii* (van Uden and Castelo-Branco) van Uden, Rev. Biol. Lisboa 3: 96. 1962.
3. *M. pulcherrima* Pitt and Miller, Mycologia 60: 669. 1968.
4. *M. reukaufii* Pitt and Miller, Mycologia 60: 671. 1968.
5. *M. zobelli* (van Uden and Castelo-Branco) van Uden, Rev. Biol. Lisboa 3: 96. 1962.

Metschnikowia includes both aquatic and terrestrial species. The first group of three species, of which none was investigated, includes weak parasites of small invertebrates:

(a) *M. bicuspidata,* attacks the crustacean *Daphnia magna* Straus, the trematode *Diplostomum flexicaudum,* the brine shrimp *Artemia salina* L., and *Castolia odorata;* (b) *M. krissii,* isolated from sea water and proved to be pathogenic to experimental *Daphnia magna;* and (c) *M. zobelli,* isolated from sea water, decomposing giant kelp *Macrocystis pyrifera* (L.) Agardh, and two fish species, *Atherinopis affinis-littoralis* Hubbs (topsmelt) and *Trachurus symmetricus* (Ayers) (the Pacific Jack mackerel), also pathogenic to experimental *D. magna.*

The second group of two species, *M pulcherrima* and *M. reukaufii,* is terrestrial. These species have been known as asexual yeasts from floral nectar for over 50 years as *Candida pulcherrima* and *C. reukaufii,* respectively. I investigated these species during 1968 and 1969 in conjunction with a general decline of the valuable alkali bee, *Nomia melanderi* Cockerell, in the Pacific Northwest. Both were found in fresh floral nectar, from the honey stomachs of foraging bees and from the larval provisions of several species of soil-dwelling bees. Apparently healthy larvae of the Costa Rican bee *Ptiloglossa* were observed (personal communication from Radcliffe Roberts, Oregon State University) to feed on a semiliquid diet of pollen, nectar, and *Candida reukaufii.*

Metchnikowia pulcherrima and *M. reukaufii* can be readily identified by their characteristic vegetative cells (the structures called "chlamydospores," or potential proasci). In the first species they are highly refractile, thick-walled, globose cells with a single large oil drop. These were called "pulcherrima" cells by earlier workers. In the second species, *M. reukaufii,* the vegetative cells are arranged as a "cross" or are airplane shaped in the freshly isolated cultures and the proasci are ellipsoid rather than globose. The shape of proasci correlates well with the shape of mature asci formed from them. In *M. pulcherrima* the asci are "sphaeropeduculate" and in *M. raukaufii* they are "ellipsoidopeduculate." Both species have two needle-shaped ascospores per ascus. They are apparently related to each other, for the haploid isolates of suitable mating types form hybrid proasci. However, the latter do not form asci. For a complete synonymy of the two species the paper by Miller and van Uden *(123)* may be consulted.

A Key to the Species of Metschnikowia

1. Asci formed by differentiation of large, thick-walled refractile chlamydospores; terrestial 2
1. Asci formed from small, thin-walled vegetative cells; . . . . . 3[4]

2. Asci formed from spheroidal cells ("pulcherrima" cells), asci sphaeropedunculate. Red pulcherrimin pigment usually present ..... *M. pulcherrima* p. 35
2. Asci formed from ellipsoidal to cylindroidal cells ("reukaufii" cells), asci ellipsoidopedunculate to clavate. Pulcherrimin pigment absent ..... *M. reukaufii* p. 37
3. Asci ellipsoidopedunculate, with one ascospore, that pointed at one end only 4
3. Asci clavate, with two acicular ascospores, visible when ascus crushed, pointed at both ends ..... *M. bicuspidata*
4. Glucose fermented, galactose and D-glucitol assimilated ..... *M. zobellii*
4. Glucose not fermented, galactose and D-glucitol not assimilated ..... *M. krissii*

Metschnikowia pulcherrima
(Figs. 14, *a* and *b*)

Metschnikowia pulcherrima Pitt and Miller, Mycologia 60: 669. 1968.

Vegetative Characters

Colonies on yeast extract malt extract agar at 25° C., at 2 weeks cellular, mucilaginous, glistening, with a uniform margin, tan to reddish brown, about 1 cm. in diameter; mycelium and pseudomycelium absent, cells blastosporic (budding type), bud formation unipolar or multipolar, cells usually broadly ellipoid or subglobose, thin-walled, subhyaline, 4μ-9μ (-12μ) X 2.0μ-4.5μ (-7.5μ), chlamydospores absent. Vegetative cells in 3-day-old cultures in Wickerham's *(193)* Difco nitrogen base broth with glucose similar to those formed on yeast extract malt extract agar.

Sexual Reproduction

Heterothallic; proasci globose to subglobose, thick-walled, chlamydospore-like, formed in liquid as well as on yeast extract malt extract agar after 3 or 4 weeks, presumably formed as a result of somatgamous conjugation between cells of two mating types, containing a single large oil globule, highly refractive, 8μ-9μ in diameter; asci sphaeropedunculate, (15μ-) 20μ-42μ (-55μ) X (4μ-) 6μ-8μ (-11μ) according to Miller and van Uden *(123)*, 2-spored; ascospores hyaline, acicular to filiform, 1-celled, 15μ-20μ X 0.5μ-15μ.

Nutritional Characters

Pellicle not formed but fine sediment present in liquid cultures; fermentation positive for glucose and galactose, negative for sucrose, maltose, lactose, and raffinose.

[4] For the description of the species under this lead consult Miller and van Uden (*123*).

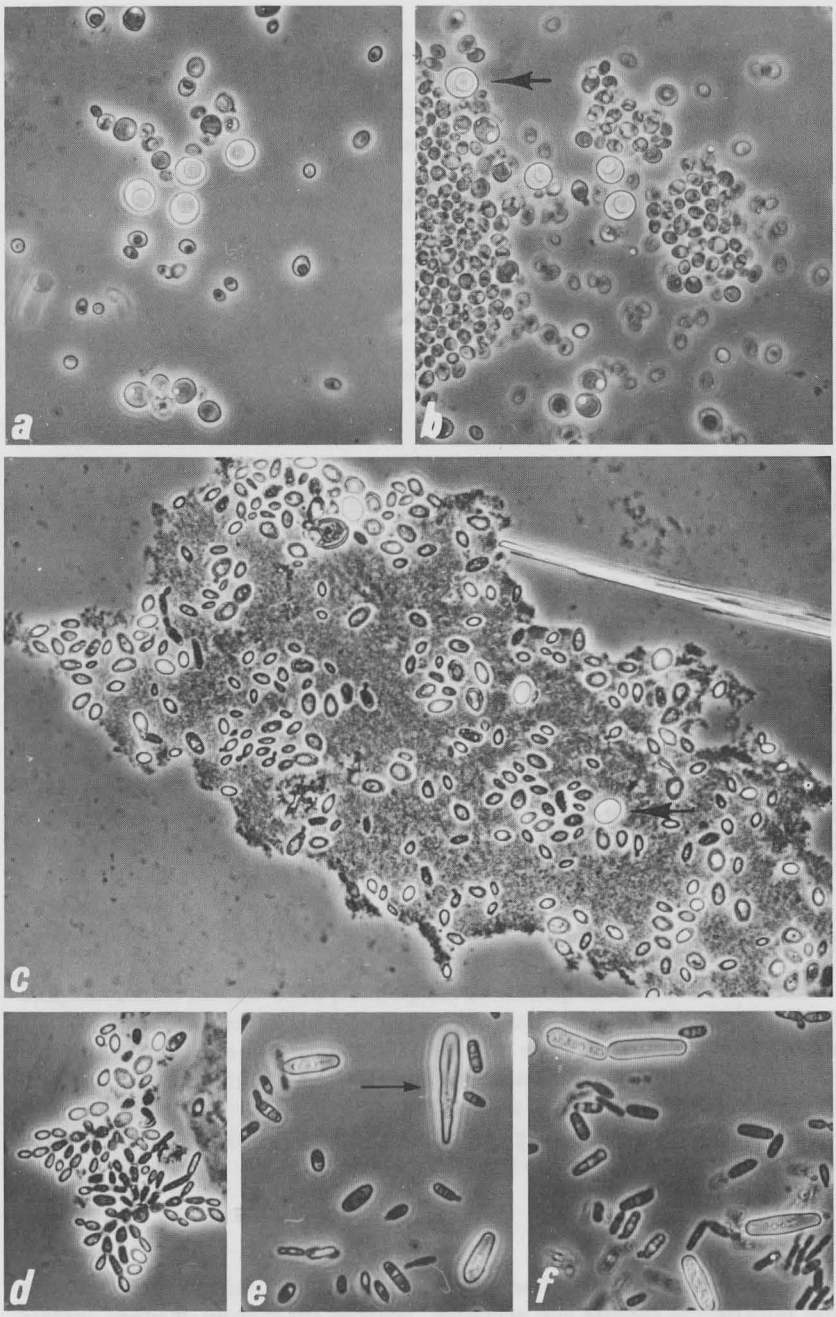

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Figure 14.—*Metschnikowia pulcherrima: a,* Vegetative cells (nonrefractive, smaller); and *b,* pulcherrima cells, or proasci (arrowed); *c-f, M. reukaufii: c,* an almost pure mass of vegetative cells and proasci (arrowed) in the honey stomach of the alkali bee, *Nomia melanderi; d,* a colony in a drop of contents of honey stomach; *e* and *f,* vegetative cells, proasci (ellipsoid large cells), and a young ascus (arrowed). All X 500.

Assimilation of carbon compounds—

glucose	+	L-rhamnose	—
galactose	+	D-glucosamine	+
L-sorbose	+	ethanol	+
maltose	+	glycerol	+
sucrose	+	erythritol	—
cellobiose	+	adonitol	+
trehalose	+	dulcitol	—
lactose	—	D-mannitol	+
melibiose	—	D-sorbitol	+
raffinose	—	c-methyl glucoside	+
melizitose	+	salicin	+
inulin	—	lactic acid	—
soluble starch	—	succinic acid	+
D-xylose	+	citric acid	+
L-arabinose	—	ethyl aceto-acetate	+
D-arabinose	—	i-inositol	—
D-ribose	+ or —		

Nitrate reduction negative; growth in vitamin-free medium negative; gelatin liquefaction negative; splitting of arbutin negative; production of "esters" negative; growth at 37° C. positive; growth on 50 percent (w./w.) glucose yeast extract agar positive.

Habitat

Nectar of flowers, honey stomachs of bees, and their larval provision.

Specimens Examined

ATCC-18,406, a subisolate of the type; BPI: 2412 A - Batra, cultures from *Medicago sativa* L., and 2412 B - Batra, spoiled pollen balls of the subterranean bee *Nomia melanderi,* Murtaugh, Idaho, Aug. 4, 1968.

Metschnikowia reukaufii
(Fig. 14, *c-f*)

Metschnikowia reukaufii Pitt and Miller, Mycologia 60: 671. 1968.

Vegetative Characters

Colonies on yeast extract malt extract agar at 25° C., at 2 weeks cream-colored, smooth, glistening, with an entire margin, about 1 cm. in diameter; mycelium and pseudomycelium absent, some cells forming rudimentary filaments that easily break up into component cells, budding multipolar, cells cylindrical or ellipsoid, thin-walled, hyaline, (6μ-)9μ-11μ(-14μ) X 2μ-3μ(-4μ). Vegetative cells in 3-day-old cultures in yeast extract malt extract broth somewhat smaller than on similar agar.

Sexual Reproduction

Heterothallic; proasci presumably formed by somatogamy in 1-month-old or older cultures, pyriform, highly refractile, containing several small oil globules, thick-walled, wall with pale-yellow pigmentation, (10μ-)13μ-20μ X 5μ-7μ(-8μ); asci ellipsoidopedunculate or clavate, hyaline, usually 2-spored, rarely 1-spored, (20μ-)30μ-37μ(-43μ) X 5μ-8μ(-9μ) according to Miller and van Uden *(123)*; ascospores acicular, hyaline, 1-celled, 15μ-30μ X 0.5μ-1.0μ.

Nutritional Characters

Growth in liquid media flocculent but a distinct pellicle not formed; fermentation positive for glucose and galactose, negative for lactose, sucrose, maltose, and raffinose.

Assimilation of carbon compounds—

glucose	+	D-ribose	+
galactose	+	L-rhamnose	—
L-sorbose	+	ethanol	+
maltose	+	glycerol	+
sucrose	+	erythritol	—
cellobiose	+	adonitol	+
trehalose	+	dulcitol	—
lactose	—	D-mannitol	+
melibiose	—	D-sorbitol	+
raffinose	—	c-methyl glucoside	+
melizitose	+	salicin	+
inulin	—	lactic acid	—
soluble starch	—	succinic acid	+
D-xylose	+	citric acid	—
L-arabinose	—	i-inositol	—
D-arabinose	—		

Nitrate reduction negative; growth in vitamin-free medium negative; gelatin liquefaction negative; splitting of arbutin negative; production of "esters" negative; growth at 37° C. positive; growth on 50 percent (w./w.) glucose yeast extract agar positive.

Habitat

In nectar of flowers, honey stomachs of bees, and their larval provision.

Specimens Examined

ATCC-18,407, a subisolate of the type; BPI, several collections (2413 A to 2413 E - Batra) from pollen balls of the subterranean bees *Nomia melanderi* and *Halictus rubicundis* (Chr.) collected Aug. 5, 1968, near Logan, Utah.

Coccidiascus

Coccidiascus Chatton, [Paris] Soc. de Biol. Compt. Rend. 20: 117. 1913.

Coccidiascus legeri
(Fig. 15)

Type Species

Monotypic: *C. legeri* Chatton (see above).

Chatton (*46*) described this fungus from the intestines of *Drosophila funebris* Fabr. whose larvae and adults feed on wine must. Approximately 10 percent of the individuals were infected. Chatton neither gave measurements of any structures of the fungus nor magnifications for his illustrations, which are reproduced in this paper as figure 15. The following account of the fungus is based on his narrative. The parenthetic comments are the writer's.

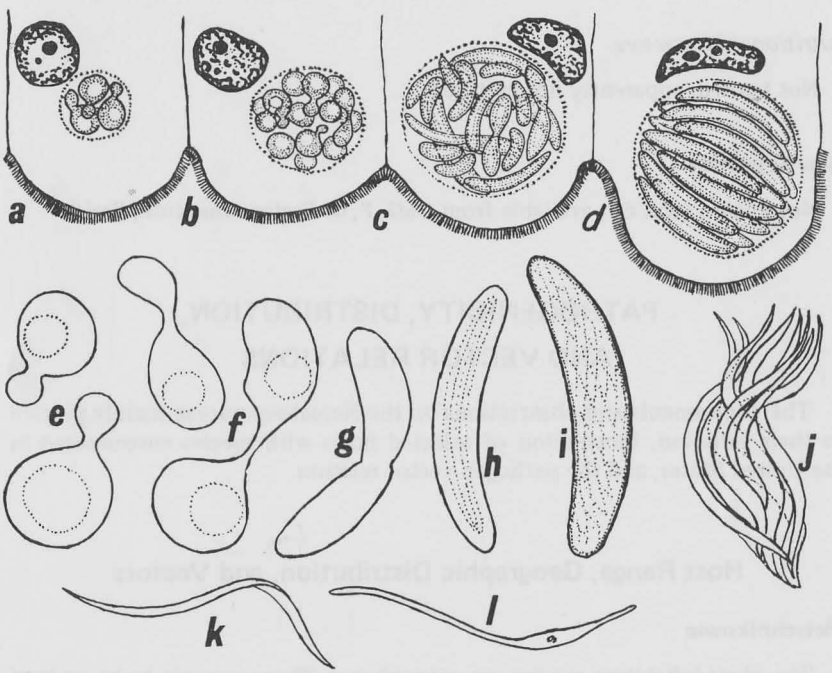

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Figure 15.—*Coccidiascus legeri*: *a-d*. Developmental stages of the thallus and asci; *e*, vegetative cells; *f* and *g*, conjugation of vegetative cells; *h* and *i*, development of asci; *j*, *k*, and *l*, ascospores. (After Chatton (*46*), magnifications not available, see text).

Vegetative Characters

Cells initially round, in older cells multiplication occurs by sprout cells (? hyaline), with a large central vacuole, thin-walled, thallus located in the vacuoles of epithelial cells of midgut (fig. 15, *a-d*), mycelium or pseudomycelium absent.

Sexual Reproduction

Asci formed after the cessation of vegetative multiplication, cylindrical, 8-spored, probably formed as a result of conjugation between cells different from bud cells and according to Chatton *(46, p. 119)*, "l est probable que ces elements gamines, qui sont tres differents des formes bourgeonnantes, representent la fecondation qui precede la fructification, phenomene dont Guilliermond a demontre l'existence et la generalite chez les Saccharomycetes." Young asci with 2 to 4 nuclei, mature asci appearing longitudinally striated because of 8 acicular ascospores helically coiled and packed with them (fig. 15, *b*); ascospores without a cytoplasmic appendage, acicular, somewhat flattened (ribbonlike), acutely pointed at both ends, (? hyaline) (fig. 15, *k* and *l*).

Nutritional Characters

Not known, apparently not cultured.

Type Specimen

Not known (was not available from CMI, P, or Pasteur Institute, Paris).

PATHOGENICITY, DISTRIBUTION, AND VECTOR RELATIONS

The experiments and observations on the Nematosporaceae mainly pertain to their isolation, inoculation of selected hosts with species encountered in the United States, and the pathogen-vector relation.

Host Range, Geographic Distribution, and Vectors

Metschnikowia

The plant-inhabiting species are saprophytes. There appears to be no host specificity. They occur in nectar and are found in the honey stomachs and in the larval provisions of many bees. They are ingested by bee larvae, but their role in the nutrition of these insects and their mutualistic relation, if any, needs further investigation.

Eremothecium

These are uncommon fungi and cause stigmatomycosis of cotton *(27)* and citrus *(41)*. They do not cause any extensive damage to either crop. Three isolates of one species, *E. ashbyi,* were available for inoculation. Five ripe fruits each of *Citrus nobilis* Lour. (King orange), *C. paradisi* Macf. (grapefruit), *C. reticulata* Blanco (small tangerine), and *C. sinensis* (L.) Osbeck (sweet orange), obtained from commercial sources, were inoculated by the needle-puncture technique or with a hypodermic syringe. After 20 days incubation in glass jars at 25° and 30° C., the fruits were cut open. They showed no visible symptoms; however, the pulp around the inoculation point tasted somewhat unpleasant. Each isolate grew well and formed ascospores in *Citrus* spp. except for *C. paradisi.*

Similar inoculation work was undertaken to test the pathogenicity of *E. ashbyii* to *Gossypium hirsutum* var. *Acala* 4-42. Five- to forty-day-old unopened bolls, each with one green leafy bract, were removed from the greenhouse-grown plants and their pedicel were immediately immersed in water. Twenty bolls were inoculated with the Jaffa orange isolate, and five bolls were used as controls. They were incubated at room temperature under a bell jar for the first 24 hours and subsequently were on a laboratory bench. The inoculated and the noninoculated controls were illuminated by a 60 incandescent lamp placed 45 cm. from the boll level. Four to seven days after inoculation, 19 bolls, including some controls, abscissed. Some bolls from each category turned grayish brown, and most of these were 5 to 19 days old at the time of inoculation. Five 42-day-old inoculated bolls matured and opened normally but showed a slight diffused yellowing of lint around the puncture site.

Each of the inoculated bolls that turned brown was sectioned, stained, and examined microscopically. In all, only eight bolls showed traces of fungus mycelium among the ovule cells, including the fiber initials. A few ascospores were present, but these may have been part of the inoculum since the accompanying mycelium was still young and without asci. The fungus was not observed within the cells. Many knobbed hyphae and yeast cells (not reported for *Eremothecium* before) were often observed in the inoculated tissue. Such cells, when plated, gave rise to normal mycelium of *E. ashbyii.*

These observations are too fragmentary to determine whether or not the fungus is pathogenic to cotton. Additional experiments with bolls attached to the plant should be conducted to verify the initial field reports *(72, 73)* of heavy losses due to *E. ashbyii.* In the meantime, the organism should be transported in accordance with the recommendations of the quarantine authorities.

Nematospora spp. and Ashbya gossypii

Nematospora spp. and *Ashbya gossypii* are by far the most important pathogenic Nematosporaceae. They often occur side by side and attack a variety of hosts (table 1) and are most widely distributed (fig. 1). It has been experimentally confirmed that insect vectors, predominantly those with piercing-sucking mouthparts such as Hemiptera, are responsible for their

transmission (*3, 50, 60, 104, 119, 137, 138, 163, 166, 175*). The fungi are rare on the exterior of sound fruits or the exterior of the vectors. *Nematospora coryli* occurred in soybeans at Beltsville, Md., in 1968 and in cotton in the Imperial Vallley, Calif., in 1970. In both localities the vectors were also present. However, field-collected *Nezara* sp. allowed to crawl on petri dishes at Beltsville and *Leptoglossus zonatus* and *Lygus* spp. collected at Brawley, Calif. yielded no *Nematospora* even though a subsequent examination of the interior of the mouthparts of the same insects revealed viable inoculum (table 2). These findings are consistent with those reported earlier *(6, 77, 104).* The low percentage of infested vectors with viable inoculum (12 to 20 percent) is also not unusual *(5, 88, 105).*

Stigmatomycosis of Some Crops Caused by *Nematospora* spp. and *Ashbya*

The following account of symptoms is based on the literature and on herbarium specimens or on fresh specimens collected in the field. From the very beginning (*3, 27, 130-133, 156*) a distinction was made between the damage caused by the vector alone and that caused by Nematosporaceae. In both cases, a varying degree of discoloration occurs in the host tissue. Generally speaking, however, the injury by a fungus-free insect, or 'stigmose' is localized rather than diffused as with damaged caused by living fungus, or by its sterilized, cell-free, culture broth. The fungus-caused discoloration is often referred to as 'stigmatomycosis', or 'yeast spot.'

A limited number of fruits were inoculated with *N. coryli, N. lycopersici,* and *A. gossypii,* all isolated from the United States, over a period of 3 years at Beltsville, Md. The cross inoculation data for various hosts are summarized in table 3. The following fruits were used: lima beans, french beans, and tomatoes 60 specimens each; cotton bolls and sweet oranges 36 specimens each. The oranges were obtained from commercial sources, and all other fruits were grown at Beltsville.

Cotton

Each of the three species *(N. coryli, N. lycopersici,* and *A. gossypii)* when inoculated into 8- to 42-day-old, green, unopened bolls, after 6 to 10 days caused yellow to yellowish-brown discoloration of the fibers or fiber initials. As in the experiment with Eremothecium (p. 41), many inoculated and uninoculated control bolls abscissed during this period. *Nematospora lycopersici* caused the least discoloration and *A. gossypii* the most. Each fungus was reisolated from the diseased tissues.

Pearson (*138*) at Barberton, S. Africa, inoculated Upland cotton "U-4/920" bolls attached to the plants with pure cultures of *N. coryli* (det. as *'N. phaseoli'*) and *A. gossypii* and proved their pathogenicity, assumed by earlier workers (*103, 124, 133, 149*), on the basis of field observations. Some of his observations are summarized in the following tabulation:

Age at inoculation	*Symptoms and observations*
1 and 2 weeks	Growth completely arrested, seeds killed, bolls either shed or abort, lint becoming a dark membrane.
3 and 4 weeks	Boll size reduced, lint heavily stained, carpels open, contorted and partially reflexed, center of lock 'hard,' not expanding, and appearing webbed.
5, 6, and 7 weeks	Little or no effect on boll size, lint intact, without webbing but stained from dark brown to straw yellow, stain intensity decreasing with age.
8 and 9 weeks	No effects other than traces of discoloration.

Beans

Half-ripe, surface-disinfested seed pods of *Phaseolus vulgaris* L. (french beans) and *P. lunatus* L. (lima beans) were inoculated with *N. coryli, N. lycopersici,* and *A. gossypii* through the pericarp by the needle-puncture technique. They were incubated in moist chambers at 25° and 30° C. and periodically examined for 2 weeks.

Phaseolus vulgaris showed no disease symptoms in response to the inoculum of any species from our collections. A pale-yellow, translucent, cylindrical plug about 0.5 mm. long protruded at the site of all punctures after 72 hours. A similar plug was also present on the inner wall of the pericarp abutting against the seed at the inoculation point. *Phaseolus lunatus* also did not adversely respond to *N. lycopersici* or *A. gossypii,* but *N. coryli* attacked a significant number of beans. Seventy-two hours after inoculation, the younger seeds, 5 to 6 mm. long, developed water-soaked, pale-greenish, depressed lesions at the inoculation site. They were visible as light-green patches through the pericarp. The seedcoat above the lesion was somewhat loose, and a porous, white friable mass of cells underneath contained abundant vegetative cells, asci, and ascospores of *N. coryli.*

A large number of Leguminosae are susceptible to *N. coryli.* In addition to *Phaseolus vulgaris* and *P. lunatus,* it attacks *P. atropurpureus* DC., *P. aureus* Roxb., *P. limensis* Macf., *Cajanus cajan* (L.) Millsp., and *Vigna sinensis* (Torner) Savi. (table 1). Except for the intensity of discoloration of the seedcoats and cotyledons, the symptoms on different beans generally appear similar. The affected areas in lima beans are pale, but in *P. atropurpureus,* an African crop, they are darker than the rest of the seed. Soybeans turn grayish brown, but cowpeas, french beans, and mung beans show no discoloration. In all, however, the affected tissues of cotyledons are sunken and covered with the wrinkled seedcoats, which may remain intact or rupture with age *(174, 176, 181, 197).*

Table 1.—Occurrence of Nematospora *and* Ashbya, *their hosts, and vectors*

Occurrence of[1] —							
A. gossypii	*N. phaseoli*[2]	*N. coryli*	*N. lycopersici*	Vector	Host[3]	Locality	Reference
				Africa			
x	—	x	—	*Antestia cincticollis* (Schaum) ...	*Gossypium ?hirsutum*	Congo	(*8*)
–	—	x	—	*Antestiopsis clymeneis* Kirkaldy ..	"Fruits"	Malagasy	(*43*)
x	—	—	—	.. do	*Gos. ?hirsutum*	Dahomey	(*100*)
–	—	x	—	*A. faceta* (Germar)	*Coffea arabica* L.	Congo	(*85, 86, 87*)
x	—	x	—	*A. lineaticollis* (Stal)	*Coffea* sp., *Acacia* sp., *Gos. ?hirsutum, Anacardium occidentale* L.	Tanzania	(*7, 95, 96, 152, 172, 173, 174, 175, 177, 183, 184*).
–	—	x	—	.. do	*Cof. arabica*	Uganda	(*78, 79, 81*)
x	—	x	—	.. do	.. do	Congo	(*85, 86, 87, 108*).
x	—	x	—	*Antestiopsis* spp	*Cof. arabica, Phaseolus vulgaris* L.	Kenya	(*114, 115, 128*)
x	—	x	—	.. do	*Coffea* sp., *Gos. ?hirsutum*	Malawi	(*195*)
x	—	—	—	.. do	*Gos. ?hirsutum*	Kenya	(*114*)
x	—	x	—	.. do	*Pha. vulgaris, Crotalaria juncea* L.	Malawi	(*192*)
—	—	x	—	*Aspilocoryphus fasciativentris* (Stal).	*Cro. juncea*	Rhodesia	(*91, 93*)

–	—	x	–	. . do	*Gos. ?hirsutum, Pha. vulgaris*	. . do	(*92, 93, 151, 192*)
x	—	x	—	*Calidea duodecimipunctata* var. *dregei* Germar.	*Gos. ?hirsutum, Hibiscus* spp	Zambia	(*2*)
x	—	—	—	*Dysdercus cardinalis* Staecker . . .	*Gos. ?hirsutum*	Ethiopia	(*38*)
x	—	—	—	*D. fasciatus* Signoret	. . do	Mozambique	(*124*)
x	—	—	—	. . do	*Gos. hirsutum* L., *Gos. herbaceum* L., *Gos. peruvianum* Cav.	Nigeria	(*67, 103, 119*)
x	—	x	—	. . do	*Gos. ?hirsutum*	Congo	(*11, 15, 16, 160, 162, 164*)
x	—	x	—	. . do	*Gos. ?hirsutum, Hib. vitifolius* L.	South Africa .	(*13, 137, 144*)
x	—	x	—	. . do	*Gos. hirsutum, Thespesia rogersii* Soland.	Rhodesia	(*4*)
x	—	x	—	. . do	. . do	Zambia	(*2*)
x	—	x	—	*D. intermedius* Distant	*Gos. ?hirsutum, Hib. vitifolius* . .	South Africa .	(*137*)
x	—	x	—	. . do	*Gos. hirsutum, Adansonia digitata* L.	Rhodesia	(*4*)
x	—	x	—	. . do	. . do	Zambia	(*2*)
x	—	—	—	*D. melanoderes* Karsch	*Gos. ?hirsutum*	Nigeria	(*119*)
x	—	x	—	*D. nigrofasciatus* Stal	*Ada. digitata, Sterculia* spp.	South Africa .	(*3*)
—	—	x	—	. . do	*Bauhinia galpinii* N.E	. . do	(*56*)
x	—	—	—	. . do	*Gos. ?hirsutum*	Mozambique .	(144)

See footnotes at end of table.

Table 1.—Occurrence of Nematospora *and* Ashbya, *their hosts, and vectors—Continued*

Occurrence of[1] —				Vector	Host[3]	Locality	Reference
A. gossypii	*N. phaseoli*[2]	*N. coryli*	*N. lycopersici*				
				Africa—(Continued)			
x	---	x	---	*D. nigrofasciatus* Stal	*Hib. vitifolius*, "Wild cotton"....	South Africa .	(*3, 137*)
x	---	---	---	*D. superstitiosus* (Fabricius).	*Gos. hirsutum, Gos. herbaceum, Gos. peruvianum, Gos. ?arboreum* L. or *herbaceum.*	Nigeria	(*67, 103, 104, 119, 191*)
x	---	c	---	..do	*Gos. ?hirsutum*	Congo	(*15, 16*)
x	---	x	---	..do	*Gos. ?hirsutum, The. rogersii*	Zambia	(*2, 8*)
x	---	x	---	..do	*Hib. cannabinus* L	Rhodesia	(*4*)
x	---	---	---	*Dysdercus* spp	*Abutilon* sp	Uganda	(*80*)
--	---	x	---	..do	*Gos. ?hirsutum*	Malagasy	(*54*)
x	---	---	---	..do	..do	Malawi	(*10, 53, 117, 150*)
---	---	x	---	..do	..do	Rhodesia	(*89, 90*)
x	---	x	---	..do	*Gos. ?hirsutum, Persea gratissma* Gaertn., *Hib. cannabinus, Hib. esculentus* L.	Uganda	(*75, 76, 80, 158*)
x	---	x	---	*Helopeltis* spp	*Ana. occidentale*	Tanzania	(*152*)
--	---	x	---	Hemipterous spp	*Cajanus cajan* (L.) Millsp	..do	(*152, 179*)

–	—	x	—	..do	*Cro. juncea*	..do	(*152, 182, 185*)
–	—	x	—	..do	*Cro. striata* L	..do	(*176*)
–	—	x	—	..do	*Dolichos lablab* L., *Citrus sinensis* (L.) Osbeck.	..do	(*152*)
–	—	x	—	..do	*Gos. ?hirsutum*	..do	(*7, 171, 180*)
–	—	x	—	..do	*Pha. acutifoliatus* A. Gray	..do	(*178*)
–	—	x	—	..do	*Pha. aureus* Roxb., *Pha. mungo* L. var. *radiatus, Pha. lunatus* L., *Pha. vulgaris.*	..do	(*152, 176, 181, 182, 186*)
–	—	x	—	..do	*Vigna sinensis* (Torner) Savi	..do	(*179*)
x	—	—	—	*Odontopus confusus* Distant	*Sterculia rogersii* N.E	South Africa	(*5*)
x	—	x	—	None reported	*Centrosema plumieri* Benth	Congo	(*161*)
–	x	—	—	..do	*Glycine max* (L.) Merr	..do	(*165*)
–	—	x*	—	..do	*Gos. ?hirsutum*	Gambia	
x	—	x	—	..do	..do	Ivory Coast	(*148*)
x?	—	x?	—	..do	*Persea gratissma*	Congo	(*18*)
–	—	x*	—	..do	*Pha. atropurpureus* DC	Uganda	
–	x?	x?	—	..do	*Pha. vulgaris*	South Africa	(*1*)
–	—	x*	—	..do	*Tephrosia* sp.	Tanzania	
				South America			
x	—	—	—	*Dysdercus* sp	*Gos. ?hirsutum*	Brazil	(*39, 99, 113, 121*)

See footnotes at end of table.

Table 1.—Occurrence of Nematospora *and* Ashbya, *their hosts, and vectors—Continued*

Occurrence of[1] —				Vector	Host[3]	Locality	Reference
A. gossypii	*N. phaseoli*[2]	*N. coryli*	*N. lycopersici*				
				South America—Continued			
–	—	x	—	*Dysdercus* sp.	*Hibiscus* sp., *Sida* sp	Brazil	(*99*)
–	—	x	—	*Nezara viridula* (L.)	*Cit. sinensis*	Peru	(*36*)
–	—	x	—	..do	*Lycopersicum esculentum* Mill	..do	(*21*)
–	—	x	–	..do	*Vigna sinensis*	Brazil	(*99*)
				Caribbean			
x	—	—	—	*Dysdercus discolor* Walker	*Gos. barbadense* L	St. Vincent	(*27, 82*)
x	—	—	—	*Dysdercus* spp	..do	Antigua, Nevis	(*27*)
x	—	x*	—	..do	..do	Jamaica	(*27*)
x	—	—	—	..do	*Gos. barbadense, Datura metel* L.	Montserrat	(*27*)
x	—	—	—	..do	*Gos. barbadense, Ascelpias curassavica* L.	Trinidad	(*27, 29, 167*)
–	—	x	—	*Nezara viridula*	*Cajanus* sp., *Dolichos* sp., *Phaseolus* sp., *Vigna* sp.	St. Vincent	(*27*)
–	—	x	—	..do	*Pha. lunatus*	Bermuda	(*187*)

–	—	—	x	None reported	*Gos. hirsutum*	Dominican Republic ...	(*68*)
–	—	—	x	..do	*Lyc. esculentum*	Cuba	(*157*)
–	x	—	—	..do	*Pha. lunatus, Pha. vulgaris*	Puerto Rico ..	(*49, 57*)
				North America			
–	x	—	—	*Acrosternum hilare* (Say)	*Cit. nobilis* L.	Florida	(*188*)
–	x	—	—	..do	*Glycine max*	Missouri	(*51*)
–	x	—	—	..do	..do.	North Carolina	(*107*)
x	—	—	—	..do	*Gossypium* sp	Texas	(*40*)
–	x	—	—	..do	*Pha. limensis* Macf	Mississippi ...	(*136*)
–	x	—	—	..do	*Pha. lunatus*	Alabama, Tennessee	(*83*)
–	x	—	—	..do	..do.	Illinois	(*24, 84*)
–	x	—	—	..do	..do.	Maryland	(*47, 83*)
–	x	—	—	..do	*Pha. lunatus, Vigna sinensis, Ipomoea batatas* (L.) Lam., *Pha. vulgaris.*	Virginia	(*197*)
–	—	x	—	*Leptoglossus zonatus* (Dallas) ...	*Cit. limon* (L.) Burm. f., *Cit. sinensis, Gos. ?hirsutum, Punica granatum* L.	California	(*60, 61*)
–	—	x?	—	*Lygus* spp.	*Pha. lunatus*	..do	(*28*)

See footnotes at end of table.

Table 1.—Occurrence of Nematospora *and* Ashbya, *their hosts, and vectors—Continued*

Occurrence of[1] — *A. gossypii*	*N. phaseoli*[2]	*N. coryli*	*N. lycopersici*	Vector	Host[3]	Locality	Reference
				North America—Continued			
--	--	x	--	*Oebalus pugnax* (Fabricius)	*Oryza sativa* L.	Missouri	(*52*)
--	--	x	--	"Sucking bugs"	*Carya illinoensis* (Wang.) K. Koch.	Florida	(*189*)
--	--	x	--	.. do.	*Cit. grandis* (L.) Osbeck or *Cit.* paradisi Macf., *Cit. nobilis, Clethra alnifolia* L.	.. do	(*188*)
--	--	x	--	.. do.	*Glycine Max, Pha. aureus*	Oklahoma ...	(*102, 145*)
--	--	--	x	.. do.	*Lyc. esculentum*	Florida	(*188*)
--	--	x	--	*Thyanta custator* (Fabricius), *Euschistus tristigmus* (Say), *E. servus* (Say), *E. variolarius* (Palisot de Beauvois), *E. servus* subsp. *euschistoides* (Vollenhoven).	*Glycine max*	Missouri	(*51*)
--	--	x*	--	None reported	*Cit. sinensis*	Florida	
--	--	--	x	.. do	*Lyc. esculentum*	California, ... Mexico	(*157*)
--	x	--	--	.. do	*Pha. lunatus*	Illinois	(*24*)
--	x	--	--	.. do	do	Virginia	(*196*)

Europe							
–	—	x	—	None reported	*Corylus avellana* L	Italy, Sicily	*(139, 140, 141).*
--	---	*x*	---	. . do	*Zea mays* L	Italy	*(142)*
Asia							
–	—	x	—	*Cappaea taprobanensis* (Dallas), *Leptoglossus membranaceus* (Fabricius), and *Rhnychocboris poseidon* Kirkaldy.	*Cit. limon, Cyphomandra betacea* Sendt, *Lyc. esculentum.*	Indonesia	*(127, 170)*
x	—	x	—	*Dysdercus cingulatus* (Fabricius). .	*Gos. ?hirsutum*	Burma	*(48, 149, 153)*
–	—	x	—	*Dysdercus* spp., *Leptoglossus zonatus, Nezara viridula.*	*Citrus* sp	China	*(110)*
x?	—	x?	—	None reported	*Cit. nobilis*	Burma	*(14)*
--	—	x	—	. . do	"Fruits"	Taiwan	*(110)*
--	—	x	—	. . do	*Gos. ?arborecum* or *herbaceum*	Iraq	*(23)*
–	—	x	—	. . do	*Gos. ?hirsutum*	India	*(44, 50)*
x	—	—	—	. . do	. . do	Russia	*(94)*
x?	—	x?	—	. . do	*Gos. ?hirsutum* or *herbaceum*	Iraq	*(22)*
–	—	x	—	. . do	*Gos. ?hirsutum, Phaseolus* spp	Burma	*(153, 154)*
x	—	—	—	. . do	*Hib. cannabinus*	Russia	*(168)*
–	—	—	x	. . do	*Lyc. esculentum*	China	*(109, 190)*

See footnotes at end of table.

Table 1.—Occurrence of Nematospora *and* Ashbya, *their hosts, and vectors—Continued*

Occurrence of[1] —				Vector	Host[3]	Locality	Reference
A. gossypii	*N. phaseoli*[2]	*N. coryli*	*N. lycopersici*				
				Asia—(Continued)			
–	—	x	—	None reported	*Pha. lunatus*	Ceylon	(*134, 135*)
–	—	x	—	..do	*Pistachia* sp	Iran	(*129*)
–	—	x	—	Rhynchota spp	*Cit. aurantium* L., *Citrus* sp.	Java	(*41*)
–	—	x	—	..do	*Cit. mitis* Blanco, *Cit. nobilis, Cit. sinensis.*	China, Japan, Philippines	(*106*)
				Oceania			
–	—	x*	—	None reported	*Macadamia ternifolia* F	New Zealand	

[1] *, new record, based on specimens examined; ?, species identification, as reported, is uncertain.

[1] This species not recognized in the text, reported here as it appeared in the original reference.

[3] ?, species identification, as reported, is uncertain.

Table 2.—Number and percentage of insect vectors harboring Nematospora coryli *in an infested locality in California and in Maryland*

Locality, vector, and date	*Insect exterior*				*Insect interior*			
	Insects examined	Infested		With viable	Infested		With viable cells	
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*	*Number*	*Percent*
Beltsville, Md.								
Nezara viridula:								
Aug. 20-25, 1968	25	4	16	0	5	20	5	20
Sept. 20-28, 1970	50	0	–	0	0	–	0	–
Brawley, Calif.								
Leptoglossus zonatus,								
Sept. 1-14, 1970	25	0	–	0	6	24	3	12
Lygus spp.,								
Sept. 1-14, 1970	25	0	–	0	0	–	0	–

Table 3.—Pathogenicity of Ashbya *sp. and* Nematospora *spp. to selected fruits in the laboratory*

Host	*Pathogenicity of indicated fungus, source of cultures, and isolate (in parenthesis)*				
	A. gossypii cotton (2,553)	*N. coryli* beans (10, 661)	*N. coryli* host (?) (10,647)	*N. coryli* soybeans (2,558)	*N. lycopersici* tomatoes (2,518)
Gossypium hirsutum var. *Acala* 4-42 (glandless).	+, ov +, F+	NT	NT	+, ov +, F+	+, ov—, F—
Citrus spp.	+	+	+	+	+
Lycopersicum esculentum "globe."	+	+	+	—	+
Phaseolus lunatus	—	—	—	+	—
P. vulgaris	—	—	—	—	—

+, Organism caused visible damage and thus is inferred to be pathogenic; —, not pathogenic; ov + or ov —, fungus observed or not observed in the ovule, respectively; F+ or F—, fibers infected or not infected, respectively; NT = not tested.

Citrus

Each of the three fungi *(N. coryli, N. lycopersici,* and *A. gossypii)* was inoculated into ripe fruits of *C. nobilis* L. (King orange), *C. reticulata* (small tangerine), and *C. sinensis* (sweet orange). The pH of these fruits varied from 3.5 to 4.5. The fungi were also grown on sliced halves of the fruits in a moist chamber. In both instances they grew well, sporulated, and caused a general softening of the pulp and rotting of juice bags, which now could be easily detached from the section walls. Three weeks after inoculation the pulp of treated fruits appeared drier than the control. An unpleasant odor emanated [no bacterial contamination] from all fruits and they tasted somewhat bitter rather than sweet, as were the controls.

Lee *(106)* gave an account of dry rot of *Citrus* spp. caused by *N. coryli* in the Philippines. He observed mealiness and thickening of section walls of fruits of *C. aurantium, C. nobilis, C. mitis,* and *C. sinensis* attached to the tree. The individual juice vesicles were dried, wrinkled, and atrophied and could be easily separated from each other. They had relatively thicker walls than those of normal vesicles. Lee *(106)* specifically mentions lack of discoloration in *C. aurantium* from the Philippines. He concluded that the ripe fruits were more susceptible than younger, green ones.

According to Fawcett *(60, 61), N. coryli* causes brownish to reddish-brown staining of the pulp sections of ripe oranges, grapefruits, and tangerines. In time the pulp deteriorates and dries without any visible symptoms of deterioration on the exterior of the rind. In addition to these symptoms, Weber *(189)* also observed protuding of oil glands in affected areas of the rind of Satsuma oranges *(C. nobilis* var. *unshiu)* and the presence of conspicuous white spots underneath them.

Tomatoes

During mid-October 1970, 120 globe tomatoes var. (?) Homestead in various stages of ripeness (pH of juice 3.8-4.2) were inoculated with *N. coryli* (three isolates), *N. lycopersici* (one isolate), and *A. gossypii* (one isolate). The fruits were divided into two groups: approximately one-half were kept attached to the vines whose cut ends were immersed in water, and the other one-half were harvested and inoculated in dishes. There was no difference in the symptomatology of the two groups.

Green-mature tomato fruits 48 hours after inoculation with *N. coryli* and *N. lycopersici* appeared to be water-soaked and darker green around the needle punctures than the controls. Development of red pigment around the inoculation point was delayed for 2 to 5 days in green-, pink-, and orange-colored fruits. The younger the fruit, the more pronounced was the inhibition of red pigmentation at maturity. The affected areas in fruits inoculated at full maturity were dull red, initially sunken, and had loose skin, but eventually the fruits distended and cracked open (fig. 12, *B*).

Tomatoes of all ages when inoculated with *A. gossypii* exhibited delayed pigmentation, remained firm throughout, and showed wartlike swellings rather than depressions in affected tissues. Internally, the diseased tissues of

ripe fruits were permeated throughout with hyphae and the seed cavities were packed with asci and ascospores. However, the fruits inoculated with *Nematospora* spp. were often watery and compact knots of fungal matter often appeared in the seed cavities.

Symptoms caused by the two isolates of *N. coryli,* ATCC-10,647 and ATCC-10,661 (labelled in the collection as *'N. phaseoli'*) were different from those just described. These isolates did not cause typical depressions at the puncture sites nor did they inhibit pigment development. Distinct white streaks or blotches (fig. 12b) appeared in the skin instead, and the fruits gave an appearance of what was appropriately described as the 'cloudy spot' by Weber *(188).* The tomatoes (Cultivar Roma x Plant Introduction No. 272,636), collected by Thomas Barksdale from South Carolina, also exhibited similar symptoms.

DISCUSSION AND CONCLUSIONS

The Nematosporaceae include several potentially destructive pathogens of important plants. In certain African, Caribbean, and South American countries they have been the most destructive pathogens of cotton and coffee and continue to cause extensive damage. The genera *Ashbya, Nematospora,* and the non-phytopathogenic *Metschnikowia* are the most common and the best known of the group. *Ashbya gossypii, N. coryli,* and *N. lycopersici* occur in the United States and have occasionally caused extensive damage to citrus, cotton, and soybeans in small endemic areas. Some damage to pecans, soybeans, and tomatoes seemingly goes undetected or is attributed to bugs alone.

Distribution and Host Range

A perusal of table 1 and 3, figure 1, and lists of specimens examined for each of the Nematosporaceae illustrates several points.

1. The polyphagous insect vectors and the fungi attack hosts from widely separated families of the flowering plants. However, they appear to be more common on Malvaceae (the genera *Gossypium, Hibiscus,* and *Adansonia*) and Leguminosae (the genera *Phaseolus, Glycine, Crotalaria, Cajanus, Vigna,* and *Tephrosia*).

2. There appears to be little host specificity—the hosts include herbs, shrubs, and trees. Also, there appears to be no species specificity between the fungi and the insect vector, although *Ashbya gossypii* appears to be predominantly transmitted by *Antestia* and *Dysdercus* spp.

3. It has been repeatedly demonstrated in experimental material *(3-5, 17, 60, 77, 118, 137)* and it is now beyond any reasonable doubt that the fungi are transmitted by these insects.

4. The insect vectors are exclusively from the families Coreidae, Lygaeidae, Miridae, Pentatomidae, and Pyrrhocoridae.

5. Whereas, many of the vectors are of worldwide distribution, the phytopathogenic Nematosporaceae are restricted to the warmer parts of the world.

Vector-Fungus Relation

Critical observations on many aspects of the vector-fungus relation are still fragmentary. Alternate hosts support insect populations and are a major source of the primary inoculum of *Nematospora* and *Ashbya* in the tropics. However, the source of such inoculum in the temperate areas is unknown. These fungi were neither found on the exterior of fruits nor were they isolated from the soil. Whether or not the overwintering adult vectors serve as a source of primary inoculum is not known (see below).

None of the phytopathogenic Nematosporaceae directly penetrate healthy fruits or seeds *(60, 77)* nor do they normally enter through the feeding punctures of uninfested vectors *(77)*. They are transmitted only by insects that have fed on previously infected tissues (*77, 137*). Fungus-free laboratory-reared insects do not cause infection typical of stigmatomycosis.

The number of fungus-infested insects in a population in an infested area varies considerably, and not all instars are equally infective (*5, 88, 105, 125*). In Barberton, Republic of South Africa, where critical studies were undertaken concerning the transmission of *Ashbya gossypii* to cotton, only 13 percent of the field-collected *Dysdercus nigrofasciatus* and *D. intermedius* were infective when caged with experimental plants. However, such a population caused severe damage as a result of repeated feeding (*5*). In Maryland and California only 20 and 24 percent of the insects, respectively, were infested (table 2) with *N. coryli.*

Pearson *(137)* in Republic of South Africa demonstrated that only adults collected in the field carry the fungi and are capable of their transmission and subsequent infection of cotton bolls. These findings were in part confirmed (*103*) at Barberton where only fourth and fifth instars, but not the second and third, caused infection. On the contrary, Muller (*127*), working with Citrus in Indonesia, observed that the bugs *Rhynchoris serratus, Cappaea taprobanensis,* and *Leptoglossus membranaceus* could tranfer *N. coryli* after one feeding on diseased fruits immediately after hatching. Most nymphs of the first two species retained their infectivity throughout their lives irrespective of molting. *Rhynchoris serratus,* fed once on diseased fruits and daily thereafter on healthy ones, was still infective after 65 days. He considered that the fungus was carried in the intestines.

Contrary to some reports *(6, 104),* anatomical investigations of infested insects reveal the presence of fungus inoculum on and within their mouthparts *(62, 124, 169)*. Frazer *(62)* described and illustrated a special enlargement within the oral cavity of *Dysdercus* sp. where yeast cells were tightly packed. Daugherty in 1967 *(51)* also observed inoculum within the mouthparts although he does not give any anatomical details. On numerous occasions I also found viable inoculum in the mouth and in the alimentary canal (but not in the excreta) of *Nezara viridula.* However, no discrete enlargements or structures similar to those described by Frazer *(62)* were observed. Internal transmission of the inoculum, a wide geographic distribution of the numerous vectors and hosts involved, and the lack of fungus-vector specificity are among the most important reasons why the Nematosporaceae are to be considered as potentially dangerous to agriculture in the United States.

Morphology, Taxonomy, and Relations With Other Hemiascomycetes

The ascus fungi, or the class Ascomycetes, have been traditionally divided into two subclasses, the Hemiascomycetidae and the Euascomycetidae. This classification therefore suggests that the hemiascus and the euascus are derived from a common sexual apparatus. Much additional information is needed to substantiate this assumption. The Nematosporaceae are included in the Hemiascomycetidae. Whether or not the latter are related to the 'true ascomycetes' does not impair the classification of the family.

The primary characteristics used for separating the genera of the Nematosporaceae are morphological, and the nutritional and caryological characteristics are secondary. The acicular or elongate ascospores, relatively large asci, and poor ability or inability to ferment are characteristics common to all. A majority of them thrive in flowers, seeds, or fruits and are transmitted by hemipterous insects. All the Nematosporaceae form buds although the buds do not always germinate to give rise to new thalli.

Except for *Guilliermondella selenospora* Nadson and Krassilnikov (=*Endomycopsis selenospora* (Nad. & Krassil.) Dekker), which has lunate ascospores, no other Hemiascomycetes have elongate-acicular spores. Only the asci of *Dipodascus (30)* and *Ascoidea (34, 35)* attain dimensions comparable with those of this family. These two Hemiascomycetes are multispored and open at the apex by gradual deliquescence. The remaining Hemiascomycetes have globose to subglobose or ellipsoid, usually 1-8 spored asci.

An important characteristic of the Hemiascomycetes, not by itself but in conjunction with others, is the stage in the life cycle when the sexual plasmogamy takes place. This was stressed by Guilliermond *(71)* and to some extent by Gäumann *(65, 66).* The ascospores in three genera of the Nematosporaceae are themselves gametosporous or the buds formed by them conjugate. In *Ashbya* and *Nematospora* the plasmogamy occurs between two sister protoplasts of the same ascospore or, rarely, between two ascospores.

The two sex nuclei fuse immediately after plasmogamy and the conjugant cell gives rise to a diploid (2n), filamentous or cellular thallus. In *Metschnikowia (123)* the ascospore gives rise to buds that may remain haploid and reproduce asexually or may conjugate and give rise to a diploid thallus. The diploid thallus in *M. pulcherrima* and *M. reukaufii* gives rise to characteristic thick-walled proasci, but in other species of the genus such structures may be thin-walled. The stage at which sexual plasmogamy takes place in the two *Eremothecium* spp. is not known. The ascospores are uninucleate and give rise to a multinucleate, filamentous thallus, which probably contains diploid and haploid nuclei in the same cell.

The species usually may be separated on the basis of nutritional characteristics, but morphological characteristics, such as cell shape, filamentous versus cellular thallus, and size of such structures are more useful. Species of *Nematospora* and some *Metschnikowia* spp. are rather similar nutritionally, but can be readily separated on the basis of cell shape. The reliability of nutritional characteristics of fungi in storage for sometime has been often

questioned, since some strains change their pattern of assimilation and fermentation. Lodder (*111 p. 10*) however considers that:

> ". . . the easy acquisition of new properties by a strain [genetic clone] should be accepted as characteristic of this strain; and when a number of strains in a species show the same phenomenon, it may be considered a species characteristic."

Relation Among the Genera and Species Concept

Except for *Nematospora,* the species of other genera are well characterized. In 1969 and 1970 species concept in *Metschnikowia* was revised by Miller and van Uden *(123)* and Pitt and Miller *(143).* They investigated a large number of isolates from many substrata and several geographic areas (see p. 34). The two species of *Eremothecium, E. ashbyii* and *E. cymbalariae* can be easily separated from each other on the basis of ascus form and its location on the thallus (see p. 10). *Ashbya gossypii* is a segregate of *Nematospora* and is so characteristic that it cannot be confused with any other species of the older genus.

The species of *Nematospora* need further investigation to establish whether or not *N. coryli* and *N. lycopersici* hybridize with each other. Several other questions also deserve attention: Is the fungus described and well illustrated as *'N. phaseoli'* a distinct species or was Wingard *(197)* dealing with a variant of one of the other two species just mentioned? Was Peglion *(140, 141)* dealing with one or two fungi? My investigations indicate that cell shape, i. e., round versus ellipsoid, appears to be characteristic for different isolates. These isolates also differ from each other nutritionally and in the ablity to elicit responses from various hosts. Isolates from various sources are being hybridized, so far unsuccessfully, to shed additional light on speciation in *Nematospora.* These isolates are also being tested serologically to ascertain similiarities and differences among them.

Wickerham *(194)* believes that *Metschnikowia* is probably the most primitive member of the Nematosporaceae. All species of this genus are cellular; whereas, the family also contains filamentous species. *Metschnikowia* is the only genus in the family that lives in both haploid and diploid states. To assume these characteristics to be primitive or advanced would be pure speculation. A new approach to substantiate or refute Wickerham's *(194)* hypothesis would be to investigate the cell-wall composition of several filamentous as well as cellular Hemiascomycetes and study them serologically. Also, analyses of the base composition of deoxyribonucleic acid of such forms may be useful. It appears that groups of closely related yeasts usually have the same base ratio of guanine + cytosine / guanine + cytosine + adenine + thymine and dissimilar DNA base compositions are found only with unrelated groups. This would prove to be a very useful approach to ascertain relation between fungi such as *Ashbya* and *Eremothecium.* Both produce copius amounts of several related flavinoid compounds. The former is certainly a close relative of *Nematospora* (see p. 22), but the taxonomic position of the latter has been uncertain (72-74).

Significance of the Nematosporaceae to Crops in the United States

The phytopathogenic Nematosporaceae were first described from the Caribbean area. They, together with their vectors, made it virtually impossible to grow cotton in many of the islands. They have been intermittently found in the United States since 1916. However, except for one severe but endemic infection of cotton in the Imperial Valley, Calif., in 1928-29 *(60),* they have not caused appreciable damage to the crops in this country.

Since 1964 a sizable acerage of soybeans in Kansas and Missouri has been found to be infected with *Nematospora coryli* and *N. lycopersici.* These two fungi were occasionally also found on other hosts (table 1). Furthermore, *Ashbya gossypii,* currently a destructive pathogen in many African countries, was reported from the 1970 crop of soybeans and cotton in Missouri and South Carolina, respectively.

There are many potential insect vectors of the phytopathogenic Nematosporaceae in this country (table 1), and fungus-infested populations were found in several localities *(51, 52).* Susceptible clones of several crops and weeds also exist. Several cultivars of *Gossypium hirsutum* of United States origin, when screened overseas in areas naturally infested with the Nematosporaceae, proved to be susceptible. Laycock and Jones *(104)* in Nigeria found cultivar *Allen* of *G. barbadense* to be more susceptible to *A. gossypii* than the indigenous cultivars *Meko* and *Isban.* Likewise, Rhind *(149)* observed that the cultivar *Cambodia* of *G. hirsutum* was more susceptible to *Nematospora* spp. than the local cultivars *Wagaye* and *Wagale.*

Robertson *(153),* Steyaert *(160),* Rainey *(147),* and others *(12, 20)* also evaluated several cultivars of *G. hirsutum* in Africa, including the American cultivars *Clevewilt, Dixie Triumph, Farm Relief, Lone Star, Triumph Big Boll,* and *Wonder Dixie* of American origin. Their work, however, was conducted primarily by artificially inoculating bolls detached from the plants with *N. coryli, A. gossypii,* or both. Except for a few clones derived from *Triumph Big Boll,* almost all cultivars proved to be susceptible to stigmatomycosis. These varieties are no longer commercially grown in the United States although some of the present-day clones do share gene pools from the older stocks.

Many details of phytopathogenicity of the Nematosporaceae have been worked out. However, many questions concerning the epidemiology of these micro-organisms still remain unanswered. What factors prevent or facilitate the spread, ingress, and establishment of these fungi? What is the primary source of inoculum? Where and in what form do the organisms overwinter in the temperate zone? Does inoculum overwinter within the adult vectors or do they fortuitously acquire it from the overwintered plant debris or infected seeds planted in the field? Is it possible that the inoculum is annually transported by the vectors from areas with mild climate into those with severe winters? Answers to these and many other questions must be sought because *Nematospora* and *Ashbya* threaten the third (soybean) and fourth (cotton) most important crops, in terms of dollar value, in the United States.

The presence of a large number of susceptible crops with a wide range of alternate hosts serving as reservoirs of inoculum; of numerous vector species, many with worldwide geographic distribution and a polyphagous nature; and of the internal transmission and multiplication of the Nematosporaceae in the vectors may in the future prove these fungi to be some of the most destructive phytopathogens in the United States.

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☆ U. S. GOVERNMENT PRINTING OFFICE : 1973 O - 504-855