CONTENTS

Morphology and Variability of the Cucurbit Black Rot Fungus (Key No. Wis.-169) - - - - - - - - - - - 81
W. F. CHIU and J. C. WALKER

Relation of Specific Gravity to Shrinkage and of These Factors to Growth in Yellow Poplar (Key No. W. Va.-17) - - - - - - - 103
HARVEY D. ERICKSON

Decay Resistance of Seven Native Oaks (Key No. G-1399) - - - 129
THEODORE C. SCHEFFER, GEORGE H. ENGLERTH and CATHERINE G. DUNCAN

Chemical Removal of Encrustants From Dew-Retted Hemp Fiber (Key No. G-1400) - - - - - - - - - - - 153
LYLE E. HESSLER

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MORPHOLOGY AND VARIABILITY OF THE CUCURBIT BLACK ROT FUNGUS

By W. F. Chiu, formerly fellow, and J. C. Walker, professor in plant pathology, Wisconsin Agricultural Experiment Station

INTRODUCTION

The cucurbit black rot fungus was first described by Fautrey and Roumeguère (17) on a Chinese variety of cucumber in France in 1891 and designated as Ascochyta cucumis. A little later in the same year Passerini (18) described the fungus Didymella melonis on Cucumis melo L. in northern Italy without knowledge of its association with Ascochyta, while Chester (2) described the same fungus on Citrullus vulgaris Schrad. in Delaware under the name Phyllosticta citrullina without recognizing the ascigerous stage. After further study in Delaware, C. O. Smith (19) (1905) named it Ascochyta citrullina. When he discovered the ascigerous stage, he described it as Sphaerella citrullina. Grossenbacher (4) designated the imperfect stage of the fungus a member of the genus Diplodina, and referred the ascigerous stage to the genus Mycosphaerella. Potebnia (14) reported a fungus on Cucumis melo L. under the name Ascochyta melonis which was considered to be closely associated with Didymella melonis Pass. He was of the opinion that A. citrullina (Chester) C. O. Sm. on watermelon was a form intermediate between A. cucumis Fauitr. and Roum. and A. melonis Poteb. As a result of further studies on Fautrey's collection, Keissler (10) considered the three names to be synonyms, and recognized Ascochyta cucumis Fauitr. and Roum. as the first valid name for the imperfect stage. It is interesting to note that Ferraris (9) published a species on Cucumis melo L. in the northern part of Italy under the name Sphaerella melonis and claimed that the asci are nonparaphysate. The description of Ferraris' Sphaerella melonis showed that it is the same fungus as Passerini's Didymella melonis and of C. O. Smith's Sphaerella citrullina. There is no specific difference between either one of the European forms and the American fungus.

The pleomorphism and variability of this fungus were the cause of some of the confusion in its early classification of the imperfect stage. Chester (2) described it as Phyllosticta citrullina on account of its continuous spores, while Keissler (10), in studying Fautrey's collection on Cucumis melo L., failed to recognize the micropycnospora.

1 Received for publication April 19, 1948.
2 The writers are indebted to W. C. Snyder for suggestions made during the course of the investigation, to J. F. Stauffer for advice and assistance in conducting the irradiation experiment, and to Eugene H. Herring for assistance in preparing the illustrations.
3 Italic numbers in parentheses refer to Literature Cited, p. 101.
stage as a phase of the fungus, and thought it to be *Phyllosticta orbicularis* Ell. and Ev. More recently, Wiant (24) pointed out that there is considerable variation in the size of pycnidia and perithecia and in the relative abundance of nonseptate spores. He also observed that the extent of sporulation varied in different isolates.

The present study is concerned with the variability of the morphology and cultural characters of the cucurbit black rot fungus. It is shown that the proper binomial of the fungus is *Mycosphaerella cucurbitae* (Eautr. and Roum.) nov. comb.

**EXPERIMENTAL RESULTS**

**SOURCE AND CHARACTERISTICS OF CULTURES**

The black rot disease was found to be endemic in a watermelon-growing area near Merrimac, Wis. Vines of the Hawkesbury variety of watermelon were collected on August 27, 1945, and from these tissue-fragment cultures were made. On September 13, 1945, specimens containing both pycnidia and perithecia were obtained from the same source. Single-spore cultures from ascospores and from pycno-

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**PYCNIDIAL STAGE**

There is evidence in certain species of *Mycosphaerella* that microconidia function as spermatia. The earliest record of the so-called spermagonium was made by Higgins (6) (1914) in *M. nigerristigma* Higgins. Klebahn (12) applied the name micropycnidia to the small
conidia in *M. hircacii* (Sacc. and Briosi) Jaap. Snyder (20) pointed out that the so-called pycnosporces of *M. brassicicola* (Fr.) Lindau did not germinate under the conditions studied and suggested that they probably were spermatia. There is no evidence as yet that micro- pycnosporces of *Mycosphaerella* having an *Ascochyta* stage function as spermatia.

Pycnidia bearing either microspores or macropycnosporces were present constantly in the sporulating cultures of *Mycosphaerella inmumis* studied. Production of one or both types on certain media varied with the strain of the organism. In general, pycnidia bearing microspores could be induced in *A* cultures by ultraviolet irradiation on potato-dextrose agar, while in *As* cultures they were produced

![Image of culture plates](image-url)

**Figure 1.**—Seven-day cultures of variants of *Mycosphaerella cucurbitis*. 

naturally on both potato-dextrose agar and oatmeal-dextrose agar. In B-a and B-1a cultures both types of pycnidia occurred on oatmeal-dextrose agar, but only pycnidia producing microspores occurred on squash-extract agar. On potato-dextrose agar, microspores predominated in B-a cultures and macrospores in B-1a cultures. The sizes of pycnidia and pycnospores are compared in table 1. It is evident that pycnidia varied greatly in size on culture media. The range of size of pycnidia from naturally infected watermelon stems was somewhat narrower (50 µ-150 µ). The size and shape of pycnidia from several sources are illustrated in figure 2.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Measurement of pycnidia and pycnospores of variants of Mycosphaerella Melonis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range and mode of size in variants indicated</td>
</tr>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Microns</td>
</tr>
<tr>
<td>Diameter of pycnidia</td>
<td>90-200</td>
</tr>
<tr>
<td>width and length of micropycnospores</td>
<td>1.5-3 x 4-8</td>
</tr>
<tr>
<td>width and length of macro pycnospores</td>
<td>2.5 x 10-13</td>
</tr>
</tbody>
</table>

1 Produced by irradiation with ultraviolet.

Most of the pycnospores obtained from naturally infected material were nonseptate, with a size range of 2.5µ-4.5µ x 5µ-10µ. Variation in septation of pycnospores of all the isolates was considerable on culture media. Pycnospores could be divided as to size into two classes, designated herein as micropycnospores and macrospores. The size range of the micropycnospores was quite similar in the variant types and on the different media used. Generally speaking, the micropycnospores were unicellular. The macrospores of B-1a were mostly uniseptate and occasionally biseptate, while the macrospores of B-a were mostly nonseptate. In a reisolate of B-1a type from overwintering watermelon stems all pycnospores were uniseptate. There was no relation between size of pycnidium and size of spore produced. Some very small pycnidia produced macrospores and some large pycnidia produced micropycnospores. A few microspores sometimes appeared in a pycnidium in which macrospores predominated but the reverse was not noted. The small pycnidia of B-a on oatmeal-dextrose agar and the larger pycnidia of B-1a on the same medium produced mostly macrospores.

The micropycnospores and macrospores did not appear to differ in function. They were equally germinative and developed
FIGURE 2.—Variation in size and shape of pycnidia. 1, As, wild type on watermelon stem; 2, As type on oatmeal-dextrose agar; 3, A type on potato-dextrose agar after ultraviolet irradiation; 4, B-1a type on oatmeal-dextrose agar; 5, B-1a type on squash-extract agar; 6, B-a type on squash-extract agar; 7, B-a type on oatmeal-dextrose agar; 8, "pseudoperithecia" of B-a type on oatmeal-dextrose agar.
into colonies true to the type from which they were taken if no mutation occurred. Later studies on the development of perithecia did not indicate that the micropycnospores were concerned with fertilization. Types of pycnospores are shown in figure 3.

ASCIGEROUS STAGE

Grossenbacher (4) studied the perithecia from diseased muskmelon and recorded their diameter as ranging from 100μ to 165μ. Recently Wiant (24) observed the perithecia from several curcurbit species and found them to vary in diameter from 71μ to 224μ. The dark-brown

![Figure 3](image-url)

**Figure 3.**—W, Old mycelium of As type on potato-dextrose agar. X, Variation in size, shape, and septation of pycnospores: 1, from naturally infected watermelon stem; 2, As type on oatmeal-dextrose agar; 3, B-a type on squash-extract agar; 4, B-1a type on squash-extract; 5, B-1a type on oatmeal-dextrose agar; 6, B-1a reisolate after overwintering on watermelon stem; 7, B-a type on oatmeal-dextrose agar. Y, Micropycnospores germinating (24 hours at 24° C.) in 2 percent orange extract. Z, Macropycnospores germinating (24 hours at 24° C.) in 2 percent orange extract.
to black color and the globose to inverted oval shape have been recorded in all descriptions since Grossenbacher (4, 13, 24). In the present investigation the perithecia were mostly globose to subglobose either on naturally infected watermelon stem or on culture media. The mature perithecia were dark brown to black. They were partially embedded or erumpent in the diseased tissue and on culture media. Measurements of perithecia, asci, and ascospores given in table 2 show that the perithecia were largest in $B-a$ and $B-la$ cultures. The sizes of asci and ascospores were about the same in all isolates.

### Table 2.—Average measurements of perithecia, asci, and ascospores on watermelon and on oatmeal-dextrose agar

<table>
<thead>
<tr>
<th>Organ</th>
<th>From watermelon stem canker</th>
<th>From the isolate indicated grown on oatmeal-dextrose agar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Microns</td>
<td>$A-a$</td>
</tr>
<tr>
<td>Perithecia</td>
<td>90-120</td>
<td>75-125</td>
</tr>
<tr>
<td>Ascii</td>
<td>6-10X36-70</td>
<td>8-10X35-88</td>
</tr>
<tr>
<td>Ascospores</td>
<td>4. 5-6X9-15</td>
<td>5-7X12-15</td>
</tr>
</tbody>
</table>

The fascicled asci were hyaline and each ascus contained eight bicelled fusiform ascospores either in one or in two series. Many mature perithecia were studied. No paraphyses were found among the asci. However, in young perithecia there were paraphysislike structures which were close to those illustrated by Klebahn (13) for Didymella lycopersici. Perithecia of different ages were picked and studied. It was found that in certain young perithecia such paraphysislike structures were more numerous than asci while in older ones they were less common or absent. Since the asci did not all mature at the same time and since abortion was common, these structures were regarded as immature or abortive asci and not as true paraphyses.

A peritheciumlike fruiting body was always found associated with the perithecia in agar cultures, usually being produced slightly earlier than the perithecia. Such structures were indistinguishable from the true perithecia in shape, color, and size ($110\mu-160\mu$). Instead of asci and ascospores, there were produced within, spores in chains which separated later. Spores were of one to three, mostly two cells, hyaline, nearly spherical, and $6\mu \times 7\mu$ to $6\mu \times 19\mu$ in size. They were discharged from the fruiting body along with granular material. Since the internal structure of the fruiting body was distinct from that of the peritheium, the name pseudoperithecium was applied. Various phases of the ascigerous and pseudoperithecial stages are illustrated in figure 4.

### DEVELOPMENT OF FRUITING BODIES

The developmental history of the fruiting bodies has been studied in other species of Mycosphaerella (6, 7, 8, 25), but the details of the development of perithecia of M. kluyveri have not been reported previously. Study of several hundred monosporic isolates revealed that this fungus was strictly homothallic. The prompt development
of pycnidia and perithecia in all fertile cultures facilitated the present studies. Observations were made on the cultures seeded at one side of oatmeal-dextrose agar plates. A young B-a culture was used because all three types of fruiting bodies were produced, aerial mycelium was absent, and the submerged mycelium grew relatively slowly. Sketches were made with the aid of a camera lucida.

Formation of pycnidia was initiated by the looping of the end of a main hypha or of its branch (fig. 5). As a rule, septation of the looping hypha increased as the size and number of coils increased. By the time three to five loops had formed, numerous slender hyphae with dense cytoplasm were developing from the inner surface of the entangled loops. These slender hyphae were possibly pycnosporal primordia which later developed into pycnosporas. When more loops developed and a young pycnidium took form, the hyaline mycelium changed to yellowish brown. As maturity was approached, the color turned darker. The loops of a pycnidium in certain cases were contributed by different hyphae from the same thallus. One characteristic of the mycelium which was destined to form pycnidial loops was the presence of many swollen cells in the older part of the thallus. Such cells were elliptical or oval in shape and had very thin walls. The contents appeared to be very dilute and a few seconds' exposure outside the culture dish resulted in their immediate collapse. The early stage of loop formation was observed in both sporulating and nonsporulating isolates. In the latter the development ceased at the formation of loops and without the formation of pycnosporal primordia.

The first step in the development of perithecia was the formation of a terminal ascogonium that was nonseptate and had very dense cytoplasm (fig. 5). The ascogonium-bearing hypha was either straight or coiled. The antheridial hypha originating from a neighboring thick hypha of the same thallus was long and slender with granular, usually hyaline, cytoplasm. As the antheridial hypha made contact with the ascogonium, the latter began to become septate and coil around the terminal swelling of the former. The behavior of the nuclei was not studied. After intensive coiling and septation of the cells below the terminal cell, a rudimentary perithecium began to take form. The color of the external cells then turned olive brown. Accessory hyphae were sometimes observed. Because of the slenderness of these accessory hyphae, they were suspected of being trichogynes characteristic of certain other species of Mycosphaerella, but they were not visible at the time of contact of antheridium and ascogonium. Mature perithecia were finally observed, presumably after fertilization of the ascogonia.

In culture, the ascogonium sometimes failed to make contact with the antheridial hypha. In such cases, the mature "perithecia" failed to produce any asci and ascospores and, instead, chains of ascosporelike structures were formed. Further study would be necessary to establish whether or not there is a positive correlation between failure of the initial to develop into a perithecium and lack of fertilization. The chains eventually separated into sporelike bodies, each
Figure 5.—Development of sexual and asexual fruiting bodies in a B–a culture on oatmeal-dextrose agar. X, Formation of pycnidium: 1, 2, fertile hyphae; 3, early stage of loop formation; 4–7, stages in development of pycnosporal primordia. Y, Formation of peritheciun and pseudoperitheciun: 1, coiling ascogonium; 2, antheridial hypha making contact with an ascogonium; 3, swelling tip of the antheridial hypha with the ascogonium coiling around it; 4, 5, 6, postfertilization stages; 7, 8, nonfertilized ascogonia developing into pseudoperithecia.
consisting of one to three cells. The cells were roughly spherical and the content was not so dense as that of ascospores. In reality these structures were homologous with pycnidia and pycnospores. In this paper the terms "pseudoperithecium" and "pseudoascospore" are applied to them.

**SPORE GERMINATION**

Pycnospores, ascospores, and pseudoascospores germinated readily in hanging drops of 2 percent orange extract (figs. 3, 4). Germination was very poor in sterile tap water.

The first step in the germination of pycnospores was the swelling of the cell to several times its original size. The second step was the formation of a hyaline germ tube. In the case of micropycnospores, a globular cell was usually formed at the tip of the germ tube, which either sent out a slender thread perpendicular to the axis of the germ tube or continued to grow and form another globular structure at the tip. In the case of macropycnospores, each cell of the bicelled spore sent out a germ tube which either remained nonseptate and nonbranched or became septate and branched immediately. Branching occurred at any point along the germ tube. Secondary sporelike structures were sometimes found on the terminals of side branches similar to those observed by Grossenbacher (4). The ascospores and pseudoascospores germinated in the same manner as pycnospores except that there was no secondary sporelike structure at the tip of hyphae.

**VARIABILITY OF CULTURAL CHARACTERS**

In his recent studies of the black rot fungus, Wiant (24) reported isolates having various sporulating capacities and different cultural characters. In this investigation spores and hyphae were stained with 1 percent crystal violet for 1 minute, and the surplus stain was removed with distilled water and blotting paper. The preparation was cleared with 1 percent picric acid and then with clove oil. The cells of ascospores and pycnospores were shown to be uninucleate and those of the mycelium multinucleate. Since the fungus was homothallic and since individual thalli arose from uninucleate cells of spores, the nature of variability of the fungus was of particular interest.

**MONOSPORIC ISOLATIONS**

The sporulating strains, As, B-a, and B-1a, were grown on oatmeal dextrose agar because of the profuse and early production of fruiting bodies. In general, pycnospores were picked from 7-day cultures and ascospores from 15-day cultures. The method was that used by Keitt and Langford (11), but no attempt was made to isolate the ascospores in their natural order.

A very dilute suspension of pycnospores was smeared over the surface of a potato-dextrose agar plate with a sterilized glass spatula. This seeded plate was incubated overnight at 24°C. When the germ tubes were beginning to protrude, spores were picked with a specially prepared glass needle under a binocular microscope. Only distinctly germinating spores were transplanted on oatmeal-dextrose agar plates. Six spores were usually arranged in a circle on one plate.
The incubation temperature was maintained at 24°. The cultural characters of the isolates were recorded and compared at the end of 1 week. Those with the same characters were grouped and given the same number.

The first picking of 100 ascospores from the As strain yielded 99 B-a colonies and 1 As colony. The single As colony was transferred to oatmeal-dextrose agar and from it 112 ascospores were picked, of which 96.4 percent produced As colonies; 1.8 percent produced A colonies; 1.8 percent were of a new type, designated as A-1, which was a sterile albino with aerial mycelium similar to that of A and As. Each of 224 pycnosporos picked from the original As strain yielded an As colony. Variants appeared to arise only from ascospores in this set of experiments, but the lack of variants from pycnosporos may have been due to the relatively small number observed.

From 108 ascospores from strain B-a, only B-a colonies developed. From 80 ascospores from a B-a culture derived from an ascospore in the first picking only B-a colonies developed. About 20 percent of the B-a colonies were lighter in color than the rest. They were designated as B-a(1) and the others as B-a(d). From 104 pycnosporos isolated from a B-a(1) colony, 95 percent yielded B-a(1) and 5 percent B-a(d) colonies. From 112 pycnosporos selected from a B-a(1) colony of the previous picking, 98 percent of the colonies were B-a(1) and 2 percent were B-a(d). It appeared that B-a(1) was a mutant from B-a(d) and tended to throw frequent B-a(d) mutants.

From 140 ascospores from a B-1a culture all colonies were B-1a. In 3 successive monopycnosporic isolations covering a total of 416 colonies, all were true B-1a type.

Since the B-b culture in its early stage of growth showed a mosaic of characters of B-a type and As type and later developed As characters entirely, it was thought that possibly it came from an As ascospore but that a B-a mutation occurred in one of its young hyphae, and the mutant character was overrun by the rapid growth of the As of the mycelium. This supposition was shown to be true by a study of monoascosporic isolations from B-b type. From 75 monosporic colonies, 23 percent were As and 77 percent were B-a. No homogeneous B-b type cultures were secured by monoascosporic isolations from As or B-a type.

A diagrammatic summary of the type of culture secured by a study of single-spore lines follows:
Isolation by the Dilution-Plate Method

In order to observe a comparatively large population during a brief period, the dilution-plate method was used. Although this method had the disadvantage that two or more sporals might grow together to form a single colony, the population that could be observed for variation was much greater than when individual spores were picked. Monosporic $A_s$ and $B$-$1a$ cultures were used in this test. The spore suspension of each culture was so adjusted that 1 milliliter of sterile water contained approximately 50 pycnospores of $B$-$1a$ or 25 spores of $A_s$. One milliliter of the prepared spore suspension was pipetted aseptically into the oatmeal-dextrose agar plates and was made to spread evenly over the surface. Twenty plates of $A_s$ and 30 plates of $B$-$1a$ were made. All plates were incubated at 24° C., for 7 days in the case of $B$-$1a$ and for 15 days in the case of $A_s$. The total number of colonies established in each plate was counted, and any colony that was slightly different from the parent culture in color and growth was recorded and isolated for further comparison both by mass transfer and by isolation of monopycnospores.

The total of resulting colonies from $A_s$ was 371, of which 3 percent were $A$ type, 1 percent were $B$-$1b$ type, and 96 percent were $A_s$ type. $B$-$1b$ type was very similar to $B$-$1a$ type, except that it assumed a deep olive-green color at an early stage of development. The difference between $B$-$1a$ and $B$-$1b$ types corresponded exactly to that between $B$-$a$ (1) and $B$-$a$ (d) types. Some monopycnosporic isolates of $B$-$1b$ were uniform for type characters. Dilution plates of a $B$-$1a$ culture yielded a total of 1,585 colonies, of which 0.4 percent was $B$-$1b$ type and 99.6 percent $B$-$1a$ type. A pycnospore suspension of a $B$-$1b$ culture was prepared as in the case of $B$-$1a$ and 100 dilution plates were made. Each of 5,126 colonies which developed was $B$-$1b$ type. From this study it was evident that in monosporic cultures of $A_s$ the type and number of mutants was greater than in $B$-$1a$ cultures. The difference in color of $B$-$1a$ and $B$-$1b$ at the early stage of development was slight, and it disappeared as the colonies grew. The results with the dilution-plate method were similar to those obtained from monosporic isolations.

Isolation of Mycelial Cells

The frequent occurrence of $A_s$ sectors and the sudden appearance of $A_s$ fruiting bodies in $A$ cultures led to consideration of the variability arising from nuclear changes in mycelial cells. Since the $A$ culture was obtained from early monosporic isolations, the purity of the culture was beyond question. The problem to be solved was the uniformity in the genotype of the cells of the mycelium that might be detected by phenotype analysis. In order to render such study feasible, the isolation of mycelial cells at random was carried out.

Two $A$ cultures from monopycnosporic isolation from the natural host substrate were available. One was 1 year old, the other was 1 month old. The mycelium of these cultures was removed and washed several times with sterile water. The washed mycelium from one plate culture of a given isolate was minced aseptically for 15 minutes in a Waring Blender. To 1 ml. of the resulting mycelial suspension was added 9 ml. of sterile water. Then with the aid of a
sterile glass spatula, one drop of the suspension was smeared over a potato-dextrose agar plate. The technique for picking spores was again used in the picking of mycelial cells. Only those pieces of hyphae which consisted of two to three cells were picked and transplanted to oatmeal-dextrose agar plates. Plates were incubated at 24° C. and records were taken at the end of the seventh day when fruiting bodies were appearing on certain colonies.

In the case of the 1-year A culture, a total of 96 pickings was made. Only 24 percent of these pickings developed into colonies. Among the developing colonies, 87 percent were As type and 13 percent A type. A total of 53 pickings was made from the 1-month A culture. All of them developed into colonies, of which 68 percent were As type and 32 percent A type. The variation of the A type culture to the As type and vice versa occurred naturally and spontaneously. It was not possible to separate these 2 types and propagate them in their own characters indefinitely.

**RELATION OF MEDIUM TO GROWTH AND SPORULATION**

In an attempt to induce sporulation of the fungus, Wiant (24) employed a number of media including corn-meal agar, oatmeal agar, and cucurbit stems. Sporulation was scant in each of these, only a few fruiting bodies appearing occasionally. In the studies just reported sporulation was governed by genetic factors. However, it was of interest to determine whether sporulation in the sterile type A could be induced by the nutrient. The agar media used are listed in table 3. Dextrose and peptone were used at the rate of 10 grams per liter. Other ingredients were used at rates indicated elsewhere (16).

**Table 3.—The effect of various agar media on sporulation of an A type culture of Mycosphaerella melonis**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Average growth rate</th>
<th>Color of the substratal mycelium</th>
<th>Aerial mycelium</th>
<th>Sporulation at 144 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mm. per hear</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potato</td>
<td>0.53</td>
<td>Pale olive</td>
<td>+</td>
<td>None</td>
</tr>
<tr>
<td>Potato-dextrose</td>
<td>0.91</td>
<td>Dark greenish black</td>
<td>+++</td>
<td>Do.</td>
</tr>
<tr>
<td>Potato-peptone</td>
<td>0.84</td>
<td>Salmon</td>
<td>++</td>
<td>None</td>
</tr>
<tr>
<td>Corn meal</td>
<td>1.75</td>
<td>Pale olive</td>
<td>+</td>
<td>A few</td>
</tr>
<tr>
<td>Corn meal-dextrose</td>
<td>0.72</td>
<td>Greenish black</td>
<td>+++</td>
<td>None</td>
</tr>
<tr>
<td>Corn meal-peptone</td>
<td>0.79</td>
<td>Salmon to brownish</td>
<td>+++</td>
<td>A few,(^2)</td>
</tr>
<tr>
<td>Czapek's solution</td>
<td>1.96</td>
<td>Colorless</td>
<td>+</td>
<td>Sparse</td>
</tr>
<tr>
<td>Czapek's solution-dextrose</td>
<td>0.79</td>
<td>Dark olive</td>
<td>+++</td>
<td>None</td>
</tr>
<tr>
<td>Czapek's solution-peptone</td>
<td>0.53</td>
<td>Salmon</td>
<td>+++</td>
<td>Sparse</td>
</tr>
<tr>
<td>Czapek's solution dextrose-peptone</td>
<td>0.86</td>
<td>Yellowish brown</td>
<td>+++</td>
<td>None</td>
</tr>
<tr>
<td>Bean</td>
<td>0.88</td>
<td>Brownish olive</td>
<td>+++</td>
<td>A few,(^2)</td>
</tr>
<tr>
<td>Bean-dextrose</td>
<td>0.97</td>
<td>Dark olive green</td>
<td>+++</td>
<td>None</td>
</tr>
<tr>
<td>Bean-peptone</td>
<td>0.67</td>
<td>Purplish</td>
<td>+++</td>
<td>Sparse,(^3)</td>
</tr>
<tr>
<td>Bean-dextrose-peptone</td>
<td>0.79</td>
<td>Brownish</td>
<td>+++</td>
<td>None</td>
</tr>
<tr>
<td>Oat-paste</td>
<td>0.92</td>
<td>Dark greenish black</td>
<td>+++</td>
<td>Abundant,(^3)</td>
</tr>
<tr>
<td>Oat-paste-dextrose</td>
<td>0.79</td>
<td>Dark greenish black</td>
<td>+++</td>
<td>Do.(^3)</td>
</tr>
<tr>
<td>Oat-paste-peptone-dextrose</td>
<td>0.79</td>
<td>Pale yellowish brown</td>
<td>+++</td>
<td>None</td>
</tr>
<tr>
<td>None</td>
<td>1.57</td>
<td>Colorless</td>
<td>-</td>
<td>Abundant,(^3)</td>
</tr>
<tr>
<td>Dextrose</td>
<td>0.82</td>
<td>Pale olive</td>
<td>+++</td>
<td>Do.(^3)</td>
</tr>
<tr>
<td>Peptone</td>
<td>0.39</td>
<td>Salmon</td>
<td>+++</td>
<td>Do.(^3)</td>
</tr>
<tr>
<td>Dextrose-peptone</td>
<td>0.90</td>
<td>Brownish</td>
<td>+++</td>
<td>Do.</td>
</tr>
</tbody>
</table>

\(^1\) Mycelium was weak and sparse.

\(^2\) — none; + to +++ + indicate increasing amounts of aerial mycelium

\(^3\) Primordial and mature perithecia also occurred
An A culture from a monopycnosporic transfer from the As type was used. Five plates of each culture medium were incubated at 24° C. for 144 hours. Sporulation, rate of growth, color of the submerged mycelium, and extent of aerial mycelium were recorded.

The results in table 3 show that the growth was most rapid when dextrose was present. In a synthetic medium, such as Czapek’s solution, best growth occurred with dextrose and peptone. The peptone-dextrose effect was less significant in media rich in organic materials. The presence of dextrose induced olive to greenish-black color while peptone induced salmon to purplish-brown color of submerged mycelium. Peptone usually induced more vigorous aerial mycelium than dextrose, but the most abundant development occurred when both were present.

Sporulation was relatively abundant only on oat-paste agar and when dextrose alone was added to the latter, but it was prevented when peptone alone was added. When dextrose was added with peptone the inhibitive effect of the latter was prevented. Sparse sporulation occurred on bean-peptone dextrose and on corn meal-peptone agar; it was rare on corn meal-dextrose and potato-peptone agars. When sporulation occurred, the cultural characters were very similar to those of As cultures. In earlier experiments it was shown that pycnidial loops occurred in all sterile cultures but development usually ceased before sporulation. It is possible, therefore, that the presence of certain nutrients or growth factors in the media mentioned enabled the A mutant to become as fertile as the wild type, As. Evidence has already been presented that the As type might exist in A cultures without expressing itself; it is possible, therefore, that the oat-paste medium promoted the growth and expression of the As type which was already present. Nevertheless, when the above media were used for culture of other sterile strains (A-1, A-2, and B-3) no sporulation was ever observed, although pycnidial loops were common. This difference may be due to a difference in the nutritional requirements of mutants.

EFFECT OF IRRADIATION ON SPORULATION

The effect of irradiation on the sporulation of fungi has been reported by Stevens (21, 22, 23) and by Ramsey and Bailey (15). In the present investigation 7-day A cultures on potato-dextrose agar plates, showing vigorous aerial mycelium, were irradiated with a mercury-quartz lamp (Westinghouse Sterilamp). The plates were placed 6 inches below the source of light with covers removed. The intervals of treatment were ½, 1, 5, 10, 15, 20, 30, and 40 minutes. After the treatment, all plates were kept in complete darkness at 24° C. The nonirradiated plates served as controls. On the fourth day after irradiation, sporulation occurred in plates irradiated 1 minute to 40 minutes, being most abundant in the 15-, 20-, and 30-minute treatments. None of the control plates showed a trace of fruiting bodies. The aerial mycelium of the irradiated cultures had all turned gray and collapsed while abundant black pycnidia appeared amongst the collapsing mycelium. The pycnidia were very similar to those of the As culture.
A series of experiments was then carried out on the media listed in table 3. In addition to sporulation on oat-paste agar, as shown earlier to occur without irradiation, the amount of sporulation was increased on those media in which it occurred only sparsely without irradiation. The most significant effect, however, was on potato-dextrose agar on which no sporulation occurred normally. A slight amount of sporulation appeared on corn-meal agar. The relative number of perithecial primordia remained constant on oat-paste agar, oat-paste-dextrose agar, and bean-dextrose agar in both irradiated and nonirradiated plates, but the maturity of the perithecia was hastened and the proportion of mature perithecia to pseudoperithecia increased with irradiation. When potato-dextrose agar plates were irradiated before they were seeded, no sporulation occurred in the subsequent culture. When \textit{As} cultures were irradiated, the amount of sporulation was not affected.

**INDUCTION OF MUTATION BY IRRADIATION**

Since spontaneous mutation was observed frequently in the \textit{A} and \textit{As} cultures, the possibility of increasing the frequency and direction of mutation by irradiation with ultraviolet rays was considered. The mercury-vapor type of lamp (General Electric AH-6,100 watts, and 500 volts alternating current) was employed. The wave length was 2,534 Å, and the constant temperature in operation was 25° C. A pycnospore suspension from an \textit{As} culture at a density of approximately 7,500 spores per milliliter of sterile distilled water was used. Ten milliliters of the spore suspension was pipetted into the radiation cell. Before irradiation, 0.2 milliliter was drawn out from the cell and diluted in 100 milliliters of sterile distilled water as a control. After starting the irradiation, 0.2 milliliter of the spore suspension was drawn out and diluted at 10-minute intervals up to 80 minutes. From each dilution of these treatments 10 plates were prepared with 1 milliliter of suspension. All plates were incubated at 24° and records were taken at the end of 6 days. Special attention was given to types of colonies that had not been observed previously.

The percentage of viable spores yielding \textit{A}, \textit{As}, or \textit{B-b} cultures decreased rapidly as the duration of irradiation increased. The mutant, \textit{A-1}, previously observed in monosporic isolations, appeared regularly throughout the treatments. \textit{B-1b} type, which had been isolated by the dilution-plate method from \textit{B-1a} cultures, appeared at 10-minute radiation. Three mutants (\textit{A-2}, \textit{A-2s}, and \textit{B-3}) which had not been observed before, were found. \textit{A-2} appeared first after 10 minutes' irradiation but was more frequent after 30 to 60 minutes. \textit{A-2s} appeared only once and then after 70 minutes' irradiation, while \textit{B-3} appeared at between 40 and 50 minutes. \textit{A-2} and \textit{A-2s} were quite similar; the former was a sterile albino, the latter sporulated and was white at first, turning brown with age. The pycnidia produced in \textit{A-2s} were peculiar in type, since they were usually grouped together to form sclerotiumlike bodies in which several pycnidia were united at the base but were distinct otherwise and had distinct ostioles. The black sclerotiumlike bodies were 0.5–3 millimeter in diameter. The mass of pycnospores discharged at the ostioles was whitish instead of the usual grayish salmon. Type \textit{B-3} grew very
slowly and had thick velvety to woolly aerial mycelium. Both aerial and submerged mycelia assumed a purplish-brown color. No mature fruiting bodies were observed, but microscopic study revealed that the loop formation characteristic of the early stage of normal pycnidial development was present. Nevertheless its development stopped before the formation of a pycnidial wall. Colonies of the mutants described above are illustrated in figure 1.

As shown in figure 6, the percentage of killing caused by irradiation increased rapidly up to 20 minutes. The differences in the percentage of killing at intervals above 20 minutes were not significant. The percentage of mutants in the surviving spores increased rapidly up to 40 minutes' exposure beyond which the rate slowed down. The dif-
ference in percentage of mutation at 70 to 80 minutes and 40 to 60 minutes is probably not significant since the population of spores which survived the two longest treatments was very small.

INDUCTION OF MUTATION BY HIGH TEMPERATURE

It was observed during very warm summer periods that A sectors appeared more frequently than usual in monosporic isolates from As cultures. Experiments were conducted, therefore, to determine whether temperature influenced the rate of mutation. Two young monosporic cultures were incubated at 24°C for about 10 days, when fruiting bodies were well formed. Then 1 of the plates was removed to an incubator at 36°C, the other to an incubator at 16°C. At the end of 1 week, 10 dilution plates for each treatment were made. All these plates were again incubated at 24°C for 1 week, and then the number and type of colonies were recorded. The data in table 4 show that the frequency of A mutations in As cultures was greatly increased after high temperature treatment. The B-la mutant also occurred after the high temperature treatment. These results, incidentally, offered an explanation of the fact that difficulty was commonly encountered in maintaining the sporulating character of the As cultures in the laboratory during warm summer periods.

### Table 4. — The effect of temperature on mutation as shown by the percentage of colonies in type classes

<table>
<thead>
<tr>
<th>Treatment °C</th>
<th>Total colonies</th>
<th>Colonies in type classes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>As</td>
</tr>
<tr>
<td>16</td>
<td>209</td>
<td>98.3</td>
</tr>
<tr>
<td>36</td>
<td>178</td>
<td>98.4</td>
</tr>
</tbody>
</table>

TAXONOMIC POSITION OF THE FUNGUS

The present study shows that the imperfect stage of the fungus is extremely variable in size of pycnidia and in size and septation of pycnospores. Moreover, considerable variation in these characters occurs as a result of genetic mutation and the segregation of mutants in culture. This accounts for the confusion in the taxonomic position of the fungus cited in the introduction of the paper. Fautrey and Roumeurer (17) published the name of the imperfect stage, *Ascochyta cucumis*, in 1891, slightly earlier than that of the perfect stage by Passerini (18) in the same year. Therefore the proper binomial of the fungus should be *Mycosphaerella cucumis* (Fautr. and Roum.) nov. comb. The synonymy follows:

*Ascochyta cucumis* Fautr. and Roum., 1891.
*Didymella melonis* Pass., 1891.
*Phylllosticta citrullina* Chester, 1891.
*Ascochyta citrullina* (Chester) C. O. Sm., 1905.
*Sphaerella citrullina* (Chester) C. O. Sm., 1905.
*Diplodina citrullina* (C. O. Sm.) Gross., 1909.
*Mycosphaerella citrullina* (C. O. Sm.) Gross., 1906.
*Ascochyta melonis* Potebnia, 1910.
*Sphaerella melonis* Ferraris, 1912.
DISCUSSION

Although Fautrey and Roumeguère (17) recognized the cucurbit black rot fungus on a variety of Chinese cucumber to be a species of *Ascochyta* and described it under the name *Ascochyta cucumis* in France as early as 1891, the pleomorphism of this fungus caused some confusion in its classification. The small continuous micropycnosporés which predominate in nature on the host apparently led Chester (2) to describe the fungus as *Phyllosticta citrullina* on watermelon and led Keissler (10) to report *Phyllosticta orbicularia* Ell. et Ev. on *Cucumis melo* L. Grossenbacher (4) stated that Chester's specimen of *Phyllosticta* might not represent the same pathogen as that described later by C. O. Smith as *Ascochyta citrullina*. The explanation of this discrepancy is found in the fact that this organism may at times produce pycnosporés which conform to the genus *Phyllosticta*. Inclusion of the perfect stage of *Ascochyta* in *Didymella* is questionable, even though Passerini (18) and Klebahn (13) claimed that paraphyses were present. Ferraris (3) and American workers (4, 19, 24, 25) did not see any paraphyses among the asci of this fungus. The present observations show that no paraphyses are present in the perithecia but that immature or abortive asci are often present among the mature asci. The abortive asci might have been interpreted erroneously as paraphyses. In view of the priority of *Ascochyta cucumis* Fautr. et Roum., the name of the perfect stage is designated herein as *Mycosphaerella cucumis* (Fautr. and Roum.) nov. comb. *Melonis (Pass.) nov. comb.*

The production of micropycnosporés and macropycnosporés varied with the strain and the media. Thalli from micropycnosporés gave rise to colonies with mature perithecia. In spite of evidence obtained in other species of *Mycosphaerella* that micropycnosporés function as spermatia, it is believed that they function as conidia in the black rot fungus.

Homothallism of this genus was first noted by Jones (9) and later proved by Hare and Walker in *Mycosphaerella pinodes* (Berk. and Blox.) Stone (5). The present pathogen also is homothallic. Fertilization of the ascogonium is accomplished by an antheridium originating from a neighboring hypha of the same mycelium.

Cells of both ascospores and pycnosporés were shown to be uninucleate, while those of the mycelium were multinucleate. Wiant's culture from Hubbard squash conforms to the *As* type described herein; his sector of the squash isolate is similar to the *B*-1a and *B*-1b types; his isolate from Puerto Rican cucumber is similar to the *B*-a type. These variants, according to the present studies, originate from the *As* wild type. Wiant also observed the sterile *A* type but failed to point out that *As* and *A* types are spontaneously and reversibly mutable.

The mutability of the *As* strain is high and the direction of mutation is various. The frequency of mutation may be increased by high temperature and by irradiation. That the repeated transferring of mycelium of species of *Ascochyta* increased the sterility of the subsequent cultures has been noted by many workers, including Yu (26). This was also observed in the *As* strain. A possible explanation is that the repeated picking of mycelium may greatly increase the probability of purifying the *A* mutant from the *As* strain, since most *As*
mycelium is producing spores and grows less rapidly than that of the mycelial mutant.

Wiant failed to induce sporulation of the culture by the use of different media (24). The sporulation of this fungus seems to be controlled by both genetic and nutrient factors as in the case of Neurospora (1). The loss of the property of synthesizing certain organic substances necessary for sporulation may be responsible for some of the sterility, and may explain the inconsistency in sporulation of the usually sterile A type. By using fertile strains, such as As and B-la, sporulation was readily obtained on all media that Wiant had tried without success.

It is interesting to note that the sterile A strain can be induced to sporulate by irradiation of a potato-dextrose culture for 1 to 40 minutes under a mercury-quartz lamp. Ramsey and Bailey (15) were able to induce the sporulation of Fusarium cepae (Hanzawa) Link and Bailey and Macrosorium tomato Cooke by irradiation, and they were of the opinion that it was not due to inhibition but rather to stimulation. Since in the present work the fruiting bodies are exclusively of the As type and since A and As are reversibly mutable, it remains to be determined whether or not irradiation increased the rate of mutation of A type to As type.

SUMMARY

The perithecia, pycnidia, and pseudoperithecia of Mycosphaerella melonis as well as the spores produced by them, are described. The perithecia varied in size within a much narrower range than the pycnidia. Two types of pycnidia are described. One produced small continuous spores (micropycnospores); the other produced larger spores (macropycnospores) which were continuous, one-septate or rarely two-septate. The types of pycnidia were indistinguishable morphologically except for the size and septation of the pycnospores produced.

Ascospores and pycnospores were uninucleate. Monosporic lines were homothallic. The developmental histories of the sexual and asexual fruiting bodies are described. There was no evidence that micropycnospores functioned as spermatia.

The wild type, As, was extremely variable. It mutated spontaneously to A, A-1, B-a, B-1a, and B-1b. A and A-1 were sterile, while B-a, B-1a, and B-1b sporulated profusely and produced mycelium sparsely. Mutant A tended to mutate back to As while A-1, B-a, and B-1a were very stable. B-1b was apparently a variant of B-1a from which it differed only in the early development of the color of the colony. The growth rate and coloration of the submerged mycelium of the fungus varied with the medium. Dextrose in combination with peptone in any kind of medium favored vigorous mycelial growth. Dextrose was responsible for the olive-green color of the submerged mycelium, while peptone induced a salmon to purplish-brown color.

Sporulation of sterile strain A was induced on oat-paste agar and by irradiation of a well-developed culture on potato-dextrose agar medium for 15 minutes,
Irradiation with ultraviolet rays induced a higher frequency of mutation and new mutants. $A-2, A-2s,$ and $B-3,$ were obtained by this means. $A-2$ and $B-3$ were sterile while $A-2s$ produced spores in sclerotiumlike groups of pycnidia.

Exposure to a temperature of $36^\circ$ C. for 1 week resulted in an increase in the number of mutants.

The confusion in earlier literature regarding the taxonomy and nomenclature of this organism is probably due largely to its pleomorphism. Size, shape, and septation of pycnospores and size of pycnidia are extremely variable. The earlier reports of paraphyses in the perithecium are probably due to erroneous interpretations of immature or abortive asci. According to the information available the proper Latin binomial for the fungus is *Mycosphaerella cucurbitae.*


(13) ——— 1921. DER PILZ DER TOMATENSTENGELKRANKHEIT UND SEINE SCHLAUCHFRUCHTFORM. Ztschr. f. Pflanzenkrank. 31: 1–16, illus.
(14) Potebnia, A.  

(15) Ramsey, G. B., and Bailey, A. A.  

(16) Riker, A. J., and Riker, R. S.  
1936. Introduction to research on plant diseases . . . 117 pp., illus. St. Louis, Mo. [Processed.]

(17) Roumeguère, C.  

(18) Saccardo, P. A.  
1891. sylloge fungorum. 9: 662. Patavii.

(19) Smith, C. O.  

(20) Snyder, W. C.  

(21) Stevens, F. L.  

(22) ———  

(23) ———  

(24) Wiant, J. S.  

(25) Wolf, F. A.  
1939. leafspot of ash and Phylosticta viridis. Mycologia 31: 258–266, illus.

(26) Yu, T. F.  