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Published on the 1st and 15th of each month. This volume will consist of 12 numbers and the contents and index.

Subscription price:
- Entire Journal: Domestic, $3.25 a year (2 volumes)
  Foreign, $4.75 a year (2 volumes)
- Single numbers: Domestic, 15 cents
  Foreign, 20 cents

Articles appearing in the Journal are printed separately and can be obtained by purchase at 5 cents a copy domestic; 8 cents foreign. When purchased in quantity of 100 or more a reduction of 25 percent in the cost will be made. If separates are desired in quantity, they should be ordered at the time the manuscript is sent to the printer. Address all correspondence regarding subscriptions and purchase of numbers and separates to the Superintendent of Documents, Government Printing Office, Washington, D. C.
STRUCTURE AND GERMINATION OF SEPTORIA SPORES

By H. G. MacMillan, senior pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and O. A. Plunkett, assistant professor, Department of Botany, University of California at Los Angeles

INTRODUCTION

An intimate knowledge of the structure of spores of pathogenic fungi is of major importance to agricultural practice because of the relation of spore structure to method of infection and possibly to the effectiveness of control measures. Such knowledge has a definite bearing on the development of effective chemical methods of preventing or controlling certain plant diseases. Current methods commonly utilize spores of different fungi as test material in determining the probable value of new and improved chemicals as fungicides. The mode of action and the effectiveness of specific substances as fungicides depend in part upon the structure and the organization of the spores with which they come in contact.

*Septoria api¿-graveolentis* Dorogin, the cause of late blight of celery (*Apium graveolens* L.), is widespread on cultivated and escaped celery in California. The general appearance of the disease on cultivated celery is somewhat different from that on escaped celery, but a study of the fungus on the two types of host failed to reveal any significant morphological differences. This study led to a further study of the spores of this species of *Septoria*, with emphasis on the detailed structure of the spores, the meaning and nature of septation, the meaning and nature of guttulae, the variation in the form of the spores, the structural changes due to growth and germination, and the meaning of maturity. The results of this study are reported herein. These observations have a bearing on the structure of *Septoria* spores in general, and this is briefly discussed.

MATERIAL AND METHODS

The spores of *Septoria api¿-graveolentis* are about 30µ to 50µ long and 1µ to 2.5µ in diameter. The spores have a highly elaborated and detailed structure, which can be observed only at high magnifications. The details of the structure cannot be delineated in the bright field alone, and it is only by the use of the dark field under the most favorable circumstances that structure is revealed more completely. Observations made in the bright field alone are incomplete and sometimes erroneous. The spores as seen under the microscope are too small to photograph or to draw satisfactorily with the aid of the camera lucida. The drawings reproduced herein represent the spores as actually seen...
and were prepared with precise attention to details of size, form, and relative position of the spore constituents. Projection at high magnification in a darkened room was a substantial aid in the work.

Celery leaves and leafstalks bearing pycnidia were selected in the field from either escaped or cultivated celery. Plants that showed reduction in vigor due to age or changes due to or symptoms of other diseases were never used. The leaves and leafstalks were put in the refrigerator at a temperature of 10° C. In a few hours spore horns (cirri) exuded from the pycnidia. Fresh spore horns provided the most uniform source of spores; they were free of extraneous matter and remarkably uniform in size and general condition. Unless spores from within the pycnidium were desired for special reasons, the spores of the spore horns were used throughout the study. After sufficient observations had been made to determine when *Septoria* spores are mature, only mature spores were used.

**THE SPORE HORN, OR CIRRUS**

The spore horn exudes from the ostiole of the pycnidium under some pressure as a threadlike structure, more or less curled. The amount of curling of the spore horn can be assigned to no specific cause, but it is believed to be due to conditions within the pycnidium and to the rapidity with which drying takes place as the horn emerges. The cross section of the horn is generally circular, but it may vary and conform somewhat to the shape of the ostiole. The length of the horns is variable. Some are only 2 to 5 mm. long, especially on thin leaves. Horns exuding from pycnidia embedded in the surface tissue of leafstalks are frequently 1 to 1.5 cm. long and occasionally as much as 2 cm. long. The color when freshly exuded is a light tan. The spores in the spore horn are held by some mucilage that is not seen. When a horn segment is placed in water the release of the spores from each other is immediate and complete, and no trace of any matrix or cementing substance can be observed. In the dry state the spores lie in the horn with their long axis parallel to that of the horn. The more slender or pointed end of the spore is the point of attachment within the pycnidium, and the spores are always oriented in the horn so that the distal or less pointed end emerges first. Although the ends of the spores are not markedly different in shape, there is ordinarily no difficulty in distinguishing the distal from the proximal end.

In an immature pycnidium the spores are bent and more crooked, sometimes with relatively abrupt bends, than they are later in the horns. This may be due to crowding, as the spores after being released from the horn and when floating free tend to become less bent or crooked. The spores usually have only a slight curvature, which is ordinarily apparent in only one plane, but the actual shape is more nearly that of a greatly stretched spiral.

**STRUCTURE OF SPORES**

The spore as observed in the bright field shows more or less conspicuous septations (p. 553) and guttulae (p. 554) as commonly described in the literature for nearly all species of *Septoria*. The spores stain readily for bright-field observation, with accentuation of the septations and some changes in the appearance of the guttulae. One of the most
suitable quick-acting stains is cotton blue. Dark-field observation was much more satisfactory in revealing the details of spore structure. The spores were commonly observed in the uninjured, unstained condition, wherein certain activities could be observed within the spore. The mature *Septoria* spore has an integument, which is regarded as the spore wall and which encloses four separate protoplasts, each surrounded by an exceedingly thin, flexible membrane. The spore is, therefore, four-celled. The integument is a thin, homogeneous membrane, without visible surface markings or structures. The inner surface is likewise smooth. It appears to be permeable, as stain and solutions pass through it readily and react with the protoplasts within but have little or no effect on the integument itself.

A number of methods were tried to stain the spore wall, including the techniques of Coleman (4), Wisselingh (15) and others, as well as the methods used with chitin and hemicellulose. None gave satisfaction. The most successful stain was obtained by the following procedure. A clean segment of freshly exuded spore horn was placed in water on a clean glass slide, the spores were spread well, and the slide was dried very slowly and lightly in the flame, thus fixing the spores to the slide. The spot of spores was then covered with a 10-percent aqueous solution of potassium hydroxide, and the slide was warmed for a few minutes. The hydroxide was rinsed off with water, the spot was covered with a saturated aqueous solution of picric acid, and the slide was warmed again for a few minutes. The picric acid solution was rinsed off, and the stained spores were mounted in water under a cover glass. This treatment stained the wall a lemon-yellow color.

Plate 1, A, represents a mature unstained spore, as seen in the bright field. It shows the unequal density of the protoplasm of the spore and the true guttulae as spots or globules of variable size of light-colored or relatively transparent material. The alleged cross walls are distinct and appear to be continuous throughout with the outside enclosing wall. The outside enclosing wall has a substantial thickness, which is uniform over the length of the spore. The spore as represented shows that the bending is approximately at the points of septation; the reason for this will appear later (p. 551).

In the dark field of the microscope the normal unstained mature spore shows other characteristics (pl. 1, B). The spore wall is reduced to a thin line, and what appear to be cross walls in plate 1, A, have lost their identity. The guttulae no longer appear as well-defined spots. The four protoplasts may be distinguished from each other by the enclosing membranes of the separate protoplasts, but in the normal state their membranes are seldom conspicuous and are not seen in every spore. The contents of the protoplasts resolve into a colorless fluidlike medium in which there are small particles or granules shining in the reflected light. The particles tend to assemble in groups, usually separate from each other, the particles in a group not being agglutinated. A few particles are much larger than others, and the light reflected from the equatorial portion causes them to appear as rings. These may be some of the more darkly shaded portions of the bright-field drawing. Many of the bright particles of the protoplast are in fairly rapid motion, which is of the nature of

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2 Italic numbers in parentheses refer to Literature Cited, p. 559.
Spores of *Septoria api-graveolentis*: A, Mature unstained spore as seen in the bright field of the microscope; B, mature unstained spore as seen in the dark field; C, mature spore plasmolyzed by heating and stained with basic fuchsin as seen in the dark field; and D, ruptured, living, unstained spore in which germination has begun, as seen in the dark field.
Brownian movement, though other closely adjacent particles in the
same group are relatively or wholly quiet.

Small particles are observed to rotate in a circle of small radius,
and then suddenly to move in a more or less straight line to another
part of the protoplast as much as a third or a half the length of the
protoplast away, where the rotating motion begins again. The
particle then returns to its former position and repeats the action
indefinitely and with some regularity. There is no streaming move-
ment of the protoplasmic contents. The grouping of the particles in
the protoplast is such that there are often single spots as large as
15 to 25 percent of the volume of the protoplast that are free from
particles, except as some may be moving across it. The groups of
particles tend to settle toward the ends of the protoplasts, though
a more general distribution is frequent. In no protoplast, stained or
unstained, has there been observed any structure that could be
construed as a nucleus. The areas within the protoplasts that are
relatively free of particles permit the light of the bright field to pass
through them with little interference and are the large guttulae so
conspicuous in some species of Septoria.

The position, size, and distribution of the particles within the
protoplasts in the dark-field view of the mature spore shown in plate
1, \( B \), are characteristic of the unstained and uninjured spore. The
points of septation are revealed very faintly at the abutment of the
protoplasts at each end, but the point of septation does not show at
the center. Though commonly absent, the cross member when
present varies in appearance from spore to spore, depending on the
position of the membranes relative to the source of illumination.
When the protoplast particles are of sufficient size they are revealed
as ringlike structures. Large particles that reflect wholly are either
misshapen globules or material of a different sort.

The spores were treated with various solutions of sugar, salt, and
other chemicals to induce plasmolysis, but without success. The cells
appeared to be wholly permeable to such solutions. By carefully
heating and drying the spores on a glass slide, the protoplasts can be
shrunk so that they will draw away from each other and from the
spore wall. Plate 1, \( C \), represents a spore in which the contents were
shrunk by heating and stained with basic fuchsin. The integument
is revealed merely as a case that encloses the four protoplasts or cells.
The protoplasts are detached from it, except as a mere matter of
contact, and the integument appears to serve merely as a confining
and protective covering. It is exceedingly thin—of the relative thick-
ness illustrated in plate 1, \( B \) and \( C \). The protoplasts have a membrane
covering, which separates the contents of one cell from those of the
next and also from the spore wall. The light reflected from the
surface of the protoplast reveals a smooth, unmarred surface of no
measurable thickness. The septa or cross walls that showed in the
spore in the bright field have entirely vanished. In other words,
there are no cross walls.

In the normal spore the protoplasts assume the shape of the integu-
ment and together completely fill it. Generally they abut at right
angles to the spore wall, and so flexible is the membrane that the
space is completely filled, as in plate 1, \( B \). Nothing separates the
protoplasts, and they lie end to end within the integument. The
transmitted light of the bright field passing through the area of contact of two protoplast membranes resolves itself into the septum or cross wall. The bending of the spore at the points of septation as shown in plate 1, A, is explained as follows: The pressure exerted by the turgidity of the protoplasts tends to stretch the integument. As the point of contact of the protoplasts, i.e., the so-called septum, is the most flexible in the spore, adjustments necessary in any spore under tension or pressure are best accommodated at these points. After the spore has been released from the confines of the pycnidium or horn its shape appears to depend on the integument. With the internal pressure relieved, as in plate 1, C, the tendency to curvature or bending is greatly lessened.

Plate 1, D, represents a spore, alive and beginning to germinate, that for some unknown reason has become ruptured. The integument has a definite break in the wall at one end. The protoplast adjacent to that end has also ruptured and collapsed, drawing the visible particle contents together within the shrunken membrane. The rest of the protoplasts, however, are alive, functioning normally, and growing. The live cell beyond the collapsed cell, released from the restraining pressure of the collapsed cell, has stretched out into the empty space without rupture or any apparent hindrance to its normal functioning. The single protoplasts seem to be units that function apart from the fate of the other units within the spore, though they commonly act in unison in germination and growth.

GROWTH AND GERMINATION OF SPORES

The Septoria spores from fresh celery material are not of uniform length, though at any single stage in their development they are relatively uniform in condition and aspect. The average length of 50 spores taken from a mature-appearing pycnidium examined within an hour after collection was 36.8μ. The spores were all three-septate and apparently wholly normal. An examination was made of 20 spores from a freshly exuded cirrus, and the same spores were followed through some successive germination stages. A suitable medium for the study of this process is celery juice, freshly extracted from petioles, filtered through paper, and used without sterilization. The slides were prepared by putting a segment of the cirrus in a drop of such juice, using a portion of the suspension as a hanging drop, and sealing and fastening the cover with rubber cement. The slides were held at room temperature, about 20° C. Instead of taking a large number of slides for observation, it was found best to use a single slide in which the conditions were uniform for all the spores under examination. As stated previously, the same 20 spores were observed throughout the period of observation, but they were not all the spores on the slide. Their average length when taken from the cirrus at the beginning of the period was 44.5μ. At the end of 4 hours their average length had increased to 50.4μ, with such a small corresponding increase in width that it could not be measured. In 5 hours the spores averaged 57.0μ in length, and in 6 hours they averaged 61.2μ.

During the next 2 hours there was no appreciable change in length, as the spores still averaged 61.2μ at the end of 8 hours. However, definite changes were then beginning. The spores were becoming
more crooked even at the end of 6 hours. Some were longer than others, but the shorter ones were swelling proportionately more than the longer ones. The swelling occurred over about one-half of the length. The contents of the spores seemed to be dividing into more protoplasts, as an increasing number of cells were apparent. At the end of 8 hours the increased diameter of the spores was very evident, bulges were appearing in many cells, and the proplasts were more coarsely vacuolated than they were previously. At the end of 10 hours there were still more noticeable increases in diameters, and the swelling of the cells had become more pronounced. The proplasts were separated slightly, and the central cells had become more heavily vacuolated. At this time the length averaged 62.5μ. During the next 2 hours growth was resumed, and at the end of that time the spores had reached an average length of 69.6μ. Branching was beginning, and it was evident that the spores had now germinated. The spore lengths of the single observation are summarized in table 1. Other series of observations on similar spore material gave like, though not identical, results.

Table 1.—Length of spores of Septoria api-graveolentis after different periods in celery juice

<table>
<thead>
<tr>
<th>Source</th>
<th>Number</th>
<th>Period</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pycnidium</td>
<td>50</td>
<td>0</td>
<td>36.8</td>
</tr>
<tr>
<td>Cirrus</td>
<td>20</td>
<td>4</td>
<td>50.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>57.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>61.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>61.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>62.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>69.6</td>
</tr>
</tbody>
</table>

Another series of slides was prepared for germination study, with raw celery juice as a medium and with spores from a freshly exuded cirrus. Spores were selected for shorter length and were more nearly like those from a pycnidium. An attempt was made to promote germination as rapidly as possible. As the spores reached the end of the allotted periods they were flushed with a solution of cotton blue, which killed, fixed, and stained them and revealed the vacuolated structure in considerable detail. The spores were observed in the bright field of the microscope. The group (pl. 2) may be regarded as stages in the germination of a hypothetical Septoria spore, which may be considered as typical. The first spore, killed immediately, is shown in plate 2, A. The spore is 38μ long, slightly over 1.5μ wide at the widest point, and well filled with protoplasm staining to appear as finely divided, foamlike globules, some brighter than others. Brightness, however, may be a matter of position, as the larger particles do not appear to differ from the smaller except in size. The dissepiments appear as definite and substantial septations, which are continuous with the outside wall.

A second spore originally of the same size and aspect as that of plate 2, A, killed and stained with cotton blue at the end of 4 hours, is shown in plate 2, B. The spore has undergone several changes. The length has increased to 46μ and the width at the widest part to
Stages in spore germination of *Septoria apii-graveolentis*.  
A, Mature spore from a freshly exuded cirrus.  
B–D, Germinating spores: B, at the end of 4 hours; 
C, at the end of 12 hours; D, at the end of 24 hours.
May 15, 1942  

Structure and Germination of Septoria Spores  

553

about 2.4μ. The two end cells have divided so that the spore is five-septate, and the tapered end of the spore has increased in length more than the other cells. The appearance of the spore contents has changed markedly from that of plate 2, A. There are larger globules of uniform-appearing material, the finely divided structure is evident in the background, and the shape and size of the larger particles suggest that they are due to a flowing together or agglutination of the smaller particles to form the larger. The contents appear to be similar throughout, and the undivided center cells show no distinction from the divided end cells.

Plate 2, C, represents a spore at the end of 12 hours. The length has increased to 62μ, and the greatest diameter is nearly 3.3μ. The two pairs of end cells of the type of spore shown in plate 2, B, have increased in length, and the ultimate cell on each end has divided again. The two center cells have shortened somewhat but have increased in diameter and apparently are under some pressure from within, as they are unequally bulged and extended. The aspect of the contents is not markedly different from that of the spore in plate 2, B. The dissepiments appear to be heavier and wider, and the several cells, except the two end ones, are becoming more crooked and unequally stretched.

The comparative spore development at the end of 24 hours is represented by plate 2, D. The two center cells of the spore of plate 2, C, have here divided, and the outer cells of each division have sent out tubes similar in all respects to the growing end cells of plate 2, C. The center cells have grown slightly, the greatest diameter being about 3.75μ. The length of this spore from tip to tip is 95μ, though a median line would be slightly longer. The contents of the extended, slim ends are very highly elaborated, with larger guttulae than in the center cells. The dissepiments now appear to be true cross walls, and not the membranes of contiguous protoplasts, as in the original spores. From this stage the germinating spore merges into mycelium and its identity becomes lost. Under the conditions of the experiment the germination appeared to be retarded somewhat in the final stages, as though staleness of the medium, or byproducts, had checked the initial rate of growth.

MEANING OF APPLIED TERMS

In the routine examination of large numbers of Septoria spores from celery and other hosts, confusing variations have appeared in certain characters. As species of Septoria have been defined in part in terms of septation and guttulae, these structures are of prime interest and should be understood more clearly. Since the spore of the celery Septoria is characterized by certain features that apply throughout the genus Septoria, it is evident that the meaning of the terms applied to the spores needs to be evaluated.

SEPTATION

The spores of Septoria are commonly spoken of as "septate," but not always with a clear understanding of the implications of the word. A great variety of structures, also designated by such names as "dividing wall," "membrane," "partition," and "dissepiment," have been called "septum." Many of the authors of texts (as Owens, 11) use the term...
"cross walls," and in this they are justified because some mycologists—Ranojević (15), Petrak (12), Diedicke (5), and others—have used the term in their descriptions. In the case of *Septoria*, however, the term "cross wall" is incorrect since the connotation would justify the belief that the spores are chambered. "Septum" is not entirely free from this meaning, though it is subject to wide interpretation. The term "dissepiment," meaning a separating tissue, characterizes with some accuracy the structure that exists. Any term employed to mean a tissue, the function of which is to divide one part from another, is misused here. The tissues that separate the cells of the spore are the abutting plasma membranes of the cells; under natural circumstances these present the aspect of a single dissepiment. The term "septate," which is founded in the designation of the genus itself and cannot be abandoned, should be used, but with an understanding of the import of the word and its modification and application to special cases.

**GUTTULA**

The accepted meaning of "guttula" as a small drop-shaped spot, and "guttulate" as having drops or spots, is universally understood. The use of the term is very common throughout the literature of *Septoria*. A close study of a *Septoria* spore reveals some confusion as to the nature of the guttula, especially when it is realized that with many species this structure occupies an important part of the spore description. Stained, the spores reveal a great many guttulae, and these are correctly named in that they are small spots or droplets of protoplasmic origin or substance. But these are not the usual guttulae of the mycologists. The guttulae of the descriptive literature are bright spots caused by the transmitted light in the microscope passing through the clear areas of the cells of the spore, which, because of structural advantages, intensify the light as do small lenses. Such clear spots are shown in plate 1, B. The single cells may have a single bright spot, or they may have two, but the authors have observed no spores of any sort in which this feature was constant within a species as a single bright spot per cell. To base specific differences on this feature does not seem justified considering its nature and the likelihood that these spots are duplicated with equal variation in a great many species. To regard them as spots is justified, but to consider them as drops is not, and for that reason the term "guttulate" should be used with some reservation. To designate the places as "bright" or "lustrous" spots would be more nearly descriptive, though it does not seem to be possible to forego the use of the anomalous term "guttulate."

**MATURITY**

There seems not to be any special definition of the term "maturity" as applying to fungi, though in most cases it is assumed to mean that the fungus structure is capable of fulfilling its physiological function and can attain its functional destiny in the proper environment. This may apply to many fungus structures, such as pycnidia, perithecia, spores of all kinds, and even conidiophores and other parts. A mature fungus spore is a reproductive entity that has terminated physiological conjunction with the thallus that produced it and is capable of germination and growth in the proper environment.
The \textit{Septoria apii-graveolentis} spore is borne on a conidiophore, first as a single-celled body; then with the formation of a single septum it becomes two-celled. The two cells divide simultaneously, so that the spore has three septa and four cells. When the spore attains three septa and four protoplasts or cells it has reached mature form, but it may or may not leave the pycnidium at this time. Until the spore is three-septate it is immature. Spores that have more than three septa have begun to grow and will germinate very soon unless growth is suspended. The interval between maturity and growth may be very short. Many spores appear to begin growth and then to suspend further activity. This is the reason that five- and seven-septate spores are sometimes found. This growth may occur while the spores are yet held in the cirrus, or a very few may be so found while in the pycnidium. There should be no spores with an even number of septa, as the manner of cell increase obviates them. The spore is mature when it becomes three-septate, and it is free to leave the pycnidium when environmental conditions permit.

\textbf{A GENERAL CONSIDERATION OF SEPTORIA SPECIES}

It is evident from the published descriptions of species of the genus \textit{Septoria} as given by Saccardo (14) that the authors were generally uncertain regarding the septation of the spores. It is frequently impossible to compare specimens with published descriptions and to arrive at definite conclusions as to their identity, largely because of the different terms used in designating septation, or the wholly vague meaning of such terms. One finds such descriptive statements about spores as the following:

<table>
<thead>
<tr>
<th>Term:</th>
<th>\textit{Saccardo} (14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subcontinuis</td>
<td>3: 481 (sp. 36)</td>
</tr>
<tr>
<td>Obsolete guttulatis</td>
<td>3: 482 (sp. 37)</td>
</tr>
<tr>
<td>Obsoleteque septatis</td>
<td>3: 482 (sp. 42)</td>
</tr>
<tr>
<td>3–7 nucleolatis</td>
<td>3: 483 (sp. 48)</td>
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<tr>
<td>Obscure nucleolatis</td>
<td>3: 484 (sp. 53)</td>
</tr>
<tr>
<td>Obscure septatis</td>
<td>3: 485 (sp. 61)</td>
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<tr>
<td>Spurie 3–4–septatis</td>
<td>3: 489 (sp. 83)</td>
</tr>
<tr>
<td>Pluri-nucleolatis</td>
<td>3: 491 (sp. 96)</td>
</tr>
<tr>
<td>Obsolete guttulato-septulatis</td>
<td>3: 495 (sp. 117)</td>
</tr>
<tr>
<td>Multiguttulatis</td>
<td>3: 511 (sp. 212)</td>
</tr>
<tr>
<td>Indistinete nucleolatis</td>
<td>3: 518 (sp. 257)</td>
</tr>
<tr>
<td>Guttulatis vel septatis</td>
<td>3: 519 (sp. 263)</td>
</tr>
<tr>
<td>Continuis v. plasmate obsolete partitis</td>
<td>25: 410 (sp. 25)</td>
</tr>
</tbody>
</table>

The situation in the genus \textit{Septoria} has long been one of confusion. Beach (2) concluded that in certain species morphological characters vary considerably under different environmental conditions and that the value of measurements now given in specific descriptions is questionable. Garman and Stevens (7) made a comprehensive study of the descriptions of \textit{Septoria} species based on those of Saccardo (14, v. 1–22). They believed that spore length was an important clue to the proper placing of species. They summarized Saccardo's descriptions, giving, among other factors, the number of septa, number of guttulae, and spore shape for 1,181 species. To this summary of the statements on septation the authors have added a digest of 231 other species from Saccardo (14, v. 25) that have been classified in the manner of Garman and Stevens. This classification is shown in table 2.
Table 2.—Supplemented summary of Garman and Stevens’ classification of Saccardo’s statements on septation in species of Septoria

<table>
<thead>
<tr>
<th>Citation</th>
<th>Classifier of statements</th>
<th>Species</th>
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<td></td>
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<td>Number</td>
</tr>
<tr>
<td>Saccardo (14, v. 1-23)</td>
<td>Garman and Stevens (7)</td>
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<tr>
<td>Saccardo (14, v. 25)</td>
<td>Authors</td>
<td></td>
<td>231</td>
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<td>Total</td>
<td></td>
<td></td>
<td>1,412</td>
</tr>
</tbody>
</table>

Table 2 represents at best only an incomplete analysis of the septation statements found in the descriptions of Septoria as given by Saccardo (14). Approximately 34 percent of the descriptions have no reference to septation whatever, and 18.6 percent of the species are said to have continuous spores (septa absent); from these last no inferences are justified. Approximately 16.9 percent have indefinite statements about septation. Of the 30.5 percent that are described in terms of septation, a further analysis reveals that in the majority of cases the specifications are vague and possibly misleading, and nothing conclusive is warranted by further segregations. It may be doubted whether many of the species with one-septate and even-number-septate spores belong to Septoria at all, if the observations recorded herein are correct. Beach (2) found that septation appeared in some of the species with which he worked, when they were properly stained, but he did not always state how many septa were revealed. Hemmi (9), in describing S. glycines, found the spores to consist of “one to three, rarely four cells,” which would indicate that they were continuous, one- or two-, and rarely three-septate, but that the septa might be easily overlooked. On germination the septa showed clearly. Wolf and Lehman (16), describing S. glycines Hemmi, illustrated one-, two-, and three-septate and continuous spores. Frank (6), studying S. avenae, found the spores to be two- to four-septate. The illustrations of S. sisymbrii given by Ranojević (13) show one-, two-, three-, four-, and five-septate spores, a condition that is untenable in the authors’ understanding of the development of mature Septoria spores.

The description given by Saccardo (14, v. 25, p. 454) for Septoria apii-graveolentis contains no mention of spores whatever. Cochran (3) studied these spores with great care and found that the number of septa varied from zero to seven. The spores studied for this character were classified by Cochran as follows:

<table>
<thead>
<tr>
<th>Number of septa per spore</th>
<th>Percent of spores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 3</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>More than 5</td>
<td>2</td>
</tr>
</tbody>
</table>

The percent of spores was calculated as follows:

- Less than 3 percent of spores: 10%
- 3 percent of spores: 60%
- 4 percent of spores: 18%
- 5 percent of spores: 10%
- More than 5 percent of spores: 2%
The 10 percent of the spores that had less than three septa undoubtedly were immature and may have consisted largely of one-septate spores, though what appeared to have been two-septate spores may have been observed. The three- and five-septate spores would be normal, especially as germination had evidently begun as shown by the presence of the five-septate and more-than-five-septate spores. The authors, however, cannot account for such a large proportion as 18 percent of four-septate spores.

DISCUSSION

The authors have examined a number of Septoria species from widely varied hosts and find that the odd-number-septate spores are characteristic throughout. The spores from Septoria species producing cirri are generally of larger size and may have a higher number of septa, as five and seven, and possibly more. Three is the number most commonly found. A few of the many species examined were Septoria dulcamarae Desm. on Solanum dulcamara L.; S. solitaria Ell and Ev. on Rhododendron occidentale Gray; S. silenicola Ell. and Mar. on Silene gallica L.; S. alnifolia Ell. and Ev. on Alnus rhombifolia Nutt. and on A. rubra Bong.; S. scabiosicola Desm. on Scabiosa arvensis L.; S. aceris-macrophylli Pk. on Acer cincinatum Pursh; S. angularis Dearn. and Barth. on Solidago latifolia L.; S. populi Desm. on Populus trichocarpa Torr. and Gray; S. rubi Westd. on Rubus vitifolius Cham. and Schlecht.; S. pentstemonicola Ell. and Ev. on Penstemon cordifolius Benth.; S. stackydis Rob. and Desm. on Stachys californica Benth.; S. scrophulariae Pk. on Scrophularia californica Cham. and Schlecht.; and S. corylina Pk. on Corylus californica (A. DC.) Rose. Septoria on celery plant parts and seeds from many parts of the world was examined.

The examination of a number of fungi now known as species of Septoria indicates that those characterized by shorter and wider spores and with one or two septa are probably misclassified in many instances and should be critically examined for possible transfer to some other genus. Some of the species examined would more properly find their places in Ascochyta, Diplodina, Hendersonia, or elsewhere; and among the many-septate forms are those that may belong in Stagonospora, Rhabdospora, and possibly Cylindrosporium. As Garman and Stevens (7), Beach (2), and others have pointed out, the greatest confusion exists in the genus Septoria and the entire genus is in need of study and revision. As a mycological study, Grove's (8) treatment of the species of Septoria is the most adequate one so far. Diedicke (5) found cause to reclassify many species of Septoria, and a detailed study of the pycnidial formation and structure would be required to establish the proper relation of these species, as pointed out by Archer (1) in the case of other Sphaeropsidales.

The manner of septation in Septoria api-graveolentis as the authors have found it and the uncertainty that results from observing these minute spores solely by means of the bright field of the microscope indicate that observation by the dark-field method is necessary for a reliable estimate of the number and kind of dissepiments in these spores. The customary manner of spore septation as it appears in
S. apii-graveolentis, by simultaneous division of the end cells after
the formation of the primary septum, indicates that an even number
of septa in these spores is anomalous. Though even-number-septate
mature spores are commonly reported to be present, the authors
have never observed one in this species. Other species also show the
odd-number-septate structure consistently, though the dark field of
the microscope usually has to be resorted to to make the odd-number
septation distinguishable.

The spores of fungi within a species are generally very uniform in
size, structure, and other characters, varying only in small percentages
from the normal. Ranojević (13), however, credited Septoria sisymbrii with spores varying in length from 19 μ to 62 μ. Leonian (10)
found that a higher food concentration in culture produces more numer-
ous pycnidia and that rich hyphal growth and pycnidial development
are parallel within a very wide range. From the authors’ study of
the celery Septoria it seems certain that the spores become mature
on attaining the three-septate condition, but not before. It is possible
that the phenomenon of suspended germination is common throughout
the genus Septoria and forms the basis for the wide variation reported
in the size and septation of the spores, multiseptation especially being
an evidence of growth after maturity. From mycologists greater
detail should be required in the definition of the conditions under
which the fungus was found and the environment to which it was
exposed. As no rest period is necessary, complete germination may
occur promptly. This is evidently a factor in the spread of the disease
caused by this fungus.

CONCLUSIONS AND SUMMARY

A detailed study has been made of the spores of Septoria apii-
graveolentis Dorogin from cultivated and escaped celery. The struc-
ture of the spore and its growth and germination are given extended
treatment. The meaning and significance of terms applied to the
spore, such as “septation,” “guttula,” and “maturity,” are analyzed.

Assuming that the spores of S. apii-graveolentis reveal the nature of
Septoria spores in general, the writers have discussed the inadequacy
and confusion existing in the descriptions of species in the genus
Septoria and have offered suggestions for greater precision in descrip-
tion and care in observation.

The following conclusions have been reached:

1. The spores of Septoria apii-graveolentis are mature when they
   consist of four cells, i.e., when they are three-septate.

2. Septation in the spores of this fungus is caused by the abutting
   membranes of contiguous cells within the integument. There is no
   wall or true septum within the mature spore.

3. The guttulae of the Septoria spores in descriptive mycological
   literature are bright spots, produced by lens action on the clear por-
   tions within the cells of the spore.

4. Germination is accompanied by cell proliferation, usually by
division of the end cells of the spore. There are changes in the
appearance of the cell contents with progressive stages of germination.
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