The economic importance of black rot of apple (Pyrus malus) and cane blight of currant (Ribes rubrum) seems to make advisable a statement of our present knowledge regarding their causal organisms even though our investigation of this group of fungi has not reached a point which warrants a detailed account of the synonymy of these species or of their life histories, genetic relationships, and distribution on the 26 hosts from which closely related fungi have already been collected and critically studied. The information at present available indicates that the ascogenous stages of these fungi are so similar as to suggest their very close relationship, and that both forms of Botryosphaeria ribis occur on apple, on which host at least one form seems to be parasitic and capable of producing a fruit rot under some circumstances.

REVIEW OF EARLIER WORK

The life history of the currant cane blight fungus was worked out by Grossenbacher and Duggar (2) who also established its parasitism. To this fungus they gave the name Botryosphaeria ribis, distinguishing between the parasite and a purely saprophytic form which occurred commonly on currant, by the fact that the parasite developed a "purplish pink color" when grown on starch paste, while the saprophyte, which was morphologically identical with the parasite, developed no such color.

The literature on the black rot of apples and its causal organism is voluminous. Until 1913, however, it dealt almost exclusively with the pycnidial form of the fungus which was usually referred to as Sphaeropsis malorum. In December of that year, both Hesler (4) and Shear (10, 14, p. 107) published the results of pure culture studies which established the relationship of Sphaeropsis malorum to its ascogenous stage. This perfect stage Hesler referred tentatively to Physalospora cydoniae Arnaud, while Shear repeated his suggestion made three years earlier (9) in describing the life history of what is apparently the same species on grape, that it was Melanops quercuum (Schw.) Rehm forma vitis Sacc. or a variety of this species.

Three years later Shear and Beckwith (11) announced that pycnospores of the Sphaeropsis malorum type had been produced in pure culture from single ascospores from a variety of hosts including apple, and that pycnospores of the type of the currant cane blight fungus, which they refer to as a Dothiorella, have

1 Received for publication Feb. 6, 1924.
2 The name Botryosphaeria is used here for species congeneric with Botryosphaeria ribis, Grossenbacher and Duggar, as represented by specimens in Fungi Columbiani No. 3409 labeled B. ribis achromogena, Grossenbacher and Duggar. As thus defined the genus comprises part of the species included by Cesati and de Notaris (1) in their original description of this genus. This name is adopted instead of Melanops because of general usage. Fungi Columbiani No. 3408 represents B. ribis chromogena of this paper.
3 Reference is made by number (italic) to "Literature cited," p. 598.

also been produced from single ascospores from apple, and other hosts. No suggestions are made as to the taxonomic status of these fungi, other than that they are closely related and that some of them have previously been referred to as forms of *Melanops quercuum*.

In 1919 Putterill (6) described a canker of apple trees in South Africa which was caused by a fungus closely resembling *Botryosphaeria ribis*. This fungus even possessed the chromogenesis (6, p. 264) described by Grossenbacher and Duggar for the currant parasite. The characters used by Putterill to distinguish his fungus, for which he proposes the name *Botryosphaeria mali*, are a difference in the width of the asci and in the size of the stromata. Recently Stevens and Jenkins (13) have demonstrated that the currant cane blight fungus occurs on horsechestnut, and certain varieties of rose, and that it is parasitic on the rose, causing cankers on the stem and sometimes killing whole canes.

**LIFE HISTORY STUDIES**

**CULTURE METHODS.**—During the last two years the writers have had under observation over three thousand cultures of fungi belonging to this group, more than ninety per cent of which have fruited. In view of the difficulty experienced by some investigators in obtaining pycnospores of these fungi in pure cultures, a brief statement of the methods used may be of interest. It is certainly true that these fungi fruit but rarely in the arid and often superheated environment furnished by many laboratories. If, however, the cultures are kept in an ordinary greenhouse the temperature of which varies through a range from 50° to 70° F. or more, they will fruit abundantly on many agar media, on sterile twigs, and on cornmeal in flasks. Summer temperatures in the vicinity of Washington, D. C., are apparently too high to permit these fungi to fruit readily but good results have been obtained during the summer in unheated wooden buildings at Wareham and Woods Hole, Mass.

Our cultures were first transferred to the greenhouse in an endeavor to secure more variable temperatures, as a result of the observations of Stevens (12), recently emphasized by Harvey (5), that the temperature of bark out of doors often fluctuates with great rapidity. There is as yet, however, no certainty that the abundant fruiting is due to temperature range or fluctuation alone. It may as well be due to the greater humidity, or to a combination of temperature and humidity, or to some as yet unrecognized and incidental factor. It is sufficient for the purpose of the present investigation to be able to secure abundant pycnospore production with reasonable certainty in pure cultures made either from mycelial transfers or from ascospores or pycnospores.

**RESULTS.**—In the course of this culture work the writers have verified the life histories recorded above many times. Within the last two years alone pycnidia of the Dothiorella type have matured in pure culture from one hundred eighty single ascospores from currant and from seventy-seven single ascospores from apple. Mature pycnidia of the Sphaeropsis type have been produced in pure culture from ninety-five single ascospores from apple.

The name Dothiorella is here used to include such pycnidial forms as are congenic with the macro-pycnidial stage of *Botryosphaeria ribis* and apparently also *Dothiorella gregaria* Sacc. Typical material is found in specimens 3407, 3408, and 3409, Fungi Columbiani. This is not *Dothiorella ribis* (Fuck.) Sacc. Number 3407 labeled “Macrophoma type” is a mere form of Dothiorella growing on young shoots and producing smaller and simpler pycnidia. The name Macrophoma has been most frequently applied to immature forms of Diplodia and Sphaeropsis, in which the spores are colorless. The name Sphaeropsis is here used to include pycnidial forms congenic with the form usually called *S. malorum* on apple and whose ascogenous stage is Physalospora.
RELATIONSHIP OF THE FUNGI HAVING PYCNIDIA OF THE 

DOTHIORELLA TYPE

The collection and growth in pure culture of so large a quantity of material for a study of the life histories of the fungi, and especially the fact that a very large proportion of the cultures fruited in less than two months from the time they were made, has enabled us to make a more careful comparison of the fungi concerned than has hitherto been possible. Although the study has included material from a variety of hosts and from widely separated localities it is proposed to discuss here only the forms which are known to occur on apple and those described by Grossenbacher and Duggar on currant.

The writers' studies of the currant cane blight fungus and its saprophytic form have extended over a period of six years and have included the more important currant-producing regions of the United States. In general, the careful work of Grossenbacher and Duggar has been fully confirmed. The parasitism of the form which is characterized by the production of a bright pink color on starchy media has been confirmed by inoculation experiments in Virginia, both outdoors and in the greenhouse, as well as in Connecticut and Massachusetts. Inoculations with the non-chromogenic form have given uniformly negative results.

Continued collecting has, as might be expected, resulted in finding that the ascospores vary through a somewhat wider range than that given by Grossenbacher and Duggar, the great majority of the spores, however, fall within the limits set by them namely, ascospores, 16–23 µ by 5–7 µ, and pycnosporas 16–31 by 4.5–8 µ. Moreover, it is apparent that the size of the stromata varies very widely and is dependent to some extent on the thickness of the bark within which
Fig. 2.—Ascospores of *Botryosphaeria ribis* and *Physalospora malorum* arranged in classes according to ratio of length to width, by percentage of total spores found in each class.
Fig. 3.—Ascospores of Botryosphaeria ribis and Physalospora malorum arranged in classes according to width, by percentage of total spores found in each class.
they grow Mature stromata of *B. ribis chromogena* have been observed on a single currant bush varying in size from $1 \times 2-3$ mm. on the smaller branches (Plate 1, C) to $3-3.5 \times 5-8$ mm. near the base of the older canes (Plate 1, D). As yet, the writers are unable to distinguish morphologically between the two forms of this species.

The most important contribution to our knowledge of *Botryosphaeria ribis* since that of Grossenbacher and Duggar has just been published (13). This is the proof that the parasitic variety which causes cane blight of currants, occurs in nature on at least two other hosts, horsechestnut (*Aesculus hypocastanum*) and several cultivated varieties of rose (*Rosa* sp.). Moreover, on rose this fungus is undoubtedly parasitic.

In view of the facts now in hand the writers are unable to escape the conclusion that the fungus found by Putterill on apple in South Africa is identical with *B. ribis chromogena* even though this fungus has not yet been reported on apple from the United States. Putterill himself points out the close resemblance of this fungus to that described by Grossenbacher and Duggar. In size and arrangement of perithecia and pycnidia his fungus agrees closely with theirs; the ascospores he finds to be $19.2-19.5 \times 6.5-8 \mu$, and the pycnospores $32.4 \times 4.8 \mu$, which are well within the limits of *B. ribis*. (See Table I and figures 1, 2, and 3.) Moreover, (6, p. 264) his fungus possessed the chromogenesis which is characteristic of the parasite on currant.

### Table I.—Ascospores arranged by classes according to length in microns

<table>
<thead>
<tr>
<th>Class (in microns)</th>
<th>Total number of spores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40</td>
</tr>
<tr>
<td>Number of spores of <em>Botryosphaeria ribis chromogena</em> on currant in each class</td>
<td>226 5 7 9 23 35 27 21 24 19 22 16 6 3 3 4</td>
</tr>
<tr>
<td>Number of spores of <em>Botryosphaeria ribis</em> on apple in each class</td>
<td>207 1 5 13 8 22 22 14 25 10 23 33 16 3 2 2 1</td>
</tr>
<tr>
<td>Number of spores of <em>Physalospora malorum</em> on apple in each class</td>
<td>223 1 1 5 8 4 11 10 11 13 31 20 28 24 30 11 6 3 2 1</td>
</tr>
</tbody>
</table>

### Ascospores arranged by class according to width in microns

<table>
<thead>
<tr>
<th>Class (in microns)</th>
<th>Total number of spores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 5 6 7 8 9 10 11 12 13 14 15</td>
</tr>
<tr>
<td><em>Botryosphaeria ribis chromogena</em> on currant</td>
<td>226 8 44 91 70 11 2</td>
</tr>
<tr>
<td><em>Botryosphaeria ribis</em> on apple</td>
<td>207 1 11 54 94 37 10</td>
</tr>
<tr>
<td><em>Physalospora malorum</em> on apple</td>
<td>223 25 31 46 68 26 23 3 1</td>
</tr>
</tbody>
</table>

### Ascospores arranged by class according to ratios of length to width in microns

<table>
<thead>
<tr>
<th>Class</th>
<th>Total number of spores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.5 2 2.5 3 3.5 4 4.5 5</td>
</tr>
<tr>
<td><em>Botryosphaeria ribis chromogena</em> on currant</td>
<td>226 10 50 78 56 22 8</td>
</tr>
<tr>
<td><em>Botryosphaeria ribis</em> on apple</td>
<td>207 1 17 74 78 26 7 2 2</td>
</tr>
<tr>
<td><em>Physalospora malorum</em> on apple</td>
<td>223 6 46 77 58 21 11 4</td>
</tr>
</tbody>
</table>
May 10, 1924  Botryosphaeria and Physalospora on Currant and Apple  595

The chief difference in the fungi, according to Putterill, lies in the fact that the asci of his form from apple are narrower than those measured by Grossenbacher and Duggar and the stromata of the apple fungus are usually about 0.5 mm. wide as contrasted with 2 mm. as the most common size on apple. Neither of these characters appears to the present writers to have significance. The asci are variable in width and are frequently found in material from currant no wider than those noted by Putterill. The stromata of this fungus, as already pointed out, vary in size with the thickness of the bark in which they grow. Mature perithecial stromata of \textit{B. ribis} on rose are often 0.3 mm. or less in diameter, yet this fungus has been proven by inoculation experiments to be the same as that producing much larger stromata on currant.

That the character of the bark in which they are produced directly influences the size of the stromata in this species seems to be fully proven by their artificial culture on sterile twigs from different species of woody plants. Plate 1, A and B, show mature pycnidial stromata which developed on dormant apple and currant twigs from subcultures of \textit{B. ribis} from currant. These cultures were started at the same time and kept near together in special culture flasks on a bench in a greenhouse from November 19, 1921, to February 22, 1922. A glance at the figures will show the relative size of the stromata on the two hosts. To be sure these are pycnidial stromata as the writers are not yet able to produce mature perithecia in quantity in pure culture. Under natural conditions, however, perithecia are often produced in the same stromata with pycnidia and on any given host the stromata bearing the two kinds of spores are of the same size.

Unfortunately the writers have not been able to secure cultures of the fungus from Africa for inoculation on currant, although Putterill has courteously tried to secure more living material for this work. Under date of January 25, 1921, he writes as follows:

"I have just returned from a visit to the infected trees at Vereening. Since my first visit there the trees have been more carefully treated, the diseased areas having been cut out and applications of coal tar made at regular intervals; I was unable to obtain fresh material for you."

In spite of the fact that inoculations can not be made, there seems to be no ground for considering the African fungus different from that which produces the cane blight of currants. Mere distance between the localities can not be considered a reason for considering the fungi different, especially when they occur on cultivated plants which are known to be shipped and carry their parasites long distances.

As to the possibility of this fungus being parasitic on apple in this country there is no evidence at present. Putterill's letter would suggest that it is sometimes not very virulent in South Africa since he was unable to find it in the orchard in which he had previously found it. That it can be made to grow on apple tissue is evident from the result of the inoculations made by Putterill on sound apples. Some years ago while checking up the results of shipping experiments on citrus for Rogers and Earle (8) the writers inoculated sound grapefruit with the currant cane blight fungus and produced a rot somewhat resembling the Diplodia stem end rot.

Since \textit{B. ribis chromogena} is an active parasite on currant, Stevens and Jenkins were able to prove the identity of the material which they collected from horse-chestnuts and rose with the cane blight fungus by actual inoculation experiments. This type of proof is not possible in the case of the saprophytic or nearly saprophytic form. There is, however, no reason to consider the nonchromogenic \textit{Botryosphaeria} which occurs on apple in this country as specifically distinct from the non-chromogenic saprophytic \textit{Botryosphaeria} on currant, since no morphological differences have been found. (See Table I, text figures 1, 2, and 3, and Plate 2, A, B, C, and K-N).
The practice of describing as "new species'' saprophytes or omnivorous parasites having practically identical morphological characters simply because they happen to be found on different hosts or in a distant locality, is to be deplored as it cumbers mycological literature with a mass of doubtful names and synonyms and greatly impedes the progress of mycological taxonomy. Real differences in essential morphological characters must be the foundation for specific segregation. What "real'' and essential differences are can only be determined by careful and thorough study of a considerable amount of good material of the organisms in hand. Moreover when it has been shown by cross inoculations or studies of the fungi in pure culture that the characters upon which species have been segregated are such as result from host or cultural conditions and vary with a change of these conditions, they must be considered specifically identical.

COMPARISON OF BOTRYOSPHAERIA RIBIS AND PHYSALOSPORA MALORUM

Physalospora malorum is here used for the fungus previously referred to as Melanops quercuum forma vitis by Shear (9) and Physalospora cydoniae Arnaud by Hesler (4), the pycnidial form of which is Sphaeropsis malorum. As there appear to be no good specimens of this species in published American exsiccati the writers will distribute what they regard as typical material of both stages to the principal large herbaria in the near future. The name Physalospora malorum is chosen as a combination of the best known names of the two stages of the fungus.

Both perithecia and pycnidia of Botryosphaeria ribis usually occur in a stroma, though single perithecia are often found and the size of the stromata varies, as has been pointed out above, with the host, and thickness of the bark. Hesler, in describing Physalospora cydoniae, the perfect stage of Sphaeropsis malorum (5), states that "the perithecia are usually scattered, standing separate from one another. Sometimes, however, from two to four fruit bodies are joined together, but no stroma has ever been observed." The writers have not yet made a sufficiently extended study and comparison of these characters to form an opinion as to their constancy and taxonomic value.

The paraphyses in both these species are very characteristic and have never, so far as we know, been correctly described or illustrated. They appear to consist of a tangled or anastomosing mass of septate, filamentous hyphae. In young perithecia the central portion is filled with a mass of pseudo-parenchyma. As the asci develops they push up into this mass, which becomes more or less filamentous at maturity, but separate paraphyses have never been observed.

The most striking difference in the perfect stages of the two organisms is the size of their ascospores. See text figures 1, 2, and 3, Table I, and Plate 2. As is evident from the Table and figures the longer spores of B. ribis equal in length the shorter ones of Physalospora malorum so that from a single spore it is not always possible to distinguish the two fungi. The great majority of ascospores of P. malorum, however, are longer than the largest yet measured from B. ribis. and in good preparations of mature ascospores the two species are easily distinguished on this basis. The same relation holds with regard to the width of the ascospores of the two fungi. The wider ascospores of B. ribis equal the narrower ascospores of P. malorum but as a whole the ascospores of P. malorum averaged about 3 μ wider. In shape as expressed by the ratio of length to width the ascospores of the two fungi are very similar indeed. See figure 3 and Table I.

The mode of liberation and ejection of ascospores has been observed in Physalospora malorum in fresh mature specimens mounted in water. The wall of the ascus ruptures transversely and the ascospores are ejected while still imbedded in a somewhat gelatinous matrix, having the same outline as the ascus and extending to the base where it appears to be attached, as illustrated in Plate 2, F. and G.
Micropycnospores are found in culture and nature associated with the macro-
pycnospores of both the Sphaeropsis and Dothiorella types. They may occur
in separate locules or be intermingled with the macrospores. They are
minute in size, about 2–3 μ long, oblong, and have never been observed to germi-
nate. See Plate 2, figure N.

An interesting and apparently constant difference between the ascospores of
the two fungi is found in their method of germination. Ascospores of Botry-
osphaeria ribis, whether from currant or from apple, characteristically develop
two germ tubes which branch before they have reached more than 8 or 10 times
(Pl. 2 O. and P.) the length of the spore. Even where only one germ tube is
produced it usually branches while rather short. Ascospores of Physalospora
malorum, on the other hand, usually develop only one germ tube which rarely if
ever branches before it reaches a length equal to 50 or 60 times that of the spore
itself. (Pl. 2, Q. and R.) So constant under the conditions of our work was
this apparently trivial character that in the case of two hundred eighty-six
ascospores of these two fungi in which the type of germination has been observed
and the development of the pycnospores in pure culture subsequently obtained,
those ascospores which germinated by means of a single, long, unbranched germ
tube have always produced pycnospores of the Sphaeropsis type and those
which germinated by means of the shorter branched germ tubes, usually two to
a spore, have always produced pycnospores of the Dothiorella type.

On many culture media early stages of the development of the two fungi
appear much alike, especially when grown in dry air at temperatures above
22° C. They may be readily distinguished, however, on beef agar made accord-
ing to the following formula:

Add 3 gm. of beef extract, 10 gm. peptone, and 5 gm. of sodium chlorid to
one liter of distilled water. Steam one hour; titrate with hydrochloric acid and
make up to plus 10, Fuller’s scale. Add enough water to make one liter, and
1½ per cent shredded agar. Steam one hour. After cooling to 60° C. add the
whites of two eggs well beaten—steam another hour, filter through cotton, tube
and autoclave 20 minutes at 15 pounds pressure.

Cultures made on agar slants of this medium from mycelial transfers show
after ten days a loose felt of short, rather fluffy mycelium on the surface of the
medium. Botryosphaeria ribis, however, leaves the color of the medium un-
changed (Baryta yellow) (7) whereas Physalospora malorum changes the medium
to a dark color, between mummy brown and black.

Cultures on corn meal in 100 cc. Erlenmeyer flasks which have reached the
stage of producing mature pycnospores are readily distinguished by the surface
character of the fungus growth. Fruiting or nearly mature cultures of Botryo-
sphaeria ribis show numerous raised knob-like stromatic bodies usually 2 to 3
mm. wide and 3 to 4 mm. high in which the pycnidia are contained. (Pl. 1, E.)

Fruiting cultures of Physalospora malorum on corn meal in flasks on the other
hand have a much more uniform surface without prominent regular elevations
of any kind, the pycnidia being almost completely buried in the mycelial growth.
(Pl. 1, F.)

If the various differences in stromata, size of ascospore, method of germina-
tion, cultural characters, and different pycnidial stages mentioned above are
constant, they furnish a good basis for generic segregation. The two generic
names which have commonly been applied to the two species on these hosts
are here used for convenience and clearness.

SUMMARY

The life histories of Botryosphaeria ribis, causing cane blight of currant, and
Physalospora malorum, causing black rot of apple, have been verified repeatedly
by the development of the pycnospores from single ascospores in pure culture.

The methods by which pycnospores of these fungi were readily produced in
pure culture are briefly described.
The differences between *Botryosphaeria* and *Physalospora*, as the names are used here, are the apparent difference in life histories, the first having Dothiorella as its pycnidial stage and the second Sphaeropsis as its pycnidial stage and the difference in ascospore sizes.

The ascogenous stages of *Botryosphaeria ribis* and *Physalospora malorum* may be distinguished by size of ascospore, method of germination of ascospores, and by certain cultural characters.

The fungus on apple in Africa described as *Botryosphaeria mali* by Putterill is apparently identical with the physiological variety of *Botryosphaeria ribis* G. and D. which the writers call *chromogena*.

The fungus commonly found on apple in this country which has Dothiorella as its pycnidial stage and closely resembles that from Africa in morphology, but is nonchromogenic, is apparently identical with *Botryosphaeria ribis* G. and D.

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(10) ——

(11) —— and Beckwith, A. M.

(12) Stevens, N. E.

(13) —— and Jenkins, A. F.

(14) Taylor, W. A.
A.—Mature pycnidial stromata of *Botryosphaeria ribis* grown in a flask on portion of sterile apple twig in greenhouse from November 19, 1921, to February 22, 1922. ×2.

B.—Mature pycnidial stromata of *Botryosphaeria ribis* grown in a flask on portion of sterile currant twig in greenhouse from November 19, 1921, to February 22, 1922. ×2.


D.—Mature pycnidial and perithecial stromata of *Botryosphaeria ribis chromogena* on trunk of same bush as that shown in C. ×2.

E.—Fruiting culture of *Botryosphaeria ribis* on corn meal in flask.

F.—Fruiting culture of *Physalospora malorum* on corn meal in flask.
PLATE 2

A.—Three asci of *Botryosphaeria ribis* on gooseberry, from host.
B.—Ascospores of *B. ribis* on gooseberry, from host.
C.—Macropycnosporo of *B. ribis*, grown in pure culture from single ascospores from gooseberry.
D.—Sporophores of macropycnosporo of *B. ribis* in culture.
E.—Ascus of *Physalospora malorum*, on apple, from host.
F.—Ascus of *P. malorum* showing mode of rupture and ejection of ascospores, from host.
G.—Upper portion of a ruptured ascus of *P. malorum* with part of the ascospores, from host.
H.—Ascospores of *P. malorum*, from host.
I.—Macropycnosporos of *P. malorum* from single ascospores from apple, cultures 2581 and 2660 from Vienna, Va., and Ambler, Pa.
J.—Macropycnosporos with sporophores from culture.
K.—Three asci of *Botryosphaeria ribis* on apple, from specimen 2805 collected at Vienna, Va.
L.—Ascospores of *B. ribis* on apple, from specimen 2805.
M.—Macropycnosporos of *B. ribis* on apple, some attached to sporophores, from culture 3106 A. from a single ascospore.
N.—Micropycnosporos and sporophores of *B. ribis* on apple, from culture 3106 A.
O.—Germinating ascospore of *B. ribis* after 18 hours at a temperature of about 12° C.
P.—Same spore held four hours after the stage shown in O—at a temperature of 22° C.
Q.—Germinating ascospore of *Physalospora malorum* after 18 hours at a temperature of about 12° C.
R.—Same spore held four hours after the stage shown in figure 1, at a temperature of 22° C. Figures O to P. ×200.