

SPECIES OF *HELMINTHOSPORIUM* AND  
*CURVULARIA* ASSOCIATED WITH  
ROOT-ROT OF WHEAT AND OTHER  
GRAMINACEOUS PLANTS.

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(With Plate IX and three text-figures.)

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The importance of *Helminthosporium* as an agent in the causation of root diseases of graminaceous plants has long been recognised, but no paper has hitherto been published in which reference is made to all species of the genus that have been found in association with root-rot of wheat. In the following paragraphs attention is drawn to the several species so far described, together with the writer's observations on the morphology of forms isolated in Australia, and reference is also made to the genus *Curvularia*, which was established by Boedijn in 1933<sup>(3)</sup> and which includes several fungi previously classified in *Helminthosporium*, *Brachysporium* and other genera.

The genus *Helminthosporium* was defined by Link in 1809,<sup>(11)</sup> and brief descriptions of numerous species of this and the related genus, *Brachysporium*, are given in the works of Saccardo. A study of the literature on fungi associated with root-rot diseases of cereals has shown, however, that seven distinct species of the genus *Helminthosporium* have been isolated by various workers from foot-rot-affected wheat. For convenience, these might be divided into (a) the large spore group, and (b) the small spore group. The species falling into each group are as follow :

(a) LARGE SPORE GROUP.

(1) *H. bicolor* Mitra. This species was described by Mitra<sup>(13)</sup> in 1930. He states that the spores are greyish

to dark greyish-smoky brown in colour, are typically cylindrical with abruptly rounded ends, are straight or slightly curved, and are 1-9 septate (av. 5); that the mean size is  $51 \times 14$  microns, the extremes being  $16.5-79 \times 10-20$  microns. The species was isolated in India, and was found to be capable of producing pronounced foot- and root-rot in wheat and barley seedlings.

(2) *H. halodes* Drechs., var. *tritici* Mitra. This new variety was also described by Mitra<sup>(13)</sup> in 1930. His observations showed that it differed from the main type in regard to spore size and septation. The extreme measurements are given as  $23-73 \times 13-20$  microns, the mean size being  $52 \times 16.5$  microns. This species was also isolated in India, and in inoculation tests proved to be virulent on seedlings of wheat and barley.

(3) *H. pedicellatum* Henry. This fungus was isolated from a diseased wheat root in Minnesota, U.S.A., by Henry, and was described in 1924,<sup>(8)</sup> the cultural and morphological features being fully outlined. The conidia measured  $65.3 \times 23.4$  microns, and were for the most part 7-septate. The species was found to be only weakly parasitic.

(4) *Helminthosporium N* Henry. A type differing from *H. sativum* sufficiently to be regarded as a distinct species was also isolated by Henry<sup>(8)</sup> in 1923, at Minnesota, principally from seeds. The conidia are stated to be much narrower and more cylindrical than those of *H. sativum*, and resemble the spores of *Podosporiella verticillata* O'Gara. No synnemata, however, developed in culture. Henry determined the means for length, width and number of septations to be 64.2 microns, 14.0 microns, and 7.28. He found in inoculation tests that this species was less virulent than *H. sativum*. The identity of the fungus was not established.

(5) *H. sativum* P.K.B. This is the most common species of *Helminthosporium* found in association with root-rot conditions in wheat. In Australia it has been isolated<sup>(10)</sup> from wheat, oats, barley, rye and several grasses, and has been obtained from material from the Federal Capital Territory and all States, excepting Tasmania. Drechsler,<sup>(6)</sup> who has critically reviewed the nomenclature of *H. sativum* and has pointed out where early workers were in error in

assigning other specific names to *Helminthosporium* isolated from diseased wheat, concludes that "for the most part a single species of *Helminthosporium*, namely *H. sativum*, is involved in the widespread infection of wheat manifested variously by such symptoms as stunting of growth, seedling

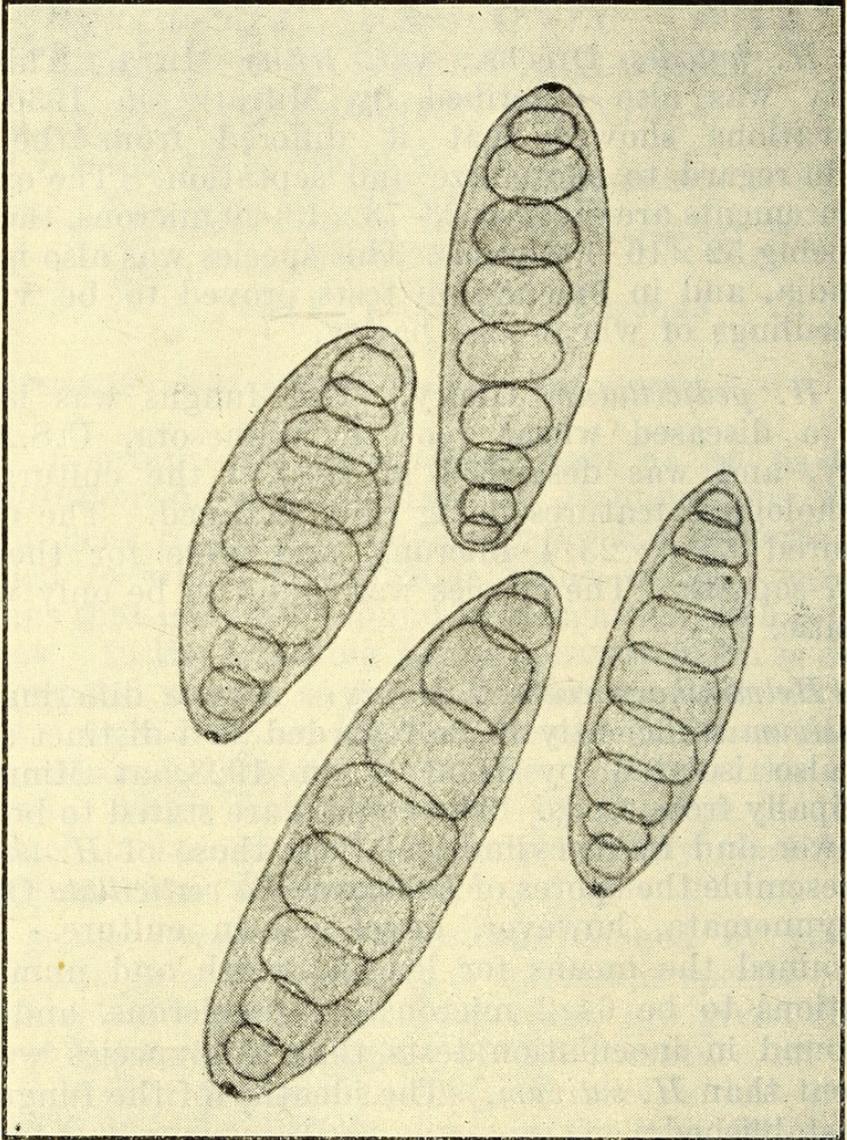


Fig. 1.—Conidia of *Helminthosporium sativum* P.K.B.  $\times 800$ .  
(From cultures on potato dextrose agar at  $20^{\circ}$  C.)

(Photo by P. R. Maguire.)

blight, basal browning, root rot, foot rot, node decay, leaf spot, stem discolouration, and black point".

This species was first described in 1910 by Pammel, King and Bakke,<sup>(14)</sup> and the morphological characters of

the species have since been closely studied by a number of workers.<sup>(4) (5) (6) (7) (13) (17)</sup>

Although *H. sativum* was first isolated in Australia in 1913, and a number of papers have been published dealing with its parasitism and control, no detailed work on the morphology of the species has so far been published in this country. The writer has made a close study of the fungus in respect of pathogenicity and cultural reactions of physiologic forms<sup>(10)</sup>, and has studied its morphology under different environmental conditions. From this work, and from the reports also of overseas authorities who have examined cultures from local material, there is no doubt that the large-spored *Helminthosporium* associated with wheat root-rot throughout Australia is predominantly, if not exclusively, *H. sativum* P.K.B. (Fig. 1.)

In studying the dimensions of conidia for comparative purposes it is important to standardise the conditions of culture. Dodsall<sup>(5)</sup> observed that while temperature exerted an effect on the mean length of spores when potato dextrose agar was used, the greatest difference in length occurred between spores produced on different sub-strata. Hence, at 24° C. the mean length of conidia produced on potato dextrose was 65·75 microns, on autoclaved ripe barley heads was 67·74 microns, and on green barley leaves was 83·14 microns.

Apart from colour, shape and mode of production, the most important features in a study of spore morphology of species of *Helminthosporium* are length, width and number of septations. Stevens<sup>(17)</sup> introduced the concepts "coefficient of longitudinal eccentricity" and "coefficient of cylindricality" for more accurate descriptions of species in respect of conidial shape, but these do not appear to have been adopted by other workers to any extent.

Therefore, for the writer's studies on conidial morphology of Australian isolates of *H. sativum*, all monosporous cultures were developed under controlled conditions, and attention was given mainly to the dimensions and number of septa. In the preliminary work, conducted several years ago, only 25 conidia of each of three isolates were studied, the spores having been produced under "standard conditions" on washed agar and wheat shoots at 25° C., as defined by Stevens.<sup>(17)</sup> The results are set out in Table 1.

TABLE 1.

Summary of Measurement Data of 25 Conidia of each of Three Monosporous Isolates of *H. tetramera* (*Curvularia spicifera*) and of *H. sativum*, Cultured on Sterilised Wheat Shoots on Washed Agar at 25° C.

Species and Strains.	Means for—		
	Length. (Microns.)	Width. (Microns.)	No. of Septa.
<i>H. tetramera</i> A .. ..	23.4	8.5	3
„ B .. ..	23.6	8.8	3
„ C .. ..	25.1	9.0	3
<i>H. sativum</i> A .. ..	76.4	21.8	7.4
„ B .. ..	69.7	21.9	7.1
„ C .. ..	62.1	19.4	5.9

TABLE 2.

Measurements of 100 Conidia of each of two Monosporous Isolates of *H. sativum* Cultured on Sterilised Wheat Heads at 20° C.

## ISOLATE 1.

Septation Class.	Frequency. %	Range. (Microns.)	Mean. (Microns.)
2	1	—	51.1 × 23.1
3	4	34.6–44.5 × 18.1–23.1	40.4 × 20.2
4	4	42.9–51.1 × 19.8–23.1	45.3 × 21.8
5	6	49.5–56.1 × 19.8–23.1	53.9 × 21.7
6	12	49.5–77.5 × 18.1–26.4	61.0 × 22.4
7	24	46.2–82.5 × 19.8–26.4	67.5 × 22.4
8	26	66.0–85.8 × 21.4–26.4	75.9 × 24.0
9	18	70.9–85.8 × 21.4–28.0	78.9 × 24.0
10	5	80.8–85.8 × 23.1–24.7	83.1 × 24.4

## ISOLATE 2.

3	2	33.0–37.9 × 14.8–16.5	35.4 × 15.6
4	2	—	52.8 × 24.7
5	3	52.8–66.0 × 19.8–24.7	57.2 × 22.5
6	14	52.8–77.5 × 18.1–24.7	60.2 × 21.4
7	30	49.5–80.8 × 18.1–26.4	66.4 × 21.7
8	29	66.0–85.8 × 21.4–26.4	76.1 × 24.4
9	16	69.3–82.5 × 19.8–26.4	77.0 × 24.3
10	3	77.5–82.5 × 23.1–26.4	80.8 × 24.7
11	1	—	85.8 × 23.1

Mean length and width of the 100 conidia of isolate 1 : 68.8 × 23.1 microns.

Mean length and width of the 100 conidia of isolate 2 : 69.5 × 23.0 microns.

A more detailed study, however, has recently been made of two isolates cultured on sterilised wheat heads in test tubes at 20° C. Since Stevens<sup>(17)</sup> has shown that humidity influences the relative variability of *Helminthosporium* spores, a representative conidial sample of each isolate for examination was obtained by taking small amounts of spores from each of three triplicate tubes and mounting them in water. The measurement data are given in Table 2, from which it will be seen that for each isolate the septation classes of highest frequency were 7 and 8, the mean dimensions for each 100 conidia being 68.8 × 23.1 microns and 69.5 × 23.0 microns. The mean length increased with increasing number of septa. These data are in agreement with results published by other workers. The method here adopted for presentation of measurements of *Helminthosporium* gives a clearer picture of spore size in relation to number of septa than in the case of methods adopted by most other investigators. The colony growth of two virulent strains of *H. sativum* on potato dextrose agar is illustrated in Plate IX.

#### (b) SMALL SPORE GROUP.

A number of small-spored types of *Helminthosporium* have been isolated by various workers from root-rot affected wheat,<sup>(2) (8) (9) (10) (12) (15) (16)</sup> but only in a few cases have specific determinations been given. It appears from a study of the literature that only two particular small-spored types have been closely studied, viz. the two species which have been used by the writer in infection studies already described,<sup>(10)</sup> and which have been designated, both here and abroad, as *Helminthosporium M* and *H. tetramera*. Each of these will now be considered in detail.

(1) *H. tetramera* McKinney. This species was isolated by McKinney from foot-rot-affected wheat in Oklahoma, U.S.A., in 1923. McKinney demonstrated that it was capable of attacking the roots and stem bases of winter wheat, causing injury similar to that produced by *H. sativum*, but he doubted whether the new fungus was an aggressive parasite. The species was named and described in 1925.<sup>(12)</sup> It has since been isolated from wheat in Australia<sup>(10)</sup> and in East Africa,<sup>(2)</sup> and the results of infection tests in each instance have shown that it is unimportant pathogenically. The cultural characters of

*H. tetramera* on different media at different temperatures have already been described by the writer.<sup>(10)</sup>

A study of the morphological characters of the fungus when grown on potato dextrose agar at 20° C. (cf. Fig. 2)

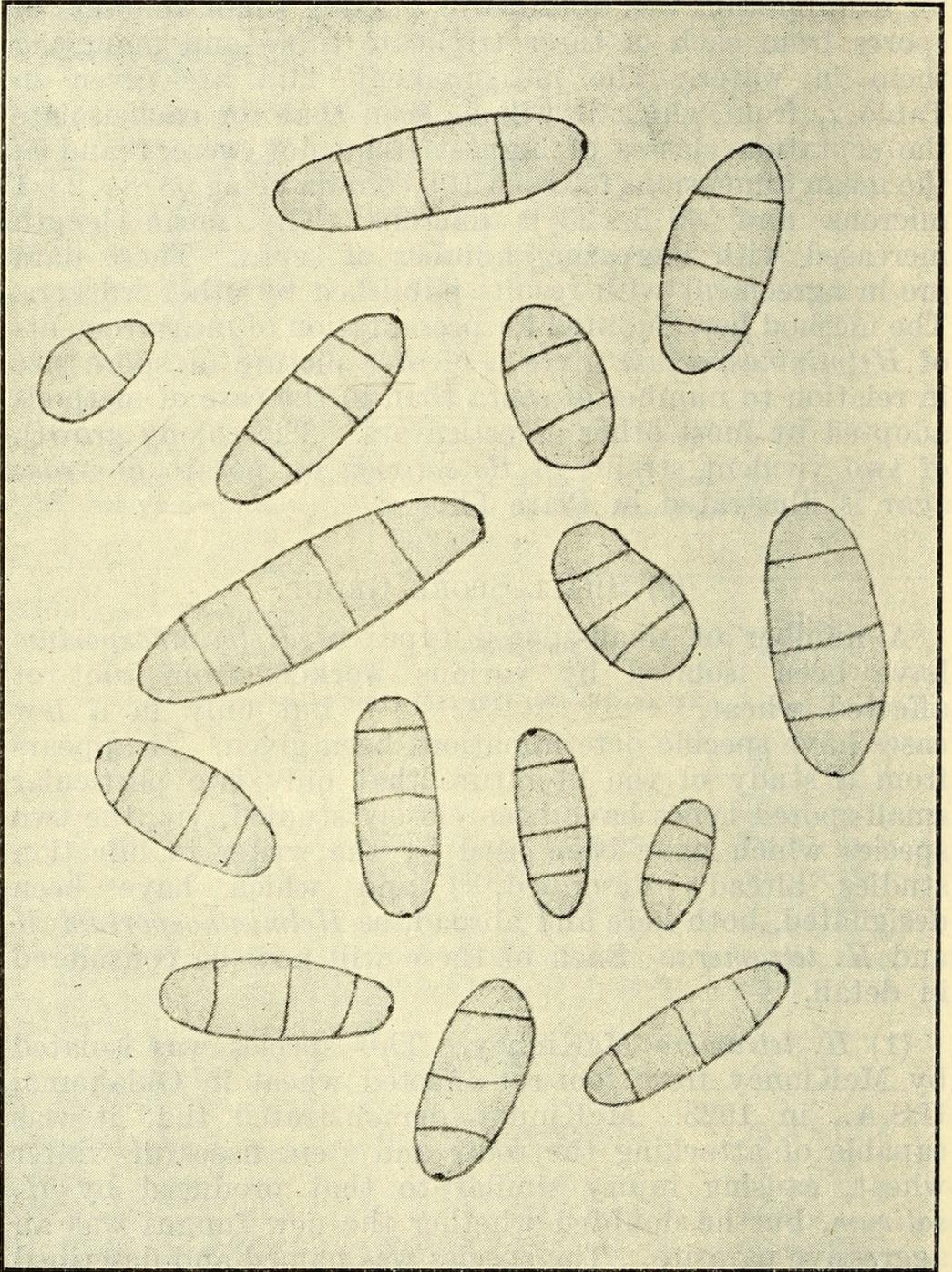


Fig. 2.—Conidia of *Helminthosporium tetramera* McKinney (*Curvularia spicifera* (Bainier) Boedijn).  $\times 800$ .  
(From cultures on potato dextrose agar at 20° C.)  
(Photo by P. R. Maguire.)

shows that the conidia are borne in clusters in varying numbers on conidiophores which are brownish in colour. The spores are oblong and symmetrical in shape, with rounded ends, and are of uniform, straw-brown colour. They are predominantly 3-septate, the septa not being as prominent as in *Helminthosporium M.*, and the spore wall not being constricted at the septa. A tiny black stipe—which connects the conidium with the conidiophore—is to be seen at the basal end of the spore. The protoplasts are smaller than in the case of *Helminthosporium M.*, so that the space occupied by the endosporium between the protoplasts and the episporium, and between the individual protoplasts is greater than with *Helminthosporium M.* The features of the episporium or outer conidial wall and the endosporium of *Helminthosporium* have been fully discussed by Stevens.<sup>(17)</sup>

The conidia germinate readily in tap water, frequently from both ends, and occasionally also from the side of the spore. Conidia kept under dry conditions in test tubes in the laboratory for 31 months germinated vigorously when placed in water.

In view of the comparative unimportance of *H. tetramera*, no extensive spore measurement studies have been made by the writer. Twenty-five conidia of each of three monosporous isolates from oats, barley and rye have, however, been measured, the spores having been produced under "standard conditions" on sterilised wheat shoots on washed agar at 25° C., as described by Stevens.<sup>(17)</sup> The data are summarised in Table 1, from which it will be seen that the approximate size of conidia of this fungus is 23·5–25 × 8·5–9 microns, based on a limited number of measurements. McKinney<sup>(12)</sup> found that "30·6 microns was the most common length" and "13·6 microns the most common width", the spores being chiefly four-celled. He does not state, however, under what conditions the conidial material was produced, nor how many spores were measured. Presumably he studied conidia taken direct from affected plants, when they would be larger than those produced on artificial media.

The fungus has been isolated by the writer from diseased roots of wheat, oats, barley and rye in New South Wales, and was first isolated in 1923. It has also been obtained from material from South Australia. Mr. E. W. Mason, of the Imperial Mycological Institute, England, has examined cultures from local material and has confirmed

the writer's determination of *H. tetramera* McKinney, but Dr. K. B. Boedijn, Java, identified the same cultures as *Curvularia spicifera* (Bainier) Boedijn, of which *Brachycladium spiciferum* Bainier is a synonym.<sup>(3)</sup> The description of this latter species was published in 1908,<sup>(1)</sup> the fungus being obtained on "brambles of dead wood". Bainier was unable to obtain perithecia or sclerotia of this fungus on artificial media, but McKinney reported that *H. tetramera* produced numerous sclerotia on potato glucose agar; no sclerotial bodies developed in the writer's cultures of *H. tetramera* on potato dextrose agar. A study of Bainier's description and drawings shows that *B. spiciferum* is identical with *H. tetramera*. The features of the new genus *Curvularia*, which was established by Boedijn in 1933, and in which *H. tetramera* is now included, are discussed later.

The species under consideration is therefore now known as *Curvularia spicifera* (Bainier) Boedijn (which falls into the "Maculans Gruppe"), the synonyms being *Helminthosporium tetramera* McKinney and *Brachycladium spiciferum* Bainier. The colony growth of two strains of the fungus on potato dextrose agar is illustrated in Plate IX. Concerning the Maculans group, Boedijn writes: "This includes forms showing conidia with three partition walls, and the two middle cells are larger and darker than the end cells. Furthermore, the conidia are here straight or merely asymmetrical. In pure cultures, stromata are never formed. The typical representative of this group is *Spondylocladium maculans* Bancroft."

(2) *Helminthosporium M.* In his investigation of root-rotting fungi, Henry<sup>(8)</sup> isolated several small-spored strains of similar character, to which he applied the name *Helminthosporium M.* The fungus was isolated from wheat seed, millet leaf, and roots of wheat and barley. Pathogenicity tests showed that some strains were rather virulent in producing foot- and root-rot in wheat, others were only weakly parasitic. Henry studied four of the strains, and found that they were of the same general morphological character, the spores being dark olivaceous, usually curved, with a small hilum at the lower end, borne in a similar manner, and germinating usually from both ends. He investigated their specific identity, and his remarks in this connection are of interest:

"The exact identity of these forms is difficult to determine. They apparently may be placed with justification in either of two genera,

namely *Helminthosporium* or *Brachysporium*. If Lindau's suggestion were followed, namely that *Brachysporium* be reserved for species whose spores are not more than twice as long as they are broad, then three of the above strains would belong in the genus *Helminthosporium*, and one in *Brachysporium*. The fungi *Brachysporium trifolii* Kauffman and *Helminthosporium inaequalis* Shear have spores which correspond rather closely in shape and size to the conidia of the forms isolated by the writer. These small-spored cultures were submitted to Dr. Charles Drechsler for identification. He was unable to place them specifically, but preferred to refer them to the genus *Helminthosporium* rather than to *Brachysporium*, and considered that it was impossible without further study to apply either a new or an old binomial to them."

The writer first isolated a small-spored type from wheat roots in New South Wales some years ago, and since it agreed in general morphological characters with *Helminthosporium M* described by Henry, the same designation was adopted for this and other isolates subsequently obtained from diseased roots of oats, barley, *Agrostis* sp., *Bromus inermis*, *Festuca rubra*, *Ischaemum laxum*, *Lolium rigidum* var. *strictum* and *Poa pratensis*. This fungus has also been isolated from material from Victoria and South Australia. Experiments dealing with its pathogenicity and cultural characters have already been described by the writer.<sup>(10)</sup> Certain strains have been shown to be extremely virulent on seedlings of wheat, oats, barley and rye.

A study of the morphology of the fungus when grown on potato dextrose agar at 20° C. (cf. Fig. 3) shows that the spores are borne in clusters on brownish-coloured conidiothores. The conidia are oblong in shape and usually are curved to a greater extent on one side than on the other, presenting a humped appearance. They are not so uniform or symmetrical in shape as those of *H. tetramera*. They are dark brown in colour and predominantly 4-septate, the spore wall being sometimes slightly constricted at the septa, which stand out much more prominently than in the case of *H. tetramera*. A tiny black stipe is to be seen on most conidia. A very striking feature is the darker colour of the large central cell or cells, and the lighter brown of the terminal segments. This was not observed in any isolate of *H. tetramera* studied. In contrast to this species also, the individual protoplasts of spores of *Helminthosporium M* are larger and occupy a greater area in each segment.

The conidia germinate readily in tap water, frequently from each end, and occasionally also from the side of the spore. Conidial material kept for 31 months in test tubes

plugged with cotton wool under laboratory conditions germinated vigorously in hanging drop cultures.

The results of measurement of 100 conidia of each of two monosporous isolates cultured on sterilised wheat heads in test tubes at 20° C. are set out in Table 3. It will be seen

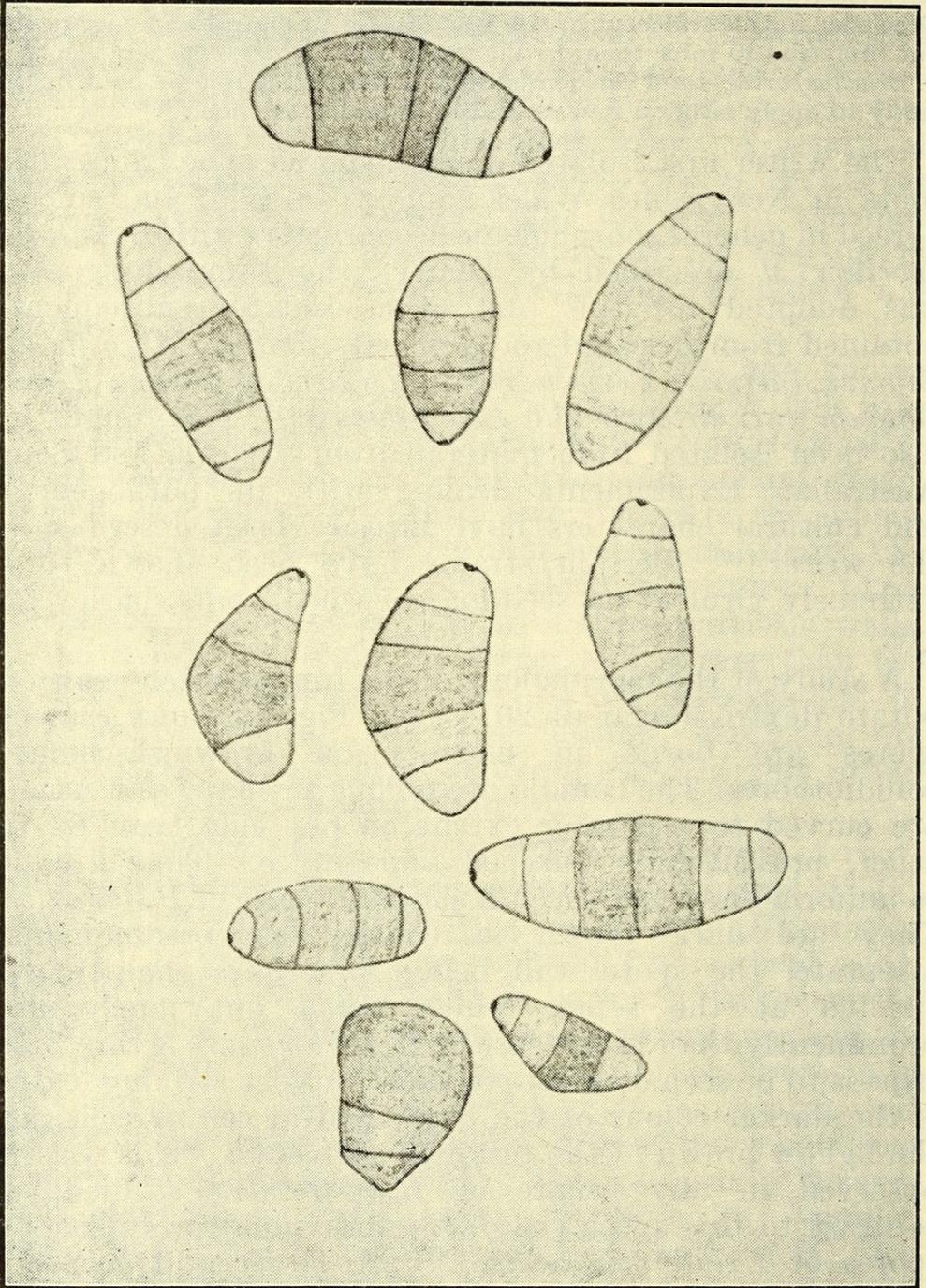


Fig. 3.—Conidia of *Helminthosporium M* (*Curvularia ramosa* (Bainier) Boedijn).  $\times 800$ .  
(From cultures on potato dextrose agar at 20° C.)  
(Photo by P. R. Maguire.)

TABLE 3.

*Measurements of 100 Conidia of each of two Monosporous Isolates of Helminthosporium M (Curvularia ramosa) Cultured on Sterilised Wheat Heads at 20° C.*

## ISOLATE 1.

Septation Class.	Frequency %	Range. (Microns.)	Mean. (Microns.)
0	1	—	11.5 × 8.2
1	4	11.5-16.5 × 6.6-11.5	14.0 × 9.0
2	6	14.8-23.1 × 8.2-13.2	19.8 × 10.2
3	17	23.1-31.3 × 9.9-14.8	26.8 × 12.0
4	48	29.7-42.9 × 11.5-16.5	35.6 × 13.8
5	19	26.4-41.2 × 9.9-14.8	37.0 × 13.6
6	5	36.3-41.2 × 11.5-14.8	39.6 × 13.5

## ISOLATE 2.

1	1	—	21.4 × 14.8
2	3	23.1-24.7 × 9.9-11.5	23.6 × 10.4
3	10	21.4-37.9 × 9.9-14.8	28.5 × 12.0
4	50	28.0-42.9 × 9.9-16.5	35.4 × 13.1
5	30	33.0-42.9 × 11.5-16.5	38.0 × 14.1
6	6	37.9-41.2 × 13.2-16.5	40.1 × 14.8

Mean length and width of the 100 conidia of isolate 1 : 32.6 × 13.0 microns.

Mean length and width of the 100 conidia of isolate 2 : 35.3 × 13.3 microns.

that the septation class of highest frequency in each case was 4; also that the mean dimensions were 32.6 × 13.0 microns and 35.3 × 13.3 microns, respectively. Henry<sup>(8)</sup> found the mean lengths of conidia of four monosporous strains cultured on sterilised wheat heads to be 26.61, 22.59, 26.7 and 25.2 microns; the mean widths to be 11.77, 11.99, 11.05 and 10.5 microns, respectively; and the mean number of septa to be 3.65, 2.88, 3.76 and 3.69, respectively. Bainier<sup>(1)</sup> observed the dimensions of *Brachycladium ramosum*—which, as indicated below, is a synonym of *Helminthosporium M*—to be 25 to 31 by 11 to 14 microns.

Cultures forwarded to Boedijn have been identified as *Curvularia ramosa* (Bainier) Boedijn, synonymous with

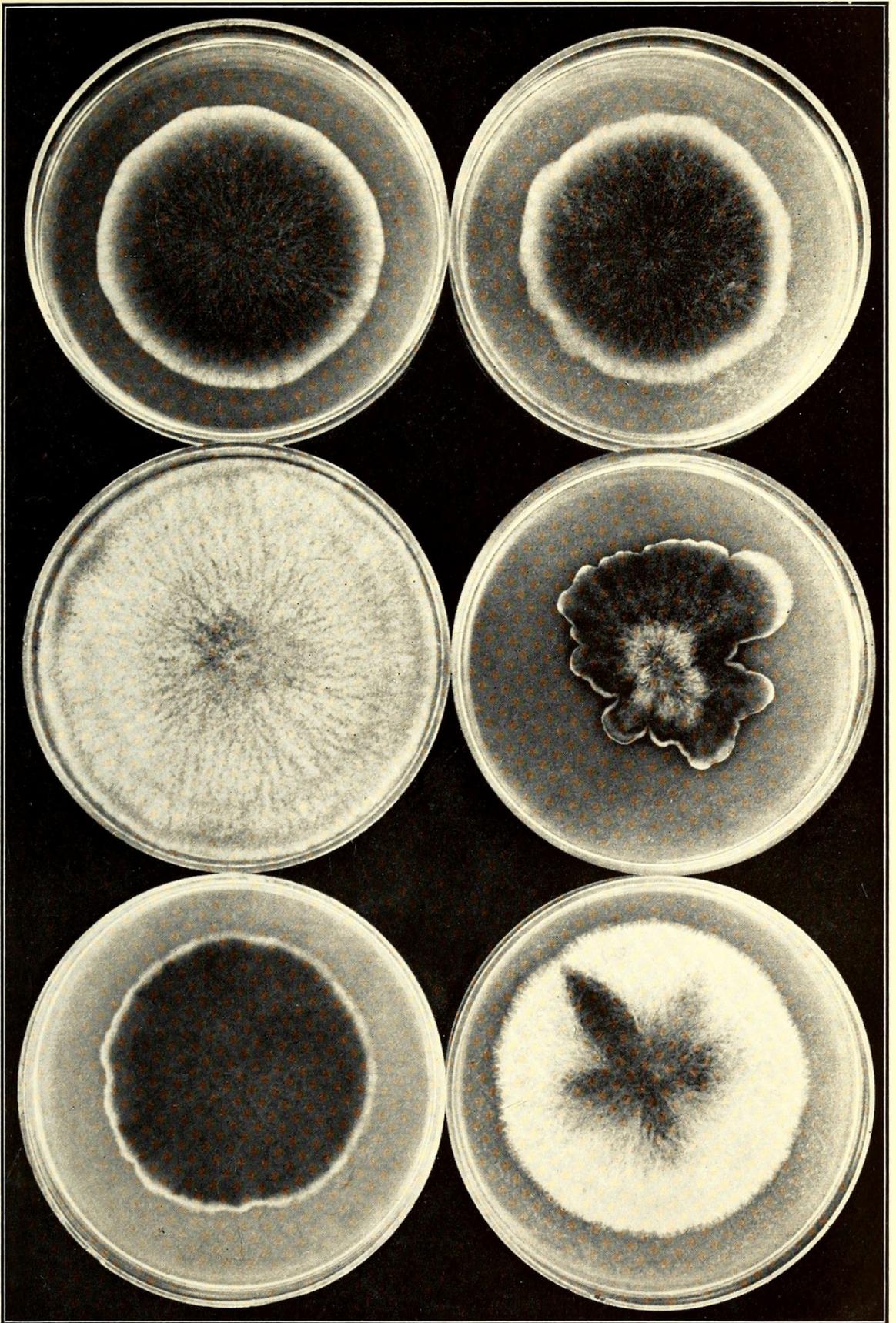
*Brachycladium ramosum* Bainier, which was described in 1908<sup>(1)</sup> from material isolated from "the dead stalks of the common nettle". From a study of Bainier's description and drawings, the writer concludes that *Helminthosporium M* is very similar to *B. ramosum*. The recent critical study of small-spored types by Boedijn<sup>(3)</sup> means that both these forms must now be classified as *Curvularia ramosa*, falling into the "Lunata Gruppe", concerning which Boedijn writes: "The conidia have three partition walls, but only one of the middle cells is larger and darker, and further, the conidia in this case are more or less heavily bent. Most species of this group form cylindrical stromata in pure culture. *Acrothecium lunatum* Wakker is an example." The colony growth on potato dextrose agar of two virulent strains of the fungus isolated by the writer is illustrated in Plate IX.

In addition to the Maculans and Lunata groups, Boedijn defines a third section, the "Genuculata Gruppe", in which he includes species with four partition walls in the conidia, and which for the most part form stromata in pure culture. He cites *Helminthosporium genuculatum* Tracy and Earle as a typical representative of the group.

#### SUMMARY.

1. Attention is drawn to the fact that five large-spore species of *Helminthosporium* have been isolated from root-rot-affected wheat by various workers. These are *H. bicolor* Mitra, *H. halodes* Drechs. var. *tritici* Mitra, *H. pedicellatum* Henry, *Helminthosporium N* Henry and *H. sativum* P.K.B. The most important species of this group, however, is *H. sativum*. The writer's studies on the morphology of Australian isolates of *H. sativum* are outlined.

2. The only small-spored species of *Helminthosporium* which appear to have been isolated from root-rot-affected wheat by various workers are *Helminthosporium M* Henry and *H. tetramera* McKinney. The writer's studies on the morphological characters of Australian isolates of each of these species are outlined. It is pointed out that as a result of Boedijn's work, *Helminthosporium M* should now be classified as *Curvularia ramosa* (Bainier) Boedijn, and *H. tetramera* as *Curvularia spicifera* (Bainier) Boedijn.





## EXPLANATION OF PLATE IX.

Colonies of parasitic strains of *Helminthosporium* spp. from foot-rot-affected wheat on potato dextrose agar at 25° C. after six days' growth.

Top: Two virulent strains of *Helminthosporium M* (*Curvularia ramosa*).

Centre: Two virulent strains of *H. sativum*.

Bottom: Two weakly parasitic strains of *H. tetramera* (*Curvularia spicifera*).

Photo by P. R. Maguire.

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