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PERICONIA CIRCINATA AND ITS RELATION TO MILO
DISEASE

By R. W. Leufel

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INTRODUCTION

In 1924 a new disease of milo \( \textit{Sorghum vulgäre} \) Pers.) was observed near Chillicothe, Tex. Later this disease was found in widely separated sorghum-growing sections in Texas, Kansas, Oklahoma, New Mexico, Arizona, and California. It proved to be a serious disease of milo and its derivatives. Losses on individual farms often were very severe, especially where milo was grown on land that previously had been cropped to milo. For a while this so-called milo disease seemed to threaten the continued growing of milo in the sorghum-growing sections of the Southwest. Apparently resistant milo plants, however, were observed occasionally in infested fields and experimental plots. Selections from such plants led to the development of resistant strains of milo long before the cause of the disease was determined \( 3, 4 \). For a number of years \( \textit{Pythium arrhenomanes} \) Drechs. was generally accepted as the causal fungus, and the disease was referred to as the pythium root rot of milo. Later experiments and observations by a number of investigators, however, created considerable doubt as to whether \( P. \textit{arrhenomanes} \) was the true cause of the disease, because typical field symptoms could not be produced by artificial inoculation of soil with this fungus. It seemed desirable, therefore, to reinvestigate the problem and to determine experimentally whether typical milo disease could be induced in susceptible milos by heavy and repeated inoculation of soil with this fungus or with some other agent associated with affected plants grown in soil from infested fields. Such experiments have been in progress since 1942. A brief description of these experiments has been published \( 10 \), and the detailed results and conclusions therefrom are presented herein.

1 Received for publication February 25, 1948.
2 The writer wishes to express his appreciation to the following members of the staff of this Bureau: To John A. Stevenson for identifying \( \textit{Periconia circinata} \) (Mang.) Sacc.; to A. G. Johnson for his helpful suggestions, for taking the photomicrographs, and for his critical review of the manuscript; to John H. Martin for information relating to the history of the milo disease; to Mrs. Flora G. Pollock for her assistance in studying the causal fungus; to R. L. Taylor for taking the photographs; and to Charles Drechsler for identifying isolates of \( \textit{Pythium arrhenomanes} \) Drechs.
3 Italic numbers in parentheses refer to Literature Cited, p. 222.
REVIEW OF LITERATURE

The milo disease was first described in 1932 by Elliott, Wagner, and Melchers (5), who called it "the root, crown, and shoot rot of milo." They attributed it to an undetermined soil-borne pathogen. In 1934 Myers (18) expressed the same belief after finding that the application of a number of soil amendments had no effect on the disease. In February 1936 Elliott et al. (3) reported that *Pythium arrhenomanes* was the causal organism and that under greenhouse conditions it produced the same symptoms on milo grown in inoculated sterilized soil as were produced in naturally infested soil. In August 1936 Wagner (22) described the reaction of different varieties, selections, and crosses of sorghum to the disease as shown in tests covering 4 years. He listed 34 susceptible, 5 segregating, and 25 resistant milo selections or crosses, 29 immune kafirs and kafir relatives, and 9 immune sorgos. He had tested these in naturally infested soil but not in soil artificially inoculated with *P. arrhenomanes*.

In June 1937 Elliott et al. (4) published a full account of their experimental investigations leading to the conclusion that *Pythium arrhenomanes* was the causal agent of the milo disease. They pictured the reaction of susceptible and resistant selections of milo grown in naturally infested soil, but they failed to show that these strains were susceptible and resistant, respectively, in soil artificially inoculated with *P. arrhenomanes*. Ezekiel (6) reported that in greenhouse experiments in April 1937 both susceptible and resistant selections of milo were killed in soil inoculated with *P. arrhenomanes* isolated from sorghum affected with the milo disease, but that in soil similarly inoculated during the following May and June no definite symptoms were produced in susceptible or resistant plants.

In 1937 Bowman et al. (1) reported the results of genetic studies on the inheritance of resistance to the milo disease in plants grown in naturally infested soil, but they did not demonstrate that this resistance applied also to plants grown in soil inoculated with *Pythium arrhenomanes*.

In May 1939 Kendrick and Briggs (9) described the milo disease as it occurred in California and reported the development of milo selections resistant to the disease. They, too, based the susceptibility and resistance of milo selections entirely on their behavior in naturally infested soil from fields in which the disease had been observed.

Melchers and Lowe in 1940 (16) and again in 1943 (17) stated that in their studies on the reaction of varieties to the milo disease and the development of resistant varieties they used only naturally infested soil. In 1944 Heyne, Melchers, and Lowe (7) reported that they also used naturally infested soil. In all these studies the selections, varieties, and crosses were classified as resistant entirely on the basis of their behavior in naturally infested soil.

In 1942 Melchers (16) expressed the opinion that *Pythium arrhenomanes* alone is not the cause of milo disease but may be one of several factors responsible for it. He reported that *P. arrhenomanes* does not produce typical symptoms of milo disease on seedlings in the greenhouse or on older plants in the field and that it is equally pathogenic to sorghum varieties resistant or susceptible to milo disease. In 1946

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Slagg and Melchers (20) reported on additional investigations indicating that *P. arrhenomanes* is not entirely responsible for the milo disease. They found that 18 of the 49 seed lots that were tested transmitted the disease although *P. arrhenomanes* had not been isolated from sorghum seed.

**MATERIAL AND METHODS**

The experiments described herein were conducted in the laboratory and greenhouse and in outdoor beds at the Plant Industry Station, Beltsville, Md. Soil from fields known to be infested with the milo disease pathogen was received from Garden City, Kans., and Dalhart, Tex. It was stored in covered metal cans in the greenhouse. Since the soil from Kansas proved to be more severely infested than that from Texas, it was used exclusively as the standard for comparison with inoculated soil and is the infested soil referred to throughout these studies. Isolations were made from diseased plants of susceptible milo selections grown in both the Kansas and the Texas soil. The fungi isolated were increased on suitable media, and these cultures were used to inoculate separate portions of greenhouse potting soil that had been steam-sterilized for 2 to 4 hours at 15 pounds' pressure.

Seed of milo selections known to be susceptible or resistant to the milo disease was planted in these lots of soil, and the growth of the plants was compared with that of these same selections in naturally infested soil and in uninoculated steam-sterilized soil. Before this seed was used, it was surface-sterilized by being soaked in water for 30 minutes, drained, and then allowed to remain covered for 6 hours. It was then soaked in a 1 to 240 formaldehyde solution for 30 minutes to eliminate seed-borne infection (20), rinsed in water several times, thoroughly dried at room temperature, and then treated with Arasan. The resistance or susceptibility of milo selections mentioned in this paper refers only to their resistance or susceptibility to the milo disease.

Every reasonable precaution was taken to prevent the inadvertent transfer of inoculum from one lot of soil to another. All implements used were flamed between operations, and the pans, boxes, and flats in which the plants were grown were first steam-sterilized. Preliminary pathogenicity tests with the isolated fungi usually were carried out by planting seed of susceptible and resistant selections of milo in metal pans, 4 by 8 by 2 inches, filled with steam-sterilized potting soil uninoculated or inoculated, with each fungus being tested and incubating separate sets of such pans at 20°, 25°, and 30° C. When results seemed to justify additional and more extensive tests, they were carried out in metal boxes, 8 by 8 by 7 inches, or in wooden flats, usually 12 by 24 by 6 inches. In the study of two of the isolated fungi, final tests were made in soil beds 5 feet square in the greenhouse and also in similar beds outdoors.

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5 The soil from Kansas was sent by Alvin E. Lowe and that from Texas by Benjamin F. Barnes.
6 These seed lots were obtained from John B. Sieglinger, Arthur F. Swanson, J. C. Stephens, and Alvin E. Lowe.
Since *Pythium arrhenomanes* had previously been considered the cause of the milo disease, naturally this fungus was the first to be tested for pathogenicity. Isolations of the fungus were made readily by the method described by Elliott et al. (4), and a number of these typical isolates were identified by Charles Drechsler as *P. arrhenomanes*. They were grown on potato-dextrose agar, corn-meal agar, and corn-meal-carrot agar and were used to inoculate soil either separately or as a composite inoculum.

*Pythium arrhenomanes* caused severe pre-emergence damping-off in both susceptible and resistant milo selections, especially at the lower temperatures. This damping-off was more severe in steam-sterilized soil inoculated with this fungus than it was in naturally infested soil similarly inoculated (fig. 1). The plants that survived the emergence stage in inoculated steam-sterilized soil were often somewhat stunted, but usually they matured. No consistent differences were observed between the growth of susceptible selections and that of resistant ones in this soil. At times, however, some resistant selections were injured more than other resistant selections. Repeated and prolonged attempts to induce typical milo disease in susceptible milos by growing them in *Pythium*-inoculated soil, both in the greenhouse and in outdoor

**Figure 1.—Dwarf Yellow milo:** A, In soil naturally infested with the milo disease organism; B, in similar soil autoclaved for 2 hours at 15 pounds’ pressure. a, Not inoculated; b, inoculated with *Pythium arrhenomanes*. 
beds, met with consistent failure. Some of the results obtained with *P. arrhenomanes* will be discussed in connection with other experiments.

**MISCELLANEOUS FUNGI**

In addition to *Pythium arrhenomanes*, many other fungi were isolated from the roots of milo plants grown in infested soil and affected with typical milo disease. These included species of *Pythium, Fusarium, Aspergillus, Penicillium, Trichoderma, Alternaria, Helminthosporium*, and a number of unidentified genera. These isolates were grown on suitable media and used as inoculum in preliminary pathogenicity tests as previously described. Some of them reduced the percentage of emergence, caused post-emergence damping-off, or produced other symptoms, but always with approximately equal severity in selections resistant or susceptible to the milo disease. Further details of these experiments need not be given since, like *P. arrhenomanes*, these various other fungi with the exception of the one discussed in the next section failed to produce results identical with those obtained when susceptible and resistant selections of milo were grown in naturally infested soil.

**PERICONIA CIRCINATA**

Isolations from the roots of diseased plants of susceptible selections of Dwarf Yellow and Colby milos grown in naturally infested soil occasionally yielded a slow-growing fungus with a light-gray to a dark-gray, fluffy mycelium (12). On potato-dextrose agar or corn-meal-carrot agar it produced abundant, large, black conidia borne on long, dark, circinate conidiophores and, under certain unfavorable cultural conditions, chains of thick-walled chlamydospores. This fungus was identified by J. A. Stevenson as *Periconia circinata* (Mang.) Sacc. (14, pp. 221–223; 19, v, 18, p. 569).

Steam-sterilized soil was heavily inoculated with cultures of *Periconia circinata* grown on corn-meal-carrot agar, and seed of susceptible and resistant Dwarf Yellow milo selections was planted in this soil and also in uninoculated steam-sterilized soil. The resistant selection emerged and grew in the inoculated soil just as in the uninoculated soil; but the susceptible selection, after fairly good emergence and early growth, showed symptoms similar to those displayed by susceptible milo plants grown in naturally infested soil. The roots were badly rotted, the seedlings were stunted, and the leaves tended to curl as if affected by drought (fig. 2). The virulence of the inoculum was so intensified by later repeated plantings in this inoculated soil that the susceptible seedlings died before making much growth.

To compare the growth of susceptible and resistant selections of Colby milo in naturally infested soil with that in soil inoculated with *Periconia circinata*, seed was planted in *Periconia*-inoculated steam-sterilized soil, naturally infested soil, and in uninoculated steam-sterilized soil. The results obtained in the first two lots of soil were almost identical. Both the susceptible and the resistant selection produced good stands in the inoculated soil; but after several weeks the plants of the susceptible selection of Colby were dead, while those of the resistant selection and the resistant and susceptible plants in the uninoculated steam-sterilized soil were growing well (fig. 3).

To produce diseased plants more nearly resembling those grown in fields less severely infested, one lot of naturally infested soil and one lot of *Periconia*-inoculated soil were diluted with steam-sterilized soil and
planted to susceptible and resistant selections of Colby milo. The results are shown in figure 4. In both lots the resistant selection grew to maturity and produced fairly good heads. The susceptible selection grown in the naturally infested soil formed a few small heads; that grown in the inoculated soil produced no heads, although the plants made a better growth than did those in previous plantings in more heavily inoculated soil.

Similar results were obtained in another experiment designed to show the relation between the quantity of inoculum in the soil and the severity of the disease. Seven boxes, 8 by 8 by 7 inches, were filled to within 1 inch of the top with steam-sterilized soil. One box was used as an uninoculated control. Five of the boxes were inoculated with Peri-
Figure 3.—Susceptible (a) and resistant (b) selections of Colby milo grown in soil: A, steam-sterilized and inoculated with *Periconia circinata*; B, naturally infested; C, steam-sterilized and not inoculated.

Figure 4.—Susceptible (a) and resistant (b) selections of Colby milo grown in soil: A, Naturally infested; B, steam-sterilized and inoculated with *Periconia circinata*

*iconia circinata* grown on potato-dextrose agar. Three petri-dish cultures were taken as a unit of inoculum, and 1, 2, 4, 6, and 8 such units were added to the soil in boxes 2, 3, 4, 5, and 6, respectively. In each case the mycelial mat relatively free of agar was removed from the petri dish. The agar remaining from the 63 petri dishes was mixed with the soil in box 7; box 8 contained naturally infested soil. Twenty seeds each of susceptible and resistant Colby milo were planted on August 4 in single rows on the right and on the left, respectively, in each box. Emergence and stand were fairly uniform in all of the boxes. On
August 12 the plants were thinned to 8 per row. After 6 weeks the relation between the quantity of inoculum and the severity of the disease in the susceptible milo was very apparent (fig. 5).

One unit of inoculum caused the least injury to the susceptible milo; and as the quantity of inoculum was increased in each succeeding box there was a corresponding decrease in the growth of the susceptible Colby and, because of less plant competition, a corresponding increase in that of the resistant Colby. The growth of susceptible Colby in the box with four units of inoculum was very similar to the growth of this selection in the infested soil. The severest injury occurred in the box to which the agar had been added. Although little fungus mycelium remained in this agar, it probably contained considerable toxic material produced by the fungus. The crowns of the diseased susceptible

![Figure 5](image-url)
plants when split showed a dark-red discoloration typical of milo disease, whereas those of the resistant plants were a healthy white.

Another experiment was designed to compare the growth of eight susceptible and resistant selections of milo in *Periconia*-inoculated soil with that of the same selections in naturally infested soil. Seed of susceptible and resistant selections of Colby, Day, Beaver, Sooner, Dwarf Yellow, Wheatland, and Pygmy milos and of darsos (*Sorghum vulgare*) was planted in flats containing *Periconia*-inoculated soil or naturally infested soil, somewhat according to the method described by Melchers and Lowe (**17**) for developing milos resistant to milo disease. The planting was made on April 8, and one of the flats containing inoculated soil was photographed on May 12 (fig. 6). The difference in

![Figure 6](image)

*Figure 6.*—Resistant (a) and susceptible (b) selections of each of four varieties of milo grown in steam-sterilized soil inoculated with *Periconia circinata*: A, Colby; B, Day; C, Beaver; D, Sooner.

the growth of the resistant and susceptible selections was evident in *Periconia*-inoculated soil just as in naturally infested soil.

In early attempts in the spring of 1945 to induce typical milo disease in plants grown in *Pythium*-inoculated soil, three outdoor beds, 5 by 5 feet, were constructed. The soil in these beds was removed, thoroughly mixed, and then steam-sterilized at 15 pounds' pressure for 4 hours on each of 2 days. The soil to be placed in one bed was then mixed with 10 percent by weight of naturally infested soil. The soil placed in the second bed was inoculated periodically for two seasons with cultures of *Pythium arrhenomanes*. The soil in the third bed was left unaltered. The soil in each bed was a foot deep and underlain by a subsoil of sand. Each bed was covered with a woven-wire cage to exclude birds and rodents. Resistant and susceptible selections of milo were grown in each of these beds in 1945 and 1946, but the milo disease developed only in the one to which naturally infested soil had been added. There was no perceptible difference between the growth of resistant and susceptible selections in the *Pythium*-inoculated soil.
In the spring of 1947 a fourth bed similar to the others was constructed, and after being steam-sterilized the 25 cubic feet of soil was inoculated with 100 petri-dish cultures of *Periconia circinata*. On June 13 seed of resistant and susceptible selections of Colby milo was planted in this and the 3 other beds. After the plants had emerged the stand was thinned to 20 plants per 5-foot row.

For 3 weeks the growth of the susceptible and the resistant selection in the four beds was rather uniform. During the fourth week the susceptible selection in the naturally infested soil and in the *Periconia*-inoculated soil began to show signs of retarded growth, which during the next few weeks gradually became more pronounced. By September 4 the leaves of the susceptible selection in these two beds were fired and the plants were about half as large as those of the resistant selection (fig. 7). In the other two beds there was no perceptible difference between the two Colby selections (fig. 8).

By October 1 the susceptible plants shown in figure 7 were dead (fig. 9). The resistant plants in these two beds, as well as the susceptible and resistant plants in the other two beds, were still growing vigorously.

Roots from the diseased plants grown in the naturally infested soil were washed in running water to remove all soil particles and then incubated on moist filter paper in a moist chamber. After 4 days conidiophores and conidia of *Periconia circinata* were produced in abundance on these roots (see fig. 12, A).

In November 1945 an indoor experiment similar to the outdoor one just described had been started in the greenhouse in three beds, 5 by 5 feet, containing fertile soil 13 inches deep on a subsoil of hard clay. The soil was removed, mixed, and sterilized as previously described for the outdoor beds. After being replaced in the beds, one lot of soil was mixed with 10 percent by weight of naturally infested soil; another lot was periodically inoculated with *Pythium arrhenomanes*; the third lot was left unchanged. The first planting of susceptible and resistant selections of milo was made on January 5, 1946, and final data were taken on April 15. A second planting was made on May 2, and final data on it were taken on September 3. As in the outdoor experiment milo disease appeared only in the bed to which naturally infested soil had been added. The disease failed to appear in the *Pythium*-inoculated soil, but both the susceptible and the resistant plants in this soil were 20 to 25 percent shorter than those in the uninoculated control.

On January 23, 1947, a fourth indoor bed, similar to the other three and containing steam-sterilized soil subsequently inoculated with *Periconia circinata*, was included in another planting of susceptible and resistant selections of Colby milo in the greenhouse. Early stands and growth were relatively poor in all four beds because of poor soil preparation, low soil temperature, and low light intensity. Typical milo disease developed in all susceptible milo plants in the *Periconia*-inoculated soil and in the naturally infested soil. A few of the susceptible plants in the *Pythium*-inoculated soil and in the uninoculated soil showed typical milo disease. Some of the darkened roots of the diseased plants from these beds were incubated in a moist chamber for several days, and they produced abundant conidiophores and conidia of *P. circinata* (see fig. 12, A). The soil in these beds, both adjacent to the *Periconia*-inoculated bed, apparently had become sufficiently contaminated with *P. circinata* to induce milo disease in a few of the susceptible plants.
Figure 7.—Susceptible (a) and resistant (b) selections of Colby milo grown in outdoor beds in soil: A, Naturally infested; B, steam-sterilized and inoculated with Periconia circinata.
FIGURE 8.—Susceptible (a) and resistant (b) selections of Colby milo grown in outdoor beds of steam-sterilized soil: A, Inoculated with *Pythium arrhenomanes* isolated from the roots of diseased milo grown in infested soil; B, not inoculated.
Before the greenhouse beds were replanted, therefore, the soil in the bed used as an uninoculated control was removed, resterilized at 15 pounds' pressure for 4 hours on 2 successive days, and replaced. Added precautions were taken to prevent its recontamination from nearby beds containing *Periconia*-inoculated or naturally infested soil. The *Pythium*-inoculated soil was not resterilized; hence it remained infested with both the *Pythium* and the *Periconia*. The soil in each of the four beds was thoroughly worked over; the lower layer and the subsoil were watered heavily to guard against subsequent drying out. All implements used were thoroughly sterilized. On June 12 one row each of resistant and susceptible selections of Colby and Day milos was planted in each bed 1 inch deep in this well-prepared soil.

The careful soil preparation, combined with seed treatment and a high soil temperature (25° to 30° C.), resulted in a uniformly good stand in all 4 beds. On June 24 the stand was thinned to 20 plants per 5-foot row. On June 30, 18 days after planting, the susceptible plants in the *Periconia*-inoculated soil began to show symptoms of milo disease. On July 7 the susceptible plants in the naturally infested soil and some of those in the *Pythium*-inoculated, *Periconia*-contaminated soil showed similar symptoms.

On August 20 the number of living and dead plants in each row and their average height were determined. These data, together with similar data taken on October 7, are shown in Table 1.

All of the susceptible plants in beds 2 (inoculated) and 4 (naturally infested) and all except six plants in bed 1 (*Pythium*-inoculated but contaminated with *Periconia*) were killed by what apparently was typical milo disease (fig. 10). When roots from these diseased plants were incubated on moist filter paper in a moist chamber for several days, an abundance of conidiophores and conidia of *Periconia circinata* appeared.
How *Periconia circinata* brings about the death of susceptible milo plants has not yet been determined. In soil heavily inoculated with the fungus, susceptible plants, as described previously, die as seedlings and the roots are almost completely rotted. In soil rather lightly inoculated, the growth of susceptible milos appears to equal that of resistant milos for a considerable period. About the time of heading, however, the susceptible plants may begin to show signs of firing. The heads fail to develop fully, and the plants die about the time when the grain of the resistant plants is in the hard-dough stage. The disease induced by soil inoculation may show a wide range in severity between these two extremes, depending on the quantity of inoculum used.

A similar range in the severity of the disease has been observed under field conditions. There are indications that the fungus produces a virulent toxin which in some way stunts the susceptible plants and may even kill them. This seems to be demonstrated by the following experiment. Five metal pans, 4 by 8 by 2 inches, were filled with steam-sterilized quartz sand. The sand in pans 1 and 2 was heavily inoculated with cultures of *Periconia circinata*, after which all of the pans were planted to Colby milo, one-half of each pan to the resistant selection and the other half to the susceptible. The pans were watered with a balanced plant-nutrient solution to maintain good growth. Because of the very heavy inoculation, the susceptible plants in pans 1 and 2 were stunted at an early stage and were much smaller than the resistant plants in the same pans.

Twelve days after the seed was planted, the sand in pans 3 and 4 was heavily inoculated with a distilled-water suspension of mycelium and spores of *Periconia circinata*. About 2 to 3 cc. of this inoculum was placed at the base of each plant. Fifteen days later all of the susceptible plants in pans 3 and 4, together with those in pans 1 and 2 (inoc-

### Table 1.—Results of growing resistant and susceptible selections of Colby and Day milos in greenhouse beds in inoculated or naturally infested soil, 1947

<table>
<thead>
<tr>
<th>Bed No. and treatment</th>
<th>Variety</th>
<th>Selection resistant (R) or susceptible (S)</th>
<th>August 20 data</th>
<th>October 7 data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number</td>
<td>Number</td>
<td>Cm.</td>
</tr>
<tr>
<td>1. Steam-sterilized, then inoculated with <em>Pythium arrhenomanes</em>, and later contaminated with <em>Periconia circinata</em>.</td>
<td>Colby</td>
<td>R</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Day</td>
<td>S</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>2. Steam-sterilized and then inoculated with <em>P. circinata</em>.</td>
<td>Colby</td>
<td>R</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Day</td>
<td>S</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td>3. Steam-sterilized and not inoculated.</td>
<td>Colby</td>
<td>R</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Day</td>
<td>S</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>4. Not steam-sterilized but naturally infested.</td>
<td>Colby</td>
<td>R</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Day</td>
<td>S</td>
<td>17</td>
<td>3</td>
</tr>
</tbody>
</table>

1 Basis incomplete because some dead plants could not be measured.
2 At 15 pounds’ pressure for 4 hours on 2 successive days.
3 From bed 2.

The susceptible plants in pans 1 and 2 were stunted at an early stage and were much smaller than the resistant plants in the same pans.
Figure 10.—Resistant and susceptible selections of milo grown in greenhouse beds, October 1947: A, In steam-sterilized soil inoculated with *Pythium arrhenomanes* and later contaminated with *Periconia circinata*; B, in steam-sterilized soil inoculated with *P. circinata*; C, in uninoculated steam-sterilized soil; D, in unsterilized infested soil.  

*a*, Resistant Day;  
*b*, susceptible Day;  
*c*, susceptible Colby;  
*d*, resistant Colby.
ulated before the seed was planted), were dead. The resistant plants in all five pans, as well as the susceptible ones in pan 5 (uninoculated), were growing vigorously.

The plants were then removed from all the pans, and pans 1 and 3 were steamed at 100° C. for 1 hour, after which the five pans were replanted as before. In 1 month the susceptible plants in pans 2 and 4 (not resteamed) were dead, whereas the resistant plants were growing normally. Of the susceptible plants in pans 1 and 3 (resteamend sand), a few had died and all of the remainder were badly stunted and deformed, presumably from the effects of a more or less thermostable toxin left in the sand from the previous crop. The resistant plants were not injured. *Periconia* could not be isolated from the roots of the dead or stunted plants in these pans, but it was found in abundance in the pans that had not been resteamed. Apparently the slight steaming had killed the fungus, but it had not destroyed the toxin produced by the fungus.

These results were duplicated in a second similar experiment. A quantity of clean quartz sand was divided into three portions of 3,300 cc. each: the first (A) served as an uninoculated control, the second (B) was inoculated with spores and mycelium of *Periconia circinata*, and the third (C) was mixed with the agar from which the inoculum in B had been taken. Three pans, 4 by 8 by 2 inches, were filled with each of these three lots of sand. One pan (a) in each group was left unaltered, another (b) was steamed for 1 hour at 100° C., and a third (c) was autoclaved for 1 hour at 15 pounds’ pressure. Seed of susceptible and resistant strains of Colby milo was then planted in each pan as described in the preceding experiment. The results are shown in figure 11.

In the uninoculated series (fig. 11, A) both selections grew equally well regardless of treatment. In the series inoculated with spores and mycelium (fig. 11, B) the susceptible milo grown in the unaltered sand (a) was completely killed, that in the steamed sand (b) was badly stunted and partly killed, while that in the autoclaved sand (c) grew normally. In the series to which the substrate had been added (fig. 11, C), the results were similar to those in figure 11, B. *Periconia* was found on the roots of the susceptible plants in the inoculated sand that had not been steamed, but it failed to develop on roots from the steamed sand.

It is possible that a toxin produced by *Periconia circinata* kills the plant cells in advance of the fungus in a manner similar to that described by Johann, Holbert, and Dickson (8) for *Penicillium oxalicum* Currie and Thom and that described by Meehan and Murphy (15) for *Helminthosporium victoriae* Meehan and Murphy. If *P. circinata* is a saprophyte, as most species of *Periconia* are, it would be able to destroy the roots of susceptible milo plants in this manner. Further study is needed on this and other phases of this disease.

The results obtained in the experiments described and those obtained in a number of similar experiments that need not be described here show beyond a reasonable doubt that *Periconia circinata* is the fungus that causes milo disease. The role of plant pathogen played by *Pythium arrhenomanes*, while not a minor one, is not restricted to varieties susceptible to milo disease or to soil that is infested with the milo disease organism. This species of *Pythium* appears to be especially inju-
Figure 11.—Susceptible and resistant selections of Colby milo grown in pans of soil: A, Not inoculated; B, inoculated with spores and mycelium of *Periconia circinata*; C, inoculated with left-over substrata of cultures of *P. circinata*. a, Not steamed or sterilized; b, steamed for 1 hour at 100° C.; c, autoclaved for 1 hour at 15 pounds' pressure. Susceptible plants in left half of each pan and resistant plants in right half.

Serious to the finer roots of most sorghum varieties regardless of whether they are susceptible or resistant to the milo disease. Its virulence in causing pre-emergence damping-off has been mentioned already and has been described also by Elliott et al. (4). It is an important cause of poor stands in most sorghums, as has been shown by Leukel and Martin (11). However, it is not necessarily a factor in milo disease; hence this disease should no longer be referred to as pythium root rot, but as periconia root rot.
DESCRIPTION OF PERICONIA CIRCINATA

*Periconia circinata* was first described in 1899 by Mangin (14, pp. 221-223), who named it *Aspergillus circinatus*. He isolated it in France from wheat plants affected with foot rot, but he did not regard it as a causal agent of that disease. In 1906 Saccardo (19, v. 18, p. 569) transferred the species to the genus *Periconia*, making the new combination:

*Periconia circinata* (Mang.) Sacc., 1906.


The different species of *Periconia* mentioned in the literature have, with few exceptions, been described as saprophytic. In 1902 Earle (2) described 11 such species of *Periconia* but did not include *P. circinata*, which at that time was still listed in the genus *Aspergillus*. In 1937 Linder (13) published a key to 12 species of *Periconia*, which included only 6 of those described by Earle. He reported that *P. toroi* was parasitic on leaves of *Machaerium moritzianum*. Stevenson and Imle (21), in 1945, described a new species, *P. heveae*, as parasitic on leaves, petioles, and twigs of *Hevea spruceana* and *H. brasiliensis*.

*Periconia circinata* grows somewhat slowly on artificial media, so that fresh isolates are readily overrun by more rapidly growing fungi, such as *Pythium*, *Fusarium*, and *Aspergillus*. This may account for its not having been discovered before in connection with studies on the milo disease. Its cardinal temperatures for growth in culture seem to be about 10°, 25°, and 40° C. When grown on potato-dextrose agar, it produces a dirty-white to mouse-gray mycelial growth, which may turn almost black with abundant sporulation, or it may produce light and dark sectors.

The mycelium consists of slender, branched, hyaline threads, 2μ to 6μ in diameter and also of larger, thicker walled, light-brown fruiting hyphae that either give rise to conidiophores or form thick-walled chlamydospores (fig. 12, D). Seemingly these chlamydospores are produced when conditions for growth in culture are more or less unfavorable owing to the type or the depth of the medium used, the presence of contaminants, or other factors. The chlamydospores vary greatly in size and shape but generally are smooth, somewhat cylindrical, 9μ to 18μ in diameter, occur singly or in chains, are terminal or intercalary, and contain conspicuous oil globules.

Conidiophores arise from the chlamydospores (fig. 12, E) or from the thick-walled mycelium. They may occur singly or in groups of 2 or 3. As the species name suggests, they usually are curved or circinate near the apex (fig. 13). They are dark brown to black, thick-walled, 150μ to 250μ long by 6μ to 8μ in diameter, and 3- to 10-septate. The apical cell of the conidiophore usually is slightly swollen and bears 1 or more (generally 3) sporogenous cells. Frequently the apical cell divides, and each division bears sporogenous cells. Some conidiophores in culture were observed to produce sporogenous cells at the first septum and less frequently at the second below the apex. These sporogenous cells may occur singly or in short chains of 2 or 3, are spherical to almost cylindrical, 5μ to 7μ in diameter, smooth, paler brown than the conidiophores, and bear conidia in basipetal succession. The mature conidia are pushed aside by the formation of new conidia, or they may adhere in short chains of 2 or 3 spores.
The conidia are dark brown to black, 15\(\mu\) to 27\(\mu\) in diameter (usually 18\(\mu\) to 21\(\mu\)), mostly spherical, and coarsely verrucose to spiny, although a few apparently smooth ones have been observed in culture. The spines on some spores are very pronounced and average 0.5\(\mu\) in length. Apparently on other spores these spines curl over and thus present a verrucose, or a warty, appearance. Mangin (14, pp. 221–223) reported that he was unable to make the conidia germinate and his experience approximates that of the writer, although extensive experiments on this phase of the problem have not been attempted. What appears to be spore germination on malt agar is shown in figure 13, \(F\).
FIGURE 13.—*Periconia ciriata*: *A*, Conidiophores on root of a diseased milo plant grown in infested soil, $\times$ 120; *B*, same, $\times$ 300; *C* and *D*, conidiophores showing septa, apical cell, and sporogenous cells, $\times$ 500; *E*, illustration of conidiophores and spores accompanying original description by Mangin (*J. Agri. Res.*, pl. 11), scale in microns; *F*, conidium germinating on malt agar.
Milo disease was first recognized in Texas in 1924 and subsequently was found to be a serious disease of milo and its derivatives in some sections of the sorghum-growing States. An unusual phase of this disease was the development and widespread use of resistant selections of milo several years before the cause of the disease was determined.

In 1936 *Pythium arrhenomanes* was reported as the cause of milo disease, but subsequently doubt was expressed as to its causal relation to the disease. Now it has been definitely established that this fungus does not cause typical milo disease because both resistant and susceptible selections of milo are equally susceptible to the injury caused by it.

In recent studies to determine the true cause of milo disease, numerous fungi including *Pythium arrhenomanes* were isolated from the roots of susceptible milo grown in naturally infested soil. These different isolates were used to inoculate separate portions of steam-sterilized soil but with one exception they failed to produce milo disease in susceptible selections grown in the inoculated soil. The one exception was a slow-growing fungus identified as *Periconia circinata*. This species produced typical milo disease in all susceptible selections or varieties tested but failed to affect those known to be resistant. The severity of the injury was more or less proportional to the amount of inoculum used.

*Periconia circinata* produces a relatively thermostable toxic by-product that kills the plant cells in the absence of the fungus mycelium. This byproduct survives steaming for 1 hour at 100° C. but is destroyed by exposure for 1 hour to 15 pounds’ steam pressure. It is possible that *P. circinata* is a saprophyte like most other species of the genus and that it grows on cells killed by the toxin.

*Periconia circinata* grows slowly on artificial culture media. It produces a dirty-white to mouse-gray mycelium of slender hyaline threads and thicker walled fruiting hyphae. The latter may produce thick-walled chlamydospores or dark conidiophores bearing large spherical somewhat verrucose spores. The chlamydospores also may give rise to conidiophores. The conidiospores do not germinate readily.

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