A COMPARISON OF SOME STRAINS OF RHIZOCTONIA SOLANI IN CULTURE[1]

By JOHN MONTEITH, JR., *Associate Pathologist, Office of Vegetable and Forage Diseases, Bureau of Plant Industry, United States Department of Agriculture,* and ARNOLD S. DAHL, *Graduate Assistant, University of Wisconsin*

INTRODUCTION

In studying the large brown-patch disease of turf, described by Piper and Coe (*6*)[2] as due to *Rhizoctonia solani*, it was found that the causal organism varied in culture in several respects from some stock cultures of *R. solani*[3] obtained from potato tubers. There was found a marked difference in their pathogenicity on grass, and it seemed possible that they might be distinguished by certain variations in growth characteristics on artificial media. The investigations here reported were undertaken in an attempt to determine to what extent such characteristics would prove reliable in distinguishing the Rhizoctonia on grass from the potato organism, either as a distinct species or as a strain of *R. solani*. Several cultures were therefore obtained from different sources to compare with the grass fungus under various cultural conditions.

SOURCE OF STRAINS

Two isolations of the Rhizoctonia producing brown patch of grass were used in these comparisons. One of these (1) was obtained from diseased bent-grass leaves growing in the greenhouse at Madison, Wis., in the spring of 1924, and the other (2) was isolated in June of the same year from grass injured by brown patch in the turf garden on the Arlington Experiment Farm, Rosslyn, Va. Since the turf growing in the greenhouse at Madison was originally sent from the Arlington turf garden, these two cultures were probably of the same origin.

Cultures 3 and 4 were obtained from B. L. Richards, isolated from potato and identified by him as *Corticium vagum* B. and C. These two represent his cultures Nos. 35 and 131, respectively. Cultures 5 and 6 were isolated by Freeman Weiss in Washington from diseased potato tubers during the summer of 1924. Culture 7 was isolated from a sclerotium on a potato tuber at Madison in January, 1925. Culture 8 was from a stock culture in the laboratory of plant pathology at Madison, Wis. This represents the culture known as Rosenbaum's strain R5, isolated in 1916 in Maine from a diseased potato stem. Culture 9 was isolated from diseased peas in the spring of 1924 by F. R. Jones in Wisconsin.

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[2] Reference is made by number (italic) to "Literature cited," p. 903.

[3] No attempt will be made to make detailed references to the extensive literature dealing with this fungus, The writers are aware of the divergence of opinion as to what constitutes a "species" or "strain" of Rhizoctonia, as expressed in such publications as those by Duggar (*2*), Peltier (*5*), Matz (*4*), Matsumoto (*3*), Briton-Jones (*1*), and Thomas (*7*).

METHODS

Differences between the fungi isolated from grass and those from potato proved to be practically the same on several culture media, so it was decided to confine the study chiefly to a single medium. Potato-dextrose agar was chosen for this purpose, since it proved to be favorable for all the cultures, besides having several other desirable features. This was prepared in the usual manner, a decoction of 200 gm. of potato per liter of the medium with 2 per cent dextrose and 1½ per cent agar being used. In pouring plates sufficient agar was used to make a layer about 5 mm. thick. This provided a sufficient reserve of moisture to take care of any small loss due to evaporation in cultures grown for several days. Tests showed that there were decided differences in initial growth, depending on whether transfers were made from old or from young cultures. In some cases inoculations made with the tips of growing mycelium had entirely crossed the plate before similar inoculations made from old cultures had started to develop. Therefore all further inoculations were made by transferring pieces of agar containing tips of growing mycelium taken from the borders of young cultures.

INFLUENCE OF TEMPERATURE ON RATE OF GROWTH

The influence of temperature on the rate of growth was studied in incubators maintained at 10°, 15°, 20,° 25°, 30°, and 35° C. In the preliminary tests, when Petri-dish cultures were piled together in one of the temperature chambers, there was frequently a noticeable difference in growth in the dishes in the center of the pile compared with those at the top or bottom. These differences were most marked in chambers where the temperatures were well above or below that of the laboratory and apparently were dependent on the time required for the plates to reach a temperature equilibrium. Therefore, in subsequent work the medium was poured and the dishes were kept in their respective incubators for several hours to bring them to the desired temperature before inoculating. Transfers were made and later observations were recorded as rapidly as possible, to avoid any serious temperature change while the material was out of the incubator.

As the work progressed it was found that at a given temperature a more vigorous growth frequently occurred if the transfer was made from a culture grown at this same temperature than if the transfer was made from a culture grown at some other temperature. Thus, if two cultures were kept at 15°, one inoculated from a culture grown at 15° and the other from one at 25°, the transfer from the 15° plate would make a much more rapid growth. This behavior was more marked in the case of certain cultures than in others. To avoid this difference, therefore, inoculations for any temperature were made from a plate previously kept at that same temperature.

When transfers were made from the same plate and even from corresponding segments of mycelium there was some noticeable difference in the rapidity with which the new cultures started growth, although when once established they grew at very nearly the same rate. To avoid this initial difference, measurements were made about 24 hours after transferring, at which time new growth had

started in most cases. Readings were again made when the mycelium had reached the edge of the plate at the optimum temperature. From these measurements the rate of radial growth per hour during this interval was calculated. Figure 1 shows the average growth for a typical temperature series, based on these calculations.

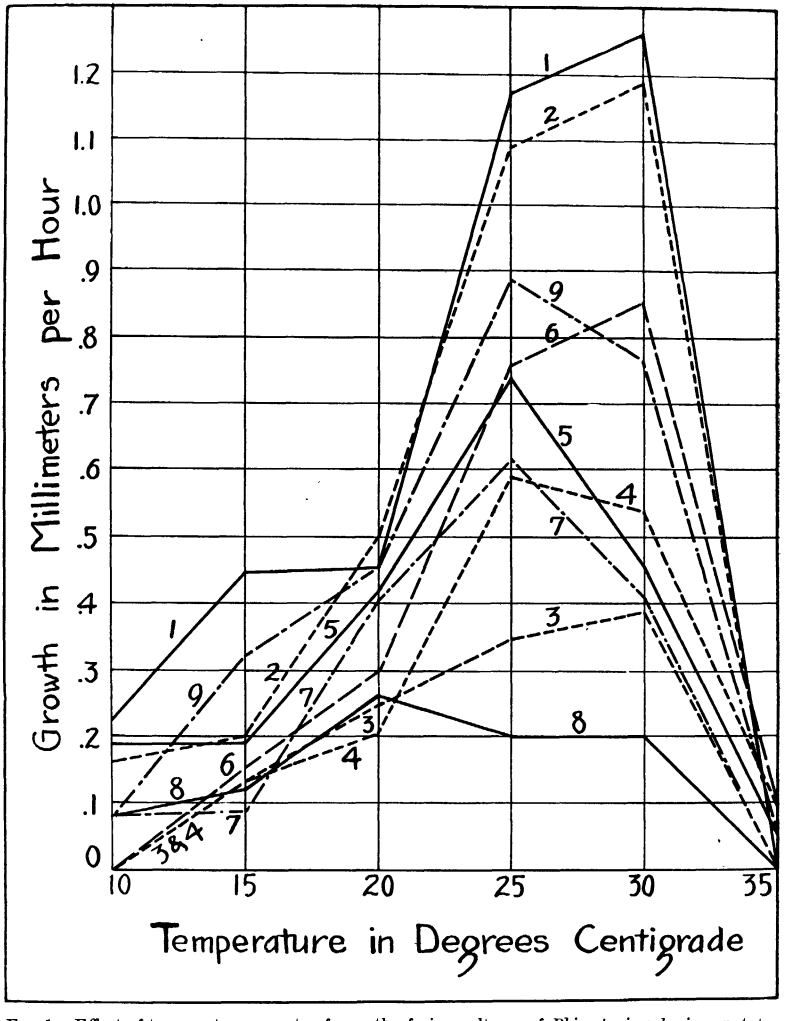

FIG. 1.—Effect of temperature on rate of growth of nine cultures of *Rhizoctonia solani* on potato-dextrose agar

It will be seen that in general the optimum temperature for growth was 25° or 30°. The two grass strains (1 and 2) showed a similar response to temperature, as might be expected, since their origin was similar. At 25° and 30° these cultures were much more vigorous than any of the potato strains. The difference between them and certain potato strains, however, was not so great as the difference existing between various strains isolated from potato tubers. In

comparing the curves it is seen that the cultures which had been kept on artificial media for the longest periods, namely, 3, 4, and 8, grew less vigorously than some of the more recent isolations. This might indicate a decrease in vigor due to continued artificial culture. However, it will be noted that the fungus representing the shortest period in culture, No. 7, was also one of the less vigorous group.

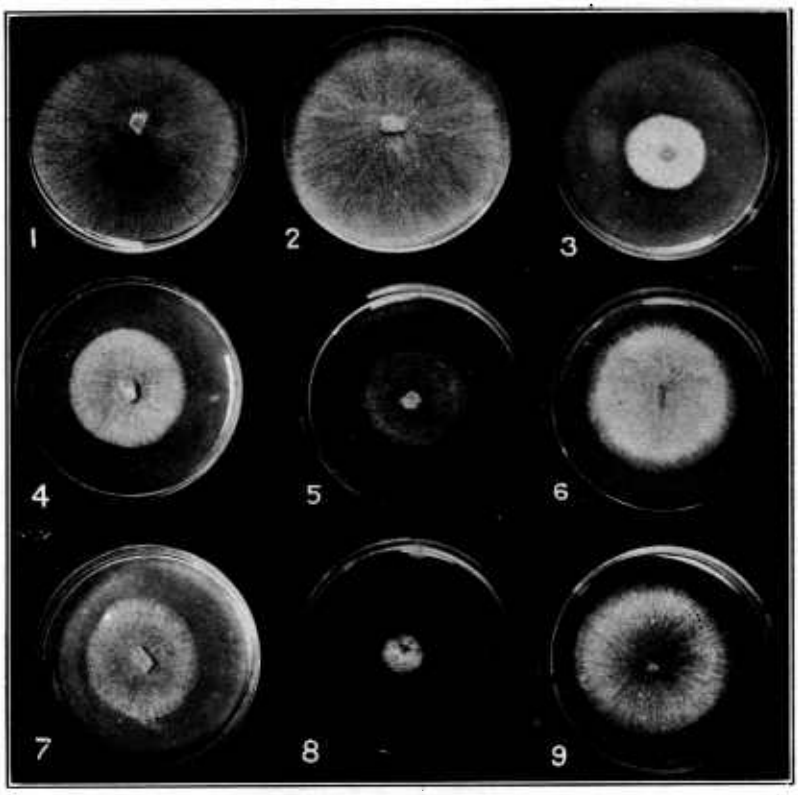

FIG. 2.—Nine cultures of *Rhizoctonia solani* grown on potato-dextrose agar at 25° C. Cultures 1 and 2 were isolated from grass, and the others were obtained from potatoes from different localities. The two from grass could readily be distinguished from the others at this temperature, but, owing to the wide variation shown in the potato strains, such differences would probably be of little value in distinguishing strains if a large collection were studied

INFLUENCE OF TEMPERATURE ON CHARACTER OF GROWTH

When the cultures were several days old there were striking differences in the appearance of the colonies at different temperatures. An attempt was made to distinguish strains based on the character of growth. For this purpose such characters as color of mycelium or sclerotia, discoloration of medium, amount and type of aerial growth, shape and size of sclerotia, and various other such characters were used.

From these observations it was evident that certain cultures could be distinguished readily from others. Figure 2 shows a group of

typical colonies of the same age grown at 25° C. This illustrates the close similarity between the two grass cultures, 1 and 2. These are readily distinguished from such potato cultures as 3 and 6. The differences in these cases, however, are no more marked than are the differences between such strains as 5 and 6, which were both isolated from potato tubers at about the same time. These macroscopic differences were furthermore complicated by changes at other temperatures; for instance, cultures 5 and 7 had very much the same appearance at 25°, but at some of the other temperatures they could be readily distinguished by their growth characters. In some cases there was a striking difference in type of growth with a change of only a few degrees of temperature. Figure 3 shows such a difference in the case of culture 6, which has a decidedly different type of growth

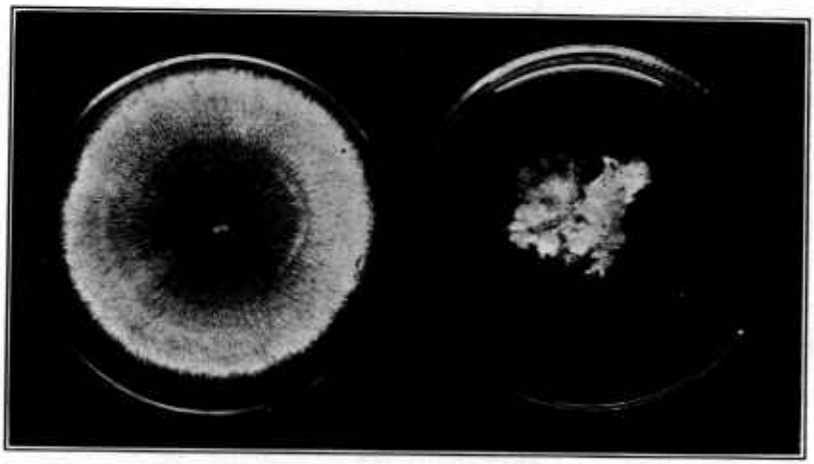

FIG. 3.—Strain No. 6, grown at 33° (left) and 35° C. (right) on potato-dextrose agar. The 33° culture was 3 days old, whereas the 35° culture had been growing 7 days

at 33° and at 35°. In other cases, as in the two grass cultures, the growth characters were sufficiently constant throughout the temperature range to enable one to distinguish them from the other cultures.

The extent to which media are discolored is a characteristic sometimes used in distinguishing strains of Rhizoctonia. Nos. 1 and 2, from grass, did not noticeably darken the agar at any temperature. No. 8 (Rosenbaum's R5 strain) darkened agar very markedly at 20°, 25°, and 30°, but caused no discoloration at 15° C. Cultures 6 and 9 did not blacken the agar at any temperature except 35°, but there the discoloration was very distinct.

Cultures 1, 6, and 7 were grown for three months in flasks containing a mixture of corn meal and sand. These were kept at approximately 15°, 25°, and 30° C. Cultures 1 and 7 produced large compact sclerotia at 15° and much smaller and less compact masses at 30°. Culture 6, on the other hand, did not produce any at 15°, but produced many small sclerotia at 30° C. Observations on color as well as on type of sclerotia failed to reveal any reliable means for distinguishing the grass strain from the fungus found on potato.

INFLUENCE OF TEMPERATURE ON DIAMETERS OF HYPHAE

To determine the extent to which the diameters of the hyphae might be used as a guide to distinguish strains, a series of measurements was made on three cultures growing at 15°, 20°, 25°, and 30° C. When these were 2 days old, cover glasses were placed over the outer edges of the colonies and measurements were made near the tips of the new hyphae, a point just back of the origin of the most recent lateral branch being selected. One hundred such measurements were made on duplicate plates for each temperature. The averages of these measurements are given in Figure 4, which shows the variation found in the diameters of the hyphae grown at different temperatures. It will be noted that the grass culture tended to increase rapidly in size with increase in temperature. This corresponds fairly closely with the increased radial growth illustrated in Figure 1. On the other hand, the other two from potatoes in general showed a tendency toward a decrease in diameter of hyphae with increase in temperature. These differences, however, are comparatively slight. A comparison of the diameter of growth at 15° and 30°, especially in the cases of 1 and 5, indicates how confusing it may be to use diameters of hyphae as a characteristic for distinguishing strains if the temperature factor is not considered.

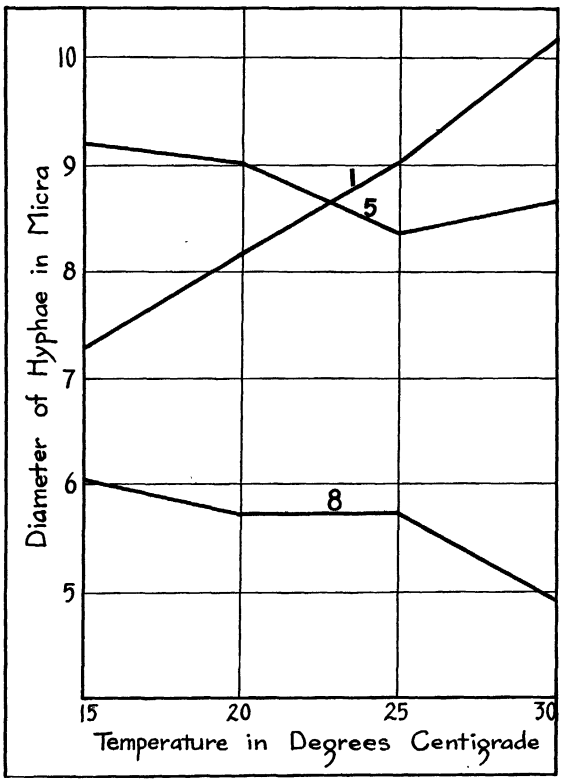

FIG. 4.—Variations in diameters of terminal hyphae in three strains of *Rhizoctonia solani* grown on potato-dextrose agar at different temperatures

INFLUENCE OF ACIDITY OF MEDIUM ON GROWTH

Rhizoctonia is apparently able to grow over a wide range in degree of acidity or alkalinity. Variations in acidity of the culture medium, as expressed on the P_H basis, produced marked variations in the growth character of different cultures. Thus, color variations, from hyaline to dark brown, were observed in mycelium, the darker color occurring usually near the neutral point. The type and color of sclerotia in each culture also showed marked variations due to degree of acidity. These differences, however, were not confined to, nor

sufficiently correlated with, any one culture or group of cultures in a way to justify their use as a key to differentiating strains.

SUMMARY AND CONCLUSION

In comparing on media cultures of Rhizoctonia isolated from diseased grass, numerous minor differences were found between them and cultures of this fungus isolated from potato. An attempt was made to determine to what extent these differences could be used to define the relationship of the Rhizoctonia found on grass to that obtained from potato or other hosts. Cultures of *Rhizoctonia solani* from different sources were therefore compared on several media under various conditions of temperature and acidity.

Using as a criterion various macroscopic and microscopic characteristics of growth such as have been used at times to define strains or even "species" of Rhizoctonia, it was found that temperature and acidity of the medium frequently so modified these characters that they appeared unreliable as dividing lines between species or even "strains" unless much detailed definition of certain environmental factors be included. Two cultures isolated from grass under most of the conditions studied could be distinguished from all other cultures of Rhizoctonia under observation. However, since each culture isolated from potato gave a somewhat different response, it is quite probable that if a sufficient number had been included there would have been found gradations covering the whole range between any of the extremes observed in this work.

The most outstanding distinction found in the grass fungus was its more rapid growth, especially at 25° and 30° C. This, however, is more likely to be a coincidence than an actual difference existing between all Rhizoctonia on grass and on potato.

From this study it is therefore concluded that the morphological and physiological differences observed between the Rhizoctonia causing large brown-patch disease on grass and stock cultures of *Rhizoctonia solani* are not sufficiently fixed and definite to serve as a reliable means for separating it from the variable fungus found on potato and other plants which is at present included in the species *R. solani*.

LITERATURE CITED

(1) Briton-Jones, H. R.
1924. strains of rhizoctonia solani kühn (corticium vagum berk. & curt.) Brit. Mycol. Soc. Trans. 9: 200–210.

(2) Duggar, B. M.
1915. rhizoctonia crocorum (pers.) dc. and r. solani kühn (corticium vagum b. & c.) with notes on other species. Ann. Missouri Bot. Gard. 2: 403–458, illus.

(3) Matsumoto, T.
1921. physiological specialization in rhizoctonia solani kühn. Ann. Missouri Bot. Gard. 8: 1–62, illus.

(4) Matz, J.
1921. the rhizoctonias of porto rico. Jour. Dept. Agr. Porto Rico, v. 5, no. 1, 30 p., illus.

(5) Peltier, G. L.
1916. parasitic rhizoctonias in america. Ill. Agr. Expt. Sta. Bul. 189, p. 281–390, illus.

(6) Piper, C. V., and Coe, H. S.
1919. rhizoctonia in lawns and pastures. Phytopathology 9: 89–92, illus.

(7) Thomas, K. S.
1925. onderzoekingen over rhizoctonia. 97 p., illus. Utrecht.